INTERNATIONAL SYMPOSIUM ON FRONTIERS IN LIFE SCIENCES: MOLECULAR BASIS OF DISEASE, PREVENTION AND TREATMENT

Qingdao (China), 20–23 September 2006
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SECTION 1: TALKS ON SPECIAL TOPICS

AVIAN INFLUENZA VIRUS - ITS THREAT TO HUMAN HEALTH
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Avian influenza H5N1 has been spreading around Asia and extending globally over the last 3 years. Its devastating effect on economy and human health is tremendous. The potential of emerging to pandemic is real and imminent. Influenza pandemic is a rare event occurred only thrice in the last century. Theoretically, it requires a cascade of events starting from the emergence of a super strain from nature, most probably the aquatic bird species, wide spread propagation among animal reservoir, repeated human exposures for adaptation and mutation, and finally gaining human-to-human transmission efficiency. How far have the current strains of H5N1 reached? How long and how likely can the virus complete the remaining steps? Avian influenza infection rarely occurs in humans because of the difference in receptor recognition for human and avian viruses. Even when avian viruses can successfully establish infection in humans, they are not necessary more severe. For instance, H9 causes mild respiratory tract illnesses, H7 mainly causes conjunctivitis. Why H5N1 infection in humans has a fatality of more than 50%? Obviously, H5N1 is not just a severe variant of human influenza. It is characterized by hypercytokinemia, haemophagocytic syndrome and multi-organ failure. These clinicological-pathological consequences cannot be explained solely by the naive host immunity. What do we know about the pathogenesis of avian influenza? Human influenza viruses preferentially recognize host receptors with a 2,6-linked sialic acid, whereas avian viruses recognize 2,3-linked sialic acid. Recent data indicate that such receptor specificity, in addition to provide a barrier for cross-species infection, may also play a role in determining the pathogenesis and transmission. There are many subtypes (16 H, 9 N) of influenza viruses circulating in nature. Apart from H5N1, are there other subtypes that we should be aware of?

SYNAPTIC TRANSMISSION IN GLOBUS PALLIDUS - AN ELECTROPHYSIOLOGICAL, IMMUNOCYTOCHEMICAL AND BEHAVIORAL STUDY OF GABA_β NEUROTRANSMISSION
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The globus pallidus occupies a critical position in the ‘indirect’ pathway of the basal ganglia and, as such, plays an important role in the modulation of movement. In recent years, the importance of the globus pallidus in the normal and dysfunction basal ganglia is emerging. However, the function and operation of various neurotransmitter systems in this nucleus are largely unknown. GABA is the major neurotransmitter used in the globus pallidus. By means of electrophysiological recording, immunohistochemistry and behavioral studies, new information on the distribution and functions of the GABA_β neurotransmission in globus pallidus has been generated. At subcellular level, immunolabelling for GABA_β₁ was localized within the cytoplasm whereas most of GABA_β₂ labelling was associated with the plasma membrane. Both GABA_β₁ and GABA_β₂ immunogold labelling was localized at pre- and postsynaptic sites. At asymmetric, putative excitatory, synapses, GABA_β₁ and GABA_β₂ immunogold labelling was found at presynaptic sites of both pre- and postsynaptic specializations. Double immunolabelling, using the vesicular glutamate transporter 2 (VGLUT2), revealed the glutamatergic nature of most immunogold-labelled asymmetric synapses. At symmetric, putative GABAergic, synapses, including those formed by anterogradely-labelled striatopallidal terminals, GABA_β₁ and GABA_β₂ immunogold labelling was found in the main body of both pre- and postsynaptic specializations. Consistent with the morphological results, whole-cell patch-clamp recordings showed that activation of GABA_β₂ receptors in globus pallidus reduces the release of GABA and glutamate by activating presynaptic auto- and heteroreceptors, and hyperpolarizes pallidal neurons by activating postsynaptic receptors. In agreement with the in vitro effect, unilateral microinjection of GABA_β₂ receptor agonist into the globus pallidus induced ipsilateral rotation in behaving animal. Furthermore, activation of GABA_β₂ system in globus pallidus completely suppressed PTZ-induced tonic seizures and reduced the mortality rate. These results suggest the existence of pre- and postsynaptic GABA_β₂ receptors in the globus pallidus which play an important role in the regulation of movement by modulating synaptic transmission.

ESTROGEN: NEUROPROTECTION IN THE NIGROSTRIATAL DOPAMINERGIC SYSTEM
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Administration of estrogen (E) prior to treatment with a nigrostriatal dopaminergic (NSDA) neurotoxin reduces the amount of striatal dopamine (DA) depletion, suggesting that E can exert a neuroprotective effect within the NSDA system. In this presentation we review the conditions under which E can function as a NSDA neuroprotectant against methamphetamine (MA)-induced striatal dopamine (DA) depletion. This capacity for E to function as a NSDA neuroprotectant against MA is sexually dimorphic. While E treatment of either intact or gonadectomized female mice is effective in diminishing the amount of striatal DA depletion resulting from MA, no such effects are observed in identically treated intact or gonadectomized male mice. This E must be administered prior to MA treatment. Treatment with E at 15, 30, 60 or 120 minutes following MA was ineffective in decreasing the amount of striatal DA depletion to this neurotoxin. In contrast, when administered as early as 30 minutes prior to MA, there is evidence for a positive effect of E in reducing striatal DA depletion to MA. Gonadectomized female mice are quite sensitive to the neuroprotectant quality of E, with doses of estradiol benzoate as low as 1 μg being effective in diminishing MA-induced striatal DA depletion. If neonatal female mice pups are masculinized by treatment with testosterone within postnatal days 2-5, E remains an effective NSDA neuroprotectant against MA-induced striatal DA depletion when these females are tested as adults. If neonatal male mice pups are feminized by gonadectomy within postnatal days 2-5, E remains incapable of showing any neuroprotection against MA-induced striatal DA depletion when tested as adults. In this way, the sexually dimorphic responses to E neuroprotection of the NSDA system against MA are not altered by neonatal manipulations which can change the sexual phenotype of the mice. On the other hand, if female mice are gonadectomized prior to puberty, E can no longer function as a neuroprotectant of the NSDA system against MA in adult female mice. Interestingly, when treated with the anti-estrogen, tamoxifen, a neuroprotective response against MA was observed in both intact female and male mice. However, if the tamoxifen was administered simultaneously with E within the gonadectomized female mouse, the tamoxifen blocked the neuroprotectant capacity of E. Taken together, these data demonstrate that E can be effective in diminishing the amount of striatal DA depletion to MA-induced striatal neurotoxicity and illustrate the conditions under which E can display this capability. Such findings can help in understanding some of the bases for gender differences in responses to MA as well as to neurodegenerative disorders of the NSDA system.

THE APPLICATION OF TISSUE ENGINEERING IN NEUROREGENERATION
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Peripheral nerve injuries are common in clinical practice due to trauma or deliberate surgical resection. A major problem related to the treatment of peripheral nerve defects is the bridging of long gaps between the cut ends of transected nerves. Recent advances in nerve tissue engineering have greatly promoted the generation of nerve graft. To bridge larger nerve defects and support nerve regeneration, research interest in artificial nerve grafts has been focused on selecting bioreorbable materials to construct nerve graft. We have developed an artificial nerve graft composed of a chitosan conduit inserted...
with longitudinal PGA filaments. To explore feasibility for peripheral nerve implantation, the artificial nerve graft was utilized to bridge dog sciatic nerve across a 30-mm long defect. The repair outcome was investigated by using a variety of histological and electrophysiological methods. After 6 months, it was observed that sciatic nerve trunk had been reconstructed with restoration of nerve continuity, functional recovery for conducting electrical impulses and transporting materials, and re-innervation of target skeletal muscle. It was observed that the sciatic nerve was able to regenerate and restore function. This study will allow improvements to meet clinical trial requirements in the future. (Supported by NSFC Grant No.30540063 and Jiangsu Province Grant No. BK2005202)

ADVANCES IN MODEL ORGANISMS OF ALZHEIMER’S DISEASE, PARKINSON’S DISEASE AND ALS TO DEFINE MOLECULAR MECHANISMS OF NEURON DEGENERATION
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Neurodegenerative diseases include a variety of central nervous system disorders that are characterized by the progressive loss of neuronal cells. It is believed that the neurodegenerative diseases are caused by genetic and environmental factors or in combination of both. Several disease-associated genes and neurotoxins have been identified recently in a small percentage of patients with Alzheimer’s disease, Parkinson’s disease and ALS. The advances in the research of model organisms will greatly help our understanding of the molecular mechanisms of these identified genetic or environmental factors, and it may also assist in finding novel genes and pathogenetic pathways. In this regards, our neurogenomic lab in collaboration with Shanghai Research Center for Bio-Model Organisms and State Key Lab of Medical Genomics of Jiao Tong University School of Medicine, has developed several model organisms platforms, which include transgenic or knock-out mice, zebrafish and e-legans models of Alzheimer’s disease, Parkinson’s disease, and ALS. Using these animal models we are investigating the genotypetype correlations and the detailed molecular pathways leading to these major neurodegenerative diseases. With these new approaches, establishing early diagnosis and identifying potential drug targets for these neurodegenerative diseases will become possible.

INVESTIGATION OF HEPATITIS B VIRAL DNA INSERTIONS IN HEPATOCELLULAR CARCINOMA
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Chronic infection of the viral hepatitis B (HBV) is a major etiologic risk factor in the development of hepatocellular carcinoma (HCC). Despite viral DNA integration into the tumor genome is a common phenomenon, the role of HBV integrants in liver carcinogenesis remains unclear. In an effort to gain insights into the viral integrants, we examined HCC cell lines and primary tumors that were derived from chronic carriers of HBV for the viral integration sites by high-throughput analysis of restriction site-PCR. Interruption of cellular genes was indicated at chromosome locations including 2q14.2, 2q35, 6p11.1, 8p11.21, 16q21 and 19q13.12. Alignment of integrants suggested the sites of integration to occur in common chromosome fragile sites and within the vicinity of important cancer-related genes, including cadherin-11 (CDH11), astrocyte-derived trophic factor 1 (ATF1), ankyrin (ANK1) and transformation/transcription domain-associated protein (TRRAP). Of interest were consistent insertions of the viral sequences were found in introns or non-coding regions of the host genome. Disruption of HBV’s sequences, on the other hand, involved breakpoints within a 500bp sequence between the core and HBx regions. In summary, viral integrants juxtaposition to cellular genes may have implication in a disrupted gene function that may confer growth advantages to infected hepatocytes. Consistent with the notion that HBx is a transactivator of host cellular genes, recurrent truncated HBx sequences observed in this study provide further support for its role in HBV-induced liver carcinogenesis.

PHOTODYNAMIC THERAPY ON MALIGNANT AND BENIGN SKIN DISEASES
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Photodynamic therapy (PDT) involves photoactivation of a photosensitizer in the presence of light and molecular oxygen. After excitation with visible light, singlet oxygen and other reactive oxygen species (ROS) are generated by either energy or electron transfer. Promising results have been shown in treating various skin malignancies and selected benign skin disorders with PDT. This review will focus on the utilization of PDT from bench to bedside in dermatology in Taiwan. We began to use 5-aminolevulinic acid (ALA) as photosensitizer and a prototype of red light emitting diode arrays as the light source to treat patients with basal cell carcinoma, squamous cell carcinoma in situ and actinic keratosis in 1998. Excellent cosmesis without tumor recurrence was noted at 4-year follow-up in more than 90% patients. In addition to PDT, in vivo fluorescence detection of tumor cells after ALA administration, a procedure known as photodynamic diagnosis, has been helpful in defining surgical margin for extramammary Paget’s disease. We recently demonstrated antimicrobial effects of PDT in treating Vibrio vulnificus infection in mice. PDT could prevent around 50% of mice from fatal wound infection. PDT also improved wound healing in patients with recalcitrant wound infections. Taking together, PDT is expected to gain more and more acceptance to physicians and the public.

A NOVEL ANTIARRHYTHMIC TARGET: M3R/IKM3
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This study was designed to explore the possible role of M3 subtype of acetylcholine muscarinic receptors (M3-mAChR) in cytoprotection of myocardial infarction. Studies were performed in a rat model of myocardial infarction and in isolated myocytes. We found that choline diminished ventricular arrhythmias during ischemia, which was achieved by correcting hemodynamic impairment, and protecting cardiomyocytes from apoptotic death. The beneficial effects of choline were reversed by the M3-selective antagonists but not by the M2-selective antagonist. Choline/M3-mAChR activated several survival signaling molecules (antiapoptotic proteins Bcl-2 and ERKs), increased endogenous antioxidant reserve (SOD), and reduced apoptotic mediators (proapoptotic proteins Fas and p38 MAPK) and intracellular Ca2+ overload. In addition, we also found that administration of choline attenuated the ischemia-induced suppression of the association between connexin 43 and M3-mAChR. We concluded that choline reduced ischemic arrhythmias via stimulating the cardiac M3-mAChRs which in turn result in alterations of multiple signaling pathways.

SYNERGISTIC FUNCTION OF SMAD4 AND PTEN IN SUPPRESSING FORESTOMACH SQUMOUS CELL CARCINOMA IN THE MOUSE
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The genetic bases underlying esophageal tumorigenesis are poorly understood. Our previous studies have demonstrated that coordinated deletion of the Smad4 and PTEN genes results in accelerated hair loss and skin tumor formation in mice (Yang L et al: Cancer Research, 2005, 65:8671-8678). Herein we exemplify that the concomitant inactivation of Smad4 and PTEN accelerates spontaneous forestomach carcinogenesis at complete penetrance during the first two months of age. All of the forestomach tumors were invasive squamous cell carcinomas (SCCs), which recapitulated the natural history and pathological features of human esophageal squamous cell carcinomas (ESCCs). A small population of
the SCC lesions was accompanied by adencarcinomas at the adjacent submucosa region in the double mutant mice. The rapid progression of forestomach tumor formation in the Smad4 and PTEN double knockout mice corresponded to a dramatic increase in esophageal and forestomach epithelial proliferation. The decreased expression of p27, p21 and p16, together with the overexpression of cyclin D1 contributed cooperatively to the accelerated forestomach tumorigenesis in the double mutant mice. Our results point strongly to the crucial relevance of synergy between Smad4 and PTEN to suppress forestomach tumorigenesis through the cooperative induction of cell cycle inhibitors.

SECTION 2: GENERAL SUBMISSIONS

NEUROSCIENCE AND NEURODEGENERATIVE DISEASES

EFFECTS OF NEUROTENSIN ON EXPRESSION OF MU OPIOID RECEPTOR mRNA - AN EXPERIMENTAL STUDY
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In the central nervous system, the opioids transmitter and non-opioids transmitter system jointly complete multiple pathophysiological process. Many studies indicated that neurotensin can effectively increase the threshold of pain, and enhance analgesia induced by morphine or acupuncture. The neuron model of rat caudate putmen culture in vitro was used to examine the effects of neurotensin and/or morphine on the expression of mu-opioid receptor mRNA. The results showed that neurotensin (10^4mol/L) promoted but morphine (10^5 mol/L) reduced the mu-opioid receptor gene expression. More interestingly, when the cells incubated by neurotensin (10^-10 or 10^-8 mol/L) plus morphine, the inhibition of mu-opioid receptor by morphine was almost missing. This study provided experimental evidence for partially explaining the mechanisms of neurotensin analgesia and neurotensin-enhanced acupuncture analgesia at molecular and receptor levels. Mouse tail reaction test was carried out to examine the effects of neurotensin on the mouse tail erection induced by morphine, and investigate the effects of neurotensin on the morphine addictive ethology. Our results showed that, mice were significantly marked by tail erection, continuously running and jumping after injection of morphine (15 mg/kg). However, the mouse tail reactions were inhibited to some extent after being pretreated with 100 μg/kg or more of NT. After morphine injection in the mice pretreated with neurotensin 300 μg/kg, no tail reaction was noted, and the mice appeared to be quiet and less active. There was a certain paralleled correlation between mouse tail reaction and the addiction of narcotic analgesia. Morphine has a strong addiction, so the mice have a higher tail erection index. Neurotensin could effectively inhibit the tail-erecting reaction at higher concentrations, which suggested that it can be partly antagonistic to morphine addiction, and this effect of neurotensin may be associated with its up-regulating mu-opioid receptor and balancing out the down-regulated mu-opioid receptor by morphine to some extent.

INFLUENCE OF NESTIN SMALL INTERFERING RNA (siRNA) ON CELL MORPHOLOGY AND PROLIFERATION OF THE CULTURED C6 ASTROCYTOMA CELLS
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The reactive astrocytes are also found by our previous studies to express nestin and nestin protein may function in cell growth and cell proliferation of astrocytoma cell line. In cultured C6 astrocytoma cells, the nestin small interfering RNA (siRNA) duplex was applied and transfection was finished within about 6 hours. After siRNA transfection, astrocytoma cell culture continued for about 72 hours and the interfering effect of siRNA on nestin expression, cell growth and cell proliferation was examined. It appeared that a single siRNA administration could suppress cell division, cell growth of these astrocytoma cells in vitro in certain extent and detailed data was in analysis. Our pilot observation has supported the hypothesis that nestin might play a role in cell survival and cell proliferation of astrocytoma, suggesting that further siRNA investigation may also enable a potential in the clinical treatment of astrocytoma patients. (Supported by NSFC Grants: 30570640, 30371572 and 30218002)

NOVEL ROLES OF THE REACTIVE ASTROCYTES IN THE BRAIN AND POTENTIAL IMPLICATIONS IN CELL THERAPEUTIC STRATEGY AGAINST PARKINSON’S DISEASE
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Parkinson” disease (PD) is a common and debilitating degenerative disease resulted from massive loss of dopamine neurons in the substantia nigra pars compacta, which is characterized by severe motor symptoms of tremor, bradykinesia, rigidity and postural instability. Protection of nigral dopamine neurons from progressive degenerative death and cell replacement of novel dopamine neurons are hopeful strategies against PD in humans. The reactive astrocytes abundantly occur in brain insults including trauma, ischemia and PD animal models. Although they were traditionally assumed to impede neuronal regeneration by forming glial scars, growing evidence has indicated that reactive astrocytes do offer crucial benefits in functional recovery of brain injuries. The reactive astrocytes can produce various neurotrophic factors for neurons to recover their function and cell replacement of novel dopamine neurons has provided an important clue to develop novel cell therapy for Parkinson’s disease. We have reported that there are significant up-regulation in numbers of reactive astrocytes found in the striatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydroxypridine (MPTP) -lesioned mice, an animal of Parkinson’s disease. The reactive astrocytes are also found by our previous studies to express nestin and...
THE EFFECT OF ACUPUNCTURE ON LEARNING AND MEMORY IMPAIRMENT IN RATS OF HYPOXIC-ISCHEMIC BRAIN DAMAGE
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In this study, we prepared the rats model of learning and memory impairment induced by hypoxic-ischemic brain damage (HIBD) (according to the method of Rice reported in 1982). The animals were divided into 3 groups: a normal control group (Con n=22), an HIBD group (HIBD n=26) and HIBD with acupuncture group (HIBD+ACU n=20). At the postnatal 8 days of animals, acupunctured “BAIHUI” and “DAZHUI” points, 1/d, 20 mins /times, total 10 days. At the postnatal 30 days, learning and memorial behaviors were investigated using the Morris water maze test. The swimming time (29.4±4.05%) and distance (21.8±4.67%) in the target quadrant of the rats of HIBD group was significantly shorter than the other groups (P<0.05). But the difference between the HIBD+ACU group and the HIBD group was statistically significant (P<0.05). In addition to behavioral test, we examined long-term potentiation (LTP) in the hippocampus slice. The changes in slope and amplitude of the population spike (PS) were expressed as percentages of the values of baseline. LTP expression was markedly impaired in HIBD animals as compared to controls (P<0.05). But after acupuncture treated, it was significantly improved as compared to HIBD rats (P<0.05). The above results indicate that the learning and memory ability in the rats of model group was deficient. However, after acupuncture application, the learning and memory capability of the rats has been improved. Acupuncture therapy partially prevents these impairments by improving LTP of hippocampus. Supported by Natural Science Foundation of China (30572416).

EFFECT OF NERVE REGENERATION FACTOR ON THE NEURITE GROWTH OF RAT HIPPOCAMPAL NEURONS
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Nerve Regeneration Factor (NRF) is an active ingredient extracted from an oral liquid of traditional Chinese medicine, named nerve growth decoction, and has been confirmed to be capable of protecting PC12 cells in serum-free medium and has been improved. Acupuncture therapy partially prevents these impairments by improving LTP of hippocampus. Supported by Natural Science Foundation of China (30572416).

DIFFERENTIAL EFFECTS OF ANTIPSYCHOTICS ON DOPAMINE TRANSMISSION IN THE MESOLIMBIC AND NIGROSTRIATAL PATHWAYS
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Although new generation antipsychotic drug aripiprazole has been used effectively to treat schizophrenia in clinic as a partial D2 receptor agonist, its neuropathological mechanism is still not clear. This study compared the effects of three generation antipsychotic drugs, aripiprazole, olanzapine and haloperidol on mesolimbic and nigrostriatal dopaminergic neurotransmissions. Rats were treated with one of the three antipsychotics or vehicle (control group) for 1 or 12 weeks. The binding densities of D2 receptors and dopamine transporters (DAT) were examined using autoradiographic techniques. Compared to control group, haloperidol significantly increased (~20-35%) D2 receptor binding density in the core of accumbens nucleus (AcbC), shell of accumbens nucleus (AcbSh) and caudate putamen (CPU) after both 1 week and 12 weeks treatments, however it significantly decreased (~30-40%) DAT binding density in the AcbC and CPU after 1 week, and in the AcbC and ventral segmental area (VTA) after 12 weeks treatments. Aripiprazole significantly decreased DAT binding density only in the AcbC (~47%) and VTA (~36%) after 1 week treatment. Furthermore, a negative correlation has been found between D2 receptor binding density and DAT binding density in the AcbC (r=0.317, P=0.047). Previously we have found that aripiprazole significantly increased D2 receptor mRNA expression and decreased TH mRNA expression in the VTA. A negative correlation between D2 receptor mRNA expression and TH mRNA expression was found in the VTA. These results suggested that reducing dopamine synthesis in the VTA is the possible mechanism for aripiprazole in controlling the symptoms of schizophrenia effectively. However, haloperidol controls the symptom of schizophrenia by blocked D2 receptors in the AcbC and AcbSh. Olanzapine had no significant influence to D2 receptors in the mesolimbic and nigrostriatal dopamine pathways.

EFFECT OF EXOGENOUS MotILIN IN AMYGDALOID NUCLEUS ON GASTRIC MOTILITY IN RATS AND ITS UNDERLYING MECHANISM
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This study aims to investigate the effect of motilin microinjection into bilateral basomedial amygdaloid nucleus (BMA) on gastric motility in rats and its underlying mechanism. Forty male Wistar rats (200-250 g) were used for four sets of experiments: Experiment (Exp) 1: Animals received bilateral injections of motilin (MT, 1 μg/side) or normal saline (NS, 0.5 μL/side) into BMA, and both intragastric pressure (IGP) and gastric motility frequency (GMF) were recorded to estimate the gastric motility (n=14). Exp 2: Pretreatment of vagotomy was carried out in 12 rats, then MT or NS injected into BMA as in Exp1. Exp3: Sixty minutes after MT or NS injection into BMA, c-fos protein expression was detected in the paraventricular nucleus (PVN) (n=7). Exp 4: Motilin contents were measured in five brain areas (hypothalamus, midbrain, pons, medulla, and pituitary gland), using Radioimmunoassay (RIA) method (n=7). Our findings were as follows: (1)Exogenous motilin in BMA enhanced the gastric motility in rats, and this action lasted about 15min. At 10, 15, 20, 25 min following motilin injection, the change in IGP percentage were 19.7±6.5% (P<0.05), 62.9±4.7% (P<0.01), 45.1±7.9% (P<0.01), 29.3±10.3% (P<0.05) ; while the changes of GMF percentage were 10.5±3.23 (P<0.05), 36.7±8.5% P<0.01, 19.5±6.0% P<0.05, 13.6±6.0% (P>0.05). No obvious changes in both IGP and GMF were observed in saline control. (2)Pretreatment of subdiaphragmal vagotomy abolished the enhanced gastric motility induced by motilin injection. (3)Motilin injection into bilateral BMA induced increased numbers of c-fos positive cells in PVN, compared with the saline control (53±48.9 vs 22±5.2, P<0.01). (4) Among five tested brain areas, the highest motilin level was found in the hypothalamus (74.3±19.6 pg/mg tissue). The motilin contents in other four areas ranged from 7.8±2.2 to 17.3±6.6 pg/mg respectively. These results suggest that exogenous motilin in BMA enhance the gastric motility in rats, an effect which might rely on the amygdala -hypothalamus -brain stem-vagus pathway.
NEUROPROTECTIVE EFFECTS OF IRON CHELATOR DESFERAL ON DOPAMINERGIC NEURONS IN THE SUBSTANTIA NIGRA OF RATS WITH IRON OVERLOAD

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The aim of the present study was to investigate whether the iron chelator Desferal prevents the degeneration of dopaminergic neurons in the substantia nigra (SN) induced by iron overload in rats. Using fast cyclic voltammetry, tyrosine hydroxylase (TH) immunohistochemistry, Perls’ iron staining, and high performance liquid chromatography-electrochemical detection, we measured the degeneration of dopaminergic neurons and increased iron content in the SN of rats overloaded with iron dextran and assessed the effects of treatment with Desferal. The results showed that iron dextran overload increased the iron content in the SN, decreased dopamine release and content, and reduced the numbers of TH-immunoreactive neurons. Treatment with Desferal prevented the increased iron content in the SN. As a result, dopamine release and content remained at almost normal levels, while the numbers of TH-immunoreactive neurons remained at control values. This study suggests that the iron chelator Desferal is neuroprotective against iron overload, so iron chelators that can cross the blood-brain barrier may have the potential to treat cases where abnormal iron accumulation in the brain is associated with the degenerative processes, as in Parkinson’s disease. This study was supported by grants from the National Program of Basic Research sponsored by the Ministry of Science and Technology of China (2006CB500704) and the National Foundation of Natural Science of China (No. 30370498; 304001399, 30570649).

NEUROPROTECTIVE EFFECTS OF BAK FOONG PILL EXTRACTS ON MPTP-INDUCED PARKINSON’S DISEASE MOUSE MODEL

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To explore neuroprotective effects and the possible mechanism of the Bak Foong Pill (BFP) extracts in protecting substantia nigra neurons from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced apoptosis in C57BL mice, C57BL mice have been given with MPTP (30mg/kg) i.p. for 5 days to establish the PD model. Bak Foong Pill extracts were given 14 days prior to MPTP in the pretreatment groups. Tyrosine hydroxylase (TH) immunostaining was used to observe the damage of dopamine neurons. The immunohistochemistry assay was used to detect the protein levels of Bax. High performance liquid chromatography-electrochemical detection (HPLC-ECD) was used to investigate the contents of DA and its metabolites dihydroxy-phenylacetic acid (DOPAC) and homovanillic (HVA) in the Str. Pretreatment with BFP extracts markedly increases TH immunostaining and reduces the expression of Bax in the substantia nigra zona compacta compared with the MPTP model group, and increases the content of DA and its metabolites DOPAC and HVA significantly. In conclusion, BFP extracts showed protective effect on dopaminergic neurons against MPTP-induced neurotoxicity in the PD model mice. This neuroprotective effect might be attributed to downregulate the expression of Bax. This project is supported by the Science and Technology Department of Shandong Province (031070125) and Science and Technology Department of Qingdao Municipal Government (04-2-35-136, 05-10-JC-97).

AMELIORATIVE EFFECT OF ACUPUNCTURE ON MEMORY IMPAIRMENT IN DIABETIC RATS WITH CEREBRAL ISCHEMIA

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The aim of this study is to explore the effect of acupuncture on memory impairment in diabetic rat with cerebral ischemia, since diabetes is a major risk factor for the development of ischemic cerebrovascular disease. In the present study learning behavior and electrophysiological measures of cerebral function were examined in streptozotocin (STZ)-induced diabetic rats with cerebral ischemia, and the ameliorative effect of acupuncture on the memory decline was also observed. Behavioral testing consisted of three parts: a passive avoidance response, an active avoidance response and a spatial learning task in water maze. Electrophysiological testing consisted of in vitro assessment of hippocampal long-term potentiation (LTP), an activity-dependent form of synaptic plasticity, which is believed to be related to the cellular mechanisms of learning and memory. The rats were divided into 5 groups: the control group, the ischemia group, the diabetic rats with sham-operation group, the diabetic rats with ischemia group and the group of diabetic rats with cerebral ischemia treating with acupuncture. Rats were tested in the water maze and the avoidance response box 4 weeks after induction of diabetes and cerebral ischemia. Next, LTP was measured in vitro in the trained rats. Both behavioral learning and LTP expression in the CA1 field of the hippocampus were impaired in diabetic rats with cerebral ischemia. In contrast, with acupuncture therapy the learning behavior and hippocampal LTP deficiency was improved in the diabetic rats with ischemia. Our results suggested that hyperglycemia and ischemia accelerated the cognitive impairment and acupuncture therapy can ameliorate it. The beneficial effect of acupuncture therapy on both the behavioral performance and hippocampal LTP indicates that acupuncture is effective in the treatment of cognitive impairment induced by diabetes with cerebral ischemia.

DETERENDED FLUCTUATION ANALYSIS OF ION SINGLE CHANNEL SIGNAL

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The gating of ion channels has widely been modeled by assuming the transition between open and closed states is a memoryless process. Nevertheless, the statistical analysis of an ionic current signal recorded from Voltage dependence K+ single channel is presented. The ionic current based on digitized signals is analyzed by means of detrended fluctuation analysis. The detrended fluctuation analysis (DFA) exponent α is significantly larger than 0.5, (DFA) exponents were calculated for four different pipette potentials in rat dorsal root ganglion neurons. A = 0.9475 ± 0.006 (n=10) for V = -30mV; α = 0.958 ± 0.004 (n=9) for V = -40mV; α = 0.966 ± 0.005 (n=11) for V = -50mV; α = 0.971 ± 0.03 (n=10) for V = -60mV. The detrended fluctuation analysis of the results provides evidence for the existence of memory. When above methods were applied to the results from simulated Markovian model, it showed that it had different DFA exponents α. The main outcome of this study indicates that long-range correlation effect is present between continue conducting states of the ion channel. The results suggest the correlation character of ion channel protein nonlinear kinetics regardless of whether the channel is in open or closed state.

THE EXCITABILITY OF CA1 PYRAMIDAL NEURONS ON RAT SLICE DURING THE EPILEPTOGENESIS INDUCED BY LOW DOSAGE OF VERATRIDINE

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The cellular mechanism of epileptogenesis has not been fully understood. PDS (paroxysmal depolarizing shift) is regarded as the marker of epileptic discharges inside the single neuron. We induced PDS-like activities on rat slice by low dosage of veratridine. When the slice epilepsy model was established, we adopted whole-cell recording by infrared visual patch clamp to observe the electroactivities of CA1 pyramidal neurons. We found that the PDS-like activities were not blocked by the synaptic transmission blocking cocktail [CNQX (5 μM) + AP-5 (12.5 μM) + Bic (10 μM)], that suggested the PDS-like activities were not synaptic. During the excellerated perfusion of low dosage of veratridine, the discharges of CA1 pyramidal were observed per 2 minutes, and a depolarizing ramp stimulations (0~300 pA, 1000 ms) were given under current clamp

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configuration to measure the AP (action potential) numbers and the threshold potential of the first AP during the stimulation. The results showed that in the epileptogenesis induced by low dosage of veratridine, the distance between membrane potential and threshold potential became increasingly smaller and triggered APs became increasingly more. On the other hand, we found 80 nm TTX could block the PDS-like activities effectively. So a series of depolarizing step (5 nV, 800 ms) were adopted under the voltage clamp configuration to measure the subthreshold TTX-sensitive INaP (persistent sodium current). The results showed the INaP became bigger when PDS-like activities occurred induced by low dosage of veratridine. Our conclusion: the excitability of CA1 pyramidal enhanced in the epileptogenesis induced by low dosage of veratridine and was associated with the enlarging INaP.

THE NEURAL MECHANISM OF LIPOPOLYSACCHARIDE-INDUCED AIRWAY HYPERRESPONSIVENESS
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The present experiment was designed to investigate the effect of repetitive intraperitoneal administration of lipopolysaccharide (LPS) on airway hyperresponsiveness (AHR) and explore its mechanism. The guinea pigs were divided into normal saline (NS)-treated control group and LPS-treated group in which the guinea pigs were injected intraperitoneally with LPS (1 mg/kg) daily for three consecutive days. On the animals anesthetized with pentobarbital sodium the respiratory rate, tidal volume, airflow, esophageal pressure, femoral arterial blood pressure and electrocardiogram (ECG) were measured, then the airway resistance (Raw) changed by inhaled aerosol of histamine (as airway responsiveness), and heart rate variability (HRV) were calculated. The nitric oxide (NO), nitric oxide synthase (NOS) activities, and total as well as differential cell counts in the bronchoalveolar lavage fluid (BALF) were measured. The airway responsiveness was significantly higher in LPS-treated group than in control group, meanwhile NO and NOS activities in BALF were obviously increased in LPS-treated group compared with control group. The power spectrum analysis of HRV showed that there was no significant difference between LPS -treated and NS -treated group, except that the total variance in LPS treated group was bigger than that in the control group. The respiratory rate, heart rate, blood pressure and cell counts in BALF did not differ statistically in LPS-treated and NS -treated guinea pigs both L NAME (non-selective NOS inhibitor), and aminoguanidine (AG), a selective inducible NOS (iNOS) inhibitor, resulted in the decrease of NO in BALF. AG significantly inhibited the persistent AHR of LPS-treated guinea pigs, but L NAME was potential to enhance the AHR induced by LPS. In conclusion, the results suggest that the inhibitory nonadrenergic noncholinergic (i-NANC) nervous system is likely to contribute to the LPS-induced persistent AHR. The vagal efferent nerve may not be involved in the development of the LPS-induced persistent AHR. The NO and NOS may play an important role in pathogenesis of AHR.

GLUCOCORTICOIDS TREATMENT OF MICROGLIA MAY IMPAIR ADAPTIVE IMMUNE RESPONSES IN THE CNS DURING STRESS
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Glucocorticoids (GC) are essential neuroendocrine regulator of the immune system during stress and prolonged psychological stress has been shown to be immunosuppressive. However, little is known about how GC influence the adaptive immune response or the role of microglia, the most potent antigen presenting cell (APC) residing in the central nervous system (CNS). Therefore, we investigated whether GC can modulate microglia function and their effects on T cell responses in vitro. In LPS-stimulated microglia, GC reduced the secretion of the pro-inflammatory cytokines IL-12, IL-6 and TNF-α. Expression of MHC class II, CD40, and CD80 on microglia was suppressed, but expression of co-inhibitor B7-DC was up-regulated. In addition, GC directly induced apoptosis of microglia. As a result, GC pretreatment of microglia reduced their ability to stimulate CD4+ Th cell proliferation in response to Con A. GC treatment also reduced the ability of microglia to promote the secretion of the type 1 cytokine IFN-γ in CD4+ Th cells while up-regulating their capacity to promote the secretion of the type 2 cytokine IL-4 and IL-10. Our data suggest that the effects of GC on microglia may contribute to the stress-induced suppression of host defenses in the CNS.

INVESTIGATION OF PROTEIN EXPRESSED PATTERN IN THE DIFFERENTIATION OF RAT BONE MARROW STROMAL CELLS INTO SCHWANN CELL-LIKE CELLS
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During the last decade, there is increasing research evidence that bone marrow stromal cells (MSC) have the potential to differentiate into neural lineages. Most of these studies show that MSC changed morphologically and expressed some proteins specific for neural cells in different conditions, there are also studies on differentiated MSC by microarray analysis. But there still have no reports about proteomic studies on differentiated MSC. In this study, we isolated MSC from adult Sprague-Dawley rat femur and tibia bone marrow and induced MSC differentiate into Schwann cells with BME, RA, FSK, bFGF, PDGF, HRG in culture. Then we investigated MSC changed expressing the protein in conditional induction to differentiate into Schwann cell-like cells by using two-dimensional gel electrophoresis (2-DE). We obtained about 792 protein spots in protein map and found 74 spots significant changed (43 spots upregulation and 31 spots downregulation) between MSC and induced MSC by PDQuest software. We analysed these 74 spots by matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) and database searching. These 74 proteins were grouped into different classes, including cytoskeleton and structure proteins (tubulin alpha, vimentin, brain-specific alpha actin 1 isofrom etc.); growth factors (gliarial neurotrophic factor, brain-derived neurotrophic factor etc.); metabolic proteins (UDP-glucose dehydrogenase, eph and elk-related kinase etc.); chaperone proteins (heat shock protein); receptor proteins (Laminin receptor 1, Ly49 stimulatory receptor 3); cell cycle proteins (p27, RAS p21 protein activator 1); calcium binding proteins and other proteins. These results suggest that MSC changed many proteins in conditional induction to differentiate into Schwann cell-like cells. These proteins also include neural and glia proteins such as BDNF, CNTF, ILGF, GFAP, synaptophysin. The results provide valuable proteomic information about differentiation of MSC into Schwann cell-like cells. This work was supported by NSFC grant NO.30540063 and NSF of Jiangsu grant NO. BK2005202.

ASSOCIATION OF NEURONAL CONNEXIN36 WITH THE PDZ DOMAIN-CONTAINING PROTEINS ZONULA OCCCLUDENS-2 (ZO-2) AND ZONULA OCCCLUDENS-3 (ZO-3)
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Gap junctional intercellular communication (GJIC) plays very important roles in maintenance of cellular homeostasis, cell growth and differentiation. Mutations in several gap junction proteins have been shown to cause human disease. To date, ten gap junction proteins (Cx30, Cx31.9, Cx32, Cx36, Cx40, Cx43, Cx45, Cx46, Cx47 and Cx50) have been reported to interact with ZO-1, a PDZ domain-containing protein. The association of neuronal Cx36 with ZO-2 and ZO-3 was investigated using cell culture, transfection and double
immunofluorescence techniques. We found that Cx36 co-localized with ZO-2 and ZO-3 in beta-TC 3 cells and in Cx36-traffected HeLa cells. Co-immunoprecipitation approaches showed that full-length Cx36 co-immunoprecipitated with ZO-2 and ZO-3, but truncated Cx36 with deletion of its last four amino acids (SAYV) did not. By in vitro binding pull-down assay, Cx36 was found to bind to the first PDZ domains of each in ZO-2 and ZO-3, while truncated Cx36 lacking its c-terminus SAYV amino acids failed to bind either ZO-2 or ZO-3. Peptide competition studies showed that a peptide containing the last fourteen amino acids of Cx36 significantly reduced the association of full-length Cx36 with ZO-2 and ZO-3. These results demonstrate that the first PDZ domains of ZO-2 and ZO-3 directly interact with Cx36 through the Cx36-c-terminus SAYV binding motif, and suggest that peptides containing the last 14 amino acids of Cx36 can interfere with Cx36/ZO-2 and Cx36/ZO-3 interaction therefore may be a valuable tool for studying the functional role of PDZ domain containing proteins in the regulation of gap junctions formed by Cx36. Supported by grants from the Canadian Institute of Health Research to JIN.

EFFECTS OF THE INTRAHIPPOCAMPAL CO-INJECTION OF BETA-AMYLOID PROTEIN1-40 AND IBOTENIC ACID ON LEARNING-MEMORY ABILITIES IN RATS
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We investigated the effects of the intrahippocampal co-injection of Aβ1-40 with ibotenic acid (Ibo) on learning and memory in normal and aging model rats and explore the mechanism underlying the effects of the co-injection. The animals were daily administrated with D-galactose (D-gal, 50 mg/kg, i.p.) for 6 weeks to produce the aging model rats. The normal and aging rats were bilaterally injected Aβ1-40 (4 μg for each side) with Ibo (2 μg for each side) into the hippocampus. The spontaneous behavior and learning-memory ability of the rats were tested by using open field, Y-maze and passive avoidance task. And the changes of membrane fluidity in hippocampal synaptosomes, the activity of superoxide dismutase (SOD) and Na+K+ATPase, and the content of MDA in hippocampus were examined. The results showed that the intraperitoneal injection of D-gal provided a significant effect for aging. The co-injection of Aβ1-40 with Ibo induced tested rats a remarkable decrease in the spontaneous behaviors and a significant decline in learning-memory ability. The neurochemical changes induced by the co-injection included a significant decrease in membrane fluidity of hippocampal synaptosomes, a significant decrease in the activity of SOD and Na+K+-ATPase, as well as a remarkable increase in the content of MDA. The results showed that co-injection of Aβ1-40 with Ibo may induce an increase of hippocampal damage by lipid peroxidation, a decrease of membrane fluidity in hippocampus, and a serious deficit in the learning and memory of the rats. The results suggested that the changes of the membrane fluidity in the hippocampus may be closely related to the ability of learning and memory. This work was supported by the Scientific Research Foundation of Qufu Normal University.

MOLECULAR BASIS FOR FETAL ALCOHOL SYNDROME, PREVENTION AND TREATMENT
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We have established a Xenopus laevis (the African clawed frog) embryo model to study the effects and molecular basis of alcohol-induced defects in fetal development. When the embryos were exposed to alcohol, the resultant tadpoles exhibited multiple and distinct morphology defects such as ocular anomalies, microcephaly, delayed gut maturation and growth retardation. We demonstrated that alcohol exerts its teratogenic effects by suppressing the expression of multiple and specific key developmental regulatory genes. Furthermore, antioxidants like catalase and PRDX can protect the embryos from developmental abnormalities by reducing the alcohol-induced overproduction of reactive oxygen species and reactive nitrogen species, which play differential and crucial roles in the downregulation of key genes as well as the alcohol-induced abnormal embryo development. Finally, natural anti-oxidant such as ascorbic acid (Vitamin C) is an effective prevention and treatment agent.

ESTROGEN: NEURODEGENERATION IN THE NIGROSTRIAL DOPAMINOGENIC SYSTEM
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Treatment with estrogen prior to administration of a nigrostriatal dopaminergic (NSDA) neurotoxin reduces the amount of striatal dopamine (DA) depletion, suggesting a neuroprotective effect. However, this capacity for estrogen to function as a NSDA neuroprotectant is not ubiquitous. Here, we show data indicating a neurodegenerative effect of estrogen. Administration of estradiol benzoate (EB, 10 μg) after an initial low dose of methamphetamine (MA) treatment (20 mg/kg×2 at 2h) increases the amount of striatal DA depletion to a second MA treatment versus that of non-EB treated gonadectomized female mice. A similar, but non-significant, trend was present when mice were treated initially with a high dose of MA (20 mg/kg×4 at 2h). No such effects were obtained with gonadectomized male mice. These results suggest that estrogen can increase the amount of striatal DA depletion in female mice with an impaired/ perturbed NSDA system. We next examined whether a lower dose of EB or related agents would exert a similar effect within the impaired NSDA system. Neither a lower dose of EB (1μg), nor any related agents-tamoxifen (TMX, 12.5μg), testosterone (5μg) and dehydroepiandrosterone (DHEA, 3μg) showed enhanced neurodegenerative effects against a second MA invasion. However, EB (10 μg) treatment prior to the first or first and second MA invasion protected the NSDA system against DA depletion. Therefore, a low dose of EB, TMX, testosterone and DHEA cannot exert neurodegenerative effects in the impaired NSDA model. However, EB administered prior to neurotoxin administration protects the NSDA system. These results suggest a basis for variations in the effects of estrogen in the NSDA disorder, Parkinson’s disease as both positive and negative effects of estrogen can be obtained as a function of the conditions of the estrogen administration and NSDA system.

EFFECTS OF NEUROTENSIN AND MORPHINE ON GENIC EXPRESSION OF MU AND DELTA OPIOID RECEPTOR
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Endogenous opioid system and non-opioid system play a very important role in the modulation of central pain sensation by close association. Our previous studies also demonstrated that neurotensin, a non-opioid modulator, can enhance analgesia induced by morphine or acupuncture. This experiment examined the effects of neurotensin or morphine on the mu, delta and kappa opioid receptor mRNA expression of rat-caduate putamen neurons cultured in vitro. The results showed that the levels of mu and delta opioid receptor mRNA decreased after administration of morphine, which persisted up to 48 h. Morphine produced a significant fall of mu opioid receptor mRNA at 10⁻⁶ mol/L, which had a dose-dependent relation within the range of 10⁻⁸ mol/L - 10⁻¹⁰ mol/L. The decreased delta opioid receptor mRNA only occurred at 10⁻⁶ mol/L. While neurotensin increased mu and delta mRNA at 10⁻¹⁰ mol/L, and the effect persisted up to 48 h. The higher dose of neurotensin, the more obvious the effect. Previous data indicated that drug dependence or addiction caused by long-term use of morphine-like analogues may be closely correlated with the down-regulation of opioid receptors. By contrast, the up-regulated receptors can enhance sensitivity to opioid drugs. In analysis of the data from our experiment, neurotensin can up-regulate mu and delta opioid receptor compared with morphine’s down-regulating. This study provided an experimental evidence for partially explaining the mechanisms of neurotensin analgesia and neurotensin-enhanced acupuncture analgesia at molecular and receptor levels: the effects of neurotensin in enhancing analgesia.
of opioid and acupuncture may be associated with up-regulation of mu and delta-opioid receptor. Moreover, it should attract our attention to whether the neurotensin effect has the antagonism for opioid addiction. Different effects of neurotensin and morphone on opioid receptors seemed to have a close association, but the route and mechanism of action remain to be further studied.

NEUROPROTECTIVE EFFECTS OF GENISTEIN ON DOPAMINERGIC NEURONS IN THE RAT MODEL OF PARKINSON’S DISEASE

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Studies provide growing evidence for marked sex differences in the incidence of Parkinson’s disease (PD) that are largely attributed to the neuroprotective effects of estrogen. Estrogen administration significantly attenuates the degree of striatal dopamine depletion to 6-hydroxydopamine (6-OHDA)-induced dopaminergic toxicity. Genistein, a well-known phytoestrogen, has long been recognized to have estrogen-like activities. However, little is known about the effects of genistein on dopaminergic neurons. The present study was performed to determine the effects of genistein or 17-β-estradiol on 6-OHDA-lesioned ovariectomized (OVX) rat female model of PD. Ovariectomized rats were treated with genistein or 17-β-estradiol intracerebroventricularly for four weeks after unilateral injection of 6-OHDA into the medial forebrain bundle (MFB). Tyrosine hydroxylase (TH) immunohistochemistry confirmed that rats injected with 6-OHDA had a massive loss of TH immunoreactivity in the ipsilateral substantia nigra (SN) compacta compared to the contralateral side (P<0.05). Genistein treatment significantly prevented 6-OHDA-induced loss of TH immunoreactive neurons (P<0.05). The same result was got for estrogen treatment. Moreover, an increase of Bcl-2, an antiapoptotic protein, was detected after genistein or estrogen treatment compared with 6-OHDA injection (P<0.05). We also detected the iron overload of SN using Perls’ iron staining. The iron staining was significantly increased in the ipsilateral SN compared to the contralateral side (P<0.05), while genistein treatment could reverse the increase of iron staining (P<0.05). Estrogen also reversed the iron overload completely. These results suggest that genistein has protective effects on the dopaminergic neurons in the 6-OHDA-induced rat model of PD and these effects are related to antiapoptosis and antioxidation. This work was supported by National Natural Science Foundation of China (30570573), and The Education Department of Shandong Province (J05L01).

ALTERATION OF MYOSTATIN MRNA AND PROTEIN IN GASTROCNEMIUS MUSCLE OF RATS AFTER SCIATIC NERVE INJURY

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Myostatin, a member of the transforming growth factor-β (TGF-β) superfamily, has been identified as an inhibitor of skeletal muscle mass. To have an insight into the expression pattern of myostatin and its potential role in skeletal muscle atrophy induced by denervation, we used two animal models of peripheral nerve injury (nerve resection and nerve crush) to examine the time-dependent changes in myostatin mRNA and protein levels in the denevated gastrocnemius muscle by using quantitative real-time RT-PCR and Western blotting respectively. The experimental results showed that myostatin mRNA and protein levels in rat gastrocnemius muscle displayed the different profiles in the two nerve injured models. After nerve resection, the expression of myostatin persistently elevated, despite a fluctuation of myostatin mRNA level at day 3 after denervation, reached their respective peaks at day 28 after denervation, and then depressed slightly until day 56 after denervation. On the other hand, in the rats endured nerve crush, over the same period, the myostatin expression traced a change pattern in a reverse parabola-like manner, which displayed that myostatin mRNA or protein gradually elevated from day 1 to day 14, and then gradually declined from day 14 to day 28, until the normal reached at day 28 after nerve crush. Furthermore, a significant correlation was found between myostatin abundance and muscle atrophy degree, suggesting that myostatin might probably play an important role in denervation-induced muscle atrophy. Our present study thus provides a new window into myostatin expression in response to different types of muscle atrophy.

EFFECTS AND MECHANISM OF NITRIC OXIDE IN PAIN MODULATION IN RAT CAUDATE NUCLEUS

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We investigated the effects and mechanism of nitric oxide (NO) in pain modulation using a pain model of potassium iontophoresis-induced tail-flick and an in vitro cultured model of caudate nucleus neurons from neonatal rats. Microdose of normal sodium (NS), L-arginine (L-Arg), D-arginine (D-Arg), N (omega)- nitro-L-arginine methyl ester (L-NAME) or methylene blue (MB) was injected into rat caudate nucleus. The changes of pain threshold (PT) were observed within 0-30 minutes after administration. The cGMP levels in the blood and caudate nucleus were detected by radioimmunoassay (RIA). The changes of caudate nucleus nNOS were observed by immunohistochemistry and the expression of caudate nucleus nNOS mRNA were detected by reverse transcription-poly-merase chain reaction (RT-PCR). The primary neurons derived from caudate nucleus of neonatal rats were cultured in vitro and the changes of cultured caudate nucleus nNOS mRNA by RT-PCR. Compared with the NS and D-Arg groups, PT significantly reduced after L-Arg administration but more significantly increased after micro-injection of L-NAME or MB. The cGMP levels in blood and caudate nucleus significantly increased after L-Arg administration. In contrast, these levels significantly decreased after MB administration. The expression of nNOS was scattered, most in cytoplasm and a little in the nerve fiber. The expression of nNOS and nNOS mRNA were increased by micro-injection of L-Arg but decreased by L-NAME. The same result was found in the primary caudate nucleus neurons cultured in vitro. Our data suggest that NO in the caudate nucleus transmits pain messages. The mechanism may be partially associated with a NO-cGMP metabolic pathway. NOS activity is one of the key factors of pain modulation induced by NO, a neurotransmitter or neuromodulator.

CENTRAL ANGIOTENSIN II REGULATES SPLENIC SYMPATHETIC NERVE OUTFLOW AND SPLENIC CYTKINE GENE EXPRESSIONS

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Bidirectional interactions exist between the immune system and the central nervous system. Neural-immune interactions provide a regulatory system required for health and disturbances in these interactions may lead to disease. Although the sympathetic nervous system is thought to play a key role in mediating neural-immune interactions, central mechanisms are not well understood. Recent evidence suggests that angiotensin II (Ang II), reactive oxygen species, and cytokines interact to influence central regulation of sympathetic nerve discharge (SND), however, the effect of these neurotransmitters/neuromodulators on splenic SND is not well established. This is a significant omission because splenic sympathetic nerves provide important communicating pathways regulating neural-immune interactions. We tested the hypothesis that central angiotensin II (Ang II) administration would activate splenic sympathetic nerve discharge (SND) which in turn would alter splenic cytokine gene expression. Experiments were completed in sinoaortic-denervated, urethane-chloralose anesthetized, splenic nerve-intact and splenic nerve-denervated, Sprague-Dawley rats. Splenic cytokine gene expression was determined using genearray and real-time RT-PCR analyses. Splenic and renal SND was significantly increased after intracerebroventricular (icv) administration of Ang II (150 ng/kg, 10 µl), but not artificial cerebrospinal fluid (aCSF). Splenic mRNA expression of IL-1β, IL-6, IL-2, and IL-16 genes was increased in Ang II-treated splenic-intact rats compared with aCSF-treated splenic-intact rats. Splenic IL-1β, IL-2, and IL-6 gene expression responses to Ang II were significantly reduced in

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spleen-denervated compared with spleen-intact rats. Splenic gene expres-
sion responses did not differ significantly in Ang II-treated spleen-denervated
and aCSF-treated spleen-intact rats. Splenic blood flow responses to icv Ang
II administration did not differ between spleen-intact and spleen-denervated
rats. These results provide experimental support for the hypothesis that Ang II
modulates the immune system through activation of spleen SND, suggesting
a novel relationship between Ang II, efferent sympathetic nerve outflow, and
splenic cytokine gene expression.

RNA INTERFERENCE FOR INHIBITION OF DMT1
EXPRESSION
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Divalent metal transporter 1 (DMT1) mediates the transport of Fe2+ into the
duodenum and out of the endosome DMT1 is also important in non-transferrin
bound iron uptake. We constructed a recombinant retroviral vector pSUPER-
DMT1 and selected a stable virus-producing cell clone that generate shRNAs
specific for DMT1. The aim of the present study is to investigate the changed
iron uptake due to the silence of DMT1. The 60nt sequences encoded for tran-
scribing DMT1 shRNA were cloned into a retroviral vector pSUPERretro with
DNA recombinant technique. The recombinant vector was identified by the
electrophoresis analysis of restriction enzyme digestion and DNA sequenc-
ing. The packaging cell PA317 was transfected with recombinant plasmid using
lipsosome-based transfection method and the stable virus-producing cell clone
was selected by using G-418 medium. The viral supernatant was used to infect
C6 cells, the expression of DMT1 was tested by RT-PCR and Western blotting.
The result of DNA sequencing demonstrated that 60bp nucleotide had been
inserted in the expected site and the insertion sequence was exactly correct.
When the recombinant vector was transfected into the packaging cell, green
fluorescent protein (GFP) was expressed. The anti-G418 positive clones were
also selected and they could excrete recombinant retrovirus. RT-PCR result
showed that high-efficiency inhibition of DMT1 mRNA and DMT1 protein
was achieved by retrovirus vector mediated RNAi in C6 cells. In conclusion,
we succeeded in constructing a recombinant retroviral vector pSUPER-DMT1
and selecting a stable virus-producing cell line. DMT1 expression in C6 cells
was specifically silenced by recombinant retrovirus pSUPER-DMT1. This
silencing system established a foundation for illustrating physiological func-
tion and relationship between the disrupted DMT1 expression and iron-related
disorders. This study was supported by grants from the National Program
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A NOVEL MECHANISM UNDERLYING THE SEDATIVE
EFFECT OF AN HERB-DERIVED COMPOUND
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Previous studies in our lab showed that a new herb-derived compound (com-
 pound B) could prolong the sleep time and improve the sleep quality in health
human and rats. The aim of the present study was to investigate the possible
mechanisms of compound B-induced sedation. We examined the effects of
compound B on the ion currents in paraventricular nucleus (PVN) neurons of
hypothalamus using a whole cell patch clamp recording method in brain slice
and on intracellular calcium concentrations in primary cultured hypothalamic
neurons using calcium imaging method. The results showed that compound B
could significantly increase the half decay time of GABA-A receptor mediated
miniature inhibitory post synaptic currents (mIPSCs) (P < 0.001). This effect
could not be blocked by flumazenil, a specific benzodiazepine binding site
antagonist. We also observed that compound B mediated prolongation of half
decay time of mIPSCs could be blocked by KN-62, a calmodulin dependent
protein kinase II (CaM-KII) inhibitor, indicating calcium/calmodulin dependent
protein kinase II pathway activation was involved in this action. Further stud-
ies demonstrated that compound B could enhance voltage-dependent calcium
currents in PVN neurons (P < 0.05). The calcium imaging results also showed
that the intracellular calcium concentration was elevated after compound B
perfusion, this effect also depends on the existence of extracellular calcium.
Based on the results we have got, a possible mechanism underlying the com-
 pound B-induced sedation could be put forward: compound B enhances volt-
age-dependent calcium currents and thereby increasing intracellular calcium
concentrations. The elevation of intracellular calcium in turns activates the
downstream signal transduction pathways and influences the GABA-A recep-
tors function therefore potentiating the GABAergic synaptic neurotransmission.

Ghrelin, a novel 28-amino acid peptide, was discovered as an endogenous lig-
and for the growth hormone secretagogue receptor (GHS-R); it is predominantly
produced in the stomach when the stomach is empty. It stimulates the release
of growth hormone and also acts at hypothalamic feeding centers to increase
hunger. GHS-Rs have also been found in many brain areas outside the hypothal-
amus, including in the hippocampus. The major emphasis of ghrelin research
has been on possible roles for ghrelin in meal initiation and energy homeostasis
in general. It is well known that icv injection of ghrelin engages the activation
of numerous subpopulations of GHS receptors. In this study, Wistar male rats
were trained in a Morris water maze after the bilateral intrahippocampal injec-
tion of either saline or rat ghrelin(0.03, 0.3 nmol) into area CA3 for 5 days.
We found that rats treated with ghrelin displayed a shorter latency and swim
distance to find the hidden platform in the hidden platform test , and treated rats
focussed its search in the quadrant that formerly contained the platform in the
probe test. Moreover, the administration of ghrelin significantly stimulated ex-
pression of NMDAR1, 2B in the dentate gyrus. These results suggest that high
level of ghrelin in rodents can modulate hippocampal function and memory
performance. This work was supported by the Natural Science Foundation of China
(No.3070467) and by the grant from the Bilateral Scientific and Tech-
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Ghrelin acts on the rat hippocampus to increase memory performance
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Department of Physiology, Qingdao University School of Medicine, 
Qingdao, 266021, P.R. China
Ghrelin is a novel 28-amino acid peptide, which is produced in the stomach when
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EFFECTS OF GHRELIN IN LHA ON GASTRIC MOTILITY
AND THE IMMUNOREACTIVE EXPRESSION OF C-FOX
PROTEIN IN HYPOTHALAMIC ARCULATE NUCLEI IN
RATS
Yan Qu, Na Han and Zheng Yao Jiang 
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Qingdao, 266021, P.R. China
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THE ROLES OF FERROPORTIN1 AND HEPHAEA splash IN THE IRON RELEASE FROM MES23.5 DOPAMINERGIC CELLS
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FERROPORTIN1 (FP1) and hephaestin (HP) are newly-discovered iron transporters. In the present study we investigated the expression, function and regulation of FP1 and HP in MES23.5 dopaminergic cell line, which is hybridized from rat mesencephalic neurons and murine neuromastoma-glioma N18T2G. Using immunofluorescent double-labeling, we observed the co-expression of FP1 and HP in MES23.5 cells. Using laser confocal scanning microscope and iron sensitive probe calcein, we observed the iron release process mediated by FP1 and HP. Antibody against FP1 almost completely blocked iron release from MES23.5 dopaminergic cells. Antibody against HP partly blocked the iron release. Ferric ammonium citrate (FAC, 100μg/ml) up-regulated the mRNA level of FP1 in MES23.5 cells while the mRNA level of FP1 did not change when treated with desferal (DFO, 100μmol/l). HP mRNA level kept unchanged in both FAC and DFO treated cells. These results suggest that FP1 and HP co-localize in MES23.5 cells and both participate in the iron release. FP1 plays a more important role in the release process. FP1 mRNA is up-regulated by the increased iron level and HP mRNA shows no response to iron levels inside cells. This study was supported by grants from the National Program of Basic Research sponsored by the Ministry of Science and Technology of China (2006CB500704) and the National Foundation of Natural Science of China (No. 30370498; 30401399, 30570649).

ENGRAFTED NEURAL PRECURSOR CELLS DERIVED FROM MOUSE ES CELLS RELIEVE MEMORY LOSS OF RATS RECEIVING A-BETA(1-40) INTRAHIPPOCAMPAL INJECTION
Jun Tang1, Hai Wei Xu1, Guang Ji Zhou1, Da Bing Li1, Li Yang1 and Xiao Tao Fan2
1Department of Physiology and 2Department of Neurobiology, Third Military Medical University, Chongqing, 400038, China
Alzheimer’s disease is characterized pathologically by insoluble extracellular deposits of β-amyloid, termed senile plaques, intracellular neurofibrillary tangles and widespread selective loss of neurons, and clinically by progressive memory loss, cognitive decline. The presence of numerous loss of neurons has attracted great interest because they appear relatively late in the course of the disease and thus provide a potential therapeutic target. The neural precursor cells (NPCs) with the properties of neural stem cells differentiated from ES cells would be suitable candidates for cell replacement. MESPU35 mouse ES cell line, which expresses enhanced green fluorescent protein that enables one to distinguish between transplanted cells and cells of host origin, was used in our experiment. Embryoid bodies (EBs) were formed and were induced to NPCs in N2 selection medium plus fibrocinet. Praxiology and immunohistochemistry methods were used to observe the survival, differentiation, and therapeutic effect of NPCs after grafted into the hippocampus of Aβ-injured rats. We demonstrate that more than 90 % of mouse ES cells differentiated into nestin-positive NPCs 5 days after the EBs were formed in the bacterial dishes and cultured in the modified N2 selective medium. The NPCs grafted in the hippocampus of Aβ-injured rats were survived and grew in colony, most of engrafted NPCs differentiated into GFAP-positive glia and some into NF200-positive neurons with long process. The learning and memory of Aβ-injured rats was relieved after the cell transplantation compared with control group. It suggests that NPCs derived from ES cells are survived and mostly differentiated into glia and a few into neurons after grafted into the hippocampus of Aβ-injured rats, and which improved the impairment of learning and memory. This work was partly supported by National Nature Science Foundation of China, No.30571770, 30500148.

THE RELATIONSHIP BETWEEN GLUTAMATE INPUTS FROM THE SUBICULAR CORTEX TO THALAMOCORTICAL PROJECTION NEURONS IN THE ANTERIOR THALAMUS OF THE RAT - A STUDY COMBINED HRP TRACT TRAinging AND IMMUNOGOLD LABELLING
Bin Wang, Xiao Kai Ma, Kai Fan and Yuan Shan Fu
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The synaptic organization and glutamatergic transmitter of the pathway linking the Anterior thalamus and the subicular complex was examined by using HRP tract tracing and post embedding immunogold technique. In the anterior thalamus, anterograde HRP labelled terminals contained round clear synaptic vesicles and several mitochondria, and established asymmetric synaptic contacts with HRP-labelled or non-HRP labelled dendrites. The highest densities of immunogold particles following glutamate immunostaining were found over HRP-labelled terminals and over similar axon terminals devoid of HRP reaction product. They established asymmetric synaptic contacts (Gray type I) with dendrites. Mean gold particle densities over such terminals were more 3 times higher than the densities over the dendrites to which they were presynaptic and more 6 times higher than the densities over the terminals establishing symmetrical synapses (Gray type II). In serial sections immunoreacted for GABA, Gray type II terminals were heavily labeled whereas Gray type I terminals displayed very low labelling. In serial sections immunoreacted for glutamate, Gray type II terminals displayed very low labelling. The result show that the terminals of projection in the pathway linking the anterior thalamus and the subicular complex are glutamergic and that the activity of the memory pathway may be modulated by GABA releasing from presynaptic axon terminals.

EFFECT OF THREE NOVEL NEUROTOXINS FROM SEA ANEMONE ANTHOPLEURA SP. ON CEREBRAL CORTEX NEURONS IN RATS BRAIN SLICES
Fang Wang, Ke Wang, Lei Wang, YunXin Zhu, Wenliang Zhou and Hui Xiang*
The Lab of Physiology and Neurobiology, School of Life Sciences, Sun Yat-Sen University, GuangZhou, 510275, P. R. of China
The three novel sea anemone neurotoxins, named HK2a, HK16a and HK7a, are composed of 47 amino acid residues, come from sea anemone Anthopleura spp. In these experiments, sea anemone neurotoxins were obtained by fusion
expression neurotoxin of genes in Escherichia coli. The aim of the present study was to determine that the function of these three recombinant neurotoxins on cerebral cortical neurons in rats' brain slices. In rat brain slices, we studied sodium currents, distinctive spike patterns, spontaneous and miniature excitatory post synaptic currents (sEPSCs and mEPSCs) in cortical neurons using whole cell recording techniques. The experiment was performed on the layer III-V neurons of sensory cortical, the neurons was identified as pyramidal neurons by their electrophysiological parameters and morphology. All three recombinant proteins could inhibit the voltage-dependent sodium currents in rat cerebral cortical neurons, the effects from strong to weak was HK7a, HK16a and HK2a. Among these toxins. Both HK7a and HK16a could prolong the decay time of sodium channel inactivation, but the effect of HK16a was weaker than HK7a. However, HK2a didn’t change the decay times of sodium channel inactivation. These results indicated that HK7a prolong the process of sodium channel inactivation, HK2a and HK16a act on the receptor sites of sodium activation. HK7a obviously changed the membrane potential from -65 mV to almost -30 mV. This could be correlated with prolong of sodium channel inactivation. The frequencies of sEPSCs and spike pattern in cortical neurons were significantly decreased by HK2a and HK16a. In summary, the recombinant production of these toxins in physiological studies may play a significant role to reveal the structure-function relationships within the proteins themselves as well as the interaction between the toxins and the sodium channels.

MODELING FOR TIMING-DEPENDENT INTEGRATION OF SYNAPTIC POTENTIATION AND DEPRESSION
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In order to study the functional relationship between synaptic modification and complex spikes, a mathematical model was put forward for describing the coactivation of the complex spikes according to the results of triple spike paradigms and quadruplets spiking paradigms in the cultured hippocampal neurons. The mathematical model can be described in the form of function:

$$W(t) = \begin{cases} 1.3 \exp(-T/59) & T > 0 \\ 1 & T < 0 \end{cases}$$

It can be said that when the pre-post spike pair appears before the post-pre pair, synaptic potentiation and depression seem to cancel; when they appear in the opposite timing, synaptic potentiation will decay in the exponential way. These results suggest that STDP follows an LTP-dominating rule in the cultured hippocampal neurons, which is quite different from the rule in the rat visual cortical slices.

THE CONSTRUCTION OF RECOMBINANT ADENOVIRUS ENCODING GENE OF HUMAN DMT1 DOMAIN 4 AND ITS EXPRESSION IN MES23.5 DOPAMINERGIC CELL LINE
Lei Wang, Hong Jiang, Hua-Min Xu, Bing Luo and Jun Xia Xie
Department of Physiology, Medical College of Qingdao University, Qingdao, China

Iron is necessary for neuronal function but in excess generates neurodegeneration. Divalent metal transporter 1 (DMT1) is the first mammalian transmembrane iron transporter, which has 12 transmembrane domains. Mutations in transmembrane domain 4 have been shown to interfere with its function. The objective of the study is to construct recombinant adenoviruses encoding gene of human DMT1 domain 4 and to detect its expression in MES23.5 dopaminergic cell line. The human DMT1 domain 4 gene was obtained by RT-PCR, then was cloned into the shuttle plasmid pAdEasy-1. The recombinant plasmid pAdEasy-1-DMT1 domain 4 was for kanamycin resistance and confirmed by multiple restriction endonuclease analyses. Paci-digested pAdEasy-1-DMT1 domain 4 was transfected into 293 cells and in which recombinant adenovirus was generated. The recombinant adenoviruses were transfected into MES23.5 dopaminergic cell line and detect its expression by RT-PCR. pAdEasy-1-DMT1 domain 4 yielded a large fragment (near 30 kb), plus a smaller fragment of 3.0kb according to different recombination. PCR showed that pAdEasy-1-DMT1 domain 4 contained gene DMT1 domain 4. GFP expression visualized by fluorescence microscopy proved that intact recombinant adenoviruses were obtained. RT-PCR analysis indicated DMT1 domain 4 mRNA was increased after transfection. The results above suggest the recombinant adenoviruses were successfully constructed and can express DMT1 domain 4 in MES23.5 dopaminergic cell line. This study was supported by grants from the National Program of Basic Research sponsored by the Ministry of Science and Technology of China (2006CB500704) and the National Foundation of Natural Science of China (No. 30370498; 30401399, 30570649).

MPP+−INDUCED GABAA RECEPTOR DYSFUNCTION: A NOVEL HYPOTHESIS OF DOPAMINE NEURON DEGENERATION IN PARKINSON’S DISEASE
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Parkinson disease is characterized by hypoactivity of the dopamine (DA) neurotransmitter system due to a loss of pigmented DA neurons in the substantia nigra pars compacta (SNc). 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) is a neurotoxicant which was found to cause a parkinsonian syndrome in humans and animals. The ultimate toxic effect of MPTP is mediated through its principal metabolite, 1-methyl-4-phenylpyridinium ion (MPP+), which is selectively taken into DA neurons by DA transporters, causes a depletion of intracellular ATP by blocking mitochondrial complex I, and finally leads to neuron degeneration. Several hypotheses have been proposed to explain the energy crisis-induced cell death by MPP+, including NMDA receptor-mediated excitotoxicity, voltage-dependent Ca2+ channel activation, or in changes intracellular calcium homeostasis. Little is known, however, about the roles of postsynaptic GABA receptors in MPP+-induced neurotoxicity although it has long been known that GABA plays a critical role in controlling DA neuron function. In the present study, we tested a new hypothesis that functional decline of GABA receptors plays a critical role in the generation of MPP+-induced acute cell degeneration using patch-clamp recordings in single DA neurons acutely dissociated from SNc. We found that the perfusion of MPP+ induced a functional rundown in GABA receptor but not in glycine or ionotropic glutamate receptors. The MPP+-induced GABA receptor rundown was mediated through cytoplasmic pathway, and was mimicked by depleting intracellular ATP and prevented by supplying intracellular ATP, suggesting that impairment of ATP production by MPP+ underlies GABA receptor dysfunction. We also found that potentiation of GABA receptor function by positive allosteric modulators significantly prevented GABA receptor functional rundown and DA neuron death. Collectively, these studies provide novel insights into an improving understanding of acute pathogenesis of MPP+-induced DA neuron degeneration. GABA receptor dysfunction at early stage during exposure to neurotoxin may cause an imbalance between excitation and inhibition in DA neurons, which may trigger glutamate neurotoxicity and Ca2+ overload, and lead to DA neuron degeneration.

DECREASED HIPPOCAMPAL CELL PROLIFERATION CORRELATES WITH OVEREXPRESSION OF BMP-4 IN THE DENTATE GRYUS OF THE APP+PSI ALZHEIMER’S DISEASE TRANSGENE MICE
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1Department of Physiology; 2Department of Neurobiology; 3Department of Medical Genetics, Third Military Medical University, Chongqing, 400038, China and 4Department of Neurosurgery, Xinqiao Hospital, Third Military Medical University, Chongqing, 400038, China

The mechanisms that regulate adult hippocampal neurogenesis have received major efforts in recent years. The stem cells' microenvironment is fundamental for understanding how a stem cell can proliferate, choose its fate and eventually integrate into mature tissue. Several candidate niche components including...
Bone morphogenetic protein-4 (BMP-4) and their antagonist/modulator have been described as critical components of this stem cell niche microenvironment. Using Congo red staining, in situ hybridization, 5'-bromo-2'-deoxyuridine Bromodeoxyuridine (BrdU) and β-amyloid peptide immunohistochemistry, we demonstrate that β-amyloid plaque was deposited at 6 months old and increased significantly at 9 and 12 months old in the cortex and hippocampus of APP*PS1 double transgenic mice (strain name, earB6C3-Tg 85Db/oJ, provided by Jackson Laboratory). The number of BrdU-labeled cells in the dentate gyrus of the transgenic mice of 9 and 12 months old, however, was decreased significantly compared with wild type mice of the corresponding age, while the number of BMP4 mRNA-positive cells in the dentate gyrus of the transgenic mice of 6, 9 and 12 months old was increased significantly compared with wild type mice of the corresponding age. There was positive correlation between the decrease of the number of BrdU labeled cells and the increase of the number of BMP4 mRNA-positive cells in the hippocampus of adult transgenic mice. It suggests that BMP4 likely plays an important role in the depressed neurogenesis of the dentate gyrus of APP*PS1 double transgenic Alzheimer disease mice. This work was supported by National Nature Science Foundation of China, No.30500148, 30571770.

THE CONSTRUCTION OF RECOMBINANT ADENOVIRUSES ENCODING DMT1 GENE AND ITS EXPRESSION IN MES23.5 DOPAMINERGIC CELL LINE
Hua Xin Xu, Hong Jiang, Lei Wang, Bing Luo and Jun Xia Xie*
Department of Physiology, Medical College of Qingdao University, Qingdao, China

Divalent metal transporter 1 (DMT1), previously known as natural resistance associated macrophage protein 2, is an important iron transport protein which transfers iron across the apical surface of intestinal cells and out of endosomes. The disturbed expression of DMT1 might be involved in iron accumulation in some neurodegenerative disorders. The aim of the present study is to construct recombinant adenoviruses encoding DMT1+IRE gene and DMT1-IRE gene and detect its expression in MES23.5 dopaminergic cell line. The human DMT1 gene including DMT1+IRE and DMT1-IRE were obtained by RT-PCR, then separately cloned into the shuttle plasmid pAdTrack-CMV containing green fluorescent protein (GFP) reporter gene. Linearization of plasmid pAdTrack-CMV-DMT1+IRE and pAdTrack-CMV-DMT1-IRE subsequent cotransformed into E.coli BJ5183 cells along with an adenoviral backbone pAdEasy-1. The recombinant plasmid pAd-DMT1 were selected for kanamycin resistance and confirmed by restriction endonuclease analysis. PacI-digested pAdEasy1-DMT1+IRE and pAdEasy1-DMT1-IRE were separately transfected into 293 cells, in which recombinant adenovirus were generated. The recombinant adenoviruses were transfected into MES23.5 dopaminergic cell line and detected its expression by RT-PCR and Western blot. PCR showed that pAdEasy1-DMT1+IRE contained DMT1+IRE and pAdEasy1-DMT1-IRE contained DMT1-IRE. GFP expression visualized by fluorescence microscopy proved that intact recombinant adenoviruses encoding DMT1 gene were obtained. DMT1 mRNA and protein levels were both increased after transfection. The results above suggest the recombinant adenoviruses are successfully constructed and can efficiently express DMT1+IRE and DMT1-IRE in MES23.5 dopaminergic cell line. It offers a system to research the function of DMT1 in the iron accumulation in central nervous system. This study was supported by the National Natural Science of China (No. 30370498; 304001399, 30570649).

NEUROPROTECTIVE EFFECTS OF GINSENOSIDE RG1 ON THE DOPAMINERGIC NEURONS IN THE 6-OHDA-RAT MODEL OF PARKINSON’S DISEASE
Li Xu, Li Xing Liu, Jun Xia Xie and Wen Fang Chen
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Ginsenosides, the principal active components of ginseng have been shown to possess a variety of beneficial effects on human health, including anti-inflammatory, antioxidant and anticancer. Many studies have suggested that ginsenoside Rg1 has neuroprotective effect, but the detailed mechanism is not very clear. The present study aims at investigating the possible mechanisms involved in mediating its actions in 6-OHDA-lesioned ovariectomized (OVX) female rat model of Parkinson’s disease (PD). Two weeks after ovariectomy, 24 Wistar rats were treated with ginsenoside Rg1 or 17β estradiol intracerebroventricularly for four weeks after unilateral injection of 6-OHDA into the medial forebrain bundle (MFB). Our results clearly showed that ginsenoside Rg1 could ameliorate the rat’s rotational behavior induced by apomorphine in a dose dependent manner. The same result was got for estrogen treatment. Immunohistochemistry studies showed that both ginsenoside Rg1 and estrogen treatment could prevent the 6-OHDA-induced decrease of TH immunoreactive neurons and Bcl-2 protein expression in the ipsilateral substantia nigra (SN) compacta compared to the contralateral side (P<0.01). Moreover, Perls’ iron staining was used to examine the iron overload. The iron staining was significantly increased in the ipsilateral SN compared to the contralateral side (P<0.01), while ginsenoside Rg1 treatment could reverse the increase of iron staining in a dose dependent fashion (P<0.01). Estrogen also reversed the iron overload completely. These results provide the further evidence that ginsenoside Rg1 have a significant protective effect on the dopaminergic neurons in the 6-OHDA-induced rat model of PD, suggesting that ginsenoside Rg1 may be helpful in slowing down the PD progression. This work was supported by National Natural Science Foundation of China (30570573), The Education Department of Shandong Province (J05L01).

ELECTROPHYSIOLOGICAL EFFECTS OF NEUROTENSIN IN RAT GLOBUS PALLIDUS: AN IN VITRO STUDY
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The globus pallidus plays a critical role in movement regulation. Morphological studies have indicated that the globus pallidus receives neurotransmitter innervation from the striatum. The present study investigated the effects of activating neurotensin receptor in globus pallidus. In vivo single unit electrophysiological recordings showed that micropressure ejection of neurotensin increased spontaneous firing rate in 26 of 51 pallidal neurons and had no effect on the other 25 neurons. The concentration response curve of neurotensin was a single bell shape with peak effects at 0.1mM. The excitatory effect of neurotensin could be mimicked by the C-terminal fragment, neurotensin (8-13), in 25 of 42 pallidal neurons, but not by the N-terminal fragment, neurotensin (1-8). Local administration of the selective neurotensin type-1 receptor antagonist, SR48692, itself did not produce a significant effect on spontaneous activity but blocked the excitatory effect induced by neurotensin. These findings suggested that palidal neurotensin type-1 receptor mediated excitation of spontaneous activity in the globus pallidus. This work was supported by the National Natural Science Foundation of China (30470545) and the Research Grants Council of Hong Kong (CUHK 4175/02M).

STUDY OF THE PROTECTIVE EFFECTS OF GINSENOSIDE Rg1 ON THE DOPAMINERGIC NEURONS IN THE 6-OHDA-RAT MODEL OF PARKINSON’S DISEASE
Li Xu, Li Xing Liu, Jun Xia Xie and Wen Fang Chen
Department of Physiology, Medical College of Qingdao University, Qingdao, 266021, P.R. China

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NEUROPROTECTIVE EFFECTS OF GINSENOSIDE RG1 ON THE DOPAMINERGIC NEURONS IN THE SUBSTANTIA NIGRA IN MPTP-TREATED C57BL6 MICE
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Ginsenoside Rg1 is one of the main components of ginseng, which has the anti-aging, anti-oxidative effects and etc. In the present study, we investigate whether ginsenoside Rg1 prevents the degeneration of dopaminergic neurons in the substantia nigra (SN) in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated C57BL6 mice. Mice were randomly divided into three groups: normal group (NS, i.p.), MPTP group (30mg/kg·5d, i.p.) and Rg1 (5mg/kg·8d, i.p.) + MPTP (30mg/kg·5d, i.p.) group. Using RT-PCR, tyrosine hydroxylase (TH), anti-aging, anti-oxidative effects and etc. In the present study, we investigate whether ginsenoside Rg1 prevents the degeneration of dopaminergic neurons in the substantia nigra (SN) in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated C57BL6 mice. Mice were randomly divided into three groups: normal group (NS, i.p.), MPTP group (30mg/kg·5d, i.p.) and Rg1 (5mg/kg·8d, i.p.) + MPTP (30mg/kg·5d, i.p.) group. Using RT-PCR, tyrosine hydroxylase (TH) immunohistochemistry and HPLC, we measured the degeneration of dopaminergic neurons in the SN of MPTP treated mice and assessed the effects of treatment with ginsenoside Rg1. The results showed that the numbers of TH-immunoreactive cells decreased significantly in the SN of MPTP treated mice, as well as the contents of dopamine and its metabolites in the striatum. Treatment with ginsenoside Rg1 reversed these effects. The expression of bcl-2...
and TH mRNA in the SN also decreased significantly in MPTP treated mice (P<0.01), while the expression of caspase-3 and bax mRNA increased significantly (P<0.01). Treatment with ginsenoside Rg1 reversed the above changes. The results suggest that ginsenoside Rg1 is neuroprotective against the neurotoxin MPTP. This effect may be due to its anti-apoptotic activity. This project is supported by the Science and Technology Department of Shandong Province (031070125) and Science and Technology Department of Qingdao Municipal Government (04-2-JS-136, 05-10-JC-97).

GINKGOLIDE B PROMOTES AND PROTECTS THE GROWTH AND DEVELOPMENT OF EMBRYONIC RAT SPINAL CORD NEURONS IN VITRO
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Spinal cord injuries (SCI) lead to neuronal loss and axonal degeneration in and around the injury site that cause partial disability or complete paralysis. An important strategy in the treatment of SCI is to promote neuron survival and axon outgrowth. Using an in vitro assay, we have identified ginkgolide B, extracted from ginkgo trees, as an efficient neuroprotective agent for spinal cord neurons. GB promotes the survival of embryonic spinal cord neuronal cells on 3 DIV, 7DIV and 10DIV and promotes the development of neurons astrocytes and cholinergic cells on 7 DIV. In addition to the effects on growth of neuronal cells, GB promotes spinal neurons from excitotoxicity induced by kainic acid on 5 DIV. It promotes the survival of neuronal cells, the cell body area, the process number and the length of the longest process. With Hoechst 33258 fluorescent staining, we observed that GB can decrease cell apoptosis. Immunocytochemical staining results with the antibody against cleaved caspase-3 revealed that GB can inhibit the activation of caspase 3. These results suggest that GB is capable of promoting and protecting the growth and development of embryonic rat spinal cord neurons indicating its plasticity and potential use in treatment of SCI.

NEUROPROTECTIVE AND ANTI-APOPTOTIC EFFECTS OF GINKGOLIDE B DURING SPINAL CORD INJURY IN RATS
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Apoptosis has been implicated in secondary tissue damage after spinal cord injury (SCI). Ginkgolide B (GB), as a highly selective and competitive PAF receptor antagonist, has been proved to possess neuroprotective and anti-apoptotic capacity in vitro models and cerebral ischemia models. This prompted us to investigate whether it is also able to inhibit nerve cells apoptosis during spinal cord injury in rats. At present we applied flow cytometry (FCM) propidium iodide (PI) to determine the nerve cell apoptosis rate and applied immunohistochemical method of caspase-3 and the cleaved caspase-3 p20 to investigate the mechanism involved in the nerve cell apoptosis in modified Alle’s WD SCI model. We also applied combined Galax behavioral score (CBS) to evaluate the return of motor behavior in Wistar rats. FCM showed an increase in apoptosis rate and reached the peak on the 3rd day after SCI and still significantly increased as compared to controls on the 14th day. Intraperitoneal injection (i.p.) with GB (2mg/kg/day) could significantly reduce the apoptosis rate on the 3rd day and on the 14th day. Immunohistochemical studies showed that the numbers of immunostaining positive cell for caspase-3 and caspase-3 p20 in penumbra areas increased on the 1st day and reached peak on the 3rd day, then decreased gradually, but still higher on the 14th day after SCI as to the controls. After treatment with GB (2mg/kg/day, i.p.) the numbers of immunostaining positive cell for caspase-3 and caspase-3 p20 were significant decreased from the 3rd day to the 14th day, moreover at the 14th day there were no difference compared to controls. CBS showed that the scores were significantly decreased after SCI. After treated with GB (2mg/kg/day, i.p.) the scores were significantly increased from the 3rd day to the 14th day. The results suggest that GB inhibits nerve cell apoptosis and promotes the recovery of motor function and its anti-apoptotic capacity contributes to the mechanism of neuroprotection.

PROTECTIVE EFFECTS OF POLYSACCHARIDE FROM SPIRULINA PLATENSIS ON 6-OHDA TREATED PC12 CELLS
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Polysaccharide from spirulina platensis (PSP) is a kind of water-soluble polysaccharide extracted from spirulina platensis. It has been reported to have anti-tumor and anti-oxidative effects. The present study is to investigate its protective effects on 6-hydroxydopamine (6-OHDA) treated PC12 cells. MTT assay showed 40μg/ml PSP treatment improved the growth of PC12 cells significantly (P<0.05), while the lower concentration did not show notable effect on PC12 cells. 20μM 6-OHDA inhibited the PC12 cells growth significantly (P<0.05), the lower concentration of 6-OHDA treatment did not show notable effect on PC12 cells growth. We then investigated the effects of 10μg/ml, 20μg/ml, 40μg/ml PSP treatment on 6-OHDA treated PC12 cells by MTT assay and RT-PCR. It showed that 20 μg/ml PSP reversed the damage of 6-OHDA on PC12 cells, the number of live cells and the expression of TH (tyrosine hydroxylase) and DAT (dopamine transporter) mRNA were increased significantly, while the 10μg/ml PSP treatment did not show notable effect on 6-OHDA damaged cells. The results suggest that PSP can protect PC12 cells damaged by 6-OHDA while the mechanism need further study. This project is supported by the Science and Technology Department of Shandong Province (031070125) and Science and Technology Department of Qingdao Municipal Government (04-2-JS-136 05-10-JC-97).

EFFECTS OF EGb761 ON HIPPOCAMAL SYNAPTIC PLASTICITY OF VASCULAR DEMENTIA RATS
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Vascular dementia (VD) rats, established by cerebral ischemia/reperfusion, were randomly divided into model group (MG) and ginkgo biloba extract 761(EGb761) treated group (ETG) (both n=30), and another 30 condition-matched rats were selected as sham-operated group (SOG). The escape latency (EL) of Morris water maze task and the population spikes (PS) of granule cell layer in the dentate gyrus in vivo were observed and recorded in each group. Results showed that the EL of MG were highly longer than that of SOG and ETG (P<0.01), but the EL of ETG still longer than that of SOG at 1m, 2m and 4m after VD modeling operation (P<0.05). The incidence rates of long term potentiation (LTP) induction in MG at different time point were significantly lower than that of SOG and ETG (P<0.01), but no obvious difference was revealed between the latter groups. Meanwhile no difference of incidence rates of LTP induction was showed in cross-reference among 1m, 2m and 4m time points of each group. Amplitude of PS was significantly higher after high frequency stimulation (HFS) than before in both SOG and ETG (P<0.01, P<0.05), but unchanged in MG. The relative amplitudes of PS after HFS in 1m, 2m and 4m time points of MG were obviously reduced compared with that of the corresponding time point of SOG and ETG (P<0.01, P<0.05), and the relative amplitude of PS in 4m time point of ETG was even higher than that in the SOG. There was no obvious difference in the peak latency of PS between different time point of each group (P>0.05). These results suggested that VD model rats had a long-lasting dysfunction of learning and memory, EGb761 could accelerate the recovery of the pathological hippocamal synaptic plasticity and improve learning and memory dysfunction in VD.

EFFECTS OF EGb761 ON SYNAPTOPHYSIN EXPRESSION IN HIPPOCAMPUS OF VASCULAR DEMENTIA RATS
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The objective of this research is to investigate the effects of ginkgo biloba extract 761(EGb761) on synaptophysin (SYN) expression in hippocampus of vascular dementia (VD) rats. VD models established by repeatedly clamping the common carotid arteries (CCA) of the rat in combination with an injection of sodium nitroprusside solution(2.5mg/kg, i.p) in anesthetized SD rats, were randomly divided into two groups: model group and EGb761 treated group (both n=30), and another 30 condition-matched rats were selected as the sham-operated group in which the bilateral CCA were separated but with neither CCA occlusion nor
injection of sodium nitroprusside solution. Above 3 groups were further divided into three subgroups respectively: 1m, 2m and 4m subgroups after model-established operation. The EGB761 treated group were treated with EGB761 (50mg/kg, dg, ig), and the model and sham-operated groups given the same volume of normal solution as EGB761 treated group. At different time point, Morris water maze (MWM) task was used as the judging criteria for spatial learning and memory ability. Immunohistochemistry technique and images analysis were used to study the synaptophysin express in hippocampal formation - MWM test showed that the escape latency (EL) of model group were higher than that of the sham-operated group, while the EL of EGB761-treated group was significantly shorter than that of model group (P<0.01), but still longer than that of the sham-operated group at 1m, 2m and 4m after VD modeling operation (P<0.05).

Immunohistochemistry analysis showed that the SYN immunoreactive expres- sion in CA1 region of model group greatly decreased and mean optical density of SYN expression highly increased compared with both sham-operated group and EGB761-treated group at three time points (P<0.01). The SYN immunoreactive activity in the dentate gyrus in model group was much lower and its mean optical density was significantly higher than that of the other groups at 1m and 2m (P<0.05). These results suggested that EGB761 could increase the expression of SYN in hippocampus, which may be one of important mechanisms of EGB761 in improving learning and memory ability of VD rats.

ELECTROPHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF ZOLPIDEM IN RAT SUBSTANTIA NIGRA PARS RETICULATA
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The substantia nigra pars reticulata (SNr) constitutes one of the output centers of the basal ganglia and its abnormal activity is believed to contribute to some basal ganglia motor disorders including Parkinson’s diseases. Different lines of evidence point to a major contribution of GABA_A receptors mediated synaptic inhibition in controlling the activities of SNr neurons. A very high density of benzodiazepine binding sites containing a1 subunit has been reported in the rat SNr, indicating the modulation on GABA_A channel kinetics. Some clinical studies pointed out that the specific agonist zolpidem had antiparkinsonian effects in some types of Parkinson’s patients. To investigate the possible effects of activating benzodiazepine binding sites by zolpidem, whole-cell patch-clamp recordings were made from SNr neurons in midbrain slices of young rats. Superfusion of 100 nM zolpidem significantly prolonged the decay time of both mIPSCs and sIPSCs by 40.65%±4.41% (n=6, P<0.01) and 49.90%±5.35% (n=7, P<0.01), respectively, without any change on their amplitude and frequency. A higher concentration (1µM) of zolpidem prolonged the decay time more potently (mIPSCs: 87.84%±8.06%, n=6; sIPSCs: 65.59%±10.38%, n=9). Furthermore, 1µM zolpidem increased the amplitude of mIPSCs (18.35%±5.14%, P<0.05) significantly, which indicated that postsynaptic GABA_A receptors in SNr were not saturated by the quantal release of GABA. In the behavioral test, zolpidem microinjected into rat SNr unilaterally caused a robust contralateral rotation (32.7±2.89turns/30min, n=7, P<0.01), significantly higher than that of control animals receiving saline injection. The present findings that zolpidem significantly potentiated GABA currents in SNr provides a rationale for further investigations into its potential in the treatment of Parkinson’s disease and epilepsy. This work was supported by the Research Grants Council of Hong Kong (CUHK 4175/02M to W.H. Yung) and Foundation of Health Department of Shandong Province (2005HW021).

ACUTE ZOLPIDEM APPLICATION MODIFIED THE ARCHITECTURE OF SLEEP IN RATS
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Zolpidem is a new non-benzodiazepin sedative hypnotic for insomnia treatment. Compared with benzodiazepines (BZs), Zolpidem selectively acts on GABA_A receptor and facilitates its function. Thus zolpidem has fewer side effects than BZs which act nonselectively on both GABA_A and GABA_B receptors. Up to date, there are only a few studies of zolpidem on experimental animals. The aim of this study is to illustrate the effects of one acute dose of zolpidem on rats. Wistar rats were implanted with telemetric EEG transmitters (DSI) that allow EEG, temperature and activity recording in free moving animals. Zolpidem (5 mg/kg) was administrated by oral gavage 30min before light on. The proportion of each sleep stage during the whole 12hours light period didn’t change, but the sleep latency decreased significantly. In the first hour (1h) after light on, zolpi- dem increased NREM sleep and decreased Awake. A rebound of NREM sleep decrease and Awake increase appeared in the next 2 hours. For REM sleep, it decreased in 1-3h but increased in the rest of the time. The entries of NREM sleep increased but the durations decreased in the whole 12h. REM sleep entries was increased from 4h to 12h, but the durations wasn’t affected. The entries of Quite Awake and Active Awake were all increased with a reduction of durations. The present results indicate that Zolpidem shortens the sleep latency, increases the NREM sleep in the beginning of light-on period. The architecture of normal sleep was changed, which implicates potential side effects of zolpidem.Supported by Innovation and Technology Fund of Hong Kong SAR.

THE EFFECT OF GRX1 OVEREXPRESSION IN HEK293T CELLS ON P38 MAPK ACTIVATION INDUCED BY H2O2
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Glutaredoxin (Grx) play a critical role in the maintenance of cellular redox homeostasis and the defense against oxidation stress as an important glutathione-dependant thiol-disulfide oxidoreductase. However, the molecular mecha- nism of antioxidation effect of Grx remains unclear. We proposed that Grx1 exerted its protection against oxidation stress through inhibiting activation of the p38MAPK pathway in response to H2O2 treatment. The recombinant expression vector pcDNA3.1 (+)-hGrx1 was transfected into HEK293T cells by liposome transfection. Transient expression of recombinant hGrx1 was assayed by means of RT-PCR and western blot. Then we established the damage model of HEK293T cells by treating the cells with different concentration of H2O2. Cell viability was determined by MTT assay, and antioxidation effect of the cells was evaluated by using LDH release assay, MDA and SOD activity. The results showed that Grx1 was overexpressed in HEK293T cells and exerted obvi- ous protection against H2O2-induced damage compared with control plasmid group. To investigate the effect of Grx1 overexpression on the activation of p38MAPK pathway induced by H2O2 in HEK293T cells, we treated HEK293T cells with 100µmol/L H2O2 and detected phosphorylation level of p38MAPK using western blot at the time intervals of 5, 15, 30, 60, 90, 120min. The results revealed that p38MAPK phosphorylation level increased after 5min treatment of H2O2 and reached a maximum peak value at 15min, and gradually decreased after 15min in control plasmid group. However, p38MAPK phosphorylation level remained stable in recombinant plasmid group after the transfected cells were treated by 100µmol/L H2O2. These results suggested that hGrx1 overexpression inhibited H2O2-induced activation of p38MAPK in HEK293T cells. In sum- mary, these observations demonstrated that the Grx1 overexpression protected HEK293T cells from H2O2-induced cell damage by inhibiting the activation of p38MAPK signal transduction pathway. Our research provided some new evidence for elucidation of the antioxidation mechanism of Grx1 under oxidation stress and new idea for future research on the role of Grx1 in redox signaling.

A ROLE OF CATALPOL ON LEARNING AND MEMORY IN THE AGED RATS
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Alzheimer’s disease (AD) is the most common neurodegenerative disorder of the older, and is characterized clinically by progressive memory loss. It has been reported that Liweisi Dihuang Wan (LW) could improve learning and memory ability during AD. Catalpol, a cycloalkene ether terpenoid, is one of the main components of LW. To determine the effect of catalpol on the ability...
of learning and memory, we selected aged rats with progressive memory loss as our subjects. These aged rats were divided randomly into the saline treated group (control group, OS) and the catalpol treated group (OC) (5mg/kg/d). They were injected for successive 10 days. We demonstrated that trial time was decreased in the Y maze and the cross number and the rear number were increased in the openfield test of the catalpol treated group compared with the control group. These results suggest that catalpol promotes the ability of discrimination learning and memory and spontaneous activities. To study the mechanism by which catalpol affect learning and memory, the brain sections of the catalpol group and the saline group were stained with Hematoxylin and eosin staining (HE) and GAP-43 polyclonal immunohistochemistry. And Cav-1, GAP-43 and PKC proteins et al were checked in the hippocampus and cortex of the two groups by western blot. The results showed that there were more glial cells in the dentate gyrus of catalpol-treated group, and increased GAP-43 protein immunohistochemical labelling was in the hippocampus CA1, CA3 and dentate gyrus (DG) after catalpol treated the old rats. Immunohistochemistry and western blot results showed that higher GAP-43 and Cav-1 level in the catalpol-treated hippocampus and cerebral cortex. All these results suggest catalpol may effectively improve senescence-induced memory degeneration via glial cell and Cav-1 or GAP-43 associated signal transduction pathways.

STEM CELL RESEARCH AND INJURY REPAIRS

HUMAN MESENCHYMAL STEM CELLS DERIVED FROM BONE MARROW AND ENDOThelial CELLS DIFFERENTIATION
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The current study was designed to investigate the biological characteristics of human mesenchymal stem cells (hMSCs) derived form bone marrow correlated with the endothelial cells (ECs) differentiation. Bone marrow was obtained from 10 donors (18 - 76 years of age). All the patients had not accepted radiotherapy and chemotherapy before operation. Single-cell suspensions were centrifuged to pellet, the cells and fractionated on a 1.073g/ml Percoll and adhered to plastic dishes. Using methods of immunohistochemistry, cytochemistry, FACS, transmission electron microscopy to explore the characteristics of hMSCs in vitro. hMSCs was a highly homogeneous population; hMSCs expressed KDR which was the marker of EPC (endothelial progenitor cells) and early differentiation of ECs. However, the expression of CD34, CD31, CD54, Flt-1, VE-cadherin and V VIII in was negative. PAS reaction of hMSCs was positive and transmission electron micrograph showed glycogen-pool in ectoplasm. Meanwhile, hMSCs were rich in organell to produce abundant proteins (enzyme), such as bFGF, fibronectin, laminin, MMP-2/9, I and IV type of collagen. hMSCs had cytological basis to differentiated into ECs. Glycogen deposition was viewed to be a developmentally regulated process during morphogenesis with “plasticity”. The abundance of matrix proteins (enzyme) and growth factors which hMSCs produced facilitated to the adherence, proliferation, differentiation and signal transduction.

MURINE BONE MARROW-DERIVED MONONUCLEAR CELLS INDUCED TO DIFFERENTIATE INTO LYMPHATIC VESSEL ENDOThelial CELLS IN VITRO
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To study the differentiation of C57BL mouse bone marrow-derived mononuclear cell (BMNCs) into lymphatic vessel endothelial cells, BMNCs were generated out of bone marrow cells from C57BL/6 mouse separated by density gradient. After induction with vascular endothelial growth factor (VEGF), vascular endothelial growth factor-C (VEGFC) and basic fibroblast growth factor (bFGF) in lymphatic vessel endothelial cells conditioned medium, at a number of time points. The lymphatic vessel endothelial cells were determined by the utilization of morphological observation, vascular endothelial growth factor receptor-3 (VEGFR-3, Flt-4) and lymphatic vessel endothelial HA receptor-1 (LYVE-1) levels showed that after culture for 28 days, BDMCs were able to express VEGFR-3 and LYVE-1, and came into a vessel-like configuration. In conclusion, our data suggest that bone marrow-derived mononuclear cell could differentiate into lymphatic vessel endothelial cells after induction and in vitro. These observations may have crucial implications in the development of novel therapies using BMNCs engineered to secrete anti-cancerous agents and to antagonize tumor lymphatic metastasis.

STUDY ON TRANSPLANTATION OF THE CULTURED HUMAN UMBILICAL CORD BLOOD MONONUCLEAR CELLS FOR SPINAL CORD INJURED MODELS IN RATS
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To detect the effects of cultured stem cells derived from human umbilical cord blood (HUCB) for treating spinal cord injury (SCI). Isolated mononuclear cells from heparinized HUCB samples were isolated by centrifugation over lymphoprep (density 1.077/ml) density gradient, then the cells cultured in DMEM medium containing 20 % (V/V) fetal bovine serum. Flow cytometry showed that most of the adherent cells were CD11b+, CD34+, CD44+ and CD45-. Immunocytochemical staining showed that some of the blood stem cells were Nestin positive cells. Thirty adult rats were used, and complete transection of spinal cord at the level of T9-T11 was performed 7days before transplantation. The adult rats were randomly divided into three groups. One group was transplanted with the cultured cells labeled by bromodeoxyuridine (BrdU); PBS and non-treatment groups were used as control. Hindlimb motor function was assessed by the Basso-Beattie-Bresnahan (BBB) locomotor rating scale once a week. The survival and differentiation of transplanted cells in vivo in rat spinal cord were evaluated by histological and immunohistochemistry. Two weeks after the transplantation, locomotor function of the experimental group was improved; the paralysed quarters began to move, and had some active moments, while the control group had no sign of recovery. The transplanted cells were found to survive and extend into the surrounding spinal cord tissue after 5 weeks in the transplantation group. Some of the Brdu-labeled cells expressed the astrocytic specific protein GFAP, and some expressed neuron marker MAP-2. Our findings showed that stem cells derived from HUCB can survive, migrate to the rats injured spinal cord site and differentiate into neurons and astrocytes. So it is hopeful that stem cells derived from HUCB could be a valuable stem cell resource for the therapy of SCI. This work was supported by the undergraduate innovation foundation of Shanxi Medical University (200470).

THE CULTURE OF EMBRYONIC SPINAL CORD STEM CELLS IN VITRO AND SPECIFIC DIFFERENTIATION INTO CHOLINERGIC NEURONS
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Inductive signals and transcription factors have been proved very important in neuron generation in vivo, now the question of whether these signals can be used to direct neural stem cells to a specific neuron in vitro is raised. Here we show that rat embryonic spinal cord stem cells (ESSCCs) can generate cholinergic neurons in vitro. The neural stem cells from spinal cord of 13d fetal rats were harvested and cultivated in a serum-free limited medium which contained EGF and bFGF, and observed the cholinergic differentiation by exposing to specific factors. The results were identified by cellular immunohistochemistry. A great number of neural stem cells could be obtained from the embryonic spinal cord, and the neurospheres were formed through cultivation in limited medium. Through immunostaining analyses, we found ESCCs expressed nestin, a common neural progenitor marker, thus indicating that ESCSCs is a type of neural progenitors. Exposed to specific inducing factors, the stem cells could be induced to differentiate into cholinergic neurons. We believe that our limited medium can increase the population of ESCSCs and maintain its attributes in vitro for a prolonged period with a stable genetic background. ESCSCs can also be induced to differentiate into cholinergic neurons directly in vitro. We also prove that there are many factors that may
mutually interact in cholinergic differentiation. The cholinergic differentiation may not be determined by only one factor, and the paths of differentiation may numerous. Our findings suggest that we may be able to repeat some steps of cholinergic neurons generation in vivo. This gives us the hope of developing applications for patients with cholinergic neurons diseases. This work was supported by Natural Science Foundation of Shanxi Province (20051095), Development Program of Science and Technology in Colleges and Universities in Shanxi Province (200340) and Principal Foundation of Shanxi Medical University (20020617).

CHOLINERGIC DIFFERENTIATION OF SPINAL CORD STEM CELLS CO-CULTURED WITH BONE MARROW Stromal CELLS
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Bone marrow stromal cells (BMSCs) are multipotential cells for which they can be differentiated in culture into osteoblasts, chondrocytes, adipocytes, and even myoblasts. They also express the neural cell-adhesion molecule neuropilin and neurotrophic factors including NGF and BDNF. Embryonic spinal cord stem cells (ESSCCs) are a type of neural progenitor. Some laboratories have shown that NT-3 promotes primary differentiation of avian motoneurons. So we applied ESCSs-BMSCs cocultures to find whether the BMSCs could induce the ESCSs to differentiate into cholinergic neurons. In our co-culture system, the ESCSs and BMSCs were segregated by the Transwell membrane. We found that some ESCSs were induced to differentiate into cholinergic neurons. The neuronal differentiation activity of BMSCs on ESCSs was primarily attributed to the soluble factors secreted by BMSCs. Co-culture medium was DMEM/F12 added with B27-supplement and bFGF. The cholinergic neurons were detected by using immunofluorescence of the choline acetyltransferase after 14 d. To determine the number of cells expressing a particular antigen, 15 fields per sample were examined and totaled. Results were expressed as percentages for the numbers of positive cells for choline acetyltransferase (ChAT) compared to total numbers of progeny of ESCSs determined by counting Hoechst 33342 stained nuclei. Statistical analyses were carried out by using SPSS software. In the co-culture system, the percentages of ChAT positive cells were 20%. And in the control, no cells expressed ChAT. So we believe that BMSCs must secrete something which can induce ESCSs to differentiate into cholinergic neurons. Our results also prove that the differentiation of NSCs is influenced by signals in the cellular microenvironment. This work was supported by Natural Science Foundation of Shanxi Province (20051095), Development Program of Science and Technology in Colleges and Universities in Shanxi Province (200340) and Principal Foundation of Shanxi Medical University (20020617).

RADIOTHERAPY-RELATED TYPES IN 842 PATIENTS IN CANTON WITH NASOPHARYNGEAL CARCINOMA
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To propose the clinical radiotherapy-related typing and summarize proportional distribution of radiotherapy-related type of nasopharyngeal carcinoma (NPC), 842 cases with NPC were randomly studied. According to five years follow-up results after radiotherapy, NPC was sub-divided into four types: type I (no primary and regional recurrence and no distant metastasis), type II (primary or regional recurrence and no distant metastasis), type III (no primary and regional recurrence, and distant metastasis), and type IV (primary or regional recurrence, and distant metastasis). The proportion of the four types and relationship between this typing and Zhi-guang Xie typing were analyzed. In conclusion, distribution proportions of radiotherapy-related types of NPC were 50.6 %, 23.2 %, 20.7 %, and 5.6 % for the type I, II, III, and IV, respectively. For type D and AD of Zhi-guang Xie typing system and stage III and IV of 92 Fuzhou staging system, the proportion of type III exceeded that of type II; for type A and stage I and II, the proportion of type II exceeded that of type III. Radiotherapy-related typing, as a new clinical sub-classification, could be supplementary for previous clinical typing and staging.

ADENOVIRUS MEDIATED HUMAN BONE MORPHOGENETIC PROTEIN-2 (BMP-2) GENE EXPRESSION INDUCED OSTEOGENIC AND CHONDROGENIC DIFFERENTIATION OF RAT BONE MARROW MESENCHYMAL STEM CELLS
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In order to investigate the potential applications of human Bone Morphogenetic Protein-2 (BMP-2) gene therapy induced Osteogenic and Chondrogenic Differentiation of Mesenchymal Stem Cells, we constructed recombinant adenoviral vector carrying the human BMP-2 gene and reporter gene LacZ respectively. The chimeric virus was named Ad.LacZ.BMP-2. We constructed replication defective recombinant adenoviral vector carrying the human BMP-2 gene and reporter gene LacZ by use of in vivo recombinant. The construction of viruses was identified by enzyme digestions, PCR amplification, sequencing, light and electron microscope and electron microscope analysis. The virus stocks were propagated in 293 cells and purified by CsCl gradient centrifugation. The virus titer was up to 109 pfu/ml. After BMP-2 gene transduction mediated by adenovirus in the primary bone marrow stromal cells (MSCs) and primary skin fibroblasts of rats, ALP stain, Ca stain, protein ektrophoresis, Western Blot, immunohistologic stain, ELISA, light and electronic analysis were performed to evaluated the gene expression and induction of Osteogenic and Chondrogenic Differentiation of Mesenchymal Stem Cells. The results showed that BMP-2 gene introduction and expression mediated by adenovirus in two types of cells were achieved efficiently, and were capable of inducing osteogenic and chondrogenic differentiation of MSCs. The study also demonstrated that the BMP-2 gene introduction and expression mediated by adenovirus in the MSCs and dermal fibroblasts were achieved efficiently. The BMP-2 gene expression mediated by adenovirus may induce Osteogenic and Chondrogenic differentiation of MSCs, thus MSCs differentiation induced by BMP-2 gene has potential applications in bone and cartilage tissue engineering.

CONSTRUCTION OF TH-GDNF VECTOR FOR GENE THERAPY OF PARKINSON’S DISEASE AND ITS EXPRESSION IN VITRO
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Current gene therapeutic methods for Parkinson’s disease (PD) have focused on two treatment strategies. One is the replacement of biosynthetic enzymes for dopamine synthesis (such as tyrosine hydroxylase, TH) and the other is the addition of neurotrophic factors (such as glial cell line-derived neurotrophic factor, GDNF) for protection and restoration of dopaminergic neurons. We suppose that the gene therapy of the two strategies combined together might be more efficient than that of each alone. Our objective was to construct a vector carrying both TH and GDNF, providing a new gene therapeutic method for PD. Human TH gene obtained from the plasmid pWAV-2 was cloned into pIREs vector to construct pIRES-TH. The mouse GDNF gene, amplified by PCR technique, was cloned into pIRES-TH to construct pIRES-TH-GDNF. The two aim gene expression vectors were constructed. The results showed that TH and GDNF genes were highly expressed in C6 cells, both in mRNA level and protein level. These results suggest that the plasmid pIRES-TH-GDNF can be constructed successfully and can express TH and GDNF in vitro. This study was supported by the Key Project of Shandong Province Education Department (J04E15).
DIFFERENTIATION OF MOUSE PRIMORDIAL GERM CELLS IN ACUTE DAMAGED LIVER MICROENVIRONMENT
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Inducing stem cells to dedifferentiate into functional cells has been proposed as a potential method for enhancing endogenous regeneration in mammals. Primordial germ cells (PGCs) from gonadal ridges is another kind of embryonic stem cells and were used to investigate the milieu-dependent differentiation of PGCs from gonadal ridges of the mouse embryos in acute damaged liver microenvironment and its potential treatment. The PGCs were proliferated and labelled with 5-bromo-2-deoxyuridine (BrdU), then transplanted into the acute damaged liver (CCl4 through tail vein). Two and four weeks later, the liver was extracted and 10μm-cryostat continuous sections were obtained. The extent of differentiation of the transplanted cells was identified by immunohistochemistry, immunofluorescence double staining and PAS histochemistry for BrdU and hepatic-specific albumin (ALB), and the glycogen. The BrdU positive cells were found in the acute damaged liver, some cells were BrdU and ALB double positive and PAS positive staining, the structure of damaged liver were also significantly improved. We demonstrated that the transplantation of PGCs could be incorporated into the acute damaged liver and differentiated into hepatocytes in acute damaged liver microenvironment, and its transplantation could also compensate for acute liver failure. These results suggest that PGCs potential cellular hepatoplasly to treat damaged liver diseases.

EST ANALYSIS OF GENE EXPRESSION IN BRAIN AND SPINAL CORD OF GEKKO JAPONICUS
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To investigate the gene expression profiles in the central nervous system of Gekko japonicus, a cDNA library from the brain and spinal cord of gekko was constructed. Totally, 4108 clones were selected for sequencing. From these sequences, 2349 unique ESTs were determined which composed of 494 clusters and 1855 singletons. The 2349 unique ESTs were translated in all six reading frames and used to search for amino acid homology in the NCBI nr protein database. 54.8% (1287/2394) of ESTs were identified as known genes and 45.2% (1062/2394) ESTs as unknown genes. According to the blast results, the 2349 unique ESTs were divided into ten categories in term of the products they encoded for. Both the house-keeping genes and the nervous system related genes were present in the library. The complete open reading frame (ORF) containing clones derived from the library were identified as well, with 450 clones being obtained to date. Finally, three clones with sequence homology to endothelial differentiation-related factor (EDF)-1, myelin-associated glycoprotein precursor (MAG) and stem cell derived neuronal survival factor (SDNSF) were selected, and their expression patterns in normal and regenerating spinal cord were analyzed by RT-PCR. All three genes were expressed in the spinal cord, and the expression of SDNSF increased, while the expression of EDF-1 and MAG decreased after tail amputation. (Supported by 973 program Grant No.2003CB515306 and Jiangsu Province Education Department Grant No.05KJA31010).

EFFECTS OF BONE MARROW MESENCHYMAL STEM CELLS AFTER HYPOXIC-ISCHEMICENCEPHALOPATHY DAMAGE IN NEONATAL RATS
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Neonatal hypoxic-ischemic encephalopathy (HIE) harms the lives and health of newborn infants and children severely. Studies have shown bone marrow mesenchymal stem cells (MSCs) have therapeutic potential in nervous system disease. We have previously found that retinoid acid (RA) can enhance the neural differentiation of MSCs in vitro. Here we examine effects of rMSCs on functional recovery after HIE in neonatal rats in order to explore a new treatment strategy. HIE models were successfully established in 7-day-postnatal Wistar rats. At 5 days after hypoxia-ischemia, the rats were randomly divided into 3 groups and respectively transplanted with saline, BrdU marked rMSCs (1×10^5) or RA-induced rMSCs (1×10^5) into their lateral cerebral ventricle. Immunohistochemistry was used to identify cells derived from rMSCs. Shuttle box test was performed to evaluate the condition of functional recovery. Neurorotrophin and receptors cDNA microarray was also employed to investigate the underlying action mechanisms of rMSCs treatment. Immunohistochemistry showed rMSCs-derived cells survived, migrated in the hypoxic-ischemic brain and a few of them expressed protein characteristic of neurons and astrocytes in RA-induced group. Rats with transplantation of RA-induced rMSCs exhibited significant improvement on shuttle box test (P<0.05). The cDNA microarray analysis showed expression of 74 genes in saline control group was up-regulated, 82 genes in rMSCs group and 17 genes in RA-induced group was down regulated. Some genes changed remarkably, including IL-6, FAS, which confirmed by Real-Time PCR. In conclusion, these results suggest transplantation of RA-induced rMSCs into lateral cerebral ventricle can improves functional recovery after HIE harms in neonatal rats and some cytokines such as IL-6 may play an important role during the process.

CYTOKINE EXPANSION ENHANCES TRANSMSIION AND ADHESION OF CORD BLOOD CD34+ CELLS IN VITRO
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Cytokine expansion culture for a long time has demonstrated defective bone marrow homing of hematopoietic stem cells. Adhesion and transmigration to endothelial cells are essential to homing process. We studied the changes of adhesion and transmigration of cord blood CD34+ after stem cell factor (SCF) and interleukin-6 (IL-6) expansion culture for 48 hours. CD34+ cells were separated by Ficoll density gradient centrifugation and stimulated by SCF and IL-6 cytokines. The changes of CD49d (VLA-4), CD11a (LFA-1), CD62L (L-selectin) and CD184 (CXCR4) expressions and the CD34+ cells which migrated to stromal cell-derived factor-1 (SDF-1) in the transwell plate were analysed by flow cytometer. The capability of CD34+ adhesion to fibronectin was detected by MTT method. Results showed that the percentages of the expressions of CD49d (VLA-4), CD11a (LFA-1), CD62L (L-selectin) and CD184 (CXCR4) on CD34+ cells were significantly increased after SCF and IL-6 stimulation for 48 hours. The migrated rate of CD34+ cell to SDF-1 and the numbers of adhesion to fibronectin were also significantly increased with the control. The results indicate that SCF and IL-6 stimulation may be useful for improving the homing of hematopoietic stem cells.

CULTURE RMSCS IN THE ENVIRONMENT WHICH HAS BEEN USED FOR GROWING POLARIZED EPITHELIA TO INDUCE RMSCS TO EPITHELIAL CELLS
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Recent studies have indicated that adult bone marrow stem cells (MSCs) can be differentiated into cells of three germ layers in response to environmental cues. To induce the differentiation from rMSCs into epithelia and compare the inducing conditions to study the effects of environment on cell differentiation. We planted rMSCs onto the apical compartment of permeable filter. 4 – 5 × 10^7/cm^2

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MSCs derived from a single cell were plated onto Matrigel coated dishes or the apical compartment of permeable filter. The cells were grown in DMEM/F12 medium containing 10% FBS. Medium was changed every two days till the cells reached confluence and seed on permeable filters for 4 days. Hematoxylin staining showed that rMSCs formed a tight monolayer, and immunofluorescence showed that the tight monolayer assumed epithelial characteristics with expression of ENaC-β, Cytokeratin 5&8 and CFTF. RT-PCR results showed that the expression of ENaC-β, ENaC-γ, CFTF and ZO-1 were upregulated in the cells grown on permeable supports. The results of Isc suggest that there are tight junctions forming between cells but the ion channels of epithelial cells are immature and without function. We found that the support of the permeable filter is a critical influencing factor to epithelial differentiation. The cell-cell contacts should promote the differentiation from rMSCs into epithelia. All of these results are helpful for us to understand the mechanism of MSCs differentiation.

TRANSPLANTATION OF NEUROTROPHINS AND NEURAL STEM CELLS DERIVED FROM BONE MARROW STROMAL CELLS PROMOTES THE RECOVERY OF ANIMAL MODELS OF EPILEPSY

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It has been affirmed that bone marrow stromal cells have a therapeutic role in many degenerative diseases and we suspect they may promote the recovery of animal models of epilepsy. Our previous research has established the culture of bone marrow stromal cells and their induction into GABA neurons in vitro. Epilepsy models: lithium chloride (3 meq/kg) was administered ip 18 - 20 h before the sc injection of pilocarpine. Also 1 mg/kg methylscopolamine was administered 30 min before the pilocarpine to reduce the peripheral consequences of pilocarpine administration. Diazepam was also injected after status epilepticus, (90 min) in order to improve the survival rate. Transplantation: neurotrophins (bFGF, NT-3, BDNF) and neural stem cells derived from bone marrow stromal cells (1×10³/ml) were graft into the two sides of hippocampus (5ul per side) of epilepsy models with the add of stereotaxic apparatus. Electrophenogram showed that wave amplitudes dropped compared with only transplantation neural stem cells derived from bone marrow stromal cells. Immunohistochemistry showed that much of the cells survived and integrated into the brain. In brief, transplantation of neurotrophins and neural stem cells derived from bone marrow stromal cells has mucher superiority in the therapy of epilepsy. This work is supported by Natural Science Foundation of Shanxi Province (20051095).

IMPROVING THE REPAIR OF SKIN INJURY ON RAT AFTER AUTOGRAFTING WITH BONE MARROW MESENCHYMAL STEM CELLS

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Skin defects are sometimes life threatening. There is increasing evidence that adult stem cells are useful for tissue regeneration. Extensive stem cell research and potential clinical applications have provided new perspectives in the use of stem cells in the treatment of human skin severe burns and wounds. Bone marrow mesenchymal stem cells (MSCs) are self-renewing and are potent in differentiating into multiple cells and tissues. To explore the effect of rat bone marrow mesenchymal stem cells (rMSCs) on the repair of rat skin wounds so as to provide a new method for clinical skin repair in the future, full-thickness skin and soft tissue defects of 1 x 1 cm² in size, were excised on the backs of 48 SD rats, and then the rats were randomly divided into 2 groups: saline control and rMSCs treatment group. DAPI marked rMSCs (6×10⁵/ml, 0.3 ml) were injected to the epidermis of the wound skin in rMSCs treatment group. The progress of wound healing was observed on micro-and macro-levels post-injury respectively (6 rats per time point). An evaluation was made of wound size, histology and protein expression of transforming growth factor-β (TGF-β1), epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) at 3, 7 and 14 days after injury. The results showed that wound healing was faster with an extra reduction of 18.6 % (P < 0.01) in wound areas of the rMSCs-treated group compared to that in the control group. Histological examination and semi-quantitative analysis demonstrated that the number of vessels and fibroblasts in the rMSCs-treated wounds were significantly enhanced (P < 0.05). The expression of TGF-β1 (1.2±0.66, P < 0.05), EGF (0.9±0.45, P < 0.05) and bFGF (1.2±0.46, P < 0.01) were observed to be elevated at 3, 7, and 3 d post-injury respectively by Western blotting from the rMSCS-treated tissues. These data suggest that rMSCS accelerate cutaneous wound healing as the rMSCS transdifferentiate into the epithelium, promoting growth factor production and release in the skin wounds.

FUNCTIONAL DEVELOPMENT OF POTASSIUM CURRENT IN NEURAL STEM CELLS CULTURED IN VITRO

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Discussing the feasibility of the cells transplantation, it is important to ensure that neural stem cells must be functional after differentiation in vitro. We demonstrate that neural stem cells from newborn rat hippocampus were cultured in vitro and induced by serum and removing mitogens. In addition to morphological changes and expression of markers, functionally, after differentiation cells were cultured for 1d, 7d, 14d and 21d, and whole-cell voltage patch clamp recording was adopted respectively to detect voltage-dependant K+ current in different developing terms. As a result, the specific markers of NSCs, nestin, were expressed on 0 passage and passed cells, differentiated cells showed expression of the specific markers of neurons (NSE) and astrocytes (GFAP), which represents that cultured cells morphologically approach maturity. In the process of differentiation, after 1d culture, no current was detected, and at the 7th, 14th and 21th day after differentiation, the average-densities of K+ currents was (18.07±2.78) pA/pF, (13.09±2.74) pA/pF, (34.04±8.06) pA/pF at the test potential of +50mV. The average-densities of K+ currents on the differentiated neural stem cells increased gradually which means that the electrophysiological function of cultured cells is approaching maturity. These results suggest that the newborn rat hippocampus neural stem cells-derived neurons are capable of proliferation and differentiation, indicating their plasticity and potential use in treatment of neural degenerative diseases.

THE SIGNAL MECHEMISM OF NOTCH DURING NEURAL DIFFERENTIATION IN hMSC

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Cell transplantation has been considered as an effective strategy aiming at repairing a damaged neuronal system. Mesenchymal stem cells (MSCs) are one kind of such cell for transplantation. MSCs can differentiate into a variety of tissue lineages including osteoblasts, chondrocytes, myocardial cells, smooth muscle cells, endothelial cells, neurons and astrocytes in the presence of inductive factors. It has been found that the Notch signaling pathway plays a key role in determinating the fate of stem cells since it is attended has been paid to Notch signaling. As an evolutionarily conserved interaction mechanism, Notch signaling plays an important role in proliferation, differentiation and apoptosis of many cells during cells development. At present, most studies of human mesenchymal stem cells (hMSCs) focus...
on the cell morphology, specific protein markers and differentiation condi-
tions, nevertheless, the mechanism of differentiation to various tissue types
is poorly understand. Therefore, in the study, we intend to observe the ex-
pression of Notch-related genes in cells when induced into neuronal cells
and to elucidate the molecular mechanisms of Notch signaling pathway.
Methods: Before and after the induction, the expression of Notch1, JAG1,
PS1 and HES1 genes were detected with RT-PCR, the protein products of
Notch1, JAG1 were further tested by immunofluorescence cytochemical
method. The neural marker neuron-specific enolase (NSE) and Nissl’s body
were detected immunochemistry. The Results were as follows: There
were expressions of Notch-related genes in cells before and after the induce-
ment, but when the hMSCs were induced into neuron-like cell, the expres-
sion of Notch-related genes all showed significant down-regulation. It was
concluded that Notch signaling may have a suppression role in the process
of neural differentiation.

**EXPRESSION OF DRD2 ON THE NEURON-LIKE CELLS-
DERIVED FROM HUMAN MESENCHYMAL STEM CELLS**

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To investigate the Expression of dopamine receptor D2 (DRD2) on the Neu-
ron-like Cells-derived from Human Bone Mesenchymal Stem Cells (hBM-
SCs). hBMSCs were separated from adult bone marrow by density gradient
centrifugation and purified by adherent culture. Cells surface markers, such
as CD34, CD45, CD44 were characterized by flow cytometer (FCM). After
induction by DMSO/BHA/FSK, Silver staining was used to detect neurofi-
bra, whereas, the protein of DRD2 and NSE were determined by immunocyto-
chemistry, and the mRNA of DRD2 and NSE were measured by RT-PCR.
The results of flow cytometry displayed that hBMSCs were CD44+, CD45,
CD34 cells, and showed a typical mesenchymal-like immunophenotype. Af-
ter being induced by DMSO/BHA/FSK, their morphogenesis changed rapidly
when observed by contrast phase microscope. The majority of cells had typi-
cal morphological features of neurons, such as spherical shape and extending
processes. Analysis of silver staining confirmed that neurofibra exhibited in
these cells. RT-PCR discovered mRNA of NSE and DRD2, and immunocyto-
chemistry staining indicated that the differenced cells express the protein
of NSE and DRD2. These results suggest that neurons derived from hBMSCs
can express DRD2.

**p38 MITOGEN-ACTIVATED PROTEIN KINASE MEDIATE
INOS-INDUCED SPINAL NEURON DEGENERATION AFTER
ACUTE TRAUMATIC SPINAL CORD INJURY**

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The enhanced production of nitric oxide (NO) via inducible nitric oxide syn-
thatase (iNOS) has been implicated in the pathogenesis of neuronal apoptosis
after acute traumatic spinal cord injury (SCI). In the present study, to further
characterize the pathways mediating the synthesis and release of NO, activa-
tion of p38 mitogen-activated protein kinase (p38 MAPK) in microglia/mac-
rophages in the injured area of adult rats subjected to a complete transection
at the T10 vertebrae level was examined and its role in NO production and
neurofibra growth was determined by Reverse-transcribed Polymerase Chain
Reaction (RT-PCR), and immunocytochemistry on the expression of nestin and
insulin. Quantification of the insulin product in the medium was determined by
ELISA. CD34+ cells accounted for more than 90% after MACS.

**CELL PROLIFERATION AND BMP4 EXPRESSION
WERE INCREASED IN THE DENTATE GYRUS OF
PENTYLENETETRAZOL KINDLING ADULT RATS**

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The aim of the study was to explore the involvement of BMP4 in the cell
proliferation in the dentate gyrus of pentyleneetrazol (PTZ) kindling rats.
PTZ kindling epilepsy model has been developed to test potential antiepi-
leptic drugs and to define more precisely the possible etiology of this disor-
der. Immuno-histochemistry and in situ hybridization approach were used to
detect Bromodeoxyuridine (BrdU) labeled cells and BMP4 mRNA-positive
cells in dentate gyrus of the hippocampus. The numbers of BrdU labeled
cells and BMP4 mRNA-positive cells increased significantly during PTZ kindling
process and reached the top level 2 day after PTZ kindled, then declined to
base level 2 months later. There was positive correlation between the increase
of the number of BMP4 mRNA-positive cells and the increase of the number
of BrdU labeled cells. It suggests that BMP4 likely plays an important role
in the enhanced neurogenesis of the dentate gyrus induced by PTZ-kindling.
This work was partly supported by National Nature Science Foundation of
China, No.30571770.

**DIFFERENTIATION OF HUMAN UMBILICAL CORD
BLOOD CD34+ CELL INTO INSULIN-SECRETING CELLS
INDUCED BY NICOTINAMIDE, BETACELLULIN, BFGF
AND HGF**

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To investigate the differentiation of CD34+ cells derived from human umbili-
cord blood into insulin-secreting cells in an in vitro system supplemented
with growth factors. CD34+ cells were isolated by magnetic cell sorting
(MACS). The purity of acquired cells was determined by FACs analysis.
Purified CD34+ cells were cultured in DMEM supplemented with 5% FBS, 1x
ITS, 10-4mol/L Ascorbic acid 2-phosphate, 4.7 µg/ml linoleic acid, and a
combination of nicotinamide (10 mM/L); Betacellulin (1.5 mM/L); bFGF
(50 ng/mL) and HGF (20 ng/mL). Cultured cells were collected after 24
days to determine the extent of the cell conversion by microscopic
observation on the morphology. The mRNA expression of nestin, ngn3 and IPF-1 were
determined by Reverse-transcribed Polymerase Chain Reaction (RT-PCR),
and immunocytochemistry on the expression of nestin and insulin. Quan-
tification of the insulin product in the medium was determined by
ELISA. CD34+ cells accounted for more than 90 % after MACS. Nestin, ngn3 and
IPF-1 mRNA were detected in the differentiated cells. Immunocytochemical
staining for nestin and insulin were observed in the parts of the differentiated
cells. And the positive ratio of the differentiated cells was 9.8 % ± 2.7 %.
Insulin ELISA result show that the insulin product in culture medium was
significantly increased after inducing in comparison with control groups (p <
0.01). A conversion of CD34+ cells derived from human umbilical cord blood
into insulin-secreting cells had been observed in this experimental culture
system.
**CARCINOGENESIS, DIAGNOSIS AND TREATMENT**

**ESTABLISHMENT OF QUANTITATIVE ASSAY DR5 DOUBLE ANTIBODIES SANDWICH ELISA**

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BALB/c mice were immunized with sDR5 in CFA. We got three strains of antibody, one could induce the apoptosis of the tumor cells, and was named mDRA-6 in the laboratory. The other two strains had no apoptosis activity, but had the ability of specific binding with DR5, and had the ability to identify the different epi-position of DR5. They were named YM366EC and YM366ED, by using which we made double antibodies sandwich ELISA reagent box for detecting the solubility of DR5 with sensitivity, specificity, accuracy and repetition. And we observed the influence of factors such as ultraviolet irradiation, eluant, dilution and confining liquid on experimental results to lay a foundation for clinical application. To obtain two mAbs identifying the different epi-position of DR5 by protein G affinity chromatography. One of which was marked by HRP; another was coated plate, added serum, compared positive control with negative control, added tagged mAbs, then determined a value after coloration. Conclude DR5 contents by standard curve of DR5. Its specificity was above 98%, and sensitivity is 0.01 µg/L. Assaying of DR5 by diagnostic kit manufactured, and 50 clinic samples were detected initially. Results shows Microtiter plate treated by YM366ED, by using which we made double antibodies sandwich ELISA reagent box for detecting the solubility of DR5 with sensitivity, specificity, accuracy and repetition. And we observed the influence of factors such as ultraviolet irradiation, eluant, dilution and confining liquid on experimental results to lay a foundation for clinical application. To obtain two mAbs identifying the different epi-position of DR5 by protein G affinity chromatography. One of which was marked by HRP; another was coated plate, added serum, compared positive control with negative control, added tagged mAbs, then determined a value after coloration. Conclude DR5 contents by standard curve of DR5. Its specificity was above 98%, and sensitivity is 0.01 µg/L. Assaying of DR5 by diagnostic kit manufactured, and 50 clinic samples were detected initially. Results shows Microtiter plate treated by ultraviolet irradiation can raise sensitivity. OD value with Tris eluant and Tris dilution was higher than that with PBS, moreover the background is lower. The effect of 10 % fetal bovine serum is best; The coefficient of variation of assay DR5 double antibodies sandwich ELISA was below 5%, which showed that the reproductibility was good, recovery rate was above 85%, and the accuracy rating was high. sDR5 level of 30 cases of healthy adult was below 10 pg/ml. sDR5 level of patient of type B hepatitis was higher. Conclusion demonstrate DR5 double antibodies sandwich ELISA was established for the first time. It can be directly used to detect the content of DR5 in body fluid and to study the relationship between the expression of DR5 and tumor patient, and viral infection.

**IDENTIFICATION AND CHARACTERIZATION OF A NOVEL AND PUTATIVE TUMOR SUPPRESSOR GENE**

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TSG is a novel membrane GPI-anchored cysteine-rich protein that shares about 30% homology to urokinase plasminogen activator receptor (uPAR). Recent findings suggested that regulation of the urokinase plasminogen (uPA) system is involved at multiple steps in cancer progression and is an important factor in the process of cancer cell invasion and metastasis. To address the role of TSG in cancer progression, we have generated stable GFP-fusion TSG-expressing transfectants in hepatocellular carcinoma cell lines. uPA activity assay and RT-PCR demonstrated that TSG exerts pleiotropic inhibitory effects on pericellular uPA system without altering the steady-state levels of the respective mRNAs. Immunoprecipitation result showed that uPA is co-precipitated with TSG, suggesting a role of cell-surface receptor and potent antagonist of uPAR. Matrigel invasion assay revealed that the inhibitory effect of TSG on uPA system correlates with significant decreased cell invasion and metastasis in vitro. Gelatin zymography demonstrated that TSG significantly reduced MMP-2 and MMP-9 secretions, suggesting a role in inhibiting ECM degradation which is important for cancer metastasis. In summary, this study provides the first evidences that TSG is a potent antagonist of uPAR and suppresses tumor invasion and metastasis by regulating the uPA system.

**PRELIMINARY STUDY ON THE USE OF COMET ASSAY FOR PREDICTING THE RADIOSENSITIVITY OF NASOPHARYNGEAL CANCER IN 60 PATIENTS**

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To discuss the predicting value of comet assay in the clinical tumor radiosensitivity of nasopharyngeal cancers, the biopsies of 60 nasopharyngeal cancer patients (43 males and 17 females) were collected before radiotherapy and analyzed by comet assay. According to the 1992 nasopharyngeal cancer Fuzhou staging method, there were 13 in stage II, 29 in stage III and 18 in stage IV. The result of the comet assay was expressed as the ratio of the tail moment (Rm). All patients were examined by Spiral CT or MR before and after tumor being irradiated for 50 Gy. The maximum cross-section area of the tumor was measured as S0 and S50. The regression rate was used to evaluate the clinical tumor radiosensitivity, expressed as regression ratio Rs. Three grades of tumor sensitivity were used: low sensitivity (Rs ≤ 50 %), intermediate sensitivity (50 % < Rs < 75 %), and high sensitivity (Rs ≥ 75 %). Statistical analysis was performed using SPSS10.0 software (Pearson analytical method for correlation analysis between Rs and Rm, and Linear regression for Rs to Rm). Rs correlated positively with Rs for the whole group (P = 0.001), and the similar results were found in each group of stage II, stage III or stage IV (P < 0.05). Linear regression showed that Rs was precisely estimated by Rm (F = 13.271, P = 0.001), with a coefficient of 0.502 (95 % CI: 0.380–0.624, t =8.276, P=0.000). In three groups of tumor patients designated by Rs as high, intermediate and low sensitivity tumors, their average Rm were 4.51 (4.51 ± 0.55), 3.48 (3.48 ± 0.42) and 2.14 (2.14 ± 0.32) respectively, among which statistically significant differences was observed (F = 7.859, P = 0.001). The
TAGALSN C, A NOVEL SMALL NATURAL COMPOUND INDUCES CELL APOPTOSIS VIA MITOCHONDRIA PATHWAY
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Bcl-2 family proteins comprise of anti-apoptotic molecules such as Bcl-2 or Bcl-xL and pro-apoptotic molecules such as Bid, Bak and Bax. Their interactions play important roles in determining programmed cell death. A lot of studies demonstrated that many cancer cells express high level of Bcl-2/Bcl-xL proteins such as breast cancer, prostate cancer and B cell lymphoma etc. It is also associated with drug resistance in cancer cells. Therefore, Bcl-2/bcl-xL protein represents an attractive target for the development of a novel therapy for the treatment of cancers. Tagalasin C (TC), isolated from mangrove plant, Ceriops tagal, is a novel small compound first discovered by our Lab in the world. Here, we report that TC can markedly inhibit the growth of Bcl-2 over-expressing cells (IM9/Bcl-2). Further studies demonstrated that TC can effectively induce Bcl-2 over-expressing cell apoptosis. This was indicated by phosphatidylserine externalization, loss of mitochondrial transmembrane potential, caspase activation, chromatin condensation and DNA fragmentation. In contrast, etoposide and cisplatin, two commonly used anti-cancer agents, were not able to induce Bcl-2 over-expressing cell apoptosis. Our results demonstrated that TC is a novel small molecule inhibitors of Bcl-2/bcl-xL, which has great capabilities of cell permeability and tumor cell-killing activity, indicating a promising chemotherapeutic compound.

STUDIES ON BEVACIZUMAB INHIBITING LYMPHANGIOGENESIS IN HIGH METASTATIC POTENTIAL XENOGRAFT LCI-D20 HEPATOCELLULAR CARCINOMA
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We have investigated the effect of anti-VEGF monoclonal antibody, Bevacizumab, on growth and lymphangiogenesis of the LCI-D20 high metastatic potential hepatocellular carcinoma. The high metastatic potential hepatocellular carcinoma LCI-D20 xenograft mode in nude mice was treated with therapeutic dose Bevacizumab. Four weeks after the treatment, masses of the tumors were measured and lymphatic microvessel density (LMVD) in tumor tissues were detected by immuno-histochemistry. The difference was considered significant as P<0.05. Tumor masses and lymphatic microvessel density (LMVD) in group treated with Bevacizumab were significantly lower than that in control group (P<0.001). In conclusion, sufficient doses of Bevacizumab could effectively inhibit growth and lymphangiogenesis of liver carcinoma, which could provide a new target of treatment in liver carcinoma.

RELATIONSHIP BETWEEN RADIOSENSITIVITY OF NASOPHARYNGEAL CARCINOMA CELL LINES AND ACTIVITY OF DNA-PK
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In mammalian cells, the nonhomologous end joining (NHEJ) which DNA-PK is the dominant component is the predominant DSBs repair system. The study is design to clarify the relationship between radiosensitive of Nasopharyngeal carcinoma cell lines and activity of DNA-PK. Methods: Expression of the protein was evaluated using Western blotting analysis. Then, Siga TECT DNA-Dependent Protein Kinase Assay System was performed to evaluate DNA-PK activity which can be evaluated through detecting phosphorylation ([γ-32P] ATP) of the bionylated DNA-PK p53-derived peptide substrate using a Scintillation counter. Although the post-irradiation expression of Ku70/Ku80 DNA-PKcs had no statistic discrepancy between the different radiosensitive NPC (Nasopharyngeal carcinoma) cell lines CNE1 and CNE2, DNA-PK activity was obviously higher in the CNE1 than in the CNE2 at every protein concentration. When the protein concentration was 25ug, 50ug, 100ug; the corresponding DNA-PK activity was 25.06, 37.02, 11.7 pmolATP/min for CNE1 and 11.12, 24.75, 9.4 pmolATP/min for CNE2, respectively. DNA-PK activity was lower after irradiation than before irradiation in both of the cells detected. The radioreistant of Nasopharyngeal carcinoma cell lines is probably related to activity of DNA-PK.

ANTITUMOR EFFECT AND MECHANISM OF ISATIN ON HUMAN NEUROBLASTOMA CELL LINE (SH-SYSY)
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To investigate antitumor effect of Isatin on neuroblastoma cell and the molecular mechanism involved. Nuclear was stained with Hoechst 33258; the apoptosis was measured by Flow Cytometry; RT-PCR was employed to analyze expression of Bcl-2, Bax and VEGF mRNA. Western-blot analyzed expression of phosphorylated mitogen-activated protein kinase (pp42/pp44). S-P immunohistochemical staining detected activated caspase-3. ELISA kits determined vascular endothelial growth factor (VEGF) protein. Isatin induced SH-SYSY cell apoptosis in a time- and dose-dependent manner. Bcl-2 mRNA and VEGF mRNA were down-regulated, Bax mRNA was not changed obviously. The activity of phosphorylated mitogen-activated protein kinase was decreased after treatment of Isatin, but activated caspase-3 was up-regulated, and Isatin also inhibited expression of VEGF protein. In conclusion, Isatin promotes apoptosis of neuroblastoma cell and might be a candidate to therapy neuroblastoma.

THE STUDY OF THE TOXIC EFFECTS OF INTERFERON-ALPHA AND ANTI-DR5 ANTIBODY ON K562 CELLS
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Unified chymic therapy is a usual method in leukemia treatment, but the side effect of chymic medicines are serious, especially the restrained function to marrow can make chymic therapy intermitting. So searching anti-tumor medicines that possess higher effect and lower toxicity is the primary task in tumor treatment. So hybridomas producing anti-hDR5 monoclonal antibody (mAb) and interferon-alpha were applied to K562 cell. Our objective was to investigate the toxic effect on K562 cells after using interferon-alpha and anti-DR5 antibody (mDRA-6) so as to offer experimental data for the treatments of leukemia in clinic. We analyzed the cytotoxic effects on K562 cells by MTT after using interferon-alpha, anti-DR5 antibody, the toxic effects on K562 cells are different in every groups. Along with the decline of concentration, the toxic effects on K562 cells are from 7.21 to zero percent after using interferon-alpha, the effects of the group of anti-DR5 antibody are from 15.36 to 0.54 percent, the effects of the combination group are from 33.23 to 8.21 percent. In conclusion, the toxic effects of the two combinations on K562 cells are increased compared with individual treatment.
DIAGNOSIS OF RECURRENT OR RESIDUAL NASOPHARYNGEAL CARCINOMAS IN THE SKULL BASE AREA WITH F-18-FLUORO-DEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY
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The purpose of this study was to investigate the diagnostic value of F-18-fluorodeoxyglucose positron emission tomography for the recurrent or residual nasopharyngeal carcinomas in the skull base area. Nine post-irradiation nasopharyngeal carcinoma patients received FDG-PET scanning, CT/MRI imaging and underwent nasopharyx and skull base-biopsy under endoscopy. The results of FDG-PET were evaluated and compared with CT/MRI studies and biopsies. In 9 cases of post-irradiation nasopharyngeal carcinoma, CT/ MRI detected 7 recurrent cases and 2 suspected recurrent cases in occipital bone and clivus. All 9 cases had accumulated FDG in nasopharynx and cranial base. A definite diagnosis was made by biopsy, 3 cases were confirmed recurrence, and others 6 cases were proved mucous chronic inflammation and (or) osteoradionecrosis. The accuracy of FDG-PET was 33.3 % (3/9), and the false positive rate was 66.67 % (6/9). Diagnosis of recurrent or residual nasopharyngeal carcinomas in the skull base area with FDG-PET has high false-positive rate, final diagnosis must depend on histopathologic examination under endoscopy.

PADKDR-TK SUICIDE GENE SYSTEM TUMOR VASCULAR TARGETING TREATING NASOPHARYNGEAL CARCINOMA IN VIVO
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The purpose of this study was to evaluate pAdKDR-tk treatment NPC in vivo by tumor vascular endothelial cells targeting to destroy their microvessels. Nude mice were injected with 5 × 10^6 (0.2 ml) single cell suspension of CNE-2 at one axilla area subcutaneously. When the diameter of tumors reached 0.6 cm, the mice were separated into 3 groups randomly. 0.1 ml recombinant adenoviral liquid was injected into tumors, repeated 24 h later and the 7th day. At the second time virus injecting, the weight of mice, then PBS and GCV100 mg/kg were injected into abdominal cavity of mice simultaneously according to different group. Once daily, the mice would be successively injected for 10 days. I: PBS; II: KDR-tk+GCV and III: CMV+GCV. Tumor sizes were determined periodically. Routine pathological study and immuno-histochemistry staining for microvessels density in margin of tumors were performed in the 11th day. The results implied that the speed of tumor volume shrink was different in the three groups. The fastest was group KDR-tk, however, the tumor volume didn’t shrink but grew continuously in and group PBS. In the 5 th day after beginning therapy, a 1.0 × 1.0 cm dry sloughing ulcer was found on a mouse’s tumor in KDR-tk group. When the therapy was over, tumor volume increasing rate was +89.2 % in group I, -47.8% in group II, +37.7 % in group III. There was significant difference between each group separately (P<0.05). Histopathological examination showed the tumors in group I grew widely and invaded into the chest muscles and the foreheads whereas there was necrosis of tumor in group II and III. The center of the necrosis was located in the site of injection. The result of microvessel density examination in the margin of the tumors was highest in group PBS and lowest in group KDR-tk. There was a distinct difference between each group for microvessel density in margin of tumors (P<0.05). pAdKDR-tk suicide gene system can kill NPC cells by tumor vascular endothelial cells targeting to destroy their microvessels. This may provide a new kind of therapy for NPC treatment.

CLINICAL SIGNIFICANCE OF THE QUANTITATIVE DETECTION PLASMA EPSTEIN-BARR VIRUS DNA CONCENTRATION IN DIAGNOSIS AND PROGNOSIS OF NASOPHARYNGEAL CARCINOMA
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This study was designed to quantitatively analyze the plasma EBV DNA concentration in nasopharyngeal carcinoma (NPC) patients and their postradiotherapy, and to evaluate its application in early diagnosis, prediction prognosis and monitoring of tumor recurrence and metastasis in NPC. From Mar 2003 to June 2004, 100 patients with primary NPC, 106 cases with postradiotherapy, and 40 healthy cases in the second affiliated hospital were examined. Plasma EBV DNA was analyzed by using RT-PCR technique, and the quantity of plasma EBV DNA was compared among these groups. Plasma EBV DNA was detectable in 97.0 (97/100) of primary NPC, 96.5% (27/28) of metastasis, and 100% (18/18) of local recurrence, the detectable proportion and concentration in recurrent and metastatic NPC patients were significantly higher than that in clinical remission NPC patients and control subjects. In primary NPC patients, patients with advanced T stage had significantly higher plasma EBV DNA concentration than those of in early T stage; the sensitive rate and specific rate of diagnosis in primary NPC was respectively 97.0 and 92.0%. Three of the clinical remission NPC patients with elevated EBV DNA copy were confirmed for tumor local recurrence or metastasis after further 6-month follow-up. The results suggest that plasma EBV DNA detection may be a sensitive tumor marker for diagnosis, prognosis and monitoring tumor recurrence and metastasis of NPC patients after radiotherapy.

FAMILIAL CORRELATION OF EPSTEIN-BARR VIRUS (EBV) SEROREACTIVITY AMONG UNAFFECTED MEMBERS WITHIN SPORADIC AND MULTIPLEX NASOPHARYNGEAL CARCINOMA (NPC) FAMILIES IN CANTONESE, CHINA
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To investigate whether seropositivity to anti-EBV VCA IgA are elevated among unaffected individuals in sporadic and multiplex NPC families and whether the EBV antibody titers are correlated among unaffected individuals in both kinds of family, we collected 413 sporadic NPC families and 140 multiplex NPC families to conduct a familial correlation analysis using EBV antibody status as the outcome variable. In addition, we recruited healthy controls in Guangdong (a higher risk population for NPC) and 521 healthy controls (a lower risk population for NPC) in Henan. Results indicated the seropositivity of VCA-IgA among the first-degree relatives in multiplex families (35.75%) was similar to that of VCA-IgA in sporadic families (32.28%) and the seropositivity of VCA-IgA among the first-degree relatives in multiplex families (35.75%) was much higher than that of healthy controls recruited from Guangdong (19.16%) and Henan (10.56%). Familial correlation analysis revealed that the correlation of the seropositivity among the spouses was similar to that among the first-degree relatives. Further, we observed that the correlation among the spouses was lower than that among the first-degree relatives in multiplex families but not in sporadic families. Our results indicate that EBV status is related to the shared familial environment in sporadic NPC families and is likely to be related to both familial environment and genetic factors in high risk families.

EUKARYOTIC EXPRESSION OF BIG-H, GENE AND ITS EFFECTS ON SECRETION OF MMPS IN HUMAN 7721 HEPATOMA CELLS
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βig-h3 was first identified as a transforming growth factor-beta1-inducible gene in human lung adenocarcinoma cell line. It encodes for a secreted extracellular matrix (ECM) protein, which is thought to act on cell attachment and ECM composition. Our previous study showed that βig-h3 was highly expresses in several human hepatoma cell lines and overexpression of HAb18G/CD147 promoted the expression of βig-h3 in human 7721 hepatoma cells. In order to elucidate the mechanism of HAb18G/CD147 in the invasive and metastatic process of human hepatoma cells and the involvement of βig-h3 in signal pathways of HAb18G/CD147, we constructed eukaryotic recombinant expression plasmid βig-h3-pEGFP-C2 and transfected into human 7721 hepatoma cell lines. Full-length βig-h3 gene, cloned by reverse transcription polymerase chain reaction (RT-PCR) was inserted into the eukaryotic expression vector pEGFP-C2. The recombinant plasmid was transfected into 7721 cells with Lipofectamine2000 and Gelatin-Zymography were adopted to detect the production of MMPs in the transfected cells. Results showed that βig-h3-pEGFP-C2 recombinant expression plasmid was successfully constructed and achieved high transfection efficiency. MMPs expression of the transfected cells was promoted significantly. In the blockade experiments with βig-h3 polyclonal antibody and HAb18 monoclonal antibody, the abilities of secretion and activation of MMP-2 and MMP-9, adhesion and invasion in transfected 7721 cells decreased obviously (P<0.01). These results suggest that overexpression of βig-h3 promoted the production of MMPs, indicating that βig-h3 may be involved in HAb18G/CD147 signal pathways and play roles in the invasive and metastatic processes of hepatoma.

EXPERIMENTAL AND CLINICAL RESEARCH OF PA VACCINE INTRAVESICAL INSTILLATION TO PREVENT THE RECURRENCE OF BLADDER TUMOR

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PA vaccine is a new kind of modified Pseudomonas aeruginosa vaccine, this article discusses the effect and feasibility of PA vaccine intravesical instillation to prevent the recurrence of bladder PA tumor. Animal trial: PA vaccine was instilled into rabbit bladder, and the rabbit general responses, urine routine, the change of bladder muscosa in gross and light-copy observed and compared with those who were instilled with normal saline and BCG. Clinical trial: 10 ml PA vaccine was dissolowed into normal saline to 50 ml, which was then instilled into the bladders of 184 cases of bladder cancer, post-operation. The side effects of rabbit bladder, which instilled PA vaccine, were similar to that of BCG; no other obvious side effects were observed. Clinical trials found that NK cell, T lymphocyte and subsets, and PA vaccine broad-spectrum antibody obviously improved. Bladder mucus was invaded by lymphocytes. The recurrence rate of PA group was 7.9% (no more than the BCG group). Conclusions: PA vaccine is a new, safe and effective biology response modifier (BMR) which can be applied for intravesical instillation to prevent recurrence of bladder tumor.

EXPRESSION OF TOLL-LIKE RECEPTORS IN HUMAN PROSTATE CANCER AND PA VACCINE INDUCELMENT

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To explore the role of Toll-like receptors (TLRs) in PA-vaccine-induced immunologic response to prostate cancer. (METHODS) Human prostate cancer cell line DU145 was cultured in RPMI 1640 medium with 10% FBS. PA-vaccine and E. coli were added in various bacterium-cell ratio for culture stimulation. After stimulation for 2 h, the expression of TLR-2 and TLR-4 in DU145 were evaluated by semi-quantitative RT-PCR. The supernatant of cell culture was collected after the stimulation of bacteria for 12 h and the levels of IL-4 and IL-12 were measured by ELISA method. Not only the expression of TLR-2 and TLR-4, but also the production of IL-12 in DU145 cells was increased by the stimulation of PA-vaccine in a dose-dependent manner. The effects reached the maximal level at dose of PA-vaccine as 8 bacilli per cell. The production of IL-4 in DU145 did not show any difference after the stimulation of PA-vaccine. The stimulation of E. coli to DU145 cells did not result in any change on the expression of TLR-2 and TLR-4, as well as the production of IL-4 and IL-12. In conclusion, the expression of TLRs in prostate cancer cells may be involved with the PA-vaccine-induced immunol response to prostate tumor.

ESTABLISHMENT OF A CISPLATIN-INDUCED HUMAN ESOPHAGEAL CARCINOMA DRUG-RESISTANT CELL LINE EC9706/CDDP AND ITS BIOLOGICAL CHARACTERISTICS

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Multi-drug resistance (MDR) is considered to be a major obstacle for chemotherapy. In order to reverse tumor MDR in vitro, we established a cisplatin-induced human esophageal carcinoma drug-resistant cell line Ec9706/cDDP in culture by exposing Ec9706 parent cells to gradually increased concentration of cisplatin (cDDP) over a period of 7 months. Compared to the parent cells, Ec9706/cDDP had a prolonged doubling time, lower growth rate, lower confluent density. Its IC50 of cDDP was 5.7 times higher than that of Ec9706, and it exhibited cross-resistance to many anti-tumor agents. When Ec9706/cDDP was stored with cDDP at –196ºC for 2 month and then recovered or cultured in RPMI-1640 without cDDP for a period of 1month, its characterization of anti-tumor drug resistance was still maintained. Flow cytometry (FCM) was performed to determined cell cycle. The number of cells in S and G1-phase were significantly increased in Ec9706/cDDP while those in G2-phase decreased. Fluorescence activated cell analysis (FACS) was employed for determining the concentration of fluorescence dye of rhodamine 123 (Rh123) with the 2 different kinds of cells. The concentration of Rh123 in Ec9706/cDDP was lower than that in Ec9706 (33.71 vs 36.97). So the newly established stable cell line Ec9706/cDDP possessed typical multi-drug resistant phenotype. It could be used as an ideal model for the research of multi-drug resistant in vitro.

COMBINATION OF AGONISTIC ANTI-HUMAN DR5 MONOCLONAL ANTIBODY WITH ADRIMAYCIN OR CISPLATIN HAS SYNERGISTIC INDUCTION OF APOPTOSIS ON LEUKEMIC CELL LINES

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It was reported that monoclonal antibodies (mAbs) against TRAIL receptors (DR4, DR5) with tumoricidal activity are potential candidates for cancer therapy. We have produced an anti-human DR5 agonistic monoclonal antibody by immunizing BALB/c mice using soluble hDR5, named mDRA-6. In this article, we report studies on apoptosis of jurkat cells and HL-60 cells in the presence of mDRA-6, adriamycin or cisplatin alone, and mDRA-6 in combination with adriamycin or cisplatin. It was reported that monoclonal antibodies (mAbs) against TRAIL receptors (DR4, DR5) with tumoricidal activity are potential candidates for cancer therapy. In order to reverse tumor MDR in vitro, we established a cisplatin-resistant cell line Ec9706/cDDP by exposing Ec9706 parent cells to gradually increased concentration of cisplatin (cDDP) over a period of 7 months. Compared to the parent cells, Ec9706/cDDP had a prolonged doubling time, lower growth rate, lower confluent density. Its IC50 of cDDP was 5.7 times higher than that of Ec9706, and it exhibited cross-resistance to many anti-tumor agents. When Ec9706/cDDP was stored with cDDP at –196ºC for 2 month and then recovered or cultured in RPMI-1640 without cDDP for a period of 1month, its characterization of anti-tumor drug resistance was still maintained. Flow cytometry (FCM) was performed to determined cell cycle. The number of cells in S and G1-phase were significantly increased in Ec9706/cDDP while those in G2-phase decreased. Fluorescence activated cell analysis (FACS) was employed for determining the concentration of fluorescence dye of rhodamine 123 (Rh123) with the 2 different kinds of cells. The concentration of Rh123 in Ec9706/cDDP was lower than that in Ec9706 (33.71 vs 36.97). So the newly established stable cell line Ec9706/cDDP possessed typical multi-drug resistant phenotype. It could be used as an ideal model for the research of multi-drug resistant in vitro.
Overexpression and biological functions of prion protein in gastric cancer: Promoting G1/S phase transition by transcriptional activation of CCND and inhibiting apoptosis through Bcl-2-dependent apoptotic pathways

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Cellular prion protein (PrPc) is a normal isoform of the pathogenic peptide (PrPres) that was previously found by our lab to be widely expressed in gastric cancer cell lines, with a certain effect on drug accumulation. In order to evaluate its biological significance in human gastric cancer, we investigated its expression in a large series of gastric tissue samples by immunohistochemical staining. Compared with normal tissues (0.74 ± 0.08), gastric adenocarcinoma showed increased PrPc expression (1.80 ± 0.18) (P<0.05), correlated with the histopathological differentiation and tumor progression. To better understand the underlying mechanism, we introduced the PrPc and two pairs of RNAi into both of gastric cancer cell lines AGS, SGC7901 and found that overexpression of PrPc stimulated cell growth, cell cycle progression and tumorigenicity both in vitro and vivo. It suppressed ROS and decreased apoptosis in transfected cells. PrPc was found to be involved in cell cycle progress by promoting G1/S phase transition dependent on overexpressed cyclinD and stimulated CHK4 activity analyzed by Western blot and induced P21 in transfected gastric cancer cell lines AGS, SGC7901. Additionally, we cloned the CCND promoter containing PrPc binding sites and constructed the luciferase reporter vectors of this promoter. Luciferase reporter assay suggested that PrPc stimulated the promoter activity of the CCND promoters higher than that of pcDNA3.1 vector. These results strongly suggested that PrPc promoted G1/S phase transition of gastric cancer cells by transcriptional activation of CCND and played a role as an effective anti-apoptotic protein through Bcl-2-dependent apoptotic pathways in gastric cancer cells. Further study into the mechanism of these relationships might enrich the knowledge of PrPc, better our understanding of the nature of gastric carcinoma.

Effect of product from corynebacterium parvum at nano scale on differentiation and maturation of mouse bone marrow-derived dendritic cells

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Recent studies have demonstrated that animals treated with corynebacterium parvum at nano scale (NCPP) were more sensitive to mDRA-6 than the spreading cells by Annexin V-FTTC/PI staining, and the apoptotic rate of non-adherent cells increased by 34 % in the presence of 800 ng/mL mDRA-6. In contrast, there was no effect in the presence of 800 ng/mL mDRA-6. The results of these studies strongly suggested that Ccpp promoted G1/S phase transition of gastric cancer cells by transcriptional activation of CCND and played a role as an effective anti-apoptotic protein through Bcl-2-dependent apoptotic pathways in gastric cancer cells. Further study into the mechanism of these relationships might enrich the knowledge of PrPc, better our understanding of the nature of gastric carcinoma.

Assay cytotoxicity of naive/primed CD8+ T cells (purified by magnetic cell sorting using a commercially available CD8+ T cell isolation kit) against B16 melanoma cells to investigate antigen presenting ability of these BM-derived DCs. The data suggest that NCPP can efficiently facilitate generation of BM-derived DCs in vivo and enhance maturation of these BM-derived DCs in vitro.

Loss of cell adhesion enhances DR5 expression on the surfaces of esophageal carcinoma cells

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DR5 is a key death receptor of TRAIL’s and plays a mighty role in TRAIL-mediated apoptosis. In present study we probed into the effect of cell non-adhesion on DR5 expression on esophageal carcinoma EC9706 cell line. Detached cells were prepared from the spreading cells pretreated or untreated with brefeldin A by EDTA digestion. Apoptosis analysis showed that non-adherent cells untreated with brefeldin A were more sensitive to mDRA-6 than the spreading cells by Annexin V-FTTC/PI staining, and the apoptotic rate of non-adherent cells increased by 34 % in the presence of 800 ng/mL mDRA-6. The results indicate that DR5 was primarily located in the cytoplasm and on the surfaces of EC9706 cells, but not in the nucleuses, and DR5 expression on the surfaces of non-adherent cells untreated with brefeldin A was much higher 25 % than pretreated with brefeldin A. Immunofluorescence assay indicated that DR5 was primarily located in the cytoplasm and on the surfaces of non-adherent cells untreated with brefeldin A was much higher than spreading cells. Because it is known to inhibit protein secretion in mammalian and other eukaryotic cells by interfering with the function of the Golgi apparatus in the presence of Brefeldin A, these results suggest cell non-adhesion may promote cytoplasmatic DR5 traffic to EC9706 cell surfaces via a Golgi-dependent pathway so that non-adherent cells are more sensitive to the apoptosis induced by agonistic antibody mDRA-6 than the spreading cells. The results may provide new insights into the regulation mechanisms of DR5 expression and distribution on cell surfaces, and also provide potential instruction significance for future clinical application of agonistic anti-hDR5 antibody and TRAIL.

A study on diabetes mellitus and prognosis of nasopharyngeal cancer

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We determined the influence of diabetes mellitus on stages and long-term outcomes of patients with nasopharyngeal cancer. The study summarized 37 patients were diagnosed as having diabetes mellitus and nasopharyngeal cancer treated by radiotherapy between January and December, 1999. With a median follow-up of 34.6 months, we analyzed differences in RFS, MFS, DFS and OS between the diabetes group and non-diabetes group. 37 patients were involved in our study. According to the Chinese Fuzhou staging system for NPC which modified in 1992, 2 patients belonged to stage I, 9 stage II, 17 stage III and 6 stage IV. The 4-year RFS of diabetes group and non-diabetes group was 52.1% vs 68.2%, the 4-year MFS was 73.0% vs 72.0%, the 4-year DFS was 35.1% vs 65.1% and the OS was 67.6% vs 75.7%. The Wilcoxon (P=0.0047) test showed there was not significant difference between the two groups of RFS, MFS and OS, while the diabetes group had a worse DFS than non-diabetes group (Wilcoxon = 0.0047). Patients with diabetes had a worse DFS than non-diabetes, but statistic test didn’t show significant difference in RFS, MFS and OS, further studies should be continue to find out the results in the future.

Building of whole-body visualizing orthotopic animal model in colon cancer

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Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF superfamily with the ability to induce apoptosis in a wide variety of transformed cell lines of diverse origin. At least five receptors for TRAIL have been identified so far. Two of them, DR4 and DR5, are capable of transducing an apoptosis signal. Monoclonal antibodies (mAbs) against TRAIL receptors with tumoricidal activity are also potential candidates for cancer therapy. There are a number of agonistic mAbs against human DR4 or DR5 reported in previous studies. In our lab, we established hybridomas, that could produce anti-hDR5 monoclonal antibody (mAb) with apoptotic activity, and was named mDRA-6. Our work provides synergistic effect of Anti-human DR5 monoclonal antibody and chemotherapeutics on SMMC-7721 cell lines. To determine the synergistic damage effect of mDRA-6 and adriamycin, cis-diaminedichloroplatinum (c-DDP) on human hepatocellular carcinoma cell lines, the survival fraction and survival inhibitory rate of SMMC-7721 were measured by MTT. The apoptosis of cells was observed by way of morphological changes, flow cytometry analysis and agarose gel electrophoresis of DNA. Results showed that cells treated with mDRA-6 exhibited typical apoptotic features in morphology. Viability of cells was decreased with mAb concentration increasing by MTT analysis. Cooperative effects of 0.08 µg/ml mDRA-6 and 5.0–25.0 µg/ml c-DDP on survival fraction and inhibitory rate of SMMC-7721 and of 0.029 µg/ml mDRA-6 and 0.01–5.12 µg/ml adriamycin on survival fraction and inhibitory rate of SMMC-7721 were found. Apoptotic analysis revealed that 0.08 µg/ml mDRA-6 could enhance remarkably c-DDP-induced apoptosis of SMMC-7721 cells. In the presence 2 µg/ml mDRA-6 for 6 h by flow cytometry analysis with Annexin V-FITC/PI staining, the apoptosis rate of the SMMC-7721 cells was 35%, the apoptosis rate of the SMMC-7721 cells was 60%. In conclusion, mDRA-6 could induce SMMC-7721 cells apoptosis and the combination of mDRA-6 and chemotherapeutics exhibit synergistic effect on SMMC-7721 cells.

SYNERGISTIC EFFECT OF ANTI-HUMAN DR5 MONOCLONAL ANTIBODY AND CHEMOTHERAPEUTICS ON SMMC-7721 CELLS

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In addition to sTRAIL, monoclonal antibodies (mAbs) against death receptors of TRAIL with tumoricidal activity are also potential candidates for cancer therapy. There are some agonistic mAbs against human DR4 or DR5 reported in previous studies most of which need cross-linkers to ensure effective killing of tumor cells. In present study, we report studies on mDRA-6, a novel agonistic monoclonal antibody against human DR5. mDRA-6 was prepared by hybridoma technique and was purified by protein G affinity chromatography. It was suggested that mDRA-6 belongs to IgG1 by Sandwich-ELISA assay. Indirect-ELISA showed that mDRA-6 specificity binds only hDR5 and not other TRAIL receptors, and does not cross-react with the murine DR5. The Kd values for the binding of mDRA-6 to DR5 were estimated at 1.4 nM. Importantly, competitive ELISA showed that mDRA-6 efficiently competed with TRAIL for binding to DR5. These results establish the specificity of mDRA-6 for human DR5, and indicate that mDRA-6 might recognize an epitope within the TRAIL-binding site on DR5. Western-blot analysis and Dot-ELISA revealed that the epitope type is of conformation. Further, functional analysis showed that mDRA-6 strongly induces cell death in human Jurkat cells. Flow cytometry analysis showed that Apoptosis rate of Jurkat cells was 87.8 % in the presence of 500 ng/ml for 6 h, but the rate decreased to 32.4 % in the presence of 4 µg/ml shDR5. The pretreatment of Caspase 8 inhibitor almost completely inhibited the apoptosis of Jurkat cells treated by mDRA-6 but that of Caspase 9 inhibitor had little effect. These results further demonstrate a caspase-dependent apoptotic mechanism of mDRA-6. mDRA-6 may be used as a useful tool for investigating the therapeutic potential of DR5 targeting in cancer and exploring the functional domain of DR5.
STUDY ON THE EXPRESSION OF YWK-II PROTEIN/APLP2 IN PANCREATIC CANCER AND ITS POTENTIAL FUNCTION IN PANCREATIC CARCINogenesis
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Pancreatic adenocarcinoma is a neoplasm with high incidence and mortality. YWK-II protein/APLP2 is a membrane protein of human sperm, which was first cloned by our lab. Using subtractive display method, Ariazi et al. demonstrated that dietary treatment of rat bearing mammary carcinoma with monoterpenes and limonene, caused regression of the cancer, associated with a marked expression of the YWK-II protein/APLP2 in the tumor cells. The present study was to investigate the expression of YWK-II protein/APLP2 and its possible roles in pancreatic carcinogenesis. The expression of YWK-II protein/APLP2 in several pancreatic cancer cell lines and normal pancreatic tissue was examined by RT-PCR, Western blot and tissue chip. The results showed that the YWK-II protein/APLP2 mRNA was significantly higher in the cancer cell lines than in normal pancreatic tissue, i.e., 7.0, 4.2, 3.1, 2.0 and 14.1 folds higher in AsPC-1, Mia-PaCa-2, P3, CaPa-1 and Panc-1 cell lines, respectively. Western blot analysis validated the finding that the expression of the YWK-II protein/APLP2 was greater in pancreatic cancer cell lines than in normal pancreatic tissue, paralleling the results obtained using Real-time PCR analysis. Unlike its weak staining in normal pancreatic tissues, YWK-II protein/APLP2 stained intensely and localized typically in the cytoplasm and plasma membranes of human pancreatic tumor cells. In conclusion, the present findings that high level expression of YWK-II protein/APLP2 occurs in pancreatic cancer cells suggest its potential functional activity in pancreatic carcinogenesis.

A NOVEL HUMAN CASPASE-9 SPlice VARIANT CASP9-γ Ping Zhang Wang, Tai Ping Shi and Da Long Ma

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Apoptosis is a genetically encoded process of cellular suicide which is involved in many physiological and pathological processes, and two apoptotic signaling pathways have been well characterized and intensively studied, the death receptor (DR)-dependent (extrinsic) pathway and the mitochondrial (intrinsic) pathway. Caspase-9 bears a caspase recruitment domain (CARD) and it plays a major role in the intrinsic apoptotic pathway induced by various forms of cell stress. Two human alternative caspase-9 splice variants have been reported, caspase-9-α and -β. Caspase-9-α bears the full cysteine protease activity, whereas caspase-9-β lacks the central large subunit and interferes with caspase-9-mediated apoptotic events by blocking the formation of the apoptosisosome. Therefore, the caspase-9-β functions as an endogenous dominant-negative isoform. In the present study, we cloned and characterized a novel caspase-9 splice variant, hereby designated Casp9-γ. Analysis of the genomic arrangement revealed that Casp9-γ comprised nine exons. Casp9-γ is generated from an additional upstream alternative 3’ splice site in the fourth exon of caspase-9, resulting in a 58-nucleotide fragment insertion compared with the full-length caspase-9 α. The fragment introduces an in-frame stop codon, and the resulting open reading frame (ORF) is preterminated. The Casp9-γ comprises the deduced 154 amino acid residues containing only the caspase recruitment domain (CARD) and does not contain the large and small subunits. The Casp9-γ was recognized by the polyclonal antibody of pro-caspase-9, and its overexpression produced a target band with the relative protein molecule mass of 17 kDa. The endogenous Casp9-γ was not detected at the protein level, perhaps due to its low mRNA expression. The Casp9-γ does not promote apoptosis when overexpressed in mammalian cells. Moreover, it inhibits the cleavage of procaspase-3 mediated by proapoptotic member Bax or apoptosis inducer staurosporine. Therefore, Casp9-γ may function as an endogenous apoptotic inhibitor by interfering with the CARD-CARD interaction between Apaf-1 (apoptotic protease activating factor-1) and procaspase-9.

ADENOVIRUS-MEDIATED TRANSFER OF SIRNA AGAINST RLcrt-196, A PUTATIVE ONCOGENE
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RNA interference (RNAi) is a powerful tool to knock down specific gene expression and has potential application in cancer gene therapy studies. However, some more efficient transduction of small interfering RNA (siRNA) into target cells is needed. Our previous data showed that a new gene Rlcrt-196, which was overexpressed in lung cancer, played an important role in the tumorigenesis of human lung. We also indicate the importance of Rlcrt-196 as a potential early diagnostic and therapeutic target for human cancers. In this study, we applied the adenovirus-delivered siRNA strategy to knock down Rlcrt-196 expression in lung cancer cells. Double-strand oligonucleotide for transcription of short hairpin RNA was constructed into the adenoviral vector with human U6 promoter. The adenovirus particles were amplified and purified with cesium chloride density gradient centrifugation. Two lung cancer cells H1299 and H520 were infected with the RNAi-containing adenovirus and the silencing efficiency was detected with reverse transcriptional PCR and western blot. The results showed that the RNAi-containing adenovirus could effectively infect H1299 and H520 cell lines and suppress Rlcrt-196 expression. Knock-down of Rlcrt-196 induced apoptosis in H1299 and H520 cell lines. Taken together, we developed an adenoviral vector harboring the Rlcrt-196-siRNA expression unit which could inhibit Rlcrt-196 expression in cancer cell lines. It will be very useful in studying the therapeutic role of Rlcrt-196 for human cancers in vitro and in vivo.

ESTABLISH OF HUMAN MULTIDRUG RESISTANT LUNG ADENOCARCINOMA CELL SUBLINES (A549/P)
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Many of the discoveries of multidrug resistance (MDR) have resulted from studies using drug-resistant cultured tumor cell lines as experimental models. Paclitaxel is one of the basic drugs for chemotherapy of lung adenocarcinoma, and it is representative to establish the MDR cells sublines induced by Paclitaxel. An in vitro paclitaxel-resistant subline (A549/P) was established by exposure to stepwise increased concentrations of the drug in a cell culture medium. Biological morphology and cell cycles were analyzed by morphometry and cytometry. The chemoresistance index of cells was measured by methyl tetrazolium assay. Compared with parent cells, the resistant sublines had a slower growth rate and lower confluent density. They were smaller and with different numbers of nucleioli. Flow cytometric analyses showed that resistant cells had a greater percentage of cells in the G2/M phase. The resistant cells, A549/P, were 118 times more resistant to adriamycin and 114 times more resistant to cisplatin than the parent cells. The resistant cells also demonstrated cross-resistance to 5-Flourouracil. The Paclitaxel -induced MDR sublines presented a stable phenotype and may be used as an experimental model for the search of a means to overcome drug resistance and elucidate possible mechanisms of acquired MDR involved in human lung adenocarcinoma.

AN EXPERIMENTAL RESEARCH ON INHIBITORY EFFECT OF CARDIAC MUSCLE CONDITION MEDIUM ON GROWTH OF NASOPHARYNGEAL CARCINOMA CELL
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Malignant tumors spread and metastasize in the majority of the organs, but are very rare in cardiac muscle. This study was conducted to explore the effect of organic microenvironment of cardiac muscles on the nasopharyngeal carcinoma. Cell with high metastases potential, and to investigate the mechanism of the
THE CLINICAL SIGNIFICANCE OF EXPRESSION AND AMPLIFICATION OF CANCER ONCOGEOE EIF-5A2 IN DIFFERENT TYPES OF HUMAN EPITHELIAL TUMORS DETECTED BY TISSUE MICROARRAY

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Recently, it has been suggested that eIF-5A2, a novel gene at 3q26.2,plays an oncogenic role in ovarian carcinoma. In the present study, we utilized the methods of immunohistochemistry and fluorescence in situ hybridization to examine protein expression and amplification of eIF-5A2 by tissue microarray in 2026 primary epithelial tumors (benign and malignant) and 856 normal epithelial tissues originating from 12 different human organs or anatomical sites, and 319 metastases from 6 malignant tumors. Overall, high-level expression of EIF-5A2 protein was observed informatively in 10.6% of normal epitheliums, 23.0% of adenomas, 35.9% of primary carcinomas and 59.7% of metastases. Amplification of eIF-5A2 was examined in 9.3% of informative carcinomas and the majority of these showed high-level expression of EIF-5A2. In the carcinoma series, a significant positive association of up-regulated expression of EIF-5A2 and increased tumor cellular proliferation (through detection of Ki-67 expression) was observed (P<0.05). In addition, a significant positive association between high-level expression of EIF-5A2 and the tumors later pTNM stages were observed in primary ovarian, colorectal, lung and bladder carcinomas (P<0.05). These findings indicate that high-level expression of EIF-5A2 protein, caused by eIF-5A2 gene amplification or other so far unknown mechanisms, in several human carcinoma types is coincident with acquisition of an invasive phenotype, suggesting a potential role of eIF-5A2 in the control of tumor cell proliferation; such may be responsible, at least in part, for tumorigenesis and/or progression of a number of distinctly different types of human carcinomas.

EXPRESS SION AND SIGNIFICANCE OF ERb IN BREAST CANCER

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To study the expression and significance of estrogen receptor beta (ERβ) in breast cancer, 30 cases of primary breast cancer were collected. ERα and ERβ were detected by immunohistochemical technique. The data were analysed with clinicopathological characters. The positive rate of ERα and ERβ in 30 primary cancer tissue was 78.4% and 55.2%, there was no correlation between the expression of ERα and ERβ (P>0.01). The expression of ERβ was inversely correlated with auxillary lymph node number (P<0.01), the ERβ positive rate was 47.2% for auxillary lymph node negative group, 79.8% for auxillary lymph node positive group. There was no correlation between ERβ and age, metastasis state, tumor size and pathological type. In conclusion, ERβ expresses in primary breast cancer tissues. The expression of ERβ is correlated with auxillary lymph node.

NEW INSIGHT INTO PATHWAYS INVOLVED IN THE PROGRESS OF METASTASIS OF COLORECTAL CARCINOMA

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Metastasis-associated genomic alteration has been recognized as playing a critical role in the progression toward metastasis of colorectal carcinoma. In our experiment, primary tumor and nodal metastatic cells were implanted into subcutaneousness of nude mice and growth and metastatic potential were observed. Then, we analyzed changes of gene profiles between primary tumor cells and metastatic tumor cells using cDNA and tissue microarrays. Nodal metastatic cells had stronger ability of growth and metastasis than primary tumor cells. Nearly 400 genes were changed between primary tumor cells and metastatic cells. Pathway analysis based on gene ontology demonstrated that several pathways were changed as the progress of metastasis. These pathways included MAPK pathway, AKT pathway, RHO pathway, STAT pathway, and so on. MAPK pathway seemed to be the dominant pathway since most genes of this pathway were evaluated. Subsequent examination of selected genes by Real-time RT-PCR and immunohistochemistry substantiated the reliability of our analysis. These results suggested that metastasis-associated genes identified by cDNA and TMA microarrays in these experiments provides a large body of potentially valuable information of metastasis of colorectal tumor and MAPK pathway may play a critical role during the process of metastasis.
CLONING, SCREENING AND IDENTIFICATION OF NEW GENES POTENTIALLY INVOLVED IN HUMAN LUNG CANCER

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Lung cancer is a leading cause of death worldwide. Over the past decade, much has been learned about the molecular alterations associated with lung cancer. However, the precise mechanisms underlying lung carcinogenesis remain unresolved. In this study, 85 functionally unknown genes, which were potentially related to lung cancer, were selected from our previous suppression subtractive hybridization (SSH) cDNA libraries. Using bioinformatic analysis, we found the full open reading frame (ORF) in the cDNA sequences, and used reverse transcription-PCR and TA cloning strategy to clone them into the T-easy vector, and then into eukaryotic expression vectors. All the clones were confirmed with DNA sequencing and further screened with functional and phenotypic methods. We cloned 51 genes and performed reporter gene assay including NF-kappaB, NFAT and AP1 pathways whose abnormalities are often involved in tumorigenesis. The results showed that the new genes, rlcrt-196 and rlcrt-456, could obviously increase the luciferase activity of the NF-kappaB-luc reporter, and might be involved in NF-kappaB activation; rlcrt-292 and bhc-29 could increase the luciferase activity of the NFAT-luc reporter, and might participate in NFAT activation process. Additional phenotypic screening showed that rlcrt-108 which was cloned from the lung cancer low-expression SSH library, could remarkably reduce colony formation in H1299 lung cancer cell line. Taken together, the 51 functionally unknown genes we have cloned, which were potentially related to lung cancer, are valuable in cancer research, if they could be further screened with other functional and phenotypic methods. The candidate genes selected from the primary screening should be further investigated for their roles in the carcinogenesis of human lung.

BMI-1 IS A NOVEL MOLECULAR MARKER OF NASOPHARYNGEAL CARCINOMA PROGRESSION AND IMMORTALIZES PRIMARY HUMAN NASOPHARYNGEAL EPITHELIAL CELLS

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The Bmi-1 oncoprotein regulates proliferation and oncogenesis in human cells. Its overexpression leads to senescence bypass in human fibroblasts and immortalization of human mammary epithelial cells. In this study, we report that compared to normal nasopharyngeal epithelial cells (NPECs), Bmi-1 is overexpressed in nasopharyngeal carcinoma (NPC) cell lines. Importantly, Bmi-1 was also found to be overexpressed in 29 of 75 NPC tumors (38.7%) by immunohistochemical analysis. In contrast to NPC, there was no detectable expression of Bmi-1 in non-cancerous nasopharyngeal epithelium. Moreover, high Bmi-1 expression positively correlated with poor prognosis of NPC patients. We also report that the overexpression of Bmi-1 lead to bypass of senescence and immortalization of NPECs, which normally express p16INK4a and exhibit finite replicative life span. Overexpression of Bmi-1 in NPECs led to the induction of telomerase reverse transcriptase (hTERT) activity and reduction of p16INK4a expression. Mutational analysis of Bmi-1 showed that both RING finger and helix-turn-helix (HT) domains of it are required for immortalization of NPECs. Our findings suggest that Bmi-1 plays an important role in the development and progression of NPC, and that Bmi-1 is a valuable marker for assessing the prognosis of NPC patients. Furthermore, this study provides the first cellular proto-oncogene immortalized nasopharyngeal epithelial cell line, which may serve as a cell model system for studying the mechanisms involved in the tumorigenesis of NPC.

ANTI-HUMAN DR5 MONOCLONAL ANTIBODY INDUCES APOPTOSIS IN HUMAN HEPATOCARCINOMA CELL LINES

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Primary carcinoma of the liver is a common malignant tumor that affects health. In recent years, with progress in the pathogenesis and treatment research of liver cancer, it has been suggested that on unusual mechanism of cell apoptosis occurs in liver cancer cells, which could be of importance in developing a new treatment method. Recently, we have been trying to induce cancer cell apoptosis for anti-cancer therapy. In our lab, we cloned the human DR5 gene and prepared the soluble recombinant human DR5 protein (mDR5). Hybridomas producing anti-hDR5 monoclonal antibody (mAb) were established with apoptotic activity, and named mDRA-6. This work provides the basis for apoptosis induction effect on hepatocarcinoma cells. To evaluate the effect of a novel anti-human DR5 monoclonal antibody (mAb mDRA-6) on the apoptosis of human hepatocellular carcinoma Cell lines and human hepatocyte HL7702 cell lines, mDRA-6 cytotoxicity on cell lines was detected by MTT analysis. The influence of mAb mDRA-6 on the morpha of cells lines was observed under fluorescence microscope. The rate of apoptosis was detected by flow cytometry with Annexin V-FITC/PI staining. Results shows cells treated with mDRA-6 exhibited typical apoptotic features in morphology. Viability of cells was decreased with mAb concentration increasing by MTT analysis. In the presence of 3 mg/L mDRA-6 for 6 h by flow cytometry analysis with Annexin V-FITC/PI staining, the apoptosis rate of the HepG2 cells was 24.6 %, the apoptosis rate of the HL7702 cells was 25.5 %. The apoptosis rate of the SMMC-7721 cells was 35% in the presence 2 mg/L mDRA-6 for 6 h. In conclusion, mDRA-6 could induce SMMC-7721 cells, HepG2 cells and HL7702 cells apoptosis and has apoptotic activity.

THE INCREASED CD4+CD25+FOXP3+ REGULATORY T CELLS IN TUMOR-BEARING MICE INHIBIT THE ANTI-TUMOR EFFECT OF EFFECTOR T CELLS

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It has been reported that the number of CD4+CD25+Tregs increases in tumor-infiltrating lymphocytes or peripheral blood of patients with tumor. But there is not enough evidence regarding the relationship between the number CD4+CD25+ T cells and tumor growth. To ask this question, in present study, a tumor-bearing model was established by injecting hepatocarcinoma cell line H22 subcutaneously into BALB/c mice and then the number of CD4+CD25+ T cells was detected by Flow Cytometry and the expression of Foxp3, a marker of regulatory T cells, was analyzed by RT-PCR. The results showed the numbers of CD4+CD25+ T cells and the expression of Foxp3 gene in draining lymph nodes and spleen of the tumor-bearing mice increased, compared with that of normal BALB/c mice. More importantly, the increased CD4+CD25+ T cells had a positive relation to the tumor size. To clarify the function of the CD4+CD25+ T from the tumor-bearing mice, CD4+CD25+ T cells were purified and their proliferation to stimulation of anti-CD3 mAb with APC and their inhibition on effector T cells were by ‘H-TdR incorporation assayed in vitro. The results demonstrated that CD4+CD25+ T cell from the tumor-bearing mice showed an anergy characteristic and the activity to suppress the proliferation of CD4+CD25- T cells, similar to natural Tregs. Furthermore, the purified CD4+CD25- effectors
cells with or without CD4\(^+\)CD25\(^-\)T cells from tumor-bearing mice were injected into tumor-bearing nude mice and then the size of tumor was calculated. The size of tumor in tumor-bearing mice injected by CD4\(^+\)CD25\(^-\)T cells plus CD4\(^+\)CD25\(^+\)T cells is smaller than that injected by CD4\(^+\)CD25\(^+\)T cells alone. The results demonstrated that CD4\(^+\)CD25\(^+\)Foxp3\(^+\)T cells from tumor-bearing mice could inhibit the proliferation of effectors T cells in vitro and the anti-tumor effect of effector T cells in vivo. To detect the CD4\(^+\)CD25\(^+\) T cells in tumor-bearing individuals could be strategy for tumor treatment.

**EXPRESSION AND SIGNIFICANCE OF GLUCOSYLCERAMIDE SYNTHASE (GCS) GENE IN HUMAN GASTRIC CARCINOMA CELLS**

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GCS is one of key enzyme that regulates the metabolism of ceramide (CER). The activity level of GCS has close relationship with multidrug resistance of tumor cells. It has been reported that GSC gene expresses also in human ovarian cancer cells, human oral epidermal carcinoma cells, human melanoma cells, and human breast carcinoma cells. But until now, the expression and significance of GCS gene in human gastric carcinoma cells has never been reported. Our study showed that GCS gene expresses in human gastric carcinoma cells and drug-resistant gastric carcinoma cells is very important to the production and development of gastric carcinoma. SGC-7901/VCR and SGC-7901 cells were cultured in vitro, then MTT was adopted to evaluate drug resistance of SGC-7901/VCR cells, RT-PCR analysis was used to detect the mRNA expression of GCS, and the expression of GCS protein was detected by Western blotting and immunocytochemistry. The drug resistance of SGC-7901/VCR cells was 61 times of SGC-7901 cells. At the same time, we observed that GCS mRNA expression in SGC-7901/VCR cells was significantly increased compared with SGC-7901 cells (P<0.05) and there was significant difference of GCS protein expression (P<0.05). These results suggest that GCS gene expresses in human gastric carcinoma cells and it is very important to the production and development of gastric carcinoma cells. The expression of GCS gene in human gastric cancer cells provides a new idea to clinical tumor therapy. The high level of GCS in drug-resistant gastric carcinoma cells suggests that GCS is closely related with drug resistance of tumors. Moreover, this provides a new direction to reverse the drug resistance of tumors.

**COMBINATION OF ENDOSTATIN AND IL-12 GENE THERAPY INHIBITS GROWTH OF HEPATOCELLULAR CARCINOMA IN MICE**

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We investigated suppression of hepatocellular carcinoma tumor growth following a gene therapy which involved concomitant administration of plasmids DNA bearing the endostatin and IL-12 genes. The recombinant plasmid pVAX-sEN expressing endostatin was constructed and its expression in vitro was verified. The pVAX-sEN could inhibit the growth of ECV204 and induce apoptosis in vitro. The recombinant plasmid pUMVC3-mIL12 expressing mouse IL-12 was used in vivo. The BALb/c mice bearing H22 were injected with naked pVAX-sEN and pUMVC3-mIL12 taking pVAX-sEN, pUMVC3-mIL12 and NS as control group. In the case of combined therapy, the rate of tumor growth was lower compared to that in mice receiving pVAX-sEN or pUMVC3-mIL12 only. On the 17th day, the inhibition rate of combined therapy group VS NS group reached 57.1%. To explain this effect, one should contribute to the combined antiangiogenic capabilities. The MVD (Micro血管 Density) was 4.5 smaller than pVAX-sEN (6.7) and pUMVC3-mIL12 (9.8) groups (P<0.05). Also when considering immune response mechanisms, the pUMVC3-mIL12 stimulates proliferation of NK cells, induces synthesis of other cytokines such as IFN-γ. The NK activities detected in combined and pUMVC3-mIL12 groups were 54.78% and 52.05% which was much higher than the pVAX-sEN (25.4%) and NS (23.6%) control groups (P<0.05). The concentration of INF-γ in tumor site of the combined group, pVAX-sEN group and pUMVC3-mIL12 group was 57.2 pg/ml, 96pg/ml and 3.9pg/ml respectively. The combined group and pUMVC3-mIL12 group were much higher than the pVAX-sEN and NS control groups (P<0.05). After injection of the recombinant plasmids, histologic examination and ALT and AST analyses showed the absence of detectable toxicity in normal tissues including the liver. The study demonstrated that gene therapy based on pVAX-sEN expressing endostatin and an IL-12-containing plasmid pUMVC3-mIL12 results in significant suppression of H22 tumor growth in mice. Funded by Doctoral Foundation of Ministry of Education (20030422056).

**ANTI-DR5 MONOCLONAL ANTIBODY ENHANCED CISPLATIN-INDUCING APOPTOSIS OF HELA CELLS**

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TRAIL has been known to induce apoptosis in a variety of tumor cells and some virally infected cells but not in most normal cells. Monoclonal antibodies (mAbs) against TRAIL receptors with tumoricidal activity are also potential candidates for cancer therapy. There are a number of agonistic mAbs against human DR4 or DR5 reported in previous studies. In our lab, we established a hybridoma, that could produce anti-hDR5 monoclonal antibody (mAb) with apoptotic activity, this and was named mDRA-6. To explore if cisplatin was able to upregulate DR5 expression and anti-DR5 monoclonal antibody mDRA-6 was able to enhance cisplatin-inducing apoptosis of HeLa cells. The expression of DR5 in HeLa cells was detected by indirect immune-fluorescence assay (IFA) and flow cytometry (FCM). The cytotoxicity of HeLa cells was examined by MTT assay. Apoptosis was determined by flow cytometry after annexin V/PI staining. The morphological characteristics were observed by digital high-sensitivity fluorescence microscope. Results showed that Cisplatin was not able to upregulate DR5 expression. The cytotoxicity of HeLa cells was obviously enhanced by Cisplatin in a dose-dependent manner, whereas mDRA-6 augmented cisplatin-induced apoptosis of HeLa cells. In conclusion, mDRA-6 enhanced the sensitivity of HeLa to cisplatin induced cytotoxicity and apoptosis. The fraction of anti-tumor was increased by cisplatin with mDRA-6.
interfering RNA directed against JNK1/2. Transfection with adenovirus-mediated dominant negative c-Jun also blocked the upregulation of AChE expression. Together, these results suggest that AChE expression may be mediated by the activation of JNK pathway during apoptosis through a c-Jun-dependent mechanism.

THE EFFECT OF S100A2 ON WNT/BETA-CATENIN SIGNALING PATHWAY
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S100 protein family constitutes of nearly 20 proteins that contain the well-conserved EF-hand calcium binding domains. Several members were shown to be overexpressed in certain types of malignant human tumors, but the mechanism was not known. This article reports that S100A2 could activate Wnt/β-catenin signaling pathway, partly attributed to the interaction between S100A2 and β-catenin. First, Osteosarcoma cell line MG63 and colorectal carcinoma cell line HT-116 were treated with GST-S100A2 fusion protein, and increased β-catenin level was detected in the two cell lines by Western blot. Second, HEK 293 cells were transfected with pTOP-Luc and pFOP-Luc plasmids, respectively, then treated with GST-S100A2. The luciferase activities in pTOP-Luc group increased by 12.3 fold compared with the GST control group after 36h, while no significance between pFOP-Luc group and the GST control group. Third, pcDNA-GSK-3β-HA, pCMV-DVL-Myc and pCMV-Axin-Myc plasmids were transfected into HEK 293 cells, respectively. After 36-48 h, cell lysate was collected for Pulldown tests. The interaction between GST-S100A2 and β-catenin / GSK-3β was verified, but no interaction between S100A2 and Axin or DVL was found. In conclusion, we found S100A2 could upregulate β-catenin level and β-catenin/TCF4 activity, and the interaction between S100A2 and β-catenin / GSK-3β may account for this process. The results may be useful to understand the role of S100A2 in malignant tumors. Supported by the National Natural Science Foundation of China (No.30340039).

PRELIMINARY STUDY OF IMMUNOTHERAPY ON HUMAN BREAST CANCER BY DENDRITIC CELLS DERIVED FROM HUMAN CORD BLOOD
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To investigate the anti-tumor effects of dendritic cells (DC) derived from the human cord blood in nude mice model of human breast cancer, we proliferated and induced differentiated the cord blood mononuclear cells into DC in vitro and loaded with breast cancer tumor antigen by pulsing with breast tumor lysates. Nude mice model of human breast cancer were established by transplanting the MCF-7 cells in situ. The phenotype analysis of DC was achieved by phase microscope, Wright-giemsa staining, flow cytometry and electronic microscope, respectively and the capacity of DC was assessed by the mixed leucocyte reaction (MLR). Anti-tumor effects of the DCs were evaluated by tumor growth curve and recurrent rate. The cryopreserved CBMNC can be effectively induced to mature dendritic cell in vitro. The positive of CD1a is 34.43% ± 0.33%. T cell proliferation could be induced by the in vitro derived DCs. There was no significantly difference between the fresh and cryopreserved cord blood. The tumor suppressive effects of treatment group were significant.

FUNCTIONS AND MECHANISM OF IL-17 IN CEREBRAL ISCHEMIC INJURY
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Increasing evidences showed that the inflammatory response exacerbates the cerebral ischemic injury. Inflammatory cells and proinflammatory cytokines play an important role in the inflammatory exacerbation. The function and mechanism of IL-17 in ischemic cerebral injury is still unknown. In this study, the ischemic symptoms, pathological changes, and expression of IL-6, IL-1β, IL-10 and IL-17 in the pMCAO-operated SD rats administered IL-17 via tail vein were evaluated. Levels of IL-6 in ischemic hemispheres of MCAO and MCAO/IL-17 groups were elevated significantly and peaked at 24h and 48h. The IL-6 levels in ischemic hemisphere of MCAO/IL-17 group were significantly higher than that of MCAO group (P < 0.001). Levels of IL-10 in ischemic hemispheres of MCAO and MCAO/IL-17 groups were elevated significantly and peaked at 48h. The IL-10 levels in ischemic hemisphere of MCAO/IL-17 group were slightly lower than that of MCAO group, there was no significant difference (P > 0.05). Levels of IL-1β in ischemic hemispheres of MCAO and MCAO/IL-17 groups were elevated significantly and peaked at 24h and 48h. The IL-1β levels in ischemic hemisphere of MCAO/IL-17 group were significantly lower than that of MCAO group at 24h, 48h, and 6d (P < 0.05), there was no significant difference (P > 0.05). Results suggested that the expression of IL-6 had been up-regulated, whereas the expression of IL-1β and IL-10 had been down-regulated by external administered IL-17. Levels of IL-17 in ischemic hemispheres of MCAO and MCAO/IL-17 groups were elevated delayingly and peaked at 6d and 8d. The IL-6 levels in ischemic hemisphere of MCAO/IL-17 group were slightly lower than that of sham group at 24h, 48h, and 6d (P > 0.05), and significantly lower at 8d (P < 0.001). There was no significant difference between the IL-17 levels of MCAO/IL-17 and MCAO groups, suggesting that the internal IL-17 expression had been slightly down-regulated. Expression of IL-17, IL-6, TNF-α, and IL-1β in human brain from patients suffered from cerebral infarction were also investigated in this study. Results showed that IL-17, IL-6, TNF-α, and IL-1β were also significant highly expressed in human ischemic brain compared with the contralateral tissues. The distribution of the positive cells were identical to the focal spots of ischemic area. The peaks of IL-17 and IL-1β expression were found at 2-5d after ischemia, while IL-6 and TNF-α expression peaked within 2 days after ischemia. There were no significant different between the expression of IL-6, TNF-α and IL-1β in the ipsilateral and contralateral brain after 5d of ischemia. To prove our hypothetic conjecture about the types of IL-17 expressing cells, the ischemic cerebral sections of human and pMCAO-operated rats had been detected by GFAP and IL-17 double-staining. Results showed only GFAP-stained cells could be seen in the normal hemisphere of MCAO rats, while a lot of GFAP and IL-17 double-stained cells could be observed in the ischemic hemisphere, revealed that IL-17 can be produced by astrocytes if an ischemic signal stimulation exists. This is the first report showed that IL-17 can be produced by cells other than activating T-cells. In conclusion, IL-17 probably protects the neural cells from inflammatory injury after cerebral ischemia by up-regulating IL-6 and down-regulating IL-1β.

EPITHELIAL FUNCTION AND RELATED DISEASES
INVolvement of CFTR in the Pathogenesis of Hydrosalpinx Induced by Chlamydia Trachomatis Infection
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Genital Chlamydia trachomatis infection is a major public health problem worldwide. It has been recognized as the single most common cause of pelvic inflammatory disease (PID) leading to severe tubal damage, ectopic pregnancy, and...
infertility and hydrosalpinx, a clinical condition seen in about 30% of infertile patients characterized by distention and accumulation of fluid in the Fallopian tubes and uterine cavity. However, the molecular mechanism underlying the formation of hydrosalpinx fluid induced by *C. trachomatis* infection remains largely unknown. In the present study, using several molecular and cell biology techniques we report up regulated expression of the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-activated chloride channel that regulates epithelial electrolyte and fluid transport, in the hydrosalpinx tissues of infertile patients with detectable serum levels of *C. trachomatis* antibodies (IgG and IgA). In a mouse model, increased CFTR expression and fluid accumulation can be observed in the uterine horns infected with *C. trachomatis* elementary bodies (EB), which is reversed by antibodies treatment specific for Chlamydial infection and CFTR channel blocker. However, fluid accumulation is not observed in *C. trachomatis*–infected CFTR mutant mice. Up regulation of CFTR and fluid accumulation in uterine horns can be mimicked by administration of exogenous IL-1β, whose serum level is found to be elevated during *C. trachomatis* infection. In summary, these results suggest the involvement of CFTR in the pathogenesis of hydrosalpinx induced by *C. trachomatis* infection.

**EXPRESSION OF CYSTIC FIBROSIS TRANSMEMBRANE REGULATOR IN MICE OVARIIES**

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Cystic fibrosis transmembrane regulator (CFTR) is recognized to play an important role in reproduction. Cystic fibrosis (CF) resulting from mutation of Cystic fibrosis transmembrane regulator (CFTR) is recognized to play an important role in reproduction. Cystic fibrosis (CF) resulting from mutation of *CFTR* gene is associated with multiple and severe abnormalities. CFTR channel blocker diphenhydramine (DPB) was able to correct fluid accumulation and electrolyte composition of uterine fluid. However, the underlying mechanisms remain largely unknown. In the present study, using several molecular and cell biology techniques we report up regulated expression of the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-activated chloride channel that regulates epithelial electrolyte and fluid transport, in the hydrosalpinx tissues of infertile patients with detectable serum levels of *C. trachomatis* antibodies (IgG and IgA). In a mouse model, increased CFTR expression and fluid accumulation can be observed in the uterine horns infected with *C. trachomatis* elementary bodies (EB), which is reversed by antibodies treatment specific for Chlamydial infection and CFTR channel blocker. However, fluid accumulation is not observed in *C. trachomatis*–infected CFTR mutant mice. Up regulation of CFTR and fluid accumulation in uterine horns can be mimicked by administration of exogenous IL-1β, whose serum level is found to be elevated during *C. trachomatis* infection. In summary, these results suggest the involvement of CFTR in the pathogenesis of hydrosalpinx induced by *C. trachomatis* infection.

**COLON IN THE STRESS CONDITIONS**

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Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated anion channel expressed in many epithelial tissues including oviduct epithelium. The aim of our present study was to investigate the electrophysiological properties of CFTR in rat oviduct epithelium. Under whole-cell patch-clamp condition, oviduct cells responded to intracellular cAMP (100μM) with a rise in inward current in Gap-free mode. The cAMP-activated current exhibited a linear 1−V relationship and time- and voltage-independent characteristics. Cells were held at -70mV and then stepped from -120 to +120 mV in 10-mV increments. The current was inhibited by the Cl− channel blocker diphenhydramine 2, 2'-dicarboxylic acid (DPC) in a voltage-dependent manner. The reversal potentials of the cAMP-activated currents in symmetrical Cl− solutions were close to the Cl− equilibrium, 0mV. These electrophysiological properties of the cAMP-activated Cl− conductance in the oviduct were consistent with those reported for CFTR. In support of the functional studies, reverse transcription polymerase chain reaction also revealed the presence of CFTR mRNA in cultured oviduct epithelium. CFTR may play a role in modulating fluid transport in the oviduct.

**A NOVEL RAT ROUND SPERMATID SPECIFIC PROTEIN RS66 MAY PLAY AN IMPORTANT ROLE IN CELL CYCLE**

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In order to identify novel genes in spermatogenesis, pure population of primary spermatocytes and round spermatids had been captured and isolated from section of rat testis by Laser Capture Microdissection. The Suppression Subtracted Hybridization (SSH) library of round spermatid-specific cDNAs from those of primary spermatocytes (RSB) was constructed. A novel gene designated as *rsb66* (GenBank accession number AT121839) was obtained from RSB subtracted library. RSB66 protein was proved to be specifically expressed in rat testis by Northern blot. In the present study we tried to explore the function of RSB66 protein. A yeast two-hybrid system was used to screen its potential interacting proteins in human testis cDNA library. As a result, INCA1 (Inhibitor of CDC interacting with Cyclin A1), an interaction partner of cyclin A1/CDK2 complex was identified. The interaction between RSB66 and INCA1 was confirmed both in vitro and in vivo by GST pull-down and co-immunoprecipitation. Due to the putative role of INCA1 in cell cycle regulation, HeLa cell line which INCA1 and cyclin A1 are endogenously expressed had been chosen as a model. Prolonged G2/M phase was induced when RSB66 protein was overexpressed in HeLa cells. Moreover, this G2/M delay was accompanied with kinase activity varying of cyclinA1/CDK2 complex. Our results suggested that RSB66 may take effect in cell cycle through cyclinA1/CDK2 complex.

**REGULATORY EFFECTS OF NOREPINEPHRINE ON THE TRANSEPITHELIAL ION TRANSPORT OF LATE DISTAL COLON IN THE STRESS CONDITIONS**

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Stress plays important roles in the onset and modulation of a number of intestinal diseases, however the underlying mechanisms remain unclear. The enteric nervous system, acting as a key part of the brain-gut axis connects gut with brain by parasympathetic and sympathetic pathways. It is well known that the sympathetic-adrenergic medulla system participates in the stress response, especially in the acute stress conditions, in which noradrenaline release increases. The aim of the present study is to investigate the effects of sympathetic adrenergic neurotransmitter, noradrenaline, on the colonic ion transport of segment close to the anus (late distal colon) in the stress conditions with the short circuit current (Isc) technique. The results showed that in the acute restraint stress conditions, exogenous noradrenaline (NE 10μm, on the basolateral side) induced a decrease in Isc of -21.8±4.14μAcm<sup>-2</sup> in the late distal colon, which was much smaller than that in the control group, -38.9±5.14μAcm<sup>-2</sup> (P<0.05, n=6). When the endogenous catecholamine was exhausted with reserpine (5mg/kg, ip, 18h), the exogenous NE produced a larger decrease in Isc -56.3±11.9μAcm<sup>-2</sup> in the late distal colon, compared to the control group, -22.9±2.53μAcm<sup>-2</sup> (P<0.05, n=6). Interestingly, exogenous DA (100μm, on the basolateral side) had no significant effect on the Isc of the reserpine treated group. -54.9±12.7μAcm<sup>-2</sup> which was significantly different from that control group, -56.5±2.24μAcm<sup>-2</sup> (P<0.05, n=5). However, in the acute restraint stress, the DA-induced Isc had no significant difference with the control group. These results suggest that the sympathetic adrenergic system is involved in the late distal colonic ion transport and also plays a certain role in the stress condition. This work was supported...
ALTERED CYCLIC EXPRESSION OF CFTR IN MOUSE ENDOMETRIUM BY CHLAMYDIA TRACHOMATIS INFECTION
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Endometrium epithelial ion channels have long been proposed as the major mechanism regulating uterine fluid absorption and secretion. Differential expression of these ion channels may give rise to dynamic changes in the fluid environment. Previous studies have demonstrated CFTR expression in the sexually mature mouse endometrium varies throughout the estrous cycle under physiological condition with the maximal mRNA level in estrus and minimum in diestrus. However, the expression pattern and function of CFTR in pathophysiological condition has not been investigated. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) and short-circuit current (Isc) technique was used in this study to investigate the CFTR mRNA expression and functional channel activity upon Chlamydia trachomatis (Cs) infection, the most common cause of sexually transmitted disease in women. Female ICR mice aged from 8 to 10 weeks were selected at the early diestrus stage confirmed by vaginal smear. Cultured high endothelial venule (LPS), the most potent antigenic component of syngenic cell wall, was administered through each sides of the uterine horn to mimic the infectious condition and the same treatment with 0.9% saline as corresponding control. Uteri tissues collected 24 h later and checked still at diestrus were used for Isc and RT-PCR examination. Forskolin stimulated a sustained rise of Isc in LPS pretreatment uteri but not in the control, consistent with the CFTR mRNA expression levels in the two groups. The underlying mechanisms of up-regulated CFTR by Cs LPS in diestrus were explored with IL-1β and TNF-α, widely known as pro-inflammatory cytokines produced during inflammation. The forskolin-induced Isc was higher in IL-1β treated group than that in TNF-α group, but no response was observed in the control. The pattern of CFTR mRNA expression levels was consistent with the channel activity observed with the Isc. The present results suggested that upon Cs bacteria infection, the mRNA and functional expression of CFTR were altered throughout the estrous cycle, which are likely to be mediated by cytokines, IL-1β and TNF-α.

DENDRITIC CELLS IN MELANOMA MOUSE MODELS: ENDOSONOMIC MIGRATION, ANTIGEN PRESENTATION AND EXPRESSION OF THE CORRELATIVE FACTORS
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Although endosomatic migration pathway of dendritic cells (DC) has been investigated, the exact migration pathway and its regulatory factors are still unknown. We studied the morphological evidence of endosomatic migration pathway and biological characteristics of BrdU-labeled and S-100+ bone marrow precursor derived DC (BMDCs) and neoantigen polypeptide-loaded marrow DC cultured in vitro with GM-CSF + IL-4 in B16 melanoma tumor-bearing mice. We demonstrate BMDCs and neoantigen polypeptide-loaded marrow DC subcutaneously injected in tumor could enter the regional lymph nodes by two pathways: lymph pathway and blood pathway. High endothelial venule-like vessels and lymphatic vessels, which were adjacent to tumor, and high endothelial venule in paracortex of lymph nodes are important places of migration. Injected BMDCs and antigen-loaded DC were both in direct contact with tumor cells; then, cell fusion and tumor cells apoptosis happened. These data suggest that these DC probably play a role in directly killing tumor cells. In addition, we investigated the mechanism of endosomatic immigration of DC and modulation of adhesion molecules and chemokine by immunohistochemistry and in-situ hybridization methods. Expression of CCR-7 was relevant positively to the migration of dendritic cells; LFA-1 was relevant positively to the lymphocytes gathering in tumor; and B7-2 and IFN-γ up-regulated the expression of LFA-1 and CCR-7. These results indicate cytokines such as LFA-1, CCR-7, B7-2 and IFN-γ play an important role in the modulation of endosomatic immigration of dendritic cells, indicating their potential use in inducing high-performance DC vaccine, enhancing the endosomatic anti-tumor immunity reaction and expanding application of tumor immunotherapy.

THE CORRELATION BETWEEN THE SERUM LEVELS OF ADMA, ICAM-1 AND CORONARY COLLATERAL CIRCULATION IN PATIENTS WITH SEVERE CORONARY ARTERY STENOSIS
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It has been demonstrated that the degree of coronary collateral circulation in the patients with severe coronary artery stenosis is closely related to the function of endothelial cells. Asymmetric dimethylarginine (ADMA) is a marker of endothelial dysfunction. The mediators of inflammation—intercellular adhesion molecules-1 (ICAM-1) have also been considered as a marker of endothelial function. This prospective study evaluated the serum levels of ADMA and ICAM-1 in patients with severe coronary artery stenosis and investigated the correlation between the serum levels of ADMA, ICAM-1 and the degree of coronary collateral circulation. 85 patients with the stenosis of at least one vessel ≥95 % among three main vessels of coronary artery were consecutively enrolled in the study according to angiographic estimation in our hospital from June to November in 2005. Development of collaterals was defined as the degree of collateral branches developed. Patients were divided into two groups (poorly developed collateral group: 50 patients with grade 0 and 1; well-developed collateral group: 35 patients with grade 2 and 3). The serum levels of ADMA were determined by HPLC method and the serum levels of ICAM-1 were measured by ELISA. Compared with patients with poorly developed collateral group, the serum levels of ADMA of patients with well-developed collateral group were significantly decreased (2.23 ± 0.59 μmol/L vs 1.79 ± 0.57 μmol/L, P = 0.001). Similarly, the levels of ICAM-1 were also markedly reduced (272.4 ± 68.3 ng/ml vs 225.0±61.9 ng/ml, P=0.002). Coronary collateral circulation (CCC) degrees had a negative correlation with serum levels of ADMA and ICAM-1 (P=0.000 and P= 0.003, respectively) by Pearson correlation analysis. The serum levels of ADMA had a positive correlation with serum levels of ICAM-1 (P=0.037) by Pearson correlation analysis. In the patients with severe coronary artery stenosis, serum concentrations of ADMA and ICAM-1 were increased in patients with poor collateral circulation, and CCC degrees showed a negative correlation with serum levels of ADMA and ICAM-1.

ACROSOME REACTION INDUCED BY RECOMBINANT HUMAN ZONA PELLUCIDA 3 PEPTIDES hUZP3A22-76 AND hUZP3B177-348 AND THEIR MECHANISM
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Recombinant human zona pellucida 3 peptides huZP3a22-76 and huZP3b177-348 were expressed in E.coli. and purified, because recombinant human zona pellucida 3 may be served as an alternative tool of native ZP3 to diagnose male sterility and study immune contraceptive. We examined whether huZP3a22-76 and huZP3b177-348 may trigger acrosome reaction of human spermatozoa and explored possible mechanism which mediated acrosome reaction. By chlorotetra-cycline staining, assessment of acrosome reaction was performed. Intracellular
free calcium concentration [Ca^{2+}]_{ii} in Fura-2/AM-loaded human sperm was monitored with spectrofluorophotometer. We found that the peptides huZP3a^{22-76} and huZP3b^{177-348} were significantly capable of eliciting acrosome reaction in a dose-dependent manner, respectively. With an addition of the peptide, [Ca^{2+}]_{ii} level was raised like a peak and plateau. The acrosome reaction could be inhibited by Gi protein sensitivity pertussis toxin (PTX), EGTA and a T-type calcium channel blocker pimozone, whereas verapamil was less effective. This study suggests the peptides huZP3a^{22-76} and huZP3b^{177-348} have the role similar to human ZP, mechanism in respond to the peptides may involve in influx of calcium, Gi protein pathway and T-type calcium channel. Supported by MHR-FZP (No.2004A002) and National Science Foundation of Zhejiang Province (No.Y204490).

IDENTIFICATION AND CHARACTERIZATION OF HSD34, A NOVEL MEMBER OF THE RBCC FAMILY IN HUMAN TESTIS
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The ubiquitin-proteasome pathway plays a key role in spermatogenesis. The method of laser capture microdissection (LCM) combined with suppressive subtractive hybridization (SSH) was used to identify the differentially expressed genes during germ cell differentiation. A new gene designated as HSD34 was obtained and characterized. HSD34, containing a RING finger domain, a B-Box, and a coiled-coil motif in the N-terminal region, belongs to the TRIM (Tripartite motif) or RBCC (RING, B-Box, coiled-coil) family. The growing evidence suggests that TRIM/RBCC proteins with the highly conserved modular structure represent a novel subclass of E3 ubiquitin ligases. HSD34 is expressed specifically in human testis, especially in round spermatids. Using a yeast two-hybrid assay, we found that HSD34 interacts with the human cell cycle checkpoint-related protein Huls (hHUL1). As we known, the human Rad9, Rad1 and Hus1 form a heterotrimeric complex (called the 9-1-1 complex), which plays a role in DNA repair and cell cycle G2/M checkpoint control pathways. Furthermore, HSD34 was implicated in cAMP-response element (CRE) cell signal transduction pathway in vivo. These results suggest that the novel RBCC protein HSD34 may play an important role in the human spermatogenesis.

INTERACTION OF ZONULA OCCLUDENS-1 (ZO-1) WITH α-ACTININ-4: A PROTEOMIC BASED IDENTIFICATION OF PDZ DOMAIN INTERACTING PROTEINS
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It is widely accepted that most proteins exert their functional roles through protein interaction or binding networks. Identification of protein binding partners is an important step for elucidating cellular roles of binding networks. In the present study, we have developed an approach using recombinant PDZ protein interaction modules of the membrane-associated guanylate kinase (MAGUK) protein zonula occludens-1 (ZO-1) to pull-down and screen for proteins that interact with these modules via their PDZ domain binding motifs. Identification of proteins from pull-down material was achieved by using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). MS analysis of tryptic fragments in pull-down material revealed a number of potential ZO-1 interacting candidates, including the presence of peptides corresponding to the cortical membrane scaffolding protein α-actinin-4. Interaction of α-actinin-4 with ZO-1 was confirmed by co-immunoprecipitation of these two proteins from cultured cells as well as from brain, liver and heart, and by immunoblot detection of α-actinin-4 after pull-down with the first PDZ domain of ZO-1. Further, immunofluorescence showed co-localization of α-actinin-4 with ZO-1 in cultured HeLa and C6 glioma cells, as well as in a variety of tissues in vivo, including brain, heart, liver and lung. In contrast, we found no evidence for the association of ZO-1 with α-actinin-1. Considering the association of ZO-1 with tight junctions, adherens junctions as well as gap junctions, the interaction of ZO-1 with α-actinin-4 may provide a mechanism for linking the known protein recruitment and sequestering, signaling, transcriptional and cell-cycle regulatory activities of ZO-1 with α-actinin-4-associated plasma membrane proteins that have similar regulatory activities at cell-cell and cell-extracellular matrix contacts. Supported by grants from the Canadian Institute of Health Research to JIN.

FUNCTIONAL DISTRIBUTION OF ABSORPTIVE AND SECRETORY CELLS IN SMALL AIRWAY EPITHELIA
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Despite the fact that primary defense of the airways requires a well controlled fluid environment on the surface of the bronchioles, little is known of the fluid and electrolyte transport properties of small airways. We use the approach of microperfusion and microelectrode to examine properties of native, dissected porcine bronchioles of 1-2 mm diameter. In addition to that anion channel cystic fibrosis transmembrane conductance regulator (CFTR) and epithelial Na⁺ channel (ENaC) both play a critical role in electroconductive properties of the small airway epithelium, we have found that immunolocalization of CFTR, ENaC, and Na⁺-K⁺-2Cl⁻ cotransporter (NKCC) showed a special distribution pattern in this tissue. Data of Microelectrode experiment suggested that absorptive and secretory cells existed respectively in the epithelium. These results and properties suggest that separate populations of cells, both rich in apical membrane and basolateral membrane, not continuous, with different distribution pattern in different regions of the bronchial epithelium, perhaps in a manner analogous to the secreting crypts and absorbing villi of the intestine. Our finding may facilitate better understanding the fundamental structure/function of small airways and pathophysiology of Cystic Fibrosis and other inflammatory lung diseases.

APPEARANCE OF SEGMENTAL DIFFERENCE OF ANION TRANSPORT IN RAT DISTAL COLON
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The present study investigated forskolin-induced segmental discrepancy of anion transport on the rat distal colon and the possible underlying mechanisms. The results showed that luminal addition of forskolin, an activator of adenyly cyclase, induced an increase in short circuit current (Isc) (4.09 ± 0.66 μA/cm², n=13) in DC1 (3 cm away from the anus) and (18.84 ± 3.18 μA/cm², n=13) in DC4 (7 cm away from anus), which were blocked by apical Cl⁻ channel blocker, glybenclamide (1mmol/L) (n=5, P<0.001), as well as removal of extracellular Cl⁻ and HCO₃⁻ in two segments (n=6, P<0.001). However, forskolin-induced Isc in DC1 was not inhibited, but enhanced from 3.49 ± 0.77 μA/cm² to 15.13± 2.71 μA/cm² (n=8, P<0.01) by bumetanide (10 μM/L), an inhibitor of Na⁺-K⁺-2Cl⁻ cotransporter. Furthermore, reducing apical and basolateral Cl⁻ did not decrease, but increased forskolin-induced Isc in DC1, from 13.5±1.7 μA/cm² at 41.6±9.5 μA/cm² (n=5, P<0.001) and from 41.0±7.0 μA/cm² at 79.6±16 μA/cm² (n=14, P<0.05), respectively. The 55% of enlarged Isc in apical Cl⁻, but not in basolateral low Cl⁻ was inhibited by bumetanide. Compared to DC1, forskolin-induced Isc in DC2 was not inhibited by bumetanide and reduced under a low Cl⁻ condition. Upon real-time PCR analysis, the amounts of CFTR, SLC26A6 mRNAs were not found to be different between the two segments. The results suggest that there is a segment-specific effect on epithelial ion transport when adding forskolin to mucosal side. Forskolin-induced Isc increase in DC1 might be mediated by both Cl⁻ and HCO₃⁻ and in DC2 predominantly by electrogenic transporters.
THE ROLE OF 5-HT3 RECEPTOR IN RAT COLONIC SECRETION

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Serotonin (5-HT) plays a key role in gastrointestinal tract (GI). The present study is to investigate the action of 5-HT3 receptors in neural and non-neural pathways by short circuit current technique and cAMP measurement (ELISA). Indomethacin (10 μM, basolateral side), a cyclooxygenase (COX) inhibitor, was routinely added to abolish the effects of endogenous prostaglandin. DC1 (segment next to the anus) and DC2 (segment 5cm away from the anus) were chosen to reduce the segmental difference. The results showed that the basic electrophysiological properties of DC1 and DC2 were not affected by pretreatment with TTX (1 μM), a neuronal Na+ channel blocker. Basolateral pretreatment with MDL72222 (10 μM), a 5-HT3 receptor antagonist did not reduced, but significantly increased 5-HT-induced ΔIsc from 893.5±168.4 μA/min (n=6) to 1018±75.6 μA/min in DC1 (n=9, P<0.05) and from 1317±189.9 μA/min (n=6) to 1875±127.4 μA/min in DC2 (n=11, P<0.05). However, pretreatment with both TTX (1 μM) and MDL72222 (10 μM) significantly inhibited 5-HT-induced ΔIsc from 820.1±159.9 μA/min (n=7) to 741.4±142.5 μA/min in DC1 (n=9, P<0.05) and from 1471.0±116.4 μA/min (n=7) to 1134.0±62.4 μA/min in DC2 (n=7, P<0.05). cAMP measurement indicated that pretreatment with MDL72222 (10 μM) could also enhance 5-HT (10 μM)-stimulated cellular cAMP concentration from 11.5 pmol/ml to 29.8 pmol/ml in DC1 (n=3) and from 17.1 pmol/ml to 20.5 pmol/ml in DC2 (n=3). The results suggest that 5-HT3 receptor involves in 5-HT-induced colonic secretion via neural and non-neural pathway. In the neural pathway, an unknown neurotransmitter activating cellular cAMP pathway might be involved, which is negatively regulated by 5-HT via 5-HT3 receptor. This work was supported by the National Natural Science Foundation of China (30570672) and Scientific Research Common Program of Beijing Municipal Commission of Education (KM200610025001).

REGULATION OF Cl- SECRETION BY TRIMETHYLITIN CHLORIDE IN ISOLATED RAT DISTAL COLON THROUGH ACTIVATION OF K+ CHANNEL

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Trimethyltin chloride (TMT), an organotin, is ubiquitous in the environment. The consumption of contaminated food leads to human dietary exposure to this toxic compound. The present study investigated the regulation of ion transport across the rat distal colon by extracellular TMT using the short-circuit current (Isc). The colonic epithelium responded to both apical and basolateral application of TMT with a sustained rise in Isc in a concentration-dependent manner, with EC50 of 40.1 μM/L. Replacement of Cl- in the bathing solution reduced the Isc to TMT significantly. The increase in Isc could be blocked by DPC1, bumetanide, but not by DIDS. While pretreatment with amiloride, it did not exert any effect. In addition, the Cl- secretion induced by TMT was abolished by two K+ channels blockers, namely quinidine (100μM/L), and Ba2+ (5 mM), suggesting that basolateral K+ channels are important in maintaining the procedure. Pretreatment of colonic mucosa with BAPTA/AM, a membrane-permeable selective Ca2+ chelator, did not alter the TMT-induced Isc markedly. No additive effect of forskolin and IBMX was observed on the TMT-induced Isc. Nystatin permeabilization studies revealed a good correlation between the Isc and the basolateral K+ current rather than the apical Cl- current under TMT-stimulated conditions. In conclusion, apical and basolateral addition of TMT led to Cl- secretion through the cystic fibrosis transmembrane conductance regulator (CFTR) in the apical membrane, and challenge with TMT was essentially regulated by basolateral K+ channel. This suggested the importance of these channels in toxicity hazard.

β-ADRENOCEPTOR, BUT NOT DOPAMINE RECEPTOR(S)-MEDIATED DOPAMINE-INDUCED LATE COLONIC ION TRANSPORT

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Catecholaminergic neurotransmitters, both dopamine and norepinephrine (NE), could evoke a downwards short-circuit current (Isc) response in rat distal colon, although NE-induced current response was larger than dopamine-induced. To investigate the underlying mechanism we pretreated late distal colonic mucosa with NE and related receptor antagonists. The results showed that dopamine (100μm)-induced Isc decrease (~15.9±1.7 μA/cm2) was significantly inhibited by NE (10µm) pretreatment (1.015±1.72 μA/cm2, n=6 P<0.0001), however, the NE (1μm)-induced Isc response (~16.3±1.2 μA/cm2) was also significantly blocked (~6.5 ± 0.65 μA/cm2) by dopamine (100µm) pretreatment (n=5, P<0.0001). A common receptor target, or a common signaling pathway might exist between dopamine and NE. SCH-23390 (n=9) and Sulpiride (n=5), antagonists of D1 and D2 receptor, could not block, but further enhanced the effect of dopamine, especially SCH-23390 (P<0.005). Similarly both SCH-23390 and Sulpiride could also increase the NE-induced Isc response from -20.86±2.0 μA/cm2 to -29.7±1.8 μA/cm2 (n=6, P<0.05) and -31.7±3.5 μA/cm2 (n=7, P<0.05). Phenotolamine, an α-adrenoceptor blocker (n=7), and yohimbine, α2-adrenoceptor blocker, failed to block the dopamine-induced ISc response, but enhanced dopamine-induced Isc decrease, especially in yohimbine (n=7, ± P<0.05); however, propranolol, an inhibitor of unselective β-adrenoceptor reduced dopamine-evoked Isc response by 83.3% (n=5), andICI-118, 551, inhibitor of β2-adrenoceptor, blocked dopamine-induced Isc decrease (n=5). In conclusion, similar to NE, dopamine, as a normal enteric amine, may involve in the functional regulation of late distal colon, which might be mediated by β-adrenoceptor, but not dopamine receptor(s). This work was supported by the National Natural Science Foundation of China (30570672) and Scientific Research Common Program of Beijing Municipal Commission of Education (KM200610025001).

CYSTIC FIBROSIS-RELATED DIABETES (CFRD) MAY NOT BE DUE TO DESTRUCTION OF THE PanCREAS

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Cystic fibrosis-related diabetes (CFRD), a relative insulin deficiency generally believed to be due to destruction of the pancreas, has emerged as an important complication of CF, the most common life-threatening genetic disease characterized by defective Cl- and HCO3- secretion due to mutations of the cystic fibrosis transmembrane conductance regulator (CFTR). Here we reported that CF mice, with a mutation in CFTR similar to that of the DF508 mutation in humans, have blood levels of insulin and glucose significantly lower and higher respectively, than those observed in wild type mice. In the present study, we examined the blood levels of glucose and insulin in the CF mice (fasting for 12h) and results showed elevated glucose and reduced insulin levels in the CF mice as compared to the wild type control. However, histological examination could not detectable abnormality in the pancreatic sections of the CF mice although the examination did confirm previously observed association between pancreatic ducts and islet tissue. These results suggest that CFRD may not be due to destruction of the pancreas and defective investigation on possible influence of exocrine secretion on insulin secretion in currently undertaking.
INFECTION AND IMMUNE DEFENSE

HEPATITIS B VIRUS CORE PROTEIN IMPAIR THE TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND CYTOTOXICITY BY DOWN-REGULATING THE EXPRESSION OF DEATH RECEPTOR 4/5

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The purpose of this study was to observe the effects of hepatitis B virus core protein (HBc) on the apoptosis of hepatoma cells induced by TNF-related apoptosis-inducing ligand (TRAIL) and to explore molecular mechanisms for its effects. In order to set up a model in vitro, BEL7402-HBc cell line was established by stably transfecting pcDNA-HBc into hepatoma cell line BEL7402. In our research, CCK-8 array, TUNEL Flow Cytometry assay were performed to detect the apoptosis rates of BEL7402, BEL7402-pcDNA3 and BEL7402-HBc induced by TRAIL. In addition, phosphorothioated antisense oligonucleotide against the poly A region of HBc gene was used to block the expression of HBc in BEL7402-HBc cells and to further confirm the effects of HBc on TRAIL-induced apoptosis. The expression of TRAIL receptors was analyzed by reverse transcription PCR, Flow Cytometry and Western blot. The CCK-K and TUNEL Flow Cytometry assay indicated that under treatment of the same concentration of TRAIL, BEL7402-HBc had a lower apoptosis rate than those of BEL7402 and BEL7402-pcDNA3. Blockade of HBc expression in BEL7402-HBc cells partly upregulated the apoptosis induced by TRAIL. HBc transfection dramatically decreased the expression of TRAIL death receptors (DR) 4 and DR5 but not of DcR1 and DcR2. Additionally, HBc transfection significantly decreased the degradation level of the caspase-3,8,9 and Bid. Our data firstly confirmed that HBc could down-regulate the sensitivity of hepatoma cell line BEL7402 to TRAIL. In addition, the decreased expression of DR4 and DR5 might play a pivotal role in this resistant effect. It implied HBc might also decrease the apoptosis rate of virus infected hepatocytes and induce chronic hepatitis in vivo. This research would be useful to further clarify the roles of imbalanced apoptosis in pathogenesis of Hepatitis. Supported by the National Nature Science Foundation of China, Grant No.30371342.

THE EFFECTS OF CHRONIC HYPOXIA AND PROINFLAMMATORY CYTOKINE STIMULATION ON THE EXPRESSION OF P-ERK1/2 IN THE CAROTID BODY OF THE RAT

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The carotid body is a peripheral arterial chemoreceptor organ located the bifurcation of the common carotid artery. Recently our studies have implicated that the carotid body could sense the stimulation of proinflammatory cytokine. In this experiment, we observed the effects of chronic hypoxia and intraperitoneal injection of recombinant murine interleukin-1 beta (rmIL-1β) on the expression of p-ERK1/2 in the carotid body by using immunohistochemistry and image analysis. Twenty adult male Sprague-Dawley rats were used and divided into four groups. The rats in the T1 group were exposed to hypoxia and injected with rmIL-1β. The rats in the T2 group and T3 group were only exposed to hypoxia or injected with rmIL-1β, respectively. The rats in the T4 group were exposed to normoxia and not subjected to an injection of rmIL-1β. The results showed that p-ERK1/2 was expressed mainly in the type II cells in normal carotid body. But after a stimulation of chronic hypoxia, rmIL-1β stimulation or chronic hypoxia combined with rmIL-1β stimulation, more p-ERK1/2-like immunoreactivities were seen in type I cells. The above results indicated that ERK1/2 pathway participates in the normal function of type II cells. Chronic hypoxia and/or IL-1β stimulation can activate ERK1/2 pathway in type I cells, and this pathway might involve in the sensation of chronic hypoxia or immunostimulation in the cells.

MITOCHONDRIAL MECHANISMS UNDERLYING HEPATIC INJURY AND HEPATOPROTECTIVE EFFECTS OF ASIATIC ACID

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LPS-D-GalN induced hepatic injury models and Ce3+-induced mitochondrial swelling models were adopted in the present work to study the mechanisms underlying hepatocyte injury or death and the hepatoprotection of asiatic acid. It was found that both morphological observation and the detection of mitochondrial function showed that mitochondrial swelling, the changes in mitochondrial membrane potential and intramitochondrial calcium content occurred following hepatic injury. In the meantime, the release of mitochondrial cytochrome c and apoptosis inducing factor (AIF), and also the down regulation of voltage dependent anion channel (VDAC), the most important component protein of mitochondrial permeability transition pore, were found. The above data indicated that mitochondrial dysfunction was involved in hepatic injury and might exert principal roles in hepatocyte injury. While the pretreatment of asiatic acid could inhibit the above mitochondrial damages, which suggest that asiatic acid have significant protection on liver mitochondria. On the other hand, mitochondria separated from normal mice were induced permeability transition by calcium, and the direct action of asiatic acid on liver mitochondrial permeability transition were assessed. The results showed that asiatic acid could directly block liver mitochondrial permeability transition and protect mouse liver against toxicity through up-modulating of voltage dependent anion channel (VDAC) and inhibiting the opening of mitochondrial permeability transition pore, and finally block apoptosis or necrosis of hepatocytes.

HEPATITIS B VIRUS MHBS(T) MODULATES THE SENSITIVITY OF HEPATOMA CELL LINE TOWARDS TRAIL-INDUCED APOPTOSIS

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Institute of Medicine School, Shandong University, China C-terminally truncated middle hepatitis B surface protein (MHBS(t)), encoded by 3'-truncated preS2/S gene (which is produced during HBV DNA integration), exists in liver cells infected with HBV. MHBS(t) is an important factor for the development of HBV-associated hepatocellular carcinoma and its function of transcriptional activation has been widely studied. However, the effects of MHBS(t) on apoptosis of liver cells have not been studied. The object of our present study is to observe the effects of MHBS(t) on TRAIL-induced apoptosis and to study preliminarily molecular mechanisms for their effects. Eukaryotic expression vectors pcDNA-MHBS(t) was constructed and stably transfected into hepatoma cell line BEL7402. Then stably-transfected and control BEL7402 cells were treated with soluble TRAIL protein at various concentrations and the cytotoxicity of TRAIL was detected by Trypan Blue Exclusion Test. Moreover, apoptosis rates for cells treated with 10 μg/L of sTRAIL were determined by TUNEL. The expression of TRAIL receptors in the surface of cells were examined by FACS and Flow Cytometry. Trypan Blue Exclusion Test indicated that three groups of cells were all sensitive to TRAIL with dose-dependent cytotoxicity. Cells stably-transfected with pcDNA-MHBS(t) had a higher sensitivity to sTRAIL than BEL7402 and pcDNA3-transfected control cells. TUNEL staining showed that with treatment of 10 μg/L of TRAIL, apoptosis rate for pcDNA-MHBS(t)-transfected cells were 44% ± 9.3%, significantly higher than that of pcDNA3-transfected cells (14.2% ± 4.5%) and that of BEL7402 cells (13.8% ± 5.3%). RT-PCR and Flow Cytometry showed that there were no obvious differences in the expression level of TRAIL receptors in the surface of three groups of cells. The results indicated that MHBS(t) could enhance the sensitivity of hepatoma cells towards TRAIL, which might play important roles in modulating apoptosis of HBV-infected liver cells. But the effects are not related with the expression level of TRAIL receptors and the molecular mechanisms still needed to be further studied.
EPITHELIAL–LYMPHOCYTE CROSSTALK IN REGULATING AIRWAY HOST DEFENSE AGAINST CHLAMYDIA LIPOLYSACCHARIDE (LPS) CHALLENGE
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Respiratory infection is extremely common and a major cause of morbidity and mortality worldwide. The airway epithelium is charged with the unique task of maintaining the first line of host defense against pathogenic challenge along the respiratory tract. Together with lymphocytes, it plays an important role in the inflammatory response during bacteria infection in the lung. However, the crosstalk between airway epithelial cells and lymphocytes receives very little attention and remains poorly understood. In the present study, an epithelial-lymphocyte co-culture system was established to investigate their crosstalk. Human respiratory epithelial cell line Calu-3 with or without mouse peripheral blood lymphocyte co-culture system was established to investigate their crosstalk. An epithelial-lymphocyte co-culture system was established to investigate their crosstalk. Human respiratory epithelial cell line Calu-3 with or without mouse peripheral blood lymphocyte co-culture system was established to investigate their crosstalk.

Effect of IL-1β stimulation on cellular activities of carotid body glomus cells and firings of carotid sinus nerve
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It is well understood that the mammalian carotid body (CB) is a chemosensory organ responsible for detection of hypoxia, hypercapnia, and acidity in arterial blood. Recent evidence that the organ can also sense low blood glucose further support its role as a polymodal sensor to manifold stimuli from the blood. The present study was undertaken to assess the assumption that the CB can also sense the stimulation of blood-borne IL-1β, a proinflammatory cytokine produced by a variety of immune cells in the early state of immune challenge. At the initial step of this study, we studied the effect of IL-1β on the I<sub>K</sub> and [Ca<sup>2+</sup>]<sub>i</sub> in the glomus cell of the rat CB by using patch-clamp and calcium imaging techniques. The results showed that application of 50ng/mL IL-1β by micro-puffer system partially and reversibly inhibited the amplitude of I<sub>K</sub> and triggered a transient rise of [Ca<sup>2+</sup>]<sub>i</sub> in the glomus cells. Next, by using extracellular recording technique to monitor the firings in the carotid sinus nerve (CSN) of the rat in vivo, the afferent nerve innervated the organ, we found that topical application of IL-1β (50ng/mL) significantly increased the discharge rate in CSN, and the effect was abolished by co-administration of IL-1 receptor antagonist. Furthermore, when IL-1β was given in combination with suramin (0.1mM), a P2X blocker, the firing effect was partially abolished, whereas co-application of IL-1β with SCH23390 (D1 receptor blocker) or haloperidol (D2 receptor blocker) did not evidently affect the increase of CSN discharge rate induced by IL-1β if given alone. Consistently, IL-1β could not induce a catecholamine release from the CB, as detected with amperometry method ex vivo. The above results indicate that stimulation of CB with IL-1β induces a cascade event in glomus cells including inhibition of I<sub>K</sub>, rise of [Ca<sup>2+</sup>]<sub>i</sub>, secretion of ATP, and increased firings of CSN. The discovery from present experiment presents evidences for a novel function of CB in perception of immune stimulation and revealed a new pathway for immune-to-brain communication.

MOTILIN LOCALIZATION IN HUMAN THYROID TUMORS
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Motilin, the previous studies demonstrated that motilin has a widespread expression in different normal cells and tissues, as well as in gastric and intestinal. Motilin is most abundant in the duodenum where it is present in endocrine cells. In the current study, we detected motilin transcripts expression in human thyroid and tuourmors by means of immunohistochemical techniques. Our results surprisingly showed that motilin immunoreactive cells abandently expressed in human thyroid and colocalized with calcitonin. This suggested that motilin localized in thyroid C cells. Thyroid tumors (thyroid medullary carcinoma, acidophilic adenoma, follicular carcinoma, papillary carcinoma and nodular goiter) showed intense and diffuse immunostaining for motilin. These data provide direct morphological evidence that motilin may well be acting in paracrine fashion in the regulation of thyroid papillary follicular cell function. On the other hand, these results were confirmed by real time PCR results. It showed that the contents of motilin mRNA was higher in thyroid medullary carcinoma and acidophilic adenoma, and in turn decreased in follicular and papillary carcinoma. Overall, it is the first time showing the expression of motilin in human thyroid. The expression of motilin in human thyroid and thyroid tumors is highly suggestive of a conserved role of this molecule in the regulation of thyroid cell function and other important organs activity as well as gastrointestinal tract.
CHARACTERIZATION AND FUNCTION OF MOUSE KV1.3 CHANNELS STABLY EXPRESSED IN CULTURED COS-7 CELLS
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Voltage-gated Kv1.3 channels are the predominating potassium channels expressed in mouse, rat and human T lymphocytes. It is known that blockade of Kv1.3 channels also blocks both T lymphocytes mitogenesis and volume regulation. Therefore, Kv1.3 channels have been regarded as the therapeutic targets for autoimmune disorders. However, many details about Kv1.3 channels’ function are still scarce. Stable expression of mouse Kv1.3 channels in COS-7 cells, present work aims to study the characters and function of Kv1.3 channels through the patch clamp technique. At a holding potential of ~80 mV, Kv1.3 channel currents were evoked by depolarizing step pulses from ~50 mV to ~+50 mV with the step of 10 mV increment (of 200-ms duration). Compared to the weak current density (4.2±0.6pA/pF) of non-transfected COS-7 cells, stronger current density (368.3±72.2pA/pF) was recorded in the membrane of the COS-7 cells transfected with mouse Kv1.3 cDNA, which showed that mouse Kv1.3 channel was stably expressed at high levels in the COS-7 cell line. The half-maximal activation and inactivation potential was 15.4±3.2mV and -38.5±1.9mV; the recovery time constant was 1480±353.1ms. The resting membrane potential of non-transfected COS-7 cell was ~20.4±2.02 mV, while the heterologous expression of mouse Kv1.3 channels reset the resting membrane potential of transfected COS-7 cell to ~46.6±5.5 mV, and incubated with OSK-1, an inhibitor of Kv1.3 almost depolarized transfected COS-7 cells to the resting membrane potential of non-transfected cells. The resting membrane potential and the current density of transfected COS-7 cell showed obviously negative correction, which could be described by the linear equation $y = -2.49 - 0.11x$ (y means resting membrane potential, x means the current density). Present work showed that stable expression of Kv1.3 channels in COS-7 cells was a convenient and reliable protocol to study the characters and function of Kv1.3 channels. The resetting effect of Kv1.3 channels on the resting membrane potential of transfected COS-7 cells are in agreement with this hypothesis that one function of Kv1.3 in T lymphocytes is to stabilize the membrane potential, thereby regulating the Ca2+ signalling critical for mitogenic stimulation.

ADAPTIVE EVOLUTION OF THE SPIKINGENE OF SARS CORONAVIRUS DURING INTERSPECIES TRANSMISSION FROM ANIMALS TO HUMAN
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It is believed that transmission of severe acute respiratory syndrome (SARS) coronavirus (CoV) from animals to humans is the cause of the SARS outbreak worldwide. The spike (S) protein, one of the best characterized proteins of SARS-CoV, plays a key role in SARS-CoV overcoming the species barrier and accomplishing interspecies transmission from animals to human, suggesting that it may be the major target of evolutionary pressure. By investigating the adaptive evolution of S protein, eleven amino acid sites (75, 239, 244, 311, 479, 609, 743, 765, 778, 1148, and 1163) in the S protein were detected under positive selective pressure. Then, we divided SARS outbreak into three epidemic phases: 02-04 interspecies epidemic group and sites 75, 778, and 1163 during 03-early to mid epidemic phase were identified under positive selection, while no site was discovered during 03-late phase. During 02-04 interspecies epidemic group and sites 75, 479, 609, 743, and 765 during 02-04 interspecies epidemic group and sites 75, 778, and 1163 during 03-early to mid epidemic phase were identified under positive selection, while no site was discovered during 03-late phase. It suggests that S protein experiences variable positive selective pressures before reaching stabilization. Sites 479, 609, 743, and 765 during 02-04 interspecies epidemic group and sites 75, 778, and 1163 during 03-early to mid epidemic phase were identified under positive selection, while no site was discovered during 03-late epidemic phase, suggesting that the positively selected sites are changeable during different epidemic phases. In addition, three specific replacements (F360S, T487S, and L665S) only fixed in all 03 human strains revealed that selective sweep also drives the evolution of S genes before the SARS outbreak. Because of the association with receptor recognition and membrane fusion, these residues are likely to be the crucial residues for viral transmission from animals to humans and viral adaptation to human hosts. The variation of positive selective pressures and positively selected sites may represent the adaptive evolution of S protein from animals to human.

GENE REGULATION IN HEALTH AND DISEASE
GENISTEIN REGULATES THE TRANSCRIPTION OF INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR GENE EXPRESSION BY ESTROGEN RECEPTOR PATHWAY
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Phytoestrogens are currently receiving considerable attention as potential alternative therapy for reducing the menopausal symptoms. Clinical, in vivo and in vitro studies have shown that genistein, a natural isoflavonoid phytoestrogen, could stimulate breast cancer cell proliferation in the low circulating estrogen environment. Recent studies demonstrated that cross talk exists between estrogen receptor (ER)-dependent and insulin-like growth factor I receptor (IGF-IR)-dependent pathways in ER-positive breast cancer cells. Our previous studies have shown that physiological concentration of genistein mimics the action of estradiol (E2) in stimulation of MCF-7 cell growth by enhancement of IGF-1 signaling pathways. The present study aims at investigating the detailed molecular mechanisms involved in mediating its actions in human breast cancer (MCF-7) cells. 1 µM of genistein significantly stimulated estrogen-responsive pS2 mRNA expression (p<0.05) in MCF-7 cells. The ER binding assay showed that 1 µM of genistein could bind to ER directly just as estrogen. 1 µM of genistein not only increased the protein and mRNA expression of insulin-like growth factor 1 receptor (IGF-IR) but also increased the IGF-IR promoter activity. These effects could be completely abolished by co-treatment of MCF-7 cells with estrogen antagonistICI 180,782 (1 µM). Co-treatment of MCF-7 cells with cycloheximide (CHX) (5 µg/ml), a protein synthesis inhibitor, completely blocked the induction of IGF-IR protein and mRNA expression by genistein. Taken together, these data provide the first evidence that genistein activates the transcription of IGF-IR gene expression. The stimulation of IGF-IR expression by genistein appears to require ER as well as de novo protein synthesis.

SIGNALLING MECHANISM OF A TESTIS-SPECIFIC TRANSCRIPTION FACTOR
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Spermatogenesis is a process during which germ line stem cells (GSCs) undergo a series of proliferations and differentiations to produce mature spermatozoa. This complicated process could be divided into three phases: mitotic, meiotic and post-meiotic phase. Each phase is tightly regulated by germ cell specific transcription factors. In previous studies, we have identified and characterized a novel testis-specific transcription factor, NYD-SP24. In the present study, we aimed to study the function and the underlying signaling mechanisms of NYD-SP24. We first identified the downstream target genes of NYD-SP24 using microarray study: We found that 16 genes were up-regulated and 4 genes were down-regulated upon overexpression of NYD-SP24 in HEK293 cells. Among the 16 up-regulated genes, p21 is of particular interest due to its reported specific expression in pachytene spermatocytes and its well-known function in cell cycle control and apoptosis. The up-regulation of p21 was confirmed by RT-PCR and western blot. To determine the direct effect of NYD-SP24 on p21 transcription, the promoter and proximal elements of p21 were cloned into pGL3 vector for luciferase assay. Co-transfection of NYD-SP24 showed a significant increase.
in p21 promoter activity. To study the function of NYD-SP24 in vivo, immunodepletion of NYD-SP24 was performed by injecting specific antibody against NYD-SP24 into seminiferous tubule of mouse testis. Sperm count and motility were determined by CASA. The data showed that interfering with NYD-SP24 results in significant reduction in motility and sperm count. In conclusion, NYD-SP24 may up-regulate p21 expression through direct promoter activation, which may be critical for G2 to mitotic I transition in primary spermatocytes.

**EFFECTS OF SPERMINE ON MYOCARDIAL ISCHEMIA/REPERFUSION INJURY IN RATS**

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We have observed the effect of exogenous spermine (low concentration) on myocardial ischemia/reperfusion injury in rats. 40 Wistar rats were randomly divided into 5 groups: sham-operation (sham) group, ischemic reperfusion (IR) group, spermine (Sp) group and natural saline (NS) group. Ischemic reperfusion injury model was performed by ligating rat’s coronary artery. In Sp group, 0.5mmol/L spermine (2ml/kg) was injected slowly into rat’s vein. During the process, we traced the electrocardiogram, recorded the LV functional parameters, assayed the levels of SOD, LDH, NO and MDA in serum, and examined the ultrastructure. In IR group, the incidence of arrhythmia was 90%, myocardiul ultrastructure injured seriously, values of LVSP and ±dp/dt max decreased, levels of LDH, NO and MDA increased while that of SOD decreased (P<0.05 or P<0.01, compared with sham group). Compared with IR and NS group, all those indexes in Sp group changed significantly (P<0.05 or P<0.01). In conclusion, exogenous spermine alleviated myocardial ischemia/reperfusion injury in rats. The mechanism may be related to its antioxidant effect and relieving the injury caused by oxygen free radical.

**PROTEIN KINASE C ZETA IS IMPLICATED IN INSULIN INDUCED GLUT4 VESICLE FORMATION**

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Protein Kinase C ζ is a key factor in insulin induced GLUT4 translocation from intracellular depot to plasma membrane, wherein it inputs glucose uptake. Although it is established that PKCζ acts downstream of PI3K activation by insulin in myotubes or adipocytes, little is known about the detail of the gap between PKCζ activation and GLUT4 activity. Here, we demonstrate that PKCζ is involved in biogenesis of GLUT4 vesicle in L6 myotubes, assayed by an in vitro reconstitution of formation of GLUT4 storage vesicles. In this cell-free reaction system, small insulin-responsive vesicles obtained by centrifugation represent the major form of GLUT4 storage in these L6 myotubes. Both ecotopic expression of PKCζ and in vitro addition of GST fusion to it enhanced GLUT4 in GSV fractionated from in vitro reaction, however, decreased by its inhibitor, the cell-permeable PKCζ pseudosubstrate peptide in high concentration, 80nM or more. This result broadens PKCζ’s role in insulin signalling pathway by exposition of GLUT4 biogenesis, except for stimulation of actin remodeling which, in turn latter inducing GLUT4 fusion to plasma membrane.

**IDENTIFICATION OF A NOVEL GENE, NC65, THAT UPREGULATES NF-κB SIGNALLING PATHWAY**

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The NF-κB family of transcription factors is a key regulator of inflammation, immune responses, oncogenesis, apoptosis, and neuronal signaling. In this study, we have screened more than 600 novel human genes using the NF-κB luciferase cis-reporting system and identified a novel gene, NC65, which can obviously activate the NF-κB signalling pathway. The NC65 gene encodes a 186-amino acids-length protein and is highly conserved in evolution across different vertebrate species from mouse to human. The amino acid sequence of NC65 shows 71% similarity with the C-terminal (353-500aa) of SPATA15 (spermatogenesis associated gene 15), which indicates that NC65 may be also related to spermatogenesis. Northern blot analysis indicates that a 1.4 kb transcript specific for NC65 is weakly expressed in the examined human heart, brain, liver, pancreas, and muscle, but highly expressed in the testis. Subcellular localization test shows that GFP-fused NC65 protein is distributed both in the nucleus and cytoplasm. Further study demonstrates that overexpressed NC65 in 293T cells can promote the translocation of p65—one of the NF-κB members—from cytoplasm to nucleus. It is known that NF-κB pathway regulates the sperm output by balancing the proapoptotic and antiapoptotic signalling of spermatogenesis, so our findings suggest that NC65, as a novel regulator of NF-κB pathway highly expressed in testis, may have an important role in the process of spermatogenesis.

**EXPRESSION OF CALCIUM SENSING RECEPTOR DURING ISCHEMIA/REPERFUSION MYOCARDIAL DAMAGE AND RELATION OF CASR WITH INJURY OF MYOCARDIUM**

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The calcium sensing receptor (CaSR) that mediates the inhibition of PTH secretion has been identified and cloned from bovine parathyroid gland. CaSR is a G protein-coupled receptor, and its activation leads to intracellular calcium release. The expression of CaSR has been identified in parathyroid, thyroid, kidney, bone and GI tract, the organs involved in systemic calcium homeostasis. Calcium handling is essential for the homeostatic control of cardiovascular functions, which may not couple directly to systemic calcium homeostasis. Whether CaSR has a functional role to play in the cardiovascular system is unclear. The expression of CaSR during ischemia/reperfusion myocardial injury and disclose the relation of CaSR with myocardial ischemia/reperfusion was investigation. The experimental model was established by the 30min ligating and 1h, 2h, 4h and 6h reperfusion of the left descending coronary artery in rats. Wistar rats were then randomly divided into 5 groups: sham group, ischemia/reperfusion 1h, 2h, 4h and 6h group (IR/1h, 2h, 4h and 6h group). Results were evaluated by measured of CaSR mRNA expression by RT-PCR. Left ventricular function was recorded as the level of plasma lactate dehydrogenase (LDH), alaniladehyde (MDA) and superoxide dismutase (SOD). Changes of ultrastructure in the ischemia/reperfusion myocardium of rats by electron microscopy observed that LVSP, ±dp/dt max and SOD activity decreased gradually with the prolonging of reperfusion time. LDH and MDA peaked at 2h. The ultramicrostructur struction of the 1h and 2h was more serious than those of 4h and 6h. The expression of CaSR increased more significantly after 30min of ischemia and reperfusion of 1h and 2h, and decreased after 4h and 6h. In conclusion, CaSR may be associated with the myocardial ischemia/reperfusion injury.

**EXPRESSION OF SCNS5A IN ISCHEMIA AND HYPOXIA RAT MYOCARDIUM IN VITRO AND IN VIVO**

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The voltage-sensitive sodium channel (SCN5A) is the main determinant of Na⁺ influx and excitability in cardiac cells and the SCN5A gene is correlated with cardiac arrhythmia such as L-QT syndrome. To elucidate the mechanism of these diseases, we investigated the changes of SCN5A expression under ischemia and hypoxia pathophysiologic conditions in vitro and in vivo. Myocardial cells were isolated and cultured under normoxia (21% O₂) or hypoxia (2% O₂, 5% CO₂, balance with
N2) Compared with control group, the expression of SCN5A was significantly increased in hypoxic group of 1 and 6 hours (P<0.001), but decreased at the 12 and 24 hours hypoxia points at the level of mRNA and protein (P>0.001). Ischemia model was made via ligating the circumflex branch of left coronary artery of the rat heart. In the ischemic region, the expression of SCN5A of 3d- and 7d-ischemia group was decreased in rats as compared with control group (P<0.05), and no difference was detected in 6h-ischemia group. In the non-ischemic region, the expression of SCN5A was increased in 6h-ischemia group (P<0.01), decreased in 3d-ischemia group (P<0.05) and nearly turned to normal level in 7d-ischemia group. The expression of SCN5A of non-ischemia region was higher than that of ischemia region in 6h- and 7d-ischemia group (P<0.05), whereas there was no significant difference between them in 3d-ischemia group. These results suggested transcriptional and translational regulation of SCN5A might be complex, and further study of the regulations may be a fruitful area of exploration in arrhythmic syndromes.

GROWTH AND NUTRITIONAL STATUS OF PRE-SCHOOL CHILDREN IN CHONGQING SUBURBS, CHINA
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Micronutrient deficiency disease is a serious public healthy problem in many developing countries. China is practicing in the “7+1” nutrition fortified food program to eliminate these diseases. Our object is to evaluate the effectiveness of “7+1” micronutrientions-fortified seasoning powder to improve the growth and nutritional status of pre-schoolchildren in Chongqing. Baseline recording on about 469 pre-schoolchildren aged from 2 to 6 years, recruited from four nurseries randomly chosen from Banan District of Chongqing. Anthropometric measurements were recorded by standardized methodology and blood was collected for serum retinol acid (RA) by HPLC, zinc and iron by atomic absorption spectrum. Indexes of nutritional status were measured. Anthropometric and biochemical data of 237 boys and 231 girls were analyzed. The prevalence of stunting (height-for-age) was 5.5 % in boys and 6.0 % in girls while wasting (weight-for-height) were 2.5 % and 0.4 % respectively, and 3.8 % of boys and 3.4 % of girls were underweight (weight-for-age) compared to 2.6 % and 3.5 % of the obese respectively. Serum RA, zinc and iron of stunted were 1.13 μmol/l (95 % CI: 1.126 μmol/l), 14.1 μmol/l (95 % CI: 11.61, 16.50 μmol/l) and 19.94 μmol/l (95 % CI: 22.4, 19.6 μmol/l) respectively. In contrast, these data of the children with IAZ-score ≥ 2 were 1.22 μmol/l (95 % CI: 1.44, 1.32 μmol/l), 14.60 μmol/l (95 % CI: 13.2, 15.9 μmol/l) and 22.4 μmol/l (95 % CI: 19.9, 25.3 μmol/l) respectively, which are higher than the corresponding of the stunted, but there were no statistical difference (P>0.05). The effect of nutritional transition to children’s health is gradually evident which resulting in some emerging nutritional problems. Further investigation is needed to determine if micronutrientions supplementation can improve the growth and nutritional status in pre-schoolchildren.

SERUM VALUES OF VITAMIN A AND ITS INDICATORS IN BANAN PRE-SCHOOLCHILDREN
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Vitamin A deficiency is a serious public healthy problem in China. Vitamin A has a marked effect on level of RBP (retinol binding protein) and PA (prealbumin) also reflect the status of protein nutrition.). Our objective is to evaluate the effectiveness of “7+1” micronutrientions-fortified seasoning powder to improve the status of vitamin A and its metabolically related proteins RBP and PA in pre-schoolchildren in Chongqing. Baseline recording was conducted in about 357 pre-schoolchildren aged from 2 to 6 years, recruited from four school nurseries randomly chosen from Banan District of Chongqing. Levels of vitamin A were determined by HPLC, RBP by ELISA, PA by immunoturbidimetry. The mean serum concentration of vitamin A, PBR and PA were 1.19 μmol/l (1.2±0.3 μmol/l), 42.0 mg/l (42.0±14.9 mg/l), and 180.9 mg/l (180.9±41.6 mg/l) respectively. The prevalence of vitamin A deficiency (VAD) (<0.7 μmol/l) is 6.4 % while marginal VAD (0.7-1.05 μmol/l) is 27.7 %. Serum values of RA were significantly related to RBP and PA (r=0.27, 0.36 respectively, P<0.01). Serum values of RBP in the VAD children (37.1±19.9 mg/l) was significantly lower (P=0.045) than that of children (44.6±12.8 mg/l) with normal VA level (≥1.05 μmol/l). Similarly, the serum values of PA in VAD children (154.0±34.2 mg/l) was marked lower (P<0.001) than that of normal children (189.4±42.6 mg/l). Also, a positive co-relationship was found between the concentration of RBP and PA (r = 0.217, P<0.01). Our study suggests that serum vitamin A level was significantly related to RBP and PA, and the measurement of RBP and PA can assess the level of serum vitamin A. Further investigation is needed to determine if the supplementation of micronutrientions-fortified food can improve the protein-nutritional status in pre-schoolchildren.

EFFECTS OF MARGINAL VITAMIN A DEFICIENCY AND INTERVENTION ON LEARNING AND MEMORY IN YOUNG RATS
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The present study was to investigate whether learning and memory in young rats would be impaired due to marginal vitamin A deficiency (MVAD) beginning from embryonic period, and whether there would be difference on the recovery by vitamin A intervention at different ages. We designed 3 groups with the dams and pups in control group fed with normal diet (VA 6500 IU/kg), MVAD group with MVAD diet (VA 400 IU/kg), and in vitamin A intervention (VAI) group, the dams fed with MVAD diet while pups with normal diet from 4 weeks postnatal. Shuttle box active avoidance reaction tests, hippocampal CA1 LTP and relative intensity of fluorescence in cells in hippocampal slices were measured. The results showed that the times to reach the learning standard of VAI group (28.8 ± 4.1) and MVAD group (45.6 ± 12.1) were more than control group (17.1 ± 4.4) (P<0.01), and that of MVAD group was more than VAI group (P<0.05) in active avoidance reaction tests. The changes of field excitatory post synaptic potentials (fEPSP) slope for MVAD group (22.9 % ± 9.4 %) and VAI group (29.5 % ± 13.7 %) were less than that of control group (57.5 % ± 27.3 %) (P<0.01). No differences of relative intensity of fluorescence in cells were found before the tetanus stimulation. However, it was significant lower in MVAD (65.1 ± 7.0) and VAI (85.8 ± 17.1) groups compared to control group (113.6 ± 20.5) after the tetanus stimulation (P<0.01), and VAI group was higher than MVAD group (P<0.05). These results implied that MVAD beginning from embryonic period impairs learning, memory and LTP in young rats, and the losses might not be reversible if the vitamin A supplementation missed the critical period of hippocampus development.

EFFECTS OF MARGINAL VITAMIN A DEFICIENCY ON LONG-TERM POTENTIATION IN YOUNG RAT
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Vitamin A is an essential micronutrient for growth, especially for brain development. Marginal vitamin A deficiency (MVAD) remains a subclinical public health problem in children, but little is known about the mechanism of MVAD affects the brain development beginning from embryonic period and early postnatal period. To study the effects of MVAD on the hippocampal CA1 long-term potentiation (LTP) in young rats, we divided six female rats into two groups at random, three in MVAD group, three in control group. The rats in MVAD group were fed with VA deficient diet (400 IU VA/kg) and rats in control group were fed with VA sufficient diet (6500 IU VA/kg) respectively since three weeks before coitus. Serum VA was assayed by high performance liquid chromatography (HPLC). All the young rats were killed at day 49 and hippocampal CA1 LTP was detected by electrophysiological technique and the ultrastructure of synapses was observed by electron microscope. The results showed that The changes of field excitatory post synaptic potentials (fEPSP) slope (25.4 % ± 2.01 %) in MVAD young rats (65.1 ± 7.0) after the tetanus stimulation (P<0.01), and VAI group was higher than MVAD group (65.1 ± 7.0) and VAI (85.8 ± 17.1) groups compared to control group (113.6 ± 20.5) after the tetanus stimulation (P<0.01), and VAI group was higher than MVAD group (P<0.05). These results implied that MVAD beginning from embryonic period impairs learning, memory and LTP in young rats, and the losses might not be reversible if the vitamin A supplementation missed the critical period of hippocampus development.
IRON STATUS OF PREGNANT WOMEN IN KAI COUNTY CHONGQING CHINA
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Iron deficiency for pregnant women is an old enemy, and can result in anemia. To understand the iron state in the early-gestation group (group one) and the middle-gestation group (group tow) of pregnant women in Kai County Chongqing China and to research whether it is necessary to supply iron to them, we performed a descriptive, prospective study, nested within a randomized controlled trial. 68 and 104 pregnant women were randomly selected to form the above two groups respectively. Blood was drawn for Hb (cyanmethemoglobin assay), STR1 (immunoturbidimetric), and SF (ELISA). The results showed that means of Hb, SF and STR1 were 125.73±15.21, 37.20±28.37, and 1.06±0.42 in group one, whilst 119.74±13.91, 16.96±14.48, and 1.29±0.74 in group two. There were significant differences in the means of Hb, SF and STR1 (P < 0.05) between the two groups. The prevalence rates (PR) of ID (iron deficiency) and IDA (iron deficiency anemia) were 23.5% and 1.5% in group one while 43.1% and 5.9% in group two. Between the two groups only the difference of the PR of ID had statistical significance (chi-square test: P=0.01). We concluded that with the advancement of the pregnancy, women were likely to incur iron deficiency. Among the second-trimester there would be more women suffering from iron deficiency but the severity of the two groups was the same. In conclusion, supplementation with iron for pregnant women is recommended for pregnant women.

EXPRESSION OF ETS-1 TRANSCRIPTION FACTOR IN MOUSE UTERUS DURING THE ESTROUS CYCLE AND EARLY PREGNANCY
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Ets-1 is an important member of Ets transcription factor superfamily. A large body of studies indicated that the members of Ets family play important roles in a variety of physiological and pathological processes, including embryogenesis, cell proliferation, differentiation, cell development, signal transduction, apoptosis and tissue remodeling. The mammalian uterus is a unique dynamic organ, and undergoes dramatic cyclic growth, differentiation, breakdown and remodeling during the estrous/menstrual cycle and the establishment of pregnancy. The present study was to investigate the temporal and spatial expression of Ets-1 mRNA and protein in mouse uterus during the estrous cycle and early pregnancy by using in situ hybridization and immunohistochemistry, so as to gain insight into the biological function of Ets-1 in embryo implantation and its regulation by ovarian steroids. The results showed that Ets-1 mRNA was localized to the luminal and glandular epithelium at proestrus and estrus, and the expression level was decreased significantly from metestrus to dioestrus. During pre-implantation period, Ets-1 mRNA was predominantly expressed in luminal and glandular epithelium at much higher level; whereas it switched to stroma during peri-implantation period, and also appeared in the blastocysts and the implantation sites. The results suggested that Ets-1 might play a role in the cycling changes of mouse uterus during the estrous cycle and embryo implantation.

MALIGNANT TRANSFORMATION OF THE CULTURED HUMAN HEPATOCYTES INDUCED BY HEPATITIS B VIRUS PRES2 PROTEIN
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The PreS2 activator is one of the transcriptional activators in hepatitis B virus. In a previous study, we found that blockage of PreS2 expression downregulated the endogenous telomerase activity and repressed hTERT expression in hepatocarcinoma cells. To further understand the functional role of preS2 on hepatocytes cells, PreS2 expression plasmid pcS2 was constructed and stable transfected into HepG2 cells to establish preS2 expression cells. We primarily studied the potential alterations in cell proliferation resulting from PreS2 expression with the growth curve and colony-forming assay. Results showed that cells transfected with PreS2 proliferated faster and could form more colonies in soft agar. Furthermore, the telomerase activity and hTERT mRNA expression were assayed using a modified telomeric repeat amplification protocol (TRAP) assay and RT-PCR. In cells expressing PreS2, the telomerase activity was consistently found to be approximately two fold higher than that of the cells transfected with empty vector and of untransfected cell. As expected, hTERT mRNA was increased in cells expression PreS2. These suggest that PreS2 could increase the malignant transformation in human hepatocarcinoma cells partly because of the activation of telomerase by upregulating hTERT expression.

PROTEIN KINASE C-ZETA INVOLVES INSULIN-INDUCED ACTIN REMODELING
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Type 2 diabetes mellitus is a multigenic disease with evident genetic predisposition, and complex pathogenesis in which environmental and genetic factors interact. The disorder of body utilization glucose is a crucial reason for causing diabetes. A major physiologic action of insulin in peripheral tissues is to stimulate the translocation of GLUT4 to the cell surface, leading to enhanced glucose uptake and storage in these tissues. Actin remodeling plays a crucial role in insulin-induced GLUT4 translocation from cytoplasm to plasma membrane and glucose transport, and atypical PKCs play an important role in glucose transport by inducing insulin-mediated GLUT4 translocation to plasma membrane. We demonstrate that overexpression WT PKCζ induced actin remodeling in L6 myotubes similar to insulin, and insulin increased the change. By immunofluorescence, PKCζ redistributed and co-localized with actin structure in response to insulin in L6 myotubes, the redistribution and co-localization can be cancelled by the PI3K inhibitor wortmannin (WM). However, insulin-induced co-localization of PKCζ and actin is instantaneous, for overexpression PKCζ is not resistant to 1%Triton-100 extraction in Hela and Cos7. These results suggest that PKCζ involves in insulin-stimulated actin remodeling dependent of insulin-Pi3K pathway and the effect is an instant effect.

THE INTEGRIN-FAK PATHWAY MAY BE INVOLVED IN BETA CELL APOPTOSIS INDUCED BY P58 PITSLRE AND p110c
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It was reported that the likelihood of apoptosis of β-cell of patients with type 2 diabetes is increased significantly. Genome-wide scanning in our lab showed there are 4 susceptibility variants of type 2 diabetes in Northern Chinese population. CDC2L2 is one of the 4 candidate genes. P34cdc2-related kinases consist of many isoforms. P58 (PITSLREβ1) is one member of the cdc2L2 gene family, PITSLRE isoforms are cleaved by caspase to produce a protein that contain the c-terminal kinase domain of the PITSLRE protein (p110c) to induce apoptosis. Here, we hypothesized that P58 (PITSLREβ1) and p110c might be involved in apoptosis of RINm5F cells through regulation of the integrin- FAK pathway. To verify this hypothesis, we constructed expression vector of P58 (PITSLREβ1) and p110c and transfected into RINm5F cells. Western blotting results showed that overexpression of P58 PITSLRE or p110c in RINm5F cells could decrease the activity of FAK in different way: P58 PITSLRE decreases phosphorylation level of FAK (Tyr937) and p110c decrease the total level of FAK expression. These results suggest that P58 (PITSLREβ1) and p110c might be involved in apoptosis through regulation of the integrin-FAK pathway.
**MICROARRAY ANALYSES AND MOLECULAR PROFILING DURING HEPATOCELLULAR CARCINOGENESIS IN HBV TRANSGENIC MICE**

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Hepatitis B virus (HBV) infection contributes to the development of end-stage liver diseases, including liver cirrhosis and hepatocellular carcinoma (HCC). HBV transgenic mice invariably developed macroscopically evident tumors within the 20th month of life. We have verified this conclusion by investigating BALB/c background mice harboring HBV complete genome (subtype ayw).

Thus the HBV transgenic mouse could be used as a natural model of HCC development. To identify dynamic molecular changes involved in hepatocellular carcinogenesis, we performed differential gene expression by microarray analyses in several life stage of HBV transgenic mice and their syngeneic BALB/c mice controls. HBV transgenic mice of 6, 12 and 20-month-old and the age-matched BALB/c mice as control were sacrificed for exclusion of aging related genes. The total liver RNA was extracted and hybridized on an oligonucleotide chip containing 35,000 Mus musculus genes. Out of all murine genes screened, the number of differentially expressed transcripts was 235±32, 687±72 and 711±156, in the group of 6, 12 and 20 month, respectively. We could conclude a molecular portrait of changes in gene expression associated with different stages of hepatocellular carcinogenesis. These genes were involved with important cellular pathways, including cell growth regulation, apoptosis, immune response, metabolism, stress, and cell adhesion. We focused on several downregulated genes such as Thrp, Cpyl7a1, Dbp, C4, Ugt1a1, Tef, Cfi, Tnfap9, Csf, Tdc1 and Mbl2. We further explored the microRNA (miRNA) profiling relevant to hepatocellular carcinogenesis, for miRNAs closely associated with the process of tumorigenesis. The downregulated genes discovered in HBV transgenic mice may be regulated by let-7, miR-122a, miR-214, miR-133, miR-370 and miR-34. Identification of these miRNAs with potential roles may help in the selection of targets for novel therapies against HBV-related HCC progression. Also the dynamics of molecular profiling during hepatocellular carcinogenesis would better clarify molecular mechanisms of HCC. Supported by the National Nature Science Foundation of China, Grant No. 30440040.

**SMAD UBIQUITIN REGULATORY FACTOR 2 (SMURF2) IS IMPORTANT IN CONTROLLING TGF-B SIGNALING DURING TROPHOBAST CELL INVASION AND MIGRATION**

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Successful implantation depends on the ability of the trophoblast cells to invade the endometrium which is negatively regulated by TGF-β1. Smurf2, a HECT type E3 ubiquitin ligase, regulates TGF-β signaling via ubiquitination of TGF-β receptors and intracellular mediators Smad2 and Smad7. Whether Smurf2 plays a role in controlling TGF-β signaling during embryo implantation is currently unclear. In the present study, the spatio-temporal expression of Smurf2 in the placenta of rhesus monkey and human, and the possible regulatory mechanisms of Smurf2 on the migration and invasion of human extravillous trophoblast cells were investigated. Smurf2 mRNAs and proteins were mainly localized in the placenta villi, trophoblastic column and trophoblastic shell in rhesus monkey. Their expression in human placental villi in the 1st trimester was significantly higher than those in the 2nd trimester and term, implying a role of Smurf2 in trophoblast invasion and migration. To test this hypothesis, an extravillous trophoblast cell line HTR8/Svneo was used. Overexpression of Smurf2 down-regulated the expression of TGF-β type I receptor, while inhibition of Smurf2 by siRNA significantly increased the TGF-β type I receptor protein level. Furthermore, overexpression of Smurf2 stimulated migration and invasion of HTR8/Svneo cells. The above results provide the first evidence that Smurf2 plays an important role in regulating the migration and invasion of trophoblast cells at least in part via mediating the expression of TGF-β type I receptor.

**CHARACTERIZATION AND EXPRESSION OF CATHEPSIN L IN AMPHIOXUS BRANCHIOSTOMA BELCHERI TSINGTAUENSIS**

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Cathepsin L is involved in the multiple physiological roles in organisms. To date, little is known about the cathepsin L in the amphioxus, a cephalochordate, which has long been regarded as the extant invertebrate most closely related to the proximate ancestor of vertebrates. We cloned the cathepsin L gene from amphioxus, the amphibia, and expressed it in all tissues examined, with the most abundance in the hepatic caecum and hind-gut, suggesting the role of food digestion for the enzyme. Whole mount in situ hybridization analysis revealed that *AmphiCL* was involved in the gut development. For further study of *AmphiCL* expression and function, we cloned the cathepsin L gene from the amphioxus, a cephalochordate, and expressed it in all tissues examined, with the most abundance in the hepatic caecum and hind-gut, suggesting the role of food digestion for the enzyme. Whole mount in situ hybridization analysis revealed that *AmphiCL* was involved in the gut development.

**NS81: A NOVEL LYSOSOME AND ENDOPLASMIC RETICULUM-ASSOCIATED MEMBRANE PROTEIN INDUCES CELL AUTOPHAGY AND APOPTOSIS**

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Programmed cell death can be divided into apoptosis and autophagic cell death. In the present study, we described the biological activity of a novel lysosome and endoplasmic reticulum-associated membrane protein, provisionally named NS81. The novel protein has several interesting and unique characteristics. Functional studies showed that overexpression of NS81 markedly inhibited clonogenic formation both in HEK293 and HeLa cells. Simultaneously, typical morphological characteristics of autophagy were observed by transmission electron microscopy, including extensive autophagic vacuolization and enclosure of cell organelles by double-membrane structures. Further experiments confirmed that the overexpression of NS81 increased the extensive punctate distribution of monodansylcadaverine (MDC) staining and LC3-GFP in HeLa cells, both in number and fluorescence intensity, as well as upregulation of LC3-I/LC3-I proportion. On the other hand, HeLa and HEK293T cells transfected with NS81, succumbed to cell death with hallmarks of apoptosis such as phosphatidylserine externalization, loss of mitochondrial transmembrane potential, caspase activation and chromatin condensation. Kinetic analysis revealed that the appearance of autophagy-related biochemical parameters prior to the nuclear apoptosis in NS81 transfected HeLa cells. These results showed for the first time that NS81 involved in both cell autophagy and apoptosis. The novel protein may play an important role in cell growth and cell death, and have important applications in the treatment and diagnosis of some diseases, such as cancer. Further experiments should be conducted to explore the molecules that interact with NS81 and may influence programmed cell death.

**REGULATION OF PROSTATE-SPECIFIC ANTIGEN EXPRESSION BY CMTM3 IN PROSTATE CANCER LNCAP CELLS**

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CFTR is involved in mediating the bicarbonate entry important for sperm capacitation

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HCO3- is one of the critical factors for sperm capacitation leading to the final acquisition of their fertilizing capacity. We hypothesized that sperm CFTR may be involved in mediating the HCO3- entry important for sperm capacitation and that defective CFTR may lead to impaired sperm fertilizing capacity resulting in infertility. This study aims to test the hypothesis using cftr-/- knockout mice model in conjunction with a number of techniques. Immunostaining and Western blot. Intracellular pH and membrane potential dye were used to monitor the intracellular pH and membrane voltage changes during the capacitation process. Immunostaining and western blot results demonstrated the expression of CFTR in human and mouse sperm membrane. In vitro study showed that the intracellular pH elevation during the process of capacitation was attenuated by CFTR inhibitor and antibody in a concentration- dependent manner. Addition of HCO3- induced a hyperpolarizing current and this process can be inhibited by CFTR inhibitor. Furthermore, a decrease in percentage of capacitated sperm and reduced fertilization were observed in heterozygous cftr-/- mice when compared to control wild types sperm. Overall, this study indicates that CFTR is expressed in sperm and involved in HCO3- transport, thus playing an important role in sperm capacitation. Supported by Strategic Investment of The Chinese University of Hong Kong.

A novel lysosome and autophagosome associated protein, NM393 induces autophagy

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Autophagy is a complex catabolic program for lysosomal degradation of proteins and other subcellular constituents. Defects in autophagy have serious consequences, as they have been linked to neurodegenerative disease, cancer, and cardiomyopathy. Here, we report the identification and characterization of NM393, a novel human functionally unknown gene. This gene encodes 393 amino acids without any obvious functional domain. The encoded protein has two putative transmembrane domains, localizes to lysosome and autophago-some as revealed by GFP-NM393 analysis. Overexpression of NM393 in HeLa cells lead to autophagic vacuolization under transmission electron microscopy, further experiments revealed enhanced and dotted monodansylcadaverine (MDC) staining, accumulation of punctuate endogenous LC3, as well as increased ration of LC3-II/LC3-I. On the other hand, knock-down of NM393 in HeLa cells inhibits autophagy induced by starvation, including reduced autophagic vacuolization, reduced intensity and dotted extent of MDC staining, as well as decreased ratio of LC3-II/LC3-I. Increasing number of studies propose that induction of autophagic cell death may be an effective mechanism to kill cancer cells, so our results suggest that NM393 as a novel autophagy promoting gene, may have potential value in diagnosis and treatment of cancers.

Expression and distribution of a novel gene in skin development and wound healing in rats

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A novel testis specific transcription factor (TSTF) has been found to have two proteins isoforms, one is a 76kDa form, belong to transcription factor involving in process of spermatogenesis, another is a 55kDa form with unknown function. However, little is known about the expression of this gene in other tissues. Our present studies show that this gene is also expressed in skin. Western blot results show that in fetal and newborn rats, both forms of TSTF protein were expressed in epidermis, however in adult rats only the 55kDa form was expressed, indicating an important role for the 76kDa form in skin development. Immunohistochemical results show that the 76kDa form was located in nucleus of the keratinocytes of all layers including the basal layer, whereas the

THE EFFECTS AND MOLECULAR MECHANISM OF THE SOY ISOFLAVONE ON THE EXPRESSION OF VEGF AND eNOS IN AGING RATS OVARY

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Phytoestrogens Soy Isoflavone (SI), which binds to the estrogen receptor (ER), are plant-derived, non-steroidal compounds with structural similarity to estrogen and can induce estrogen-dependent gene transcription. We randomly divided forty-four normal 12-month old female SD rats into five groups: aging model group, three SI treatment groups (SI 20mg/kg/d, 100mg/kg/d and 500mg/kg/d) and estrogen group (estriol valerate 0.1mg/kg/d). Eight 3-month female SD rats were used as a normal control group. The rats were treated with SI and estrogen group (estrodiol valerate 0.1 mg/kg/d). Eight 3-month female SD rats were used as a normal control group. The rats were treated with SI and estrogen group (estrodiol valerate 0.1 mg/kg/d). These results suggest that the expression of VEGF and eNOS were increased and the production of NO was decreased in aging rats ovary. Soy Isoflavones could increase the serum E2 levels in serum were measured by radioimmunoassay (RIA), and the expression of VEGF and eNOS were measured by RT-PCR and immunohistochemistry. We demonstrated that compared with the control group, the expression of VEGF was enhanced and the expression of eNOS was reduced while E2 levels in serum were reduced, too. Compared with the aging model group, the expression of eNOS was down-regulated and the expression of eNOS was up-regulated in aging rats ovaries which was treated with SI. These results suggest that the expression of VEGF was increased and the production of NO was decreased in aging rats ovary. Soy Isoflavones could increase the serum E2 level and decrease the expression of VEGF by increasing the production of NO in aging rat ovaries, which could be one of the mechanisms of delaying ovary aging by the Soy Isoflavone.
55kDa form was located in cytoplasm and highly expressed in the squamous cell layer, the granular layer and stratum corneum, indicating a possible role related to keratinocytes differentiation. Moreover, significant expression changes of the 55kDa form during wound healing in adult rats were revealed. Following wounding, the staining of this 55kDa protein was downregulated throughout the migrating epidermis at the wound edge 3 days post-wounding and, although to a lesser extent, up to 7 days post-wounding. The staining intensity returned to near basal levels by 14 days post-wounding when the wounds were full epithelialized. Consistent with the immunohistochemical pattern of decreased wound staining post-wounding, the 55kDa protein levels, as measured by western blot, were also significantly downregulated in response to wounding. The marked temporal downregulation of this 55kDa form during early wound healing suggests a role for this protein in the process of epidermal maturation.

Taken together, these results of different expression pattern of TSTF at different stages of skin development and wound healing indicate that the novel gene, TSTF may play an important role in the process of development and repair of skin. This work was supported by Strategic Investment of CUIHK.

IDENTIFICATION OF SEVERAL PROTEINS INVOLVED IN REGULATION OF SPERM MOTILITY BY PROTEOMIC ANALYSIS
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Male factor infertility is a major medical problem, while sperm motility is one of the main determinants of male infertility. In this study, we used the technique of proteomic analysis to compare the sperm protein expression profiles (proteome maps) of eight asthenozoospermic patients with those of eight normal spermatozoa donors. Seventeen differentially expressed spots were found in patient samples compared to normal samples using gel analysis software. Ten proteins corresponding to 14 spots were identified with mass spectrometry and peptide matching. These sperm proteins fall into three main categories: (1) structure associated proteins, i.e. outer dense fiber protein; (2) metabolic enzymes, i.e. isocitrate dehydrogenase subunit alpha, triosephosphate isomerase, phosphoglycerate mutase 2, glutamate oxaloacetate transaminase-1 and carbonic anhydrase II; and (3) other functional proteins. These proteins may play roles in regulation of sperm motility, and their characterization could merit further investigation on sperm motility related male infertility.

EXPRESSION OF PACAP mRNA IN DEVELOPING STAGES OF RAT OVARY AND ITS REGULATION
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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide. Recently, line of evidence has shown that PACAP also exists in human ovaries, participating in the regulation of ovarian function. We demonstrate that specific signals of PACAP transcripts were detected in the granulosa cells and some theca cells of large prefollicular antral follicles and preovulatory follicles. Almost no signal was detected in the corpus luteums of hCG-treated ovaries. After culturing granulosa cells with PAF for 6h, no significant change of PACAP mRNA levels was observed compared with the levels of those cultured in the medium alone. However, PAF could stimulate PACAP mRNA expression by enhancing the effect of hCG. The activator of adenylate cyclase forskolin, the activator of protein kinase C, PMA, and the inhibitor of protein-tyrosine kinases pathway, genistein, were administered to granulosa cells that had been incubated in the media alone for 24h. We found that forskolin could significantly stimulate PACAP mRNA expression. These results suggest that PACAP may play an important role in the growth of developing follicles and in ovulation as an autocrine/paracrine factor. In granulosa cells, PAF can stimulate PACAP mRNA expression indirectly by enhancing the effect of hCG and the hCG stimulation of PACAP mRNA may be mediated by cAMP.

DOUBLE STRAND BREAK MUTAGENESIS IN HUMAN LYMPHOBLASTOID CELLS RESPONSE TO INHIBITING EXPRESSION OF Ku80 BY RNAi
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DNA double-strand breaks (DSBs) are important pre-mutagenic lesions, which may be caused either by a number of external agents or by several normal cellular processes, and may lead to the partial or complete loss of a chromosome or to the rearrangement of genetic information. I-SceI, as a powerful tool, was widely used for research on specific-site DSB in eukaryote. In the present study, we developed a system of “loss or gain of function” to explore the mutagenic effects of specific DSB at a normal human tk gene. We analyzed (1) whether induction of a DSB at the Alu sequence location of the intron 2 affected mutation in human tk gene and (2) whether silencing expression of the Ku80 by siRNA affected the DSB repair. Using siRNA targeting following I-SceI expression vector transfection, we have demonstrated that mammalian cell frequently repair DSBs by the non-homologous end joining (NHEJ) pathway. Firstly, cell lines for studying DSB repair were established by gene targeting, and DSBs were produced by the I-SceI nuclease expression; Secondly, the effect of DSB induced by I-SceI transfection on mutation at normal human tk gene were tested and DSB at different TK loci intron 2 cause different mutation frequency. Thirdly, mutation frequency was decreased for the comparison of mock treated with siRNA, especially after Ku80 knockdown following the induction of DSB. Our data in this paper imply that DNA double-strand breaks are produced in cells following I-SceI induction, that the non-homologous end-joining repair pathway is involved in their repair and that they are produced with sufficient frequency to have biological significance.

OTHERS

FEATURE SELECTION OF EFFUSION CELL BASED ON GENETIC ALGORITHM
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Quantitative cytology is valuable to discriminate malignant pleural effusions by analyzing the features of cell. Automatic image cytology can analyze cells in effusions quantitatively, quickly and accurately, which can overcome the disadvantages of conventional methods. Usually, more than 100 features of cells, including geometry, optical and physics, etc., can be got by cytology. Diagnosis result can be got on the basis of analyzing and induction the properties of these features. In fact, this procedure is a pattern recognition program, i.e. designing a classifier according to the features of cells. Obviously, there must be redundancy in the features, which is deleterious to the diagnosis of pleural effusions. So feature selection is necessary to improve the performance of classifier and explain the biology background of malignant pleural effusions more exactly. Genetic algorithm (GA) has been used as a method for classifiers to adaptively evolve solutions for classification problems. In this paper, feature selection is explored with GA-based classification to find less relevant features in the input domain of each class. By removing these features, it is aimed to reduce the dimensionality of classification problems and accelerate the classification or diagnosis. The experiment results show that GA-based classification can be used to find less relevant features and help achieve faster classification due to the feature space dimension reduced.

ROLE OF CAMP-PKA PATHWAY IN MEDIATING THE EFFECT OF ADENOSINE ON THE K CHANNELS IN BASOLATERAL MEMBRANE OF THE THICK ASCENDING LIMB OF RAT KIDNEY
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We used the patch-clamp technique to examine the effect of adenosine on the basolateral 50-pS K channels in the thick ascending limb (TAL) of rat kidney. Application of 5 μM cyclohexyladenosine (CHA), an adenosine analog, stimulated basolateral 50-pS K channel activity and increased channel activity, as identified as NP (o), from 0.25 to 0.49. The stimulatory effect of CHA is absent in the presence of 8-(3-chlorostyryl) caffeine (adenosine A2a receptor antagonist) but not affected by 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX, adenosine A1 receptor antagonist). 5 μM CHA increased NP (o) of the K channels from 0.19 to 0.42 in the presence of DPCPX. This suggests the effect of CHA is the result of stimulation of A<sub>2α</sub> adenosine receptor rather than A<sub>1</sub> adenosine receptor. The stimulatory effect of CHA on the basolateral 50-pS K channels was blocked by H8 (an inhibitor of protein kinase A). This indicates that the effect of CHA on the K channels is mediated by a cAMP-PKA-dependent pathway. We conclude that CHA activates the 50-pS K channel in the basolateral membrane of the TAL and the stimulatory effect of CHA is mainly mediated by a PKA-dependent pathway via A<sub>2α</sub> adenosine receptor that is present in the TAL.

**CLONING, MOLECULAR CHARACTERIZATION OF HELICOBACTER PYLORI CAGA AND CONSTRUCTION OF A CAGA-DELETED MUTANT STRAIN**

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The CagA protein of Helicobacter pylori, which is injected from the bacteria into bacteria-associated gastric epithelial cells, is associated with gastric carcinoma, while its pathogenesis remains unclear. In this study, we established a PCR method for amplifying cagA gene in full length, and constructed and identified a deficiency mutant of Chinese Helicobacter pylori strain of MEL-Hp27 without cagA gene, in order to provide the research basis for clarifying the role of cagA/CagA in the pathogenesis of H. pylori infection. cagA gene of MEL-Hp27 without 5′ UTR of 649 bp and 3′ UTR of 476 bp, coding 1169 amino acids, was cloned. The box and -35 box were found locating in the upper stream of cagA gene. Sequence analysis showed that the coding region of cagA gene of MEL-Hp27 shared 96% homology to East Asian H pylori strains while about 86% to Western strains. There was also the heterogeneity in the untranslated region of cagA gene between MEL-Hp27 and Western strains. 5′ and 3′ UTR of cagA gene fragments and a selectable kanamycin resistance marker between them were engineered into pb-luescript plasmid (pBS-cagA mutant). Electrotansferation of H. pylori cells with cagA deleted derivative plasmid pBS-cagA mutant resulted in isolation of kanamycin resistance H. pylori transfectants. The deleted status of mutants of Chinese H. pylori was identified by their failure to yield PCR products with primers specific for internal regions of cagA. We cloned the cagA gene of Chinese Helicobacter pylori strain of MEL-Hp27 in full length, and constructed a cagA-deleted mutant of Chinese H. pylori strain, which are essential steps for further investigation of the role of cagA/CagA in the pathogenesis of H. pylori infection.

**LEPTIN INHIBITS EXPRESSION OF PREPROINSULIN mRNA IN RATS**

Qiang Li, Hong Qiao, Jin Chao Zhang, Yu Qian Sun and Wei Liang

In vitro, isolated rat islets from noninjected rats were incubated in the absence or presence of recombinant rat leptin (100 ng/ml) for 2 or 24 h at either 5.6 mM or 20 mM glucose; in vivo, islets were isolated from leptin-injected or PBS-injected rats. The levels of preproinsulin mRNA were measured by RT-PCR. Firstly, leptin did not change preproinsulin mRNA levels at normal glucose concentration. Secondly, preproinsulin mRNA was not inhibited by leptin at 2 h at glucose concentrations of 20mM. Thirdly, leptin reduced preproinsulin mRNA at 24 h and 20 mM glucose concentration. In conclusion, leptin inhibits insulin synthesis in rat islets directly. Leptin resulted in a time-dependent decrease in preproinsulin mRNA levels. There is a glucose-dependent inhibition of preproinsulin gene expression by leptin.

**EXPRESSION OF THE LONG AND SHORT LEPTIN RECEPTOR ISOFORMS IN MONONUCLEAR CELLS**

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Leptin receptors which include the long isoform, the short isoform and the soluble leptin receptor are present in hypothalamus and many peripheral tissues in human. In this experiment, we would mainly demonstrate the expression levels of the long isoform leptin receptor (OB-R<sub>L</sub>) and the shortest membrane bound variant (OB-R<sub>S</sub>) in mononuclear cells from the obese individuals and the lean individuals. Peripheral blood was obtained from 50 healthy individuals (30 obese individuals, BMI 18.9 – 25 kg/m<sup>2</sup>; 20 lean individuals, BMI 25 – 47.2 kg/m<sup>2</sup>). Mononuclear cells were isolated from peripheral blood with lymphocyte isolated reagent. We report the quantification by reverse transcriptase-polymerase chain reaction (RT-PCR). Leptin levels were measured in the serum from all individuals using a human leptin radioimmunoassay kit. OB-R<sub>L</sub> was expressed in all individuals. OB-R<sub>S</sub> was expressed in 38 individuals, OB-R<sub>L</sub> was not expressed in 12 individuals who were obese with BMI=39kg/m<sup>2</sup>. The predominance of OB-R<sub>S</sub> over OB-R<sub>L</sub> was apparent in all samples and ranged from 4- to 27-fold. There was no significant difference in the expression of either isoform between men and women. The relative expression of both OB-R<sub>L</sub> and OB-R<sub>S</sub> isoforms were significantly lower and the serum leptin levels was significantly higher in the obese subjects (BMI=25 kg/m<sup>2</sup>), compared with the lean subjects (BMI=25 kg/m<sup>2</sup>). In conclusion, both OB-R<sub>L</sub> and OB-R<sub>S</sub> were coexpressed in peripheral blood mononuclear cells from the lean and the medium obese subjects with a consistent predominance of OB-R<sub>S</sub>. This several-fold higher expression of the OB-R<sub>L</sub> over the OB-R<sub>S</sub> leptin receptor splice variant was observed in both men and women, whether the lean or the obese individuals. Compared with the lean individuals, the expression of OB-R<sub>L</sub> and OB-R<sub>S</sub> leptin isoforms appears to be reduced in human mononuclear cells from the obese individuals, with OB-R<sub>L</sub> remaining the predominant leptin receptor isoform. There was an overall significant inverse correlation of both leptin receptor transcripts with the BMI and the serum leptin levels.

**THE EFFECT OF INSULIN ON THE SYNTHETIC AND SECRETION OF LEPTIN IN ADIPOSE CELLS OF RAT**

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Earlier study suggested that insulin play a vital role in increasing expression and secretion of leptin. The location of leptin in adipose cells and whether insulin can directly increase the release from these cells by double-labeling immunofluorescence in vitro were studied in this paper. Adipose cells were extracted and purified from SD rat epididymal fat pads and incubated in vitro in presence or absence of insulin for 0,15,60 min respectively. These cells were incubated with primary polyclonal anti-calanexin and anti-leptin antibodies and then with fluorescence-labeled secondary antibodies in time order. Change of fluorescence staining of these cells and analysis quantity by scanning imaging system was carried out. After 15 min, whether insulin treatment was given or not, fluorescence staining was weaker than that at beginning, but no difference was found between the two groups. However, after 60 min of insulin treatment, the amount of cell-associated leptin was higher than that of non-insulin treated and at 0 time. There was no obvious effect of insulin on acute decreasing of leptin amount in adipose cells in vitro. But we can conclude that insulin increases leptin production if we prolong the observation time, i.e. insulin cannot directly increase leptin release from adipose cells, but can directly increase leptin secreted by stimulating production.

**EXPRESSION AND DISTRIBUTION OF THE FUNCTIONAL LEPTIN RECEPTOR MRNA**

Qiang Li, Zhe Liu, JinChao Zhang, Hong Fu, ShuYun Zhang, Wei Liu

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OF DYSMENORRHOEA alleviate dysmenorrhoeal symptoms by altering uterine quiescence was therefore symptoms, but the mechanism is largely unknown. The possibility that BFP could alleviate dysmenorrhoeal symptoms by altering uterine quiescence was therefore investigated. Results demonstrated that ethanol extract of BFP was able to initiate uterine relaxation following both oxytocin and KCl induced contractions ex-vivo. Attempted inhibition of BFP’s relaxatory response with the use of L-NAM, a non-selective nitric oxide inhibitor, and MDL122330A, an adenylyl cyclase inhibitor, however, had no significant effect, suggesting that most of BFP’s relaxatory response was not due to increases in NO or cAMP. Alterations in extracellular calcium levels however, did alter the ability of BFP to induce uterine relaxation, with high extracellular calcium levels needing higher concentrations of BFP to induce relaxation than normal calcium levels following KCl induced contractions. The parallel and sigmoidal nature of the high extracellular and normal extracellular calcium solutions curves suggested that BFP competes with calcium via inhibition of a receptor system. Indeed, further studies on tetramethylpyrazine (TMP), a major active ingredient of BFP, indicated that TMP could inhibit the Thapsigargin-sensitive SERCA calcium pump, thus preventing increases in intracellular calcium levels. In conclusion, the present study demonstrated BFP could concentration dependently cause relaxation of isolated mouse uteri. The overall mechanism of uterine relaxation of BFP is unlikely to be due to oxytocin antagonism, increase in cAMP or NO with modulation of calcium uptake most likely being responsible.

BAK FOONG PILLS PROMOTE UTERINE RELAXATION BY MODULATING CALCIUM: IMPLICATION IN TREATMENT OF DYSMENORRHOEA

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During menstruation, women who suffer from dysmenorrhoea can experience the same type of low frequency contractions as experienced during labour, thus leading to the high intrauterine pressure, causing extreme pain. Since contractility of the uterus appears to be the major source of pain during dysmenorrhoea, alleviation of the contractions are believed to be a possible treatment strategy. Bak Foong Pills (BFP), a traditional Chinese formulation for use in gynaecological disorders, has long been thought to be effective in the treatment of dysmenorrhoeal symptoms, but the mechanism is largely unknown. The possibility that BFP could alleviate dysmenorrhoeal symptoms by altering uterine quiescence was therefore investigated. Results demonstrated that ethanol extract of BFP was able to initiate uterine relaxation following both oxytocin and KCl induced contractions ex-vivo. Attempted inhibition of BFP’s relaxatory response with the use of L-NAM, a non-selective nitric oxide inhibitor, and MDL122330A, an adenylyl cyclase inhibitor, however, had no significant effect, suggesting that most of BFP’s relaxatory response was not due to increases in NO or cAMP. Alterations in extracellular calcium levels however, did alter the ability of BFP to induce uterine relaxation, with high extracellular calcium levels needing higher concentrations of BFP to induce relaxation than normal calcium levels following KCl induced contractions. The parallel and sigmoidal nature of the high extracellular and normal extracellular calcium solutions curves suggested that BFP competes with calcium via inhibition of a receptor system. Indeed, further studies on tetramethylpyrazine (TMP), a major active ingredient of BFP, indicated that TMP could inhibit the Thapsigargin-sensitive SERCA calcium pump, thus preventing increases in intracellular calcium levels. In conclusion, the present study demonstrated BFP could concentration dependently cause relaxation of isolated mouse uteri. The overall mechanism of uterine relaxation of BFP is unlikely to be due to oxytocin antagonism, increase in cAMP or NO with modulation of calcium uptake most likely being responsible.

BIOLOGIC EFFECTS OF THREE VANADIUM COMPLEXES

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The new vanadium complexes VO(ph-acac)(HB(pz)2)(pz:C6H4N2) (2), VO(O2)2(HB(pz)3)(pz) (3) and VO(HB(pz2Me2)2)(2)SCN (4) with poly-pyrazolylborate ligand were synthesized. To explore if the complexes act as medicine for diabetes, we tested the toxic effect of the vanadium complexes on 3T3-L1 pre-adipocytic cells and rat CBRH-7919 hepatoma by methyl thiazolyl tetrazolium (MTT)-reduction assay. The results indicated the liveability of 3T3-L1 cells was improved in the medium with lower concentration (<10μM) of complexes (1), (4) for 36 h, while decreased obviously in that with higher concentration (>100μM) of them. The liveability of 3T3-L1 cells all reach maximum in the four vanadium complexes of 10μM. With same concentration of the complexes (10μM) for 24h post-treatment, the effect of the new vanadium complexes on 3T3-L1 cells was more serious than that on CBRH-7919 cells, which presented the different response of that (compound 4) > (compound 1) > (compound 2) > (compound 3). It is therefore suggested that 3T3-L1 cells are more sensitive to the complexes with CBRH-7919 cells.