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The 3rd International Conference for Cancer Metabolism and Therapy was successfully held at the South Hospital Conference Center of Shanghai First People's Hospital, nearly 200 international experts from the field of cancer metabolism and therapy and about two thousand local scientists attended the conference. The conference was sponsored by the Yangtze River Delta Group City Hospital Synergistic Development Strategic Alliance, the Chinese Anti-Cancer Association Cancer Metabolism Professional Committee, the Chinese Association for Cancer Metabolism and Therapy under Chinese Medical Doctoral Association-Clinical Precision Medicine, and co-organized by the First People's Hospital Affiliated to Shanghai Jiaotong University, and Shanghai Jiao Tong University School of Basic Medicine Undertake, Translational Medicine Network, Shanghai Anti-Cancer Association Youth Council, Fudan University Affiliated Tumor Hospital, University of California, Los Angeles, Agi Hirshberg Center for Pancreatic Diseases and Hirshberg Foundation for Pancreatic Cancer Research, Dalian University of Technology, New York-Presbyterian, American Cancer Research Association (AACR). The theme of the conference was "Inheritance, Innovation, Excellence, Leading" and its aim is to create a high-end academic exchange platform to discuss new technologies, new methods, and new products in tumor metabolism, tumor immunity, tumor markers and other fields. The conference involves cancer metabolism reprogramming, metabolism and tumor microenvironment, lipid metabolism, non-metabolic function of metabolic enzymes, metabolism and epigenetics, clinical transformation, new technologies for tumor immunotherapy, clinical application of tumor immunotherapy, emerging targeted therapy, new technologies for tumor immunotherapy, clinical transformation, new technologies for tumor immunotherapy, clinical application of tumor immunotherapy, emerging targeted therapy, PD-1/PD-L1 technology, CAR-T technology, novel tumor protein markers, novel tumor methylation markers, ctDNA, CTC, etc. The meeting ended in a lively discussion among scientists from different levels who truly benefit from the sessions about cancer metabolism and treatment. The next meeting is planned to be held October 2 through October 6, 2019 in Los Angeles, Calif. The meeting venue will be announced accordingly in the meeting web site (www.cmt.org).

ABSTRACTS PRESENTED

Targeting Purinergic Receptor P2Y2 Prevents the Growth of Pancreatic Ductal Adenocarcinoma by Inhibiting Cancer Cell Glycolysis

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Objective: Extensive research has reported that the tumor microenvironment components play crucial roles in tumor progress. Thus, blocking the supports of tumor microenvironment is a promising approach to prevent cancer progression. We aimed to determine whether P2RY2 could be a potential therapy target in PDAC.

Methods: Expression of P2R Y2 were determined in 264 pancreatic ductal adenocarcinoma (PDAC) patient samples, and correlated to survival. P2R Y2 were inhibited in human PDAC cell lines by antagonist and shRNA, respectively, and cell viability, clonogenicity and glycolysis were determined. RNA sequencing of PDAC cell line was applied to reveal underlying molecular mechanisms. Multiple PDAC mouse models were used to assess the effect of the P2R Y2 inhibition on PDAC progression.

Results: P2R Y2 was upregulated and associated with poor prognosis in PDAC. Activated P2R Y2 by increased extracellular ATP in tumor microenvironment promoted glycolysis and growth of PDAC. Further studies showed that the agonist-activated P2R Y2 crosstalked with PDGFR mediated by Yes1, triggering P3K/AKT-mTOR signaling that resulted in elevating expression of c-Myc and HIF1, which subsequently enhanced cancer cell glycolysis. Genetic and pharmacological inhibition of P2R Y2 impaired tumor cell growth in vivo as well as delayed tumor progression in inflammation-driven PDAC model. Additionally, synergy was observed when AR-C118925XX, the selective antagonist of P2R Y2 receptor, and gemcitabine combined, resulting in prolonged the survival of xenografted PDAC mice.

Conclusions: These findings revealed the roles of the P2R Y2 in PDAC metabolic reprogramming, suggesting that P2R Y2 may be a potential metabolic therapeutic target for PDAC.

Interrelationship of Pancreatic Cancer With Diabetes, Pancreatitis and Obesity

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Pancreatic cancer (PC) is a challenging malignancy with increasing incidence and high mortality rate. It is projected that by 2030, it will be the second leading cause of US cancer mortality. Pancreatic cancer has a complex relationship with
diabetes, pancreatitis, and obesity. The mechanism is partially investigated and their contribution to pancreatic carcinogenesis are not fully understood. Diabetes can be both a risk factor and early manifestation of PC, while obesity and pancreatitis are linked to increased risk of PC. The research gaps and opportunities have been reviewed in the recent National Institutes of Health (NIH) conference to guide the investigators and funding agencies. Areas reviewed include the role of altered energy metabolism in PC risk, evidence that PC can be caused by diabetes, obesity as a cause and risk factor for PC and inflammation and immune system dysfunction as a critical mechanism to PC development, progression, and therapeutic resistance. These research gaps and opportunities were summarized in the conference report (Pancreas. 2018;47:516–525). To address these research gaps and to foster multidisciplinary collaborations to better diagnose and characterize this interrelationship, the NIH launched the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer (CPDPC). The goal was to gain insight into the pathophysiology and relationship between these pancreatic diseases and to develop better methods for diagnosis, prevention, monitoring, early detection and therapy. The CPDPC will conduct studies on chronic pancreatitis in children and adults and factors that increase the risk of PC with chronic pancreatitis, T2DM and in patients who are newly diagnosed with diabetes (Pancreas. 2018;47:1208–1248).

Osteoblast-Derived PKD1 Promotes the Dormancy of Prostate Cancer Cells in the Tumor Microenvironment

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Objectives: Disseminated tumor cells (DTCs) remain in the distant organs of prostate cancer patients for a prolonged period of time, therefore they are resistant to chemotherapy targeting tumor cells dormancy division. Protein kinase D1 (PKD1) belongs to a family of serine/threonine protein kinases that play a key role in bone formation. How osteoblast regulates tumor cells dormancy remains unclear. Here we show that osteoblast-derived PKD1 contributes to the prostate cancer cells dormancy in the tumor microenvironment. Overexpression of PKD1 via lentivirus infection in pre-osteoblast cell line, MC3T3-E1 and primary osteoblast dramatically reduced the proliferation or enhanced the dormancy of co-cultured prostate cancer cells, manifested by downregulation of CDK1, PCNA, and Ki-67 in PCa cells, while upregulation of p38 phosphorylation and p21 expression. In contrast, the dormancy of prostate cancer cells was significantly reduced by osteoblast-derived PKD1 depletion during the co-culture. Interestingly, expression of dormancy related genes such as GAS6, TGF-β2, and BMP7 in osteoblast were upregulated by osteoblast-derived PKD1, while specific blockade of dormancy related proteins in osteoblast limited the ability of the osteoblasts to induce the dormancy of prostate cancer cells.

Conclusions: These findings suggest that osteoblast-derived PKD1 may play a significant role in resistance to chemotherapy through induction of prostate cancer cells dormancy in the bone marrow of tumor microenvironment.

SOCS3 Interacted With ACADVL Promotes Hepatocellular Carcinoma Metastasis

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Objectives: Suppressors of cytokine signaling 3 (SOCS3) plays crucial roles in JAK-STAT signaling pathway, signaling for negative feedback to STATs (Signal Transducer and Activator of Transcription), and is found to be inversely correlated with STAT3 expression. SOCS3 has been observed in certain human cancers, and the protein expression of SOCS3 relatively reduces in cancer tissues. However, SOCS3 expression in HCC (Hepatocellular Carcinoma) is paradox. Methods: Cellular energy metabolism was detected by ATP assay. The mRNA expression levels were investigated by real-time PCR analysis. The expression of proteins was investigated by western blotting, immunofluorescence staining and immunohistochemical staining. We also stably knocked down the SOCS3 protein using siRNA system.

Results: Our results showed that in HCC tumor tissues, SOCS3 showed an increased expression compared with that in the adjacent non-tumor tissues, and also was high-expression in HCC/LM3 cell. Then, we found that SOCS3 could interact with ACADVL, a mitochondrial protein, which catalyzes the first step of mitochondrial beta-oxidation for long-chain fatty acids. We extracted the mitochondria and found that SOCS3 decreased in the cell with knocking down TOM70, which means SOCS3 maybe enter the mitochondria by TOM70. After knocking down SOCS3, the STAT3 and ATP did not increase a lot, respectively. Conclusions: SOCS3 expressed in HCC tissues is different from other researches. The reason is that the hypermethylation of SOCS3 gene is found in only 30% of hepatocellular carcinoma (HCC) tissues, but the methylation-directed silencing of SOCS3 gene promotes cell growth and migration in human HCC. SOCS3 can interact with ACADVL, but the mechanism is still not elaborated.

Identification of Prognostic Biomarkers Regulated by the KEAP1-NRF2-CUL3 Axis in TCGA-Head and Neck Squamous Cell Cancer (HNSCC)

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Objectives: NRF2 is the key regulator of oxidative stress in normal cells and aberrant expression of the NRF2 pathway due to genetic alterations in the KEAP1 (Kelch-like ECH-associated protein 1)-NRF2 (nuclear factor erythroid 2-like 2)-CUL3 (cullin 3) axis leads to tumorigenesis and drug resistance in many cancers including HNSCC. The main goal of this study was to identify specific genes regulated by the KEAP1-NRF2-CUL3 axis in HNSCC patients, to assess the prognostic value of this gene signature in different cohorts, and to reveal potential biomarkers.

Methods: RNA-Seq V2 level 3 data from 279 tumor samples along with 37 adjacent normal samples from patients enrolled in the Cancer Genome Atlas (TCGA)-HNSCC study were used to identify upregulated genes using two methods (altered KEAP1-NRF2-CUL3 versus normal, and altered KEAP1-NRF2-CUL3 versus wild-type). We then used a new approach to identify the combined gene signature by integrating both datasets and subsequently tested this signature in 4 independent HNSCC datasets to assess its prognostic value. In addition, functional annotation using the DAVID v6.8 database and protein-protein interaction (PPI) analysis were performed to further characterize the expression.

Results: A signature composed of a subset of 17 genes regulated by the KEAP1-NRF2-CUL3 axis was identified by overlapping both the upregulated genes of altered versus normal (251 genes) and altered versus wild-type (25
genes) datasets. We showed that increased expression was significantly associated with poor survival in 4 independent HNSCC datasets, including the TGCA-HNSCC dataset. Furthermore, GO, KEGG, and PPI analysis revealed that most of the genes in this signature are associated with drug metabolism and glutathione metabolic pathways.

Conclusions: Altogether, our study emphasizes the discovery of a gene signature regulated by the KEAP1-NRF2-CTL3axis which is strongly associated with tumorigenesis and drug resistance in HNSCC. This 17-gene signature provides potential biomarkers and therapeutic targets for HNSCC cases in which the NRF2 pathway is activated.

The Effect of IRX6 Hypermethylation in the Pathogenetic of Pancreatic Cancer

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Objectives: Pancreatic cancer is a kind of highly malignant tumor. In-depth study of the molecular mechanism and identifying novel therapeutic targets are the preconditions for pancreatic cancer treatment. The inactivation of gene expression caused by aberrant methylation of tumor suppressor gene is an important cause of promoting tumor development. Therefore, DNA methylated genes is promising to serve as a new target for the treatment of pancreatic cancer.

Methods: We predicted genes with abnormally high methylation and low expression in pancreatic cancer by bioinformatics analysis. Of 13 promising genes, we focused our studies on IRX6, a tumor suppressor gene in pancreatic cancer, for further downstream analysis, including methylation and expression. In order to further study the tumor suppressing effect of IRX6 in pancreatic cancer cells, we constructed IRX6 overexpression and knock-down cell lines through lentivirus expression vectors.

Results: We identified that IRX6 is significantly down-expressed and methylated in pancreatic tumor tissues compared to normal tissues in tissue arrays (P < 0.05). Through the further analysis, the low expression of IRX6 caused by the DNA methylation was proved to be negatively correlated with pancreatic tumor grade (Rs = -0.229, P = 0.042). Functional assays of IRX6 gene in pancreatic cancer cells indicated overexpression with IRX6 full-length gene induced significantly reduction in cell proliferation and colony formation rate, as well as the promoting effect on the cell migration, invasion and sensitivity to Gemcitabine (P < 0.05). Through the detection of EMT-related markers, we found that reducing the expression of IRX6 promoted the EMT process.

Conclusions: IRX6 may have tumor-suppressive effects in human pancreatic cancer and is promising to serve as a new target for the treatment of pancreatic cancer.

A Novel Detection Tool Being Used for miRNA Detection in Human Serum

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Objectives: Pancreatic cancer (PC) is one of the most malignant cancers with poor prognosis and high mortality. Therefore, the early diagnosis a crucial in the prevention and treatment of PC. Detection of PC in clinic traditionally relied on medical history, physical examination, pathological morphology, and imaging diagnosis such as CT, etc., which demonstrated less sensitive approaches be used clinically. Considering the specific expression of miRNA in PC at different stages suggested miRNAs being developed as a novel diagnostic biomarker. In this study, a novel technique of multiplex reverse transcriptase quantitative polymerase chain reaction (mRT-qPCR) was developed for detection of multiple miRNAs in a reaction in human serum.

Methods: RNA extraction from serum of subjects was performed by using phenol extraction method. Unique primers and probes were designed by using Beacon. Optimization of the mRT-qPCR was performed in various specimen.

Results: With different optimization of the probes and experimental conditions, a miRT-qPCR assay was developed successfully, which can detect simultaneously four different genes in a reaction including U6, miR159, cel-39 and a specific miRNA, including miR220, miR30, miR24a, miR23b, and miR132a. The mRT-qPCR assay was assessed based on the following parameters including accuracy, sensitivity, specificity and repetitability of detection in clinical serum samples. Currently, more than 80 clinical samples have been tested by the mRT-qPCR assay. As compared to a classical single RT-qPCR, the newly developed mRT-qPCR is superior to the signal RT-PCR in detection of miRNA in serum with an improved CV (<5%), and lower FDR (<1%), and high accuracy (100% coincidence to a reference lab).

Conclusions: The newly developed miRT-qPCR is proved to be an effective way for detection of multiple genes in a reaction for analysis of miRNA in human serum.

Characterization of Hollow Hematite Sub-micron Spheres Prepared by Sol-gel

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Objectives: In this work, we report the preparation by sol-gel and the characterization of hollow sub-micron hematite spheres (α-Fe2O3).

Methods: For the preparation, ferric nitrate and citric acid were diluted in water and agitated to form the gel precursor without the use of templates. The oxidation was performed by annealing at different temperatures up to 600°C. The characterization was performed by X-ray diffraction (XRD) and Small angle X-ray scattering (SAXS) which revealed the phase evolution from magnetite to pure hematite. This is confirmed by thermo gravimetric analysis.

Results: Scanning electron microscope images shows the formation of the hollow spheres, with ~800 nm external diameter and ~60 nm shell thickness suggest they are promising for encapsulation applications. The Morin transition, which is a typical property on the magnetism of hematite, is studied after annealing the samples. The change of strong ferromagnetism to weak ferromagnetism is observed indicating pure hematite.

Co-regulation of STAT3 mRNA Localization and Translation by TDP-43 and Fmrp Promotes Hepatocellular Carcinoma Metastasis

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Objectives: Transactive response DNA-binding protein 43Kd (TDP-43) is a ubiquitously expressed RNA-binding protein required for early development, and it has been implicated in multiple cellular processes including cell cycle progression, apoptosis, RNA processing, alternative splicing, etc. Due to its central role in neurodegenerative disease pathogenesis, most research recently has focused on its role associated with neurodegeneration disease, however, how it affects cancer progression is still unknown.

Methods: We used RNA IP assay and FISH combined with IF to verify the relationship between TDP-43 and STAT3 mRNA. We also stably knocked down the TDP-43 protein using siRNA system, then, RNA extraction, RT-qPCR array, FISH, IF, western blotting were conduct to test the expression and localization of Stat3. Using co-immunoprecipitation, RTCA, transwell, immunohistochemical analysis and establishing a in situ liver cancer model to find the role of TDP-43 in HCCLM3 cells and human liver cancer tissue.

Results: Our results showed that TDP-43 was interacted with STAT3 mRNA and Fmrp protein in HCCLM3 cell. Meanwhile, TDP-43 can interact with many different translocation complexes, such as elf4G and elf4E. By silencing TDP-43, we saw the most of STAT3 mRNA molecules of HCCLM3 cell were located in the cytoplasm and the Stat3 expression was increased, we also found that significant inhibition of cell invasion and metastasis in HCCLM3 cells. After knocking down TDP-43, the levels of metastasis in the lungs of nude-mouse are decreased. Furthermore, our results have shown that the high expression level of TDP-43 was expressed in hepatocellular carcinoma tissue than para-carcinoma tissue.

Conclusions: In this study, we identified STAT3 mRNA as a new TDP-43 binding target which involved STAT3 mRNA translation through interacting with Fmrp. Knocking down TDP-43 decrease the HCCLM3 metastasis, and the TDP-43 protein level is elevated in hepatocellular carcinoma tissue, suggesting that TDP-43 may affect the hepatocellular carcinoma metastasis through interacting with Fmrp to regulate expression of Stat3.
Lappaconitine Sulfate Suppresses Proliferation of Human Cancer Cells by Regulating the PI3K/Akt Signaling Pathway

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Objectives: Lappaconitine (LA), an important diterpenoid alkaloid, can be isolated from many plants of aconitum species such as Aconitum sinomontanum Nakai. Since lappaconitine was extracted, it has exhibited a wide range of biological activities. Lappaconitine hydrobromide (LH) is a major drug widely used in clinical treatment for treatment of neuropathic pain as a non-addictive drug in China and as an antiarrhythmic in Uzbekistan. Recently, some researches have showed it has anti-tumor cancer effect. However, the potential molecular mechanisms are unclear.

Methods: In this study, we investigated the molecular mechanisms of lappaconitine sulfate (LS) against HeLa cells.

Results: Our results suggested that LS significantly suppressed the proliferation of HeLa cells in a dose-dependent manner. Specifically, LS inhibited mitotic spindle formation through disruption of microtubule assembly and induced G1 phase cell arrest through down-regulation of the cyclin D1 level. LS also induced cell apoptosis by decreasing MDM2 expression and the ratio of Bcl-2/Bax. Additionally, LS led to constitutive downregulation of the PI3K/Akt signaling pathway and decreased the protein levels of its downstream factors, including p53, p21, p65, and caspases.

Conclusions: Collectively, the findings of our studies are that LS exhibits antiproliferation, cell cycle arrest and apoptosis-inducing effects by regulating the PI3K/Akt signaling pathway in human cervical cancer cells.

Expression of Collagen Triple Helix Repeat Containing-1 (CTHRC1) in Prostate Cancer and Correlation With Clinical Parameters

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Objectives: To investigate the expression and clinical significance of CTHRC1 gene in prostate cancer.

Methods: CTHRC1 gene expression in PCa was analyzed by gene chip, GEO database, and oncomine database. The Cancer Genome Atlas (TCGA) was analyzed by chipobtah to evaluate the prognostic role of CTHRC1 for overall survival (OS), disease-free survival (DFS). Correlations between CTHRC1 expression and clinical characteristics were assessed in 119 patient samples. To investigate CTHRC1 expression traits in PCa, we comparatively analyzed the CTHRC1 protein and mRNA profiles in PCa cell lines and primary human normal prostate epithelial cells (HPEpiC).

Results: The expression levels of CTHRC1 were significantly up-regulated in prostate cancer tissues in two gene chip data and five oncomine datasets. In analyses of gene expression of human PCa tumor samples deposited in TCGA databases, up-regulation of CTHRC1 in human PCa patients correlated significantly with lower overall survival (OS) and disease-free survival (DFS). Immunohistochemical results showed that the positive expression rate of CTHRC1 in high-grade prostate cancer and low-grade prostate cancer was significantly higher than that in prostate intraepithelial neoplasia (PIN) and benign prostatic hyperplasia (BPH) (P<0.05). Clinical data analysis showed that there were a significant correlation between the expression intensity of CTHRC1 and the total PSA level, TNM stage, and Gleason score. ROC curve shown that as a diagnostic factor between PCa and BPH, CTHRC1 has a good positive value (P<0.01). However, as a diagnostic factor between PCa and PIN, there was no significant difference (P>0.05). In addition, CTHRC1 combined with PSA can improve the ability to detect PCa (P<0.01). The data showed that the CTHRC1 protein and mRNA was significantly up-regulated in PCa cell lines compared with HPEpiC, indicating that CTHRC1 may play an important role in prostate cancer development and progression.

Conclusions: The expression of CTHRC1 in prostate cancer is related to the degree of malignancy and prognosis of prostate cancer, which is expected to be a new diagnostic marker and therapeutic for prostate cancer.

Mechanistic Study of OLA1-Induced Oral Cancer Metastasis

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Objectives: Oral cancer metastasis is a life threat phenomenon worldwide. An effective targeted therapy is particularly important for patients with oral cancer. Our recent study suggested that OLA1 may be an effective drug target for therapy of oral cancer. This study further addressed the molecular mechanism of OLA1 in oral cancer metastasis.

Methods: Western blot was used to detect differences in OLA1 and its targeting proteins in oral cells, and qPCR was used to detect differences in RNA levels of individual proteins. Wound assay, Transwell assay, and Flow cytometric assays were used to study the function of the OLA1 in oral cancer metastasis. Immunoprecipitation and Mass spectrometry were used to determine the mechanism by which OLA1 is involved.

Results: The endogenous level of the OLA1 in oral cancer cell lines was significantly lower than that in normal oral cells, and that in high metastatic cell line UM-1 was lower than that in the carcinoma cell line UM-2. Elevated expression of OLA1 resulted in a reduced ability of metastasis in UM-1, and the weakened resistance to paclitaxel. Knocked down OLA1 in UM-2 enhanced cell migratory ability and differentiated expression of EMT markers, suggesting that OLA1 may regulate the metastatic ability of oral cancer through the EMT pathway.

Conclusions: The above data suggest that OLA1 may be a potential target for the treatment of oral cancer and play an important role in the prognosis of oral cancer.

SIRT1/MRPS5 Axis Enhances the Metabolic Flexibility of Liver Cancer Stem Cells

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Objectives: Metabolic reprogramming endows cancer cells the ability to adjust metabolic pathway to support heterogeneous biological processes. Previous studies focus on metabolic transformation accompanied malignant degeneration of normal cells or tumor metastasis. However, the underlying regulator of the metabolic reprogramming during cancer stem cells (CSCs) differentiation remains unclear.

Methods: Here we show that liver CSCs transform mitochondrial-dependent energy supply to Warburg phenotype accompanied by the dual function of mitochondrial ribosome protein 5 (MRPS5).

Results: Deacetylated MRPS5 locates in mitochondria to enhance the function of Complex-I, which increases the generation of NAD+ to enhance the oxidative phosphorylation (OXPHOS) and activate the UPRmt to treat the negative byproduct ROS, processes involved in maintenance of cancer stem cells. Conversely, acetylated MRPS5 gather in nuclei to increase the expression of glycolytic proteins and promote the Warburg Effect. The acetylation status of MRPS5 is directly regulated by deacetylase SIRT1, which is abundant in liver CSCs and decreases during differentiation.

Conclusions: Thus SIRT1/MRPS5 axis participates in metabolic reprogramming to facilitate tumor progression and may become a promising cancer therapeutic target.

Low-Dose Microcystin-LR Antagonizes AFB1 Induced Hepatocarcinogenesis With Decrease of Premalignant Lesion and DNA Adducts Decrease

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Objectives: Aflatoxin B1 (AFB1) and microcystin-LR (MC-LR) are hepatotropic pollutants universally found especially in developing countries including China. People are exposed to those two biotoxins directly through drinking water and indirectly through food chain. Many lab experiments have proved that AFB1 and MC-LR co-exposure at different ratios, detected their carcinogenic effects in SD rats using liver histopathological analysis, examined their tumorigenic effects in cells using CCK-8, cloning formation and animal xenograft models, and evaluated the possible mechanism by measuring liver tissues AFB1-DNA adduct formation.

Results: Our results revealed that co-exposure to AFB1 and MC-LR significantly decreased the carcinogenic effect compared with AFB1 alone as observed in the SD rat liver pathological H&E staining and transmission electron microscope, and measured by the levels of serum liver function indexes (ALT, AST, GGT) and hepatocarcinoma clinical biomarkers (AFP, and area of GSTPositive foci). Meanwhile, AFB1 and MC-LR in combination significantly reduced the SD rat liver pathological H&E staining.

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Roles of the Specific circRNAs as Novel Biomarkers for Prostate Cancer

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Objectives: To determine whether the differentially expressed circRNAs in prostate cancer can serve as novel biomarkers for prostate cancer diagnosis and make clear the roles of the specific circRNAs as novel biomarkers for prostate cancer during the period of early diagnosis, progression and prognosis.

Methods: A total of 173 tissue samples from the patients including 78 cases of BPH tissues and 95 cases of prostate tumor tissues were collected. We screened differentially expressed circRNAs in the SBC-eRNA (4*180K) array using 4 pairs of paracancerous and prostate tumor tissues. The function pathways of differentially expressed circRNAs was analyzed by GO and KEGG for the host gene of circRNAs. The relative expression of circRNAs was detected by using Reverse Transcription and quantitative Real-time Quantitative Polysensm Chain Reaction (RT-qPCR), and the correlation between circRNAs expression and clinicopathological features was analyzed. To predict the target miRNAs of differentially expressed circRNAs and its relevant mRNAs, we also analyzed the coexpression networks of prostate cancer according to the pattern of circRNA-miRNA-mRNA using Arraystar's homemade miRNA software, IPA, and Cytoscape 3.5.1 software.

Results: We demonstrated that 1021 differentially expressed circRNAs, of which 904 circRNAs were repressed significantly and 117 circRNAs were up-regulated. Pathway analysis showed that the host genes of differentially expressed circRNAs were mainly involved in cell adhesion, gonadotropin response, regulation of blood platelet degranulation, vitamin metabolism and amino acid metabolism. RT-qPCR analysis also confirmed the expression of hsa_circ_0062019, hsa_circ_0057558 and SLC19A1 in prostate cancer tissues were significantly up-regulated (P < 0.01). Further, hsa_circ_0057558 was found to be positively correlated with total cholesterol and triglyceride level, and significantly correlated with triglyceride level. The ROC curve analysis showed that the area under the ROC curve of hsa_circ_0057558 and hsa_circ_0062019 were 0.729 and 0.828, respectively. The result of ROC curve analysis for the combination of PSA level and two differentially expressed circRNAs showed that AUC, sensitivity and specificity were significantly increased, which were 0.938, 84.5%, and 90.9%, respectively.

Conclusions: Our results found that differentially expressed circRNAs (hsa_circ_0062019 and hsa_circ_0057558) can be used as potential tumor markers for PCa. The depth analysis of the regulation network of the differential circRNAs in PCa will provide a direction for further understanding the mechanism and clinical diagnosis and evaluation to differential circRNAs in prostate cancer. The circRNA-miRNA-mRNA network showed that hsa_circ_0034467 and hsa_circ_0060252 regulated hsa_circ_0062019 and hsa_circ_0057558 regulated miR_6084, hsa_circ_0060252 and hsa_circ_0062019 regulated miR_5008. The host gene of SLC19A1 of hsa_circ_0060219 is also involved in the regulatory network, and the specific regulation mode is hsa_circ_0060219 - mir_5008-5p-SLC19A1, hsa_circ_0060252-mir_5008-5p-SLC19A1 and hsa_circ_0034467 - miR_6721-5p - SLC19A1.

Lappaconitine Sulphate (LS) Down-regulated PI3K/Akt/GSK3β Pathway and Induced Apoptosis in Human Colon Cancer Cells

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Objective: This study intended to investigate the role of lappaconitine sulfate (LS) in proliferation of human colon cancer HT-29 cells and to explore the potential molecular mechanism.

Methods: Cell proliferation was detected by CCK-8 assay and EdU proliferation assay. Cell morphological change was expressed by Hoechst 33258 staining. Expression of apoptosis related proteins were detected by Western blotting. In addition, the effect of LS on cell cycle was detected by flow cytometry.

Results: LS exhibited anti-proliferative activity and induced apoptosis in HT-29 cells in a dose-dependent manner. LS induced expression of p53, p65, and Bax, cleaved PARP, cleaved-caspase-3/7/9, and inhibited Bcl-2 expression. LS also affected cyclin D1 and p21 expression, inducing cell cycle arrest in the G0/G1 phase.

Conclusions: Our findings demonstrate that LS induced cell apoptosis, arrested cell cycle in G0/G1 phase, and suppressed the PI3K/Akt/GSK3β signaling pathway of HT-29 cells.
overexpression is been suppressed. In vivo, overexpression of TSPAN8 promotes tumor metastasis. If EPSPS3 is knockdown, tumor metastasis is been suppressed. **Conclusions:** These findings uncover a previously uncharacterized mechanism underlying TSPAN8 enter into nucleus and EPSPS3 regulation by TSPAN8 for remodeling of cytoskeleton and promote cell migration.

**GABRP Regulates Chemokine Signaling, Macrophage Recruitment, and Tumor Progression in Pancreatic Cancer Through Tuning KCNN4-Mediated Ca2+ Signaling in a GABA-Independent Manner**

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**Objectives:** Neurotransmitter system has a fate-determining role for tumor progression and clinical outcome, especially for pancreatic ductal adenocarcinoma (PDAC). Here, we aimed to uncover neurotransmitter-initiated signaling pathway in PDAC cells.

**Methods:** The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) datasets were used to identify differentially expressed neurotransmitter receptors. The expression pattern of Gamma-aminobutyric acid type A receptor pi subunit (GABRP) in human and mouse PDAC tissues and cells was studied by immunohistochemistry and western blotting. The in vivo implications of GABRP in PDAC were tested by subcutaneous xenograft model, lung metastasis model, and KrasG12D/+/Trp53R172H/+Pdcox1-Cre (KPC) mice. Bioinformatics analysis, transwell experiment, and orthotopic xenograft model were used to identify the in vitro and in vivo effects of GABRP on macrophages in PDAC. The enzyme-linked immunosorbent assay, Co-immunoprecipitation, proximity ligation assay, electrophysiology, promoter hiocerase activity, and quantitative real-time PCR analyses were used to identify molecular mechanism.

**Results:** GABRP expression was remarkably increased in PDAC tissues and associated with poor prognosis, contributed to tumor growth and metastasis. GABRP was correlated with macrophage infiltration in PDAC and pharmacological deletion of macrophages largely abrogated the oncogenic functions of GABRP in PDAC. Mechanistically, GABRP interacted with KCNN4 to induce Ca2+ entry, which leads to activation of NF-κB signaling and ultimately facilitates macrophages infiltration by inducing CXCL5 and CCL20 expression. Genetically inhibition of GABRP or KCNN4 suppressed macrophage infiltration and slowed growth in KPC mice.

**Conclusions:** Overexpressed GABRP exhibits an immunomodulatory role in PDAC in a neurotransmitter-independent manner. Targeting GABRP or its interaction partner KCNN4 may be an effective therapeutic strategy for PDAC.

**MicroRNA-34a Enhances Drug Sensitivity by Regulating Cell Stemness**

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**Objectives:** Pancreatic cancer is a life-threatening disease worldwide due to high death rate. Drug resistance challenges the effective therapy of pancreatic cancer. The exact molecular mechanism underlying drug resistance is still unknown.

**Methods:** Two cell lines including PANC-1 (less sensitive to gemcitabine) and SW1990 (most sensitive to gemcitabine) were used in this study. The sensitivity of different pancreatic cancer cell lines to gemcitabine with and without miR-34a treatment was screened by MTT assay. Stem cell surface markers CD44 and CD133 were analyzed by using qPCR and flow cytometry. Cell assays for clony formation, migration, invasion were analyzed. The EMT pathway markers and notch1 were also studied by qPCR.

**Results:** The expression of miR-34a in SW1990 was significantly higher than in PANC-1. Overexpression of miR-34a in those cell lines enhanced significantly the sensitivity of pancreatic cancer cells to gemcitabine. Results from qPCR and flow cytometry showed that the stemness of pancreatic cancer cells was significantly reduced in cells with overexpressed miR-34a. In cell lines with the overexpressed miR-34a, gemcitabine increased inhibitory effects on pancreatic cancer cells. Overexpressed miR-34a decreased expression of the cell surface markers CD44+/CD133+, and the ability of clony formation, cell migration and invasiveness, as well as the reduced expression of the EMT markers. Target analysis further showed that notch1 is targeted by miR-34a.

**Conclusions:** miR-34a may affects pancreatic cancer drug-resistance by regulating cell stemness through EMT pathway.
Conclusions: Our data showed that CTAB may overcome drug resistance of breast cancer through the AMPK-HIF-1α-P-gp pathway. Our study sheds a light on development of a novel drug lead reversing drug-resistance.

CD44-α6β4 Complex Promotes Pancreatic Cancer and Metastasis

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Objectives: Pancreatic cancer is the fourth most lethal malignancy in the world. It is difficult to be detected in early phase and has remote metastatic spread. The mechanisms regulating pancreatic cancer metastasis are so far poorly understood. CD44 is a transmembrane glycoprotein expressed on a subset of pancreatic cancer cells, regulating cell migration, adhesion and many other cellular mechanisms. Integrin is a family of transmembrane receptors related to cell-extracellular matrix adhesion, intracellular skeleton and so on, which plays a role in cell signaling transduction of cell cycle, α6β4, as a subunit of integrin, was found a receptor of CD44. Thereby, our study was to answer the question whether the CD44-α6β4 complex may lead the activation of EMT associated downstream genes so as to promote the pancreatic cancer development and metastasis.

Methods: Based on the data in TCGA database, the CD44 expression level between normal population and pancreatic carcinoma patients and the survival curve of patients with high or low/medium CD44 expression levels were explored, obviously showing that patients with high CD44 expression levels had poor prognosis.

Results: The expression of CD44 in pancreatic cancer was positively correlated with α6β4 according to the result of western blot of blood serum samples. Outcomes from databases and clinical samples suggest that CD44 might promote tumor metastasis via contacting with α6β4. These findings showed that CD44 was found to be an independent predictor of prognosis in tumor and survival analysis. EMT is an essential cellular process in carcinoma pathogenesis. It plays an important role in forming highly malignant tumors which have evident motility, invasiveness and higher cellular mechanisms. Integrin is a family of transmembrane receptors related to cell-extracellular matrix adhesion, intracellular skeleton and so on, which plays a role in cell signaling transduction of cell cycle, α6β4, as a subunit of integrin, was found a receptor of CD44. Thereby, our study was to answer the question whether the CD44-α6β4 complex may lead the activation of EMT associated downstream genes so as to promote the pancreatic cancer development and metastasis.

Conclusions: Our data showed that CTAB may overcome drug resistance of breast cancer through the AMPK-HIF-1α-P-gp pathway. Our study sheds a light on development of a novel drug lead reversing drug-resistance.
Objectives: Starfish extract was previously evaluated by our research group for its wound restoration performance. Due to its complex structure of star fish tissue regeneration capacities, attempts have been made to analyze its bioactive components from amputated tissue extracts for anticancer and antioxidant efficacy. Our prior studies have shown the significant effects of starfish extract in regenerating damaged tissue in zebra fish. However, the active fractions and unique cellular mechanisms of starfish regenerated tissues extracts have yet to be elucidated. The goal of this study was to examine the anti-proliferative and antioxidant effects of bioactive compounds from *L. maculata* and dissect its mechanism of action.

Methods: Cytotoxicity on human carcinoma KB cells was measured by MTT assay. To gain more insight of the mode of anti-proliferative impact of starfish extracts, we quantified intracellular ROS levels by DCFH-DA, mitochondria membrane ability alterations by Rh-123 staining, oxidative DNA damage through comet assay and apoptotic morphological changes through AO/EtBr dual staining technique. The pharmacologically active components have been characterized by HPLC, GC-MS/MS, and FTIR.

Results: Our key findings showed that the bioactive fraction acquired from HPLC induced apoptosis, enhanced ROS levels, altered the mitochondria membrane potential and increased the oxidative DNA damage in KB cells. The up-regulation of Bax/Caspase 3 protein expression was negatively correlated with the expression of Bcl-2 protein and the proliferation of cyclin D1 associated markers in active components treated KB cells. Furthermore, 35 fractions with predominant anticancer compounds have been determined as anticancer compounds by GC-MS, including 5α-Cholest-7-en-3β-ol, Hexadecanoic acid, Myristic acid, Palmitic acid, and 9,12-Octadecadienoic acid, which could be responsible for the anti-proliferative impact of KB cells.

Conclusions: Our data suggest that bioactive compounds from regenerated tissues of *L. maculata* starfish extracts exhibits potent anticancer effect in KB cells, which could be promising assets of anticancer, antioxidant and wound healing agents in biomedicine.

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Detection of HER2 Amplification Using Multiple Reference Genes by 3-color Digital PCR

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Approximately 20% of current HER2 testing may be inaccurate, partly because of polymersy 17 and centromeric amplification. Here three reference genes were assessed using 3-color digital PCR to evaluate HER2 amplification in FFPE samples. The concordance rate of HER2 amplification examined in FFPE samples with HER2/CEP17 dPCR assay and IHC/FISH was 84% (3 out of 19), and those with HER2/3-ef and IHC/FISH was 94.7% (1 out of 19). It demonstrated that this dPCR method was as effective as IHC/FISH and therefore might present an effective way to determine the true HER2 amplification status for guiding HER2-targeted therapy.

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A Next Generation Sequencing Panel (CNSeq) for Molecular Classification of Central Nervous System Tumors

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Objectives: The molecular classification of CNS tumors is important for clinical management. Methods: A targeted panel (CNSeq) covering 348 amplicons for SNVs, MGMT methylation and 1p19q LOH derived from 13 genes frequently aberrant in different glioma types were designed. Results: Performance of CNSeq assay were evaluated in 43 CNS tumors, and CNSeq assay correctly identified 43/43 (100%) SNVs and methylation variation and 42/43 (97.7%) 1p19q LOH known to be present by conventional techniques, including IDH1, IDH2, BRCA1/2, 1p19q LOH and MGMT methylation.

Conclusions: Our targeted panel CNSeq can be used as a powerful tool for the molecular classification of CNS tumors.

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Up-regulation of OLA1 Promotes the Stemness and EMT Phenotypes in Chemoresistant Pancreatic Cancer Cells

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Objectives: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignant disease with a 5-year survival rate less than 5% mostly because of the drug resistance. Cancer stem cells (CSCs) and epithelial–mesenchymal transition (EMT)-type cells are considered as potential causes of chemoresistance, tumor recurrence and metastasis in pancreatic cancer. Ong like AtPase 1 (OLA1) is a newly cloned member of the Ong family P-loop GTPases, which is overexpression in many malignant tumors. In this study the potential role of OLA1 in the acquired drug resistance of pancreatic tumor was investigated.

Methods: Methyl thiazolyl tetrazolium (MTT) assay, qRT-PCR and Western blot (WB) analysis were used to verify the relationship between OLA1 and chemoresistance. Flow cytometric assay (FCA), tumor sphere formation, clonogenicity, qRT-PCR and WB assay were used to detect the stemness and Epithelial–Mesenchymal transition (EMT) marker in the OLA1 regulated cell lines.

Results: In the present study, we focused on investigating how OLA1 involved in the chemoresistance of pancreatic cancer. We found that OLA1 overexpression in the chemoresistant patients indicated OLA1 induced chemoresistance through EMT pathway. Down-regulated OLA1 improved the sensitivity of Gemcitabine and reduced stem cell markers (CD44+, CD133+, c-Met), and EMT (snail + slug, E-cadherin) process in pancreatic cancer cells. These phenomena indicate that OLA1 confers the chemoresistance of PDAC and may serve as a potential target for therapy of pancreatic cancer.

Conclusions: Results observed-above suggest that OLA1 was associated with Gemm resistance of pancreatic cancer. Exact molecular mechanism underlying OLA1 regulation in pancreatic cancer resistance is still under investigation.

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Mitochondrial Matrix Peptidase ClpP Regulates ROS-mediated MAPK Activation to Promote Esophageal Squamous Carcinoma Tumorigenesis

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Objectives: Esophageal squamous carcinoma (ESCC) is one of the most aggressive malignancies in digestive system with high rate of mortality worldwide. ClpP as an important mitochondrial protease, the inhibition of ClpP can impair tumor growth and drug resistance in some cancer types. However, the role of ClpP in ESCC development remains largely unknown.

Methods: Western blot and qRT-PCR were used to detect protein and mRNA alteration of indicated molecules. Seahorse XF96 was performed to determine aerobic glycolysis and oxygen consumption rate. IHCwas used to analyze ClpP protein level in ESCC tumor tissues and adjacent non-cancerous tissues. Kaplan-Meier survival analysis was applied to analyze survival rate post-operation.

Results: In the present study, we focused on investigating how ClpP involved in the development of ESCC. We found that ClpP overexpression in most ESCC patients, which play a protective role in tumor progression. Down-regulated ClpP can inhibit cell growth and migration. Additionally, we found that ClpP down-regulated in ESCC cells decreased cellular bioenergetics as determined by measuring aerobic respiration and glycolysis using extracellular flux analyzer. Furthermore, depletion of ClpP can suppress autophagy and MAPK pathway by down-regulating ROS production. We also demonstrated that ClpP was an independent prognostic factor for overall survival of ESCC.

Conclusions: Taken together, these findings indicate that ClpP contributes to the development of ESCC and may serve as a potential biomarker for diagnosis and prognosis of ESCC and therapeutic target for patients with ESCC.

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In Vitro Metabolic Diagnosis of Early Stage Lung Cancer Using Plasmonic Gold Chips

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Objectives: In vitro diagnostics (IVD) contributes to ~66% of clinical diagnosis serving as the “eye” of medical doctors. IVD analysis relies on rationally designed materials for direct metabolite detection with minimal sample treatment procedures for practical diagnostic applications. However, current metabolic analysis face major challenges including (a) the low molecular abundance and high sample
complexity of metabolites in biofluids; and (b) construction of diagnostic tools for real case application in clinics.

**Methods:** We constructed a series of chips coated with plasmonic gold nanoshells for metabolic fingerprinting of biofluids and exosomes in lung cancer diagnostics by laser desorption ionization mass spectrometry (LDI MS). The surface roughness of chips was achieved through controlled particle synthesis, dip-coating, and gold sputtering for mass production. We integrated the optimized chip with microarrays for laboratory automation and micro-/nanoscaled experiments, affording sensitive, selective, multiplex, and quantitative metabolic fingerprinting using 500 nL of serum, cerebrospinal fluid (CSF), urine and exosomes. We further utilized these optimized plasmonic chips for in vitro metabolic diagnosis of early stage lung cancer patients using serum and exosomes.

**Results:** We constructed the plasmonic chip through a three step process including particle synthesis, dip-coating, and gold sputtering. The as-made chip demonstrated reproducible and high-throughput metabolic analysis using biosamples ranging from 400 nL down to 400 pL. The distinct surface roughness on-chip present due to the specific nanogaps and nanocrevices of gold shells selectively trapped small metabolite molecules and transfer the laser energy, toward advanced metabolic analysis of complex biosamples in real case. The on-chip metabolic fingerprinting of serum/CSF/urine and exosomes, by direct LDI MS demonstrated the selectivity toward small metabolites in a complex bio-mixtures compared to conventional methods. We further differentiated the early stage non-small cell lung cancer (NSCLC) patients from healthy controls by on-chip metabolic analysis of serum and exosomes, and anticipated these key m/z values to serve as potential metabolic markers.

**Conclusions:** We have introduced plasmonic gold chips as new substrates for direct LDI MS detection of small metabolites in biofluids and exosomes, and further constructed a novel platform technology for metabolic fingerprinting based IVD. Our work makes solid contributions to the design of materials (gold nanoshells) and device (plasmonic chips) for advanced metabolic analysis toward precision medicine, and initiates the development of various personalized diagnostic tools for diverse diseases including but not limited to lung cancer in the near future.