PG. Cyp7a1 hepatic expression was induced in PG (3.6-fold) and CMT (7.9-fold). This was associated to a reduced ileal Fgf15 gene expression in both groups (relative mRNA levels: 0.38±0.1 in PG and 0.08±0.01 in CMT vs. 1.10±0.23 in CG, p<0.05). Of note, CMT ileal gene expression of Fgf15 was significantly lower than PG (p<0.05). Conclusions: We confirmed the occurrence of down-regulation of export and import biliary genes and an upregulation of hepatic Cyp7a1 gene expression during pregnancy in mice. Ileal down-regulation of Fgf15 gene expression is likely contributory to the observed pregnancy-associated upregulation of Cyp7a1 gene expression in the liver. In a setting of decreased canalicular export, increased expression of Cyp7a1 may raise bile salt levels inside the hepatocyte and contribute to cholestasis during pregnancy. (FONDECYT grant #1110455 to MA).

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Circulating liver-specific miR-122 as a novel potential biomarker for diagnosis of cholestatic liver injury

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Background: Circulating microRNA-122 (miR-122) has been increasingly reported to be a potential biomarker for drug-, viral-, alcohol- and chemical-induced liver injury. The present study was initiated to determine the potential of circulating miR-122 as a biomarker for cholestatic liver injury. Methods: Both bile-duct ligation (BDL) mice and patients with biliary calculi were employed as cholestatic liver injury models, and serum miR-122 level was determined by stem-loop real-time reverse-transcription PCR (SLqRT-PCR). All quantitative PCR values were normalized to those for U6 RNA and calculated with the 2^-ΔΔCt method. Results: Serum miR-122 increased significantly after BDL-induced cholestatic injury and showed a similar time course to ALT concentrations. Compared with the sham controls, BDL mice had increased serum levels of miR-122 by 24.36 ± 12.86, 423.63 ± 322.89, 4.43 ± 2.02 and 12.23 ± 8.92 folds after 1, 3, 7 and 14 days, respectively. Moreover, serum miR-122 level was substantially higher in patients with biliary calculi than that in the healthy control group. In addition, patients with severe liver injury showed significantly higher levels of serum miR-122 when compared with healthy controls or patients with mild or moderate liver injury. Furthermore, serum miR-122 was found to show significant diagnostic value for biliary calculi by yielding an AUC (the areas under the receiver operating characteristic curve) of 0.931 with 77.4% sensitivity and 96.4% specificity in discriminating biliary calculi from healthy controls. Conclusion: Collectively, these data suggest that serum miR-122 has strong potential as a novel, specific and noninvasive biomarker for diagnosis of cholestasis-induced liver injury.

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Genome-wide analysis of stepwise hepatocarcinogenesis using next generation sequencer

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Early hepatocellular carcinoma (HCC) consists of small hepatocytes with little cellular atypia but with structural atypia. This type of very well-differentiated cancerous tissue is usually characterized by indistinct margins and many portal tracts within the tumor, and is clinically detected as hypovascular tumor by imaging modalities. Early HCC usually becomes hypervascular over time and develops into classical HCC. To identify sequential genomic changes in multistep hepatocarcinogenesis, we analyzed fusion genes and mutations using next generation sequencer and methylation status of CpG islands using 450K array about 40 early and 96 advanced HCCs. First, exome sequence showed that base sequence aberrations were more frequent in advanced HCC than early HCC (307.5 vs 90.5 genes per tumor). Mutations including p53 (30.0% vs 41.6%) and WNT signaling pathways (27.5% vs 30.2%) were recurrently observed in both early and advanced HCCs. On the other hand, mutations in chromatin remodeling genes were more frequently observed in advanced HCC (19.7%) than early HCC (5.0%). Consistent with our previous report homozygous deletions near CSM1 and CDKN2A were also found in 8 and 5 cases, respectively, only in advanced HCC. Next, we detected 4 types of fusion gene, including interchromosomal (translocation), intrachromosomal (deletion), intrachromosomal (translocation), and inversion by RNA sequencing. We also found that every HCC harbored 2 to 5 fusion genes, which were more frequent in advanced than early HCC, although there were no recurrent rearrangements in neither advanced nor early HCCs. Especially chromathripisises, in which 10 to 100 rearrangements were localized in the specific genomic regions and genomic features imply chromosome breaks occur in one-off crisis, was the event observed only in advanced HCC. Finally, we integrated methylation status of CpG islands and expression data from RNA sequencing and identified silenced genes by promoter methylation through stepwise hepatocarcinogenesis. Taken together, genomic aberrations including mutation, expression alteration, rearrangement, and methylation status were more frequent in advanced HCC, suggesting early HCC originates in the chronic liver disease and progresses into advanced HCC with accumulation of such genomic aberrations.

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Reciprocal Regulation of miR-122 and c-Myc in Hepatocellular Cancer: Role of E2F1 and TFD2

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c-Myc is a well-known oncogene frequently upregulated in different malignancies whereas liver specific miR-122, a bona
fide tumor suppressor, is downregulated in hepatocellular cancer (HCC). Here, we explored the underlying mechanism of reciprocal regulation of these two genes. Northern blot and real-time RT-PCR analysis demonstrated reduced expression of the primary, precursor and mature miR-122 in c-MYC induced HCCs but not in the benign livers, indicating transcriptional suppression of miR-122 upon Myc overexpression. Indeed, chromatin immunoprecipitation (ChiP) assay showed significantly reduced association of RNA polymerase II and H3K9Ac, markers of active chromatin, with the miR-122 promoter in tumors relative to the c-Myc uninduced livers, indicating transcriptional repression of miR-122 in c-Myc overexpressing tumors. ChiP assay also demonstrated significant increase in c-Myc association with the miR-122 promoter region that harbors several c-Myc binding sites, in tumors compared to the livers. Ectopic expression and knockdown studies showed that c-Myc indeed suppresses expression of primary and mature miR-122 in hepatic cells. Significant increase in miR-122 promoter driven luciferase reporter activity in cells following knockdown of endogenous c-Myc by siRNA and its reversal after deletion of the c-Myc binding sites from the promoter confirmed direct suppression of miR-122 gene expression by c-Myc. Additionally, among the LEFTs the level of Hnf1α and Hnf3β that upregulate miR-122 expression, was also downregulated in tumors. Notably, miR-122 also repressed c-Myc gene expression by targeting transcriptional activator E2f1 and coactivator Tfdp2, as evident from ectopic expression and knockdown studies and luciferase reporter assays. Collectively, these results show that c-Myc represses transcription of miR-122 gene by directly associating with its promoter whereas miR-122 indirectly inhibits c-Myc transcription by targeting E2f1 and Tfdp2. In essence, these results suggest a double-negative feedback loop between a tumor suppressor (miR-122) and an oncogene (c-Myc).

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390 Discovery of a tyrosine kinase fusion gene in Intrahepatic Cholangiocarcinoma
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INTRODUCTION: Intrahepatic Cholangiocarcinoma (ICC) is a rare bile duct cancer with dismal prognosis. Fusion events are among the most potent oncogenic drivers, and recent studies report dramatic therapeutic responses blocking these targets in melanoma and lung cancer. We aimed to identify fusion events that meaningfully contribute to ICC pathogenesis. METHODS: We analyzed a cohort of 115 ICC cases; 7 ICC fresh-frozen paired samples were screened for fusion events using RNA-seq (HiSeq2000sequencer), and 108 paraffin-embedded tissues were used to validate the finding by RT-PCR and sanger sequencing. To identify fusion events, raw cDNA reads were aligned to a reference genome, with subsequent filters applied via in-house methods. A fusion gene was selected based on the number of supporting reads and partner genes and validated in the same patient where it was identified. Whole-genome sequencing was run in the sample with the fusion event. NIH3T3 cells were stably transfected to over-express the full fusion gene. The effect of the fusion gene on cell migration was investigated in vitro (transwell assay). RESULTS: An interchromosomal event resulting in the formation of a fusion gene comprising portions of an oncogenic tyrosine kinase receptor, FGFR2 (10p12) and a gene involved in epithelial differentiation, PPHLN1 (12q12) was identified in 1 patient. The fusion event was supported by 149 single-end reads spanning the fusion junction of exon 19 of FGFR2 to exon 4 of PPHLN1 and of hepatocyte proliferation promoters MET and YAP1. CDH1, MET, and YAP1 were independent predictors of recurrence-free survival by Cox regression when corrected for tumor stage (p<0.0001). Conclusion: HCV-cirrhosis is characterized by proliferation of liver stem cells and inhibition of hepatocyte proliferation. HCC tumors in which this pattern persists have superior outcomes to those which acquire a hepatocyte proliferation signature (loss of CDH1 and MST1, gain of MET, YAP1, MCM2). Genes in this signature should be studied further for potential as tissue or serum biomarkers for patient risk stratification. CDH1 and MET are candidates for personalized therapies with targeted pharmaceutical agents.

Cox proportional hazards modeling of expression levels of proliferation genes, corrected for stage at diagnosis. The final model was highly significant (p<0.001)
was not present in the paired normal tissue. By performing broad range PCR, we verified that the first 19 exons of FGFR2 were present at the 5’ end and included an intact open reading frame and kinase domains. Whole-genome sequencing of the tumor and matched normal tissue verified the presence of an inverted translocation between the two chromosomes in the tumor but not in the normal tissue. 108 cases of ICC were analyzed by RT-PCR and Sanger sequencing for the presence of the same fusion event. The screening revealed that 21 out of 108 ICCs (19.4%) harbored the same alteration. To verify if PPHLN1 is able to mediate dimerization of the receptor, we expressed histidine-tagged fusion gene in NIH3T3 cells and verified its oligomerization, suggesting constitutive activation. NIH3T3 overexpressing the fusion gene showed enhanced migration capability (p<0.02). CONCLUSIONS: A novel fusion event including an active tyrosine kinase was discovered in 20% of ICC cases by next-generation sequencing. The identified fusion gene is caused by a DNA rearrangement and increases cell migration capability in vitro. This novel fusion may represent an appealing target for selected molecular therapies.

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Genome-wide analysis of miR-106b targets in cholangiocarcinoma cells identifies tumor suppressors Kruppel-like factor-2 and -6, characterization of an antiapoptotic microRNA

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Activating pathways associated with liver damage and necrosis in regenerating mice liver following concurrent inhibition of MET-EGFR kinase activity

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The HGF receptor MET and the EGFR receptor (EGFR) are mitogenic receptor-tyrosine kinases for hepatocytes. The MET-EGFR signaling pathway is activated within 15-30 minute following a two-thirds partial hepatectomy (Phx) in mice and rats. MET and EGFR functionally interact and there is also considerable crosstalk between the two pathways. In order to understand the role played by these two pathways during liver regeneration, we used MET-EGFR specific Tyrosine kinase inhibitors to block the receptor kinase activity. Mice were administered EGFR specific Gefitinib (300 mg/kg) and MET specific JNJ 38877605 (100mg/kg) by oral gavages. The following day, mice were administered a second dose and two hours later a Phx was carried out. Appropriate vehicle controls were also used. In mice treated with Tyrosine kinase inhibitors, pMET & pEGFR levels were significantly reduced compared to vehicle treated controls. Global changes in gene expression patterns in treated and control livers were analyzed by microarray analyses. Pathway analysis using Ingenuity and gene enrichment analyses using Metacore software indicated differential regulation of more than 30 genes associated with liver damage and liver degeneration as early as three hours post Phx in treated mice. Significant downregulation of proliferation associated genes was also observed in treated mice. Luminex based cytokines assays on mouse plasma samples obtained at various time points post Phx, from inhibitors treated and control animals were carried out. An increased expression in TNF, Fas and HGF was evident at 10 hours post Phx in mice treated with inhibitors. A survival analyses following a JO-2 antibody challenge in the treated and control mice indicated that pro survival
effects of a PHx were abrogated in the inhibitor treated mice compared to controls. In conclusion, treatment with MET, EGFR kinase inhibitors resulted in global changes in signaling pathways that seemed to promote block in proliferation and liver damage. The survival-repair pathways that are activated following a PHx are not protective in MET-EGFR inhibited mice, suggesting an absolute requirement of MET-EGFR signaling for successful liver regeneration in rodents.

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393 Enhanced Efficacy of Low Dose Adenovirus with HCC-specific RNA Replacement by hTERT-targeting Trans-splicing Ribozyme Modifiers or Derivatives at Post-transcriptional Level in Mouse Model

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We developed an adenoviral vector harboring human telomerase reverse transcriptase(hTERT) RNA-targeting trans-splicing ribozyme(TSR) and liver-specific promoter PEPPC for HCC-specific gene therapy, successfully. This ribozyme can mark cancer cells expressing hTERT and sensitize them to ganciclovir treatment by expression of therapeutic thymidine kinase(TK) gene[Ad-PEPPC-hTERT.Ribo-TK: PRT]. PEPPC promoter has tissue-specificity but weaker transcriptional activity, than CMV promoter. It is needed to enhance efficacy of ribozyme through increasing ribozyme transcript level without compromising its tissue specificity. We pursued increasing efficiency of ribozyme using low dose of adenovirus, regulated at posttranscriptional level by insertion of splice donor(SD)/splice acceptor(SA) site and woodchuck hepatitis post-transcriptional regulatory element(WPRE)[SD/SA-PRT, PRT-WPRE/SD/SA-PRT]. In this study, we investigate whether these ribozyme modified vectors show enhanced efficacy in comparison with PRT, in vitro and in vivo without hepatotoxicity. Plasmids(pSEAP-SRF[SV40 promoter + ribozyme + firefly luciferase] and pSEAP-SD/SA-SRF-WPRE) and adenoviral vectors(PRT, SD/SA-PRT, PRT-WPRE, SD/SA-PRT-WPRE) were constructed. Hep 3B, HEK 293, MCF7, AGS cells were investigated. In vivo multifocal HCC model in nude mice was established by subspenic injection of Hep3B cells and all vectors were provided systemically. When cells infected with plasmids, pSEAP-SD/SA-SRF-WPRE treatment showed enhanced transgene activity(Hep 3B; 7.5-fold, HEK 293; 2.9-fold, MCF7; 5.5-fold, AGS; 2.6-fold) than pSEAP-SRF treatment. Hep 3B cells treated with SD/SA-PRT-WPRE showed 5-fold increased cytotoxicity than PRT. This increased cytotoxicity was proved to be resulted from increased ribozyme transcriptional activity via RNA,retro assay. Hepatotoxicity was not remarkable in normal Balb/C mice when injected with SD/SA-PRT-WPRE, similar to PRT group(n=5, each). After treatment with SD/SA-PRT-WPRE(0.25x1010vp), tumor weight was 0.42±0.0mg, with PRT(n=10x1010vp), 0.37±0.0mg, representing remarkably enhanced efficacy with 1/40 dose of PRT, compared with PBS group, 4.02±2.0mg(n=7, each), P<0.0001; ANOVA), in multifocal HCC mouse model. Least hepatotoxicity was observed in SD/SA-PRT-WPRE treated group, similar to PRT group. This study represents that post-transcriptional expression regulation of ribozyme discloses remarkably enhanced antineoplastic activity, resulting in lowering the dose of adenovirus, leading to more safety as well as efficacy. Liver cancer specific gene therapy by hTERT targeting TSR with enhanced efficacy promises highly specific and efficient HCC gene therapy.

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394 Peretinoin, an acyclic retinoid, inhibits liver carcinogenesis through suppression of sphingosine kinase 1 activation in vitro and in vivo

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[Objective] A group of phospholipid plays an important role in various physiological and pathological aspects as mediator molecules among cells and organs. A sphingolipid, sphingosine-1-phosphate[S1P], is a potent bioactive lipid metabolite which could regulate carcinogenesis and progression of cancer. Both sphingosine kinase 1(SphK1) and SphK2 are the essential kinases that produce S1P. Therefore, SphK can be a therapeutic target by crucially regulating sphingolipid metabolism in several kinds of cancer. We and other groups have demonstrated that peretinoin, an acyclic retinoid, reduced the post therapeutic recurrence of hepatocellular carcinoma(HCC) in patients with chronic hepatitis C, and phase 3 study is ongoing. However, the mechanisms by which peretinoin exerts its inhibitory effects against recurrent HCC remains unclear. Because peretinoin binds retinoid X receptor and retinoic acid receptor, which are known to function as a sensor and regulator of sphingolipid metabolism, we hypothesized that peretinoin could prevent liver carcinogenesis by modifying SphK1-S1P axis. In the present study, we assessed the effect of peretinoin on SphK activation and development of liver cancer. [Method] We examined the effect of peretinoin on the expression and the enzymatic activity of SphK1 in Huh-7 cells. Next, using diet-induced NASH related liver cancer mouse model by feeding atherogenic high fat(AHF) diet, we administrated AHF diet with or without peretinoin(0.03%) in C57Bl/6J mice for 48 weeks and examined the effect of peretinoin on liver carcinogenesis and the expression of SphK1. [Results] [In vitro] After treatment of peretinoin(10 to 50 μM), it reduced mRNA and protein expression of SphK1 in Huh-7 cells in time- and dose-dependent manner. However, peretinoin did not change the expression of SphK2 expression in Huh-7 cells. Furthermore, peretinoin markedly reduced the enzymatic activity of SphK1 assessed by in vitro measured [32P]-labellled SphK1 activity. Importantly, peretinoin inhibited cell proliferation of Huh-7 cells measured by a MIT assay. In addition, activity of SphK1 expression by siRNA abolished the anti-proliferation effect of peretinoin. [In vivo] Peretinoin dramatically reduced mRNA expression for SphK1 in the liver of mice after 24 weeks of treatment. At 48 weeks of treatment, peretinoin downregulated SphK1 mRNA expression and prevented AHF diet-induced liver carcinogenesis. [Conclusion] Our data indicate that peretinoin prevents liver carcinogenesis at least partly through reduction of expression and activation of SphK1. Therefore, SphK1 activation may play a critical role which links aberrant sphingolipid metabolism to liver carcinogenesis.

Disclosures:
Remote gene therapy using AdMMP8 modifies the fibrogenic gene expression pattern and decreases fibrosis in experimental liver cirrhosis

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BACKGROUND. Cirrhosis is a consequence of chronic liver disease characterized by replacement of liver tissue by fibrosis (contains mostly type I collagen), with increase profibrogenic and proinflammatory gene expression. Some experimental protocols have shown that the liver is a muscle-producing tissue. Matrix metalloproteinase 8 (MMP-8) degrades interstitial collagens acting preferentially on collagen type I. MMP-8 is a proteolytic enzyme that must be activated to be functional. PURPOSE. Analysis of intramuscularly administering an adenoviral vector containing MMP-8 gene (AdMMP8) in the prevention of liver fibrosis. MATERIAL AND METHODS. Experimental liver fibrosis was induced in male Wistar rats by TTA administration for 7 weeks. Four groups of rats (n=15) were included: control, no fibrosis; TAA, thioacetamide-induced-fibrosis; TAA+AdGFP, vector with an irrelevant gene; TAA+AdMMP8, vector with therapeutic gene. At the beginning of the fifth week of TAA intoxication, administration of vectors in soleum muscle was accomplished. Sub-groups of rats (n=5) at the end of first, second and third week after vector administration were sacrificed. Percentage of fibrosis, liver function, gene expression of MMP8, profibrogenic genes (IL-1 beta, TNF-alpha), profibrogenic genes (collagen α1(I), CTGF and TGF-β) and antifibrogenic genes (MMP-1 and MMP-9) were determined. RESULTS. After 3 weeks of treatment: In the liver and serum, amount of MMP8 protein was sustained, fibrosis decreased up to 48%, profibrogenic genes expression was modified only at the end of the third week, profibrogenic gene expression decreased (Col α1(I) 4 times, TGF-beta 3 times and CTGF 2 times), antifibrogenic genes expression increased (MMP-9 2.8 times and MMP-1 10 times), and reduction of liver function was not statistically significant. According to Knodell score, a clearly diminution of inflammatory cells infiltration in comparison with counterpart animals treated with AdGFP, could be appreciated. CONCLUSIONS. Obtained information allows us to establish that a single dose of AdMMP8 in muscle is enough in order to obtain a stable liver MMP-8 protein expression and activity during 21 days. Degradation of collagen in the liver modifies pro and anti-fibrogenic gene expression allowing a restoration of hepatic architecture. MMP-8 protein is expressed in muscle using Advector, is released systemically and is activated in the liver, where have collagenolytic activity

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397 Hepatocytes lacking microRNAs are protected from ICC Yann Malato1, Julia Bindman1, Laure Dumont1, Donghui Wang2, Holger Willenbring1; 1 Surgery, University of California San Francisco, San Francisco, CA; 2 Preclinical Therapeutics, UCSF, San Francisco, CA

Purpose: The role of microRNA (miRNA) deficiency in the development of intrahepatic cholangiocarcinoma (ICC) is poorly understood because animal models that involve both are lacking. Methods: We crossed Albumin-Cre transgenic mice with Di George syndrome Critical Region gene 8 (DGC8R) floxed mice to obtain hepatocyte-specific DGC8R KO mice (DGC8RΔhep mice). DGC8R is an essential cofactor of Drosha involved exclusively in miRNA processing. Because these mice have non-functioning DGC8R-mediated miRNA processing in cells that express albumin, leading to global miRNA deficiency in hepatocytes, they provided an accurate model for defining the role of miRNAs in ICC formation. We used our previously established model of ICC consisting of hydrodynamic tail vein injection (HDTVI) of a cocktail of plasmids (AKT, NICD, n-RAS and LUC) stably integrated using the sleeping beauty transposase. This model induces ICC formation of hepatocyte origin in 4 weeks. Results: We monitored luciferase activity using an IVIS spectrum instrument over time and noticed that DGC8R KO mice were losing activity 3 weeks after HDTVI while WT mice had an increased luciferase activity. When we sacrificed mice at 4 weeks, the WT mice had macroscopic cystic tumors in their livers while the KO had a smooth liver. Alanine transaminases were significantly reduced in DGC8R KO compared to WT mice. In order to have an accurate overview of the liver lobes, we took serial pictures of H&E staining that we stitched in Fiji. We then confirmed that WT livers were full of cystic tumors at 4 weeks after HDTVI but KO livers displayed significantly less after counting the tumors. They were also smaller in size. Immunostaining for the liver progenitor marker osteopontin showed a significant increase in KO mice which suggested that these cells may play a role in helping the DGC8R KO mice to clear the cancer. Thus, we repeated the HDTVI experiment but in mice fed with DDC for 4 weeks prior the injection. In this condition, no matter the genotype, we could not detect any tumor 4 weeks after injection macroscopically, and found only 1 WT mouse with few nodules compared to the more than 30 nodules per lobe on the chow diet-fed WT mice. Conclusion: In conclusion and in analogy to HCC that we also found to be protected in these KO mice, our results support the hypothesis that global microRNA deficiency in hepatocytes impairs ICC formation and that one or more miRNAs are needed for ICC formation and inhibition of these specific miRNAs may have a therapeutic potential for ICC.

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398 Blockade of CXC2 suppresses tumour growth of intrahepatic cholangiocellular carcinoma Tadamichi Hirano, Hideaki Sueoka, Yugo Uda, Nobukazu Kuroda, Toshitomo Okada, Yasuake Asano, Yuchii Kondou, Kuo Nakamura, Shogo Tanaka, Seikan Hsi, Yoshi Imuro, Jiro Fujimoto; Hyogo College of Medicine, Nishinomiya, Japan

Background/Aims: Complete surgical resection is the only approach to cure for intrahepatic cholangiocellular carcinoma (ICC), however, prognosis is extremely poor. Novel therapeutic approach is urgently required. CXC chemokine receptor 2 (CXCR2) have been found to be associated with tumorigenesis and metastasis in human malignancy. In the present study, we investigated the suppressive effect of ICC growth by blockade of CXCR2. Material/Methods: Expression of CXCR2 in ICC is estimated by immunohistochemical staining using thirty three ICC specimens and investigated relevance with prognosis. The role of CXCR2 was estimated using human ICC cell lines, RBE and SSP25. CXCR2 siRNA and antagonist (SB225002) were used to block CXCR2. Proliferation assay, migration assay and invasion assay were performed to confirm the suppressive effect by blockade of CXCR2. Expression of CXC ligand (CXCL) that binds to CXCR2 were also investigated in human ICC samples and in supernatant of ICC cell lines. Subcutaneous SSP25 tumours established in athymic nude mice were administered SB225002. Results: Prognosis of patients who had higher CXCR2 expression in ICC was significantly poor (p=0.004). CXCR2 siRNA significantly suppressed CXCR2 expression both RBE and SSP25. Cell proliferation, migration and invasion was significantly suppressed by both CXCR2 siRNA and SB225002 compared with control group. SB225002 also suppressed growth of transplanted subcutaneous tumours (p=0.02). By contrast, knockdown or addition of CXC ligand 8 (CXCL8) did not affect on ICC proliferation and migration, though CXCL8 expression was confirmed in human ICC samples and in supernatant of ICC cell lines. SB225002 suppressed growth of transplanted subcutaneous tumours (p=0.02). Conclusions: Our results demonstrated that down regulation of CXCR2 markedly suppressed growth and metastasis of ICC. These results suggested that CXCR2 acts crucial role in the development of ICC and blockade of CXCR2 may represent a novel strategy for ICC.

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399 Association between IL28B T allele and reduction of mir-122 expression in patients with chronic hepatitis C who failed to respond to pegylated-interferon plus ribavirin Emille Estrabaud1,2, Martine Lapalus1,2, Philippe Broel1,2, Kevin Appourchaux1,2, Simon De Mynck1,2, Olivier Lada1,2, Michelle Martinet-Peignoux1,2, Ivan Bieche3, Dominique Valla2, Pierre Bedasso5, Patrick Marcellin1,2, Michel Vidaud1,2, 1 INSERM U773/CRB3 Bichat Hospital, Paris, France; 2 Service d’Hépatologie, Beaujon Hospital, Clichy, France; 3 Service d’Hépatologie, Beaujon Hospital, Clichy, France; 4 UMR669, University Paris-Sud, Villejuif, France; 5 UMR745 INSERM, Université Paris Descartes, Paris, France; 6 Service d’Anatomie Pathologique, Beaujon, Clichy, France

Background and aims Mir-122 is highly expressed in hepatocytes, where it represents 70% of the total miRNAs. Mir-122 binding within Hepatitis C virus (HCV) RNA stimulates its replication, in vitro. A reduction of hepatic mir-122 expression has been suggested in patients with primary non-response (pNR) to PEG-IFN/ribavirin. IL28B CC genotype [rs12979780] is strongly associated with sustained virological response (SVR). The aim of the study was to investigate, in vivo, the relationships between hepatic and serum expression of mir-122, IL28B and response to PEG-IFN/ribavirin. Patients and Methods Pre-treatment liver biopsies and serum from 133 patients with CHC were included. Eighty three men and 50 women were included in the study. Sixty six patients achieved a SVR, and 64 failed to respond to the treatment, 43 had non-response (NRS) and 21 had relapse (RR); 63 had complete early virological responders (cEVR) and 47 had pNRs. In our series 80, 12, 14, 25 and 1
patients were respectively infected by genotypes 1, 2, 3, 4 and 5. The mean viral load was 5.5±0.7 log UI/mL. Mir-122 expression was assessed in a total of 127 percutaneous liver biopsies and 83 sera by RT-q-PCR. IL28B rs12979860 polymorphism was analyzed by direct sequencing. Results A significant decrease in the mean level of hepatic mir-122 expression was observed for patients with a pNR as compared to cEVR (p=0.003) and for patients with failure to respond to the treatment (NRs+RRs) as compared to SVRs (p=0.016). Moreover, hepatic mir-122 expression was higher in CC patients when compared to CT and TT, in the total group of patients (p<0.025) and in NRs (p<0.013). Mir-122 expression in the liver and in the serum were not associated (p=0.21). An increased viral load was associated with a decreased hepatic mir-122 (p=0.02) and an increased serum mir-122 expression (p=0.001). Higher ASAT and ALAT were associated with a decreased hepatic mir-122 (ASAT, p=0.03 and ALAT, p=0.03) and an increased serum mir-122 (ASAT, p=0.009 and ALAT, p=0.004). Both serum and hepatic expression of mir-122 were strongly associated with GGT (p=0.005 and p=3.10-6). Conclusions The major novelty of our work consists in the description of a decreased hepatic mir-122 within IL28B CT and TT patients who failed to respond to the treatment (NR+RR) compared to patients carrying CC genotype. The study of modification of mir-122 expression may help to elucidate the molecular mechanism behind NR and IL28B polymorphism.

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Sorafenib shows inhibitory efficacy in a newly established murine hepatic angiosarcoma cell line

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Background: Liver angiosarcoma (AS) is a rare and highly aggressive tumor of endothelial origin with dismal prognosis. Studies of the molecular biology of AS are limited since animal models are missing. We have previously shown that knockdown of Notch1 in mice leads to spontaneous formation of hepatic AS (Gastroenterol. 2012) and we have established three cell lines from these animals. The aim of this study is 1) to study the molecular pathogenesis and therapeutic targets and 2) to evaluate the effect of targeted therapies in the treatment of AS. Methods: Mouse liver AS were studied by electron microscopy. Transcriptome analysis in AS cell lines vs. normal liver sinusoidal endothelial cells (LSEC) from control and Notch1 KO mice without AS including gene set enrichment analysis (GSEA) were performed. In one AS cell line the effects of treatment with increasing sorafenib concentrations (1-20 μM) were analyzed. Time-lapse microscopy of sorafenib exposed AS cells grown on matrigel was analyzed. Cell proliferation was monitored using the real-time cell analyser system xCELLigence. Cell viability and intracellular signalling were assessed by flow cytometry and western blotting. Results: In the transitional zone between tumor and normal tissue, ultrastructural features varied from normal to dysplastic endothelial cells with prominent nucleoli and Weibel Palade bodies. Transcriptome analysis showed massive changes in gene expression identifying FGFRs, TGFβ, met proto-oncogene, PI3K, and VEGF-A as potential drivers in malignant transformation of hepatic AS. Moreover, GSEA revealed that six of the top 20 upregulated chemical and genetic perturbation gene sets were related to myc targets (FDR<0.25). C-myc is a downstream transcription factor target of the Raf/MEK/ERK pathway, which can be blocked by sorafenib, a multikinase inhibitor. Timelapse imaging showed that sorafenib treatment dramatically reduced migration of AS cells. Differences in filopodia dynamics were significant (p=0.0201) after 6 h with a decrease in filopodial extensions. Sorafenib inhibited cell proliferation in a time and dose-dependent manner, whereas the number of apoptotic cells was only slightly elevated with increasing concentrations. In addition, sorafenib suppressed ERK phosphorylation and expression of Cyclin D2 in the AS cell line. Conclusion: We identified Notch1 as LSEC tumor suppressor gene and established three hepatic AS cell lines as a useful in vitro tool. Our data demonstrate anti-tumor activity of sorafenib in AS cells with potent inhibition of migration, filopodia formation, and cell proliferation, which support further evaluation of sorafenib as a novel treatment strategy.

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HCV core protein-induced down-regulation of microRNA-152 promoted aberrant proliferation by targeting Wnt1 in HepG2 cells

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Background: Hepatitis C virus (HCV) has been reported to regulate cellular microRNAs (miRNAs). The HCV core protein is considered to be a potential oncoprotein in HCV-related hepatocellular carcinoma (HCV-HCC), but HCV core-regulated miRNAs are largely unknown. Our preliminary experiments revealed significantly down-regulated microRNA-152 (miR-152) expression in HCV core protein-overexpressing HepG2 cells in comparison with the control Ad-EGFP infected HepG2 cells. Through target gene prediction software, the proliferation-promoting oncogene, Wnt1, was predicted to a potential target of miR-152. Thus the present study was initiated to investigate whether miR-152 are aberrantly regulated by the HCV core protein, and involved in the regulation of the aberrant proliferation of HCV-HCC cells. Methods: MiR-152 levels were examined by stem-loop real-time RT-PCR (SLqRT-PCR). Cell proliferation was analyzed by MTT and colony formation assay. Cell cycle analysis was performed by propidium iodide staining. A luciferase reporter assay was conducted to confirm miRAN-target association. Wnt1 expression was determined by real-time qPCR and Western blotting. Results: HCV core protein significantly suppressed miR-152 expression, and led to significant Wnt1 up-regulation with a concomitant aberrantly promoted proliferation. Moreover, we validated that miR-152
402 Pivotal role of miR-148a in the liver: hepatic differentiation and tumor suppression
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MicroRNAs (miRNAs) are evolutionary conserved small non-coding RNAs that post-transcriptionally regulate gene expression. Numerous studies have reported the key role of miRNAs in development, cell proliferation, apoptosis, and tumor biology. However, the implication of miRNAs in liver development is not fully understood. By using an experimental model developed by our group that allows the induced and controlled differentiation of mouse fetal hepatoblasts (MFHs) into mature hepatocytes, we identified miR-148a as a hepatospecific miRNA highly expressed in adult liver. The main finding of our study revealed that miR-148a was critical for hepatic differentiation by the direct targeting of DNA methyltransferase (DNMT) 1, a major enzyme responsible for epigenetic silencing, thereby allowing the promotion of the "adult liver" phenotype. It was also confirmed that the reduction of DNMT1 by RNA interference significantly promoted the expression of the major hepatic biomarkers. In addition to the essential role of miR-148a in hepatocyte maturation, we identified its beneficial effect through the repression of hepatocellular carcinoma (HCC) cell malignancy. MiR-148a expression was frequently downregulated in the biopsies of HCC patients as well as in mouse and human HCC cell lines. Overexpressing miR-148a led to an enhancement of albumin production and a drastic inhibition of the invasive properties of HCC cells, whereas miR-148a silencing had the opposite consequences. Finally, we showed that miR-148a exerted its tumor-suppressive effect by regulating the c-Met oncogene regardless of the DNMT1 expression level. To conclude, miR-148a appears essential for the physiology of the liver, as it promotes the hepatospecific phenotype and acts as a tumor suppressor. Most importantly, we demonstrate a functional role for a specific miRNA in liver development via the regulation of the DNMT1 enzyme.

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403 IL28B Genotype is Associated with Differential Expression of Interferon-Stimulated Genes after Liver Transplantation
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Both polymorphisms in the Interleukin 28 B (IL28B) haplotype block and hepatic interferon-stimulated genes (ISG) expression levels are known predictors of treatment response in hepatitis C patients. The two are also interrelated, with favorable CC genotype patients expressing lower ISG levels than their CT/TT counterparts. Though the relationships between IL28B, ISGs and treatment response in the non-transplant setting have been established, they remain unknown in the context of post-transplant recurrent hepatitis C treatment response. This study explores these relationships. Twenty-eight patients with recurrent hepatitis C post-transplant (genotype 1, n=23; 2, n=4; 3, n=5) who were treated over a two-year period with peg-IFN+RBV were included in the study. Overall, 78.5% of patients achieved EVR; 50% achieved SVR. All liver biopsy specimens were collected within one year prior to treatment. Patients with major complications other than recurrent hepatitis C were excluded. Native IL28B rs12979860 genotypes were as follows: 8 CC, 20 non-CC; donor IL28B genotypes: 12 CC; 16 non-CC. Analysis by recipient IL28B, 90% of CC patients achieved EVR, 80% SVR; by donor IL28B, 100% of patients achieved EVR, but only 50% achieved SVR. ISG expression was studied by qPCR of hepatic mRNA; genotyping for the IL28B SNP rs12979860 was performed with TaqMan assay. Nine ISGs (IFI44L, RSAD2, ISG15, IFI27, IFI6, LAMP3, OAS2, OAS3, Mx1) previously identified as predictive of treatment response were chosen for analysis. Results showed significant differences in the hepatic mRNA level of ISGs between native CC and non-CC genotypes, with CC genotype expressing significantly lower levels of most genes. Analysis by donor genotype revealed significant differences in only two of the studied genes (IFI27 and IFI6); however, the overall trend was similar, with donor CC expressing lower levels of ISGs. Time from transplantation, type of immunosuppression, and fibrosis stage did not relate to ISG expression levels. These results are in accordance with findings in pre-transplant populations, with IL28B CC genotype expressing lower levels of hepatic ISGs than CT/TT genotypes. Recipient IL28B genotype seems to have more influence on interferon pathways than donor genotype. In our population, it also seems to have a greater influence on treatment response. IL28B’s mechanism of action is likely affected through ISG expression. These results indicate that patient’s native IL28B genotype has a greater influence than donor genotype on relevant ISG expression, and consequently on treatment response.

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Differential microRNA expression in the bile of cholangiocarcinoma patients

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BACKGROUND & AIMS MicroRNAs (miRNAs) are an important class of small non-coding RNA molecules that bind to their complementary sequence on their target mRNAs, resulting in translational repression. MiRNAs play major roles in development, metabolism, infection, and cancer. The diagnosis of cholangiocarcinoma is sometimes difficult due to the lack of a specific tumor marker and limited sampling for histological evaluation. In this study, we analyzed miRNA expression in bile to identify predictive miRNAs for hepatobiliary malignancy.

METHODS MiRNAs were obtained from bile samples taken from 25 patients with benign hepatobiliary disease and 34 patients with malignant hepatobiliary disease, including cholangiocarcinoma and gallbladder cancer. Expression of 328 miRNAs was determined with the TaqMan real-time PCR detection system using a MicroRNA Assays Human Panel. MiRNAs were functionally evaluated in cholangiocarcinoma cell lines (Huh7, Hucl1, and KMBC) following the overexpression or knocking down of specific miRNAs using mimic-miRNA or anti-miRNA. RESULTS Among the 328 miRNAs tested, 16 were differentially expressed between the bile of patients with malignant hepatobiliary disease and those with benign hepatobiliary disease (p<0.05). From these 16 miRNAs, we focused on the two—miR-451 and miR-486—that were most significantly increased in malignant hepatobiliary disease. The area under the curve of the receiver operating characteristic curve for the prediction of malignant hepatobiliary disease using these miRNAs was 0.85, which was equivalent to that of the tumor marker CA19-9. Univariate analysis using multiple clinical parameters, including tumor markers (CEA and CA19-9) and a liver function test, revealed serum ALT and T-Bil, miR-451, and miR-486 as significant variables. Multivariate analysis identified CEA, CA19-9, and miR-486 as significant variables. Functional analysis of these miRNAs showed that miR-451 had tumor suppressive and anti-inflammatory properties by decreasing the expression of PDGFRα, KRT7, IL1, and IL6, while miR-486 was tumorigenic by increasing the expression of PDGFRα, N-cadherin, and p38MAPK. CONCLUSIONS We identified two predictive miRNAs, miR-451 and -486, in bile for the diagnosis of malignant hepatobiliary disease. Interestingly, these miRNAs are functionally different in that one has tumorigenic properties and the other functions as a tumor suppressor. These miRNAs may be useful for diagnosing biliary cancer and determining its prognosis.

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Disease progression from chronic hepatitis C to cirrhosis and hepatocellular carcinoma is associated with increased DNA promoter methylation

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Changes in DNA methylation patterns are believed to be an early event in hepatocarcinogenesis. The aim of our study is to analyze the methylation frequency of tumor suppressor genes; P14, P15, P73 and Mismatch repair gene (O6MGMT) in HCV related chronic liver disease and HCC to identify candidate epigenetic biomarkers for HCC prediction. Methods: 516 Egyptian patients with HCV-related liver disease were recruited from Kasr Alaini multidisciplinary HCC clinic from April 2010 to January 2012. Subjects were divided into 4 different clinically defined groups; HCC group (n=208), liver cirrhosis group (n=108), chronic hepatitis C group (n=100), and Control group (n=100); to analyze the methylation status of tumor suppressor genes; p14, p15, p73 and the DNA Mismatch repair gene (O6MGMT) in patients’ plasma by using Epitfect Methyl qPCR Array technology. Methylation frequency was considered to be hypermethylated if >10% and/or intermediately methylated if >60%. Result: In our series, a significant difference in the hypermethylation status of all studied genes was noted within the different stages of chronic liver disease and ultimately HCC. Hypermethylation of the P14 gene was detected in 100/208 (48.1%), 52/108 (48.1%), 16/100 (16%) and 8/100 (8%) among HCC, liver cirrhosis, chronic hepatitis and control groups respectively with a statistically significant difference between the studied groups; (p=0.008). We also reported P15 hypermethylation in 92/208 (44.2%), 36/108 (33.3%), 20/100 (20%) and 4/100 (4%) among HCC, liver cirrhosis, chronic hepatitis and control groups respectively with a statistically significant difference between the studied groups; (p=0.006). In addition, more hypermethylation frequency of P73 was detected in 136/208 (65.4%), 72/108 (66.7%), 32/100 (32%) and 4/100 (4%) among HCC, liver cirrhosis, chronic hepatitis and control groups respectively with a statistically significant difference between the studied groups; (p=0.001). Also, we detected O6MGMT hypermethylation in 84/208 (40.4%), 60/108 (55.3%), 20/100 (20%) and 4/100 (4%) among HCC, liver cirrhosis, chronic hepatitis and control groups respectively with a statistically significant difference between the studied groups; (p<0.001). Conclusion: The epigenetic changes observed in this study shows that HCC tumors exhibit specific DNA methylation signatures associated with the potential clinical applications in diagnosis and prognosis. On the other hand, methylation frequency could be used to monitor whether the patient with chronic hepatitis C will be subjected to liver cirrhosis or even HCC. So, we can conclude that methylation process in an early event in hepatocarcinogenesis.

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Jnk1/p53/miR-34a/SIRT1 as a Key Pathway Mediating
Deoxoycholic Acid-Induced Apoptosis in Primary Rat
Hepatocytes
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Hydrophobic bile acids, such as deoxocholic acid (DCA), are
known to modulate the expression of several apoptosis-related
proteins, including c-Jun N-terminal kinase (JNK), leading to
cell death. In addition, microRNAs (miRNAs or miRs) are being
increasingly implicated in cell death and in the pathogenesis of
human liver diseases. In that regard, we have recently shown
that the miR-34a/Sirtuin1 (SIRT1)/p53 pathway correlates with
non-alcoholic fatty liver disease severity and apoptosis, and
that ursoodeoxycholic acid, an endogenous hydrophilic bile
acid, counteracts this pro-apoptotic pathway. The purpose of
this study was to evaluate whether DCA-induced apoptosis of
primary rat hepatocytes occurs via miR-34a-dependent path-
ways and whether they relate with activation of JNK. Primary
rat hepatocytes were incubated with 100 microM DCA, and
transfected with a specific miRNA-34a inhibitor or precursor, or
with a p53 overexpression plasmid. p53 transcriptional activity
was assessed in nuclear extracts and by using target reporter
constructs. SIRT1 was upregulated using resveratrol, and JNK
function was evaluated by immunoblotting and silencing exper-
iments. Viability, caspase-3 activity and apoptosis were deter-
mined using the ApoTox-GloTM Triplex Assay and Hoechst
staining. Our results showed that DCA enhances the miR-34a/SIRT1/p53 pro-apoptotic signalling in cultured primary
rat hepatocytes, in a dose- and time-dependent manner. miR-
34a overexpression increased apoptosis by DCA. In turn, miR-
34a inhibition and SIRT1 overexpression significantly rescued
cells from apoptosis by DCA. In addition, activation of p53 trig-
gered the miR-34a/SIRT1/p53 pathway, further induced by
DCA. Interestingly, DCA increased p53 expression, as well as
p53 transcriptional activation of PUMA and miR-34a itself, pro-
viding a functional mechanism for its targeting of miR-34a.
Finally, JNK1, but not JNK2, was shown to be a major player,
upstream of p53, in engaging the miR-34a/SIRT1/p53 pro-
apoptotic pathway and apoptosis by DCA. In conclusion, our
results support a link between the miR-34a, hepatocyte apopt-
osis and JNK signalling, where JNK1-mediated activation of
p53 is the key mechanism behind induction of miR-34a by
DCA. The JNK/miR-34a/SIRT1/p53 pro-apoptotic pathway may represent an attractive pharmacological target for the
development of new drugs to arrest apoptosis-related liver
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A novel reducible cationic siRNA delivery agent for liver
cancer with an enhanced intracellular release mechanism
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Introduction: Development of an efficient delivery system for
siRNA is urgently needed for its therapeutic application. This
project aims to create a reducible cationic delivery agent for
siRNA based on polyethylenimine (PEI) disulfide-linked to lin-
oleylamine (LA-SS-PEI). The LA-SS-PEI was then complexed with
siRNAs by electrostatic interaction and evaluated for luciferase
siRNA delivery to SK-Hep-1 liver cancer cells stably transfect-
ed with luciferase. Methods: Linoleylamine-SS-PEI (LA-SS-PEI) was
prepared by a three-step method: 1. Linoleylamine and N-suc-
cinimidyl-3-(2-pyridyldithiol)propionate (SPDP) were combined
to produce LA-PDP. 2. PEI was combined with Traut’s reagent
reagent to produce PEI-SH. 3. The product of step 1 and 2 were
combined to produce LA-SS-PEI. LA-SS-PEI/siRNA complex was
prepared using an ethanol injection method. The physiochem-
ical properties of LA-SS-PEI/siRNA complexes, their particle
size, zeta potential, cellular uptake, reduction by glutathione,
and cytotoxicity were investigated. Luciferase silencing activity
of the complex was determined by luciferase assay. Results: Par-
ticle size of LA-SS-PEI/siRNA complex was 197.3±20.4 nm
and zeta potential was ±31.1±3.7mV. No significant toxicity
was found with LA-SS-PEI. Dissociation of the complex was
shown in the presence of 5 mM reduced glutathione, demon-
strating that the complex was reducible. Compared with
PEI/siRNA, LA-SS-PEI/siRNA was significantly more effective
with 54% greater cellular uptake and induced 58% reduction in
luciferase activity in SK-Hep-1 cells. Conclusion: In this study,
we synthesized a LA-SS-PEI conjugate and evaluated its ability
to deliver siRNA into SK-Hep-1 cells in vitro. LA-SS-PEI has the
characteristics of a multivalent polyamine-based cationic lipid
and the intracellular reduction of its disulfide bonds can medi-
ate intracellular breakdown of the complexes followed by effi-
cient release of siRNAs. Further evaluation of this agent for
siRNA delivery is warranted.

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Lee

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Association between Polymorphisms of PPM1E Gene
and Tumor Size of Hepatocellular Carcinoma Patients in
Korean Population
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Korea
Introduction Hepatocellular carcinoma (HCC) is the most com-
mon type of primary liver cancer in Korea. Recently, there is an
increasing evidence that polymorphism in genes may have a
role in altering the risk of HCC. Protein phosphatase plays a
crucial role in biological function and controls nearly every cel-
ular process. The protein phosphatase, Mg2+/Mn2+ dependent,
1E (PPM1E) inactivates multiple substrates including
5’-AMP activate protein kinases (AMPK) which inhibits the
growth and survival of cancer cells. However, no study on the
possible genetic association of PPM1E single nucleotide poly-
morphism (SNP) with HCC has been conducted yet. Patients &
Methods HCC patients (153 males and 30 females) and
healthy individuals (167 males and 224 females) were enrolled
in this study. The Age and sex of controls matched those of HCC
patients. We selected three exonic SNPs (rs16943333,
Regulation of miR-335 expression in association with methylation status of MEST promoter in hepatocellular carcinoma

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MicroRNAs (miRNAs) are expressed in a tissue-specific manner, and play important roles in development, cell proliferation, apoptosis, and oncogenesis. Some tumor-suppressive miRNAs are known to be epigenetically silenced by promoter DNA methylation in cancer. In the present study, we aimed to identify miRNA genes that are silenced by DNA hypermethylation in hepatocellular carcinoma (HCC). We screened for miRNA genes with promoter DNA hypermethylation using a genomewide methylation microarray analysis called Microarray-based Integrated Analysis of Methylation by Isoschizomers (MIAMI) in HCC cells. We compared DNA methylation profiles between three HCC cell lines (SNU449, Li-7, and PLC/PRF/5) and one normal liver tissue using the MIAMI method. The hypermethylated genes included eight miRNA genes (miR-let-7b, miR-101-2, miR-122a, miR-146b, miR-149, miR-200b, miR-335, and miR-497). Expression levels of six miRNAs (miR-let-7b, miR-101-2, miR-122a, miR-146b, miR-335, and miR-497) were lower in more than half of the 21 HCC cells than normal liver. Expression of four miRNAs (miR-101-2, miR-146b, miR-335, and miR-497) were restored with 5-aza-dCyd treatment in all three HCC cells. Further methylation assays including methylation-specific PCR, combined bisulfite and restriction analysis, and bisulfite sequencing indicated that aberrant DNA methylation within the CpG island of miR-335/MEST was evident in all three HCC cells. It was found that miR-335, which is harbored within an intron of its protein-coding host gene, MEST, was downregulated by aberrant promoter hypermethylation. The expression levels of miR-335 significantly correlated with those of MEST, supporting the notion that the intronic miR-335 is co-expressed with its host gene. The level of miR-335/MEST methylation was significantly higher in 18 (90%) out of 20 primary HCC tumors, compared to their non-tumor tissue counterparts (P<0.001). The expression level of miR-335 was significantly lower in 25 (78%) out of 32 primary HCC tumors, compared to their non-tumorous tissue counterparts (P=0.011). Since miR-335 was identified as a metastasis suppressor miRNA in breast cancer, we examined the relationship between the expression levels of miR-335 and the presence of distant metastasis in these 32 primary HCCs. The expression level of miR-335 was significantly lower in HCC tumors with distant metastasis than in those without distant metastasis (P=0.02). In conclusion, our results indicate that expression of miR-335 is reduced by aberrant DNA methylation in HCC.

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The following people have nothing to disclose: Osamu Dohi, Masayasu Jo, Yoshito Itoh

TXNDC5 Gene Polymorphism Contributes to Increased Risk of Hepatocellular Carcinoma in the Korean Male Population

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Thioredoxin domain-containing protein 5 (TXNDC5) has been found to be associated with cancer development and growth. We investigated whether TXNDC5 gene polymorphisms are associated with hepatocellular carcinoma (HCC) in Korean male population. Seven SNPs were selected based on minor allele frequency (≥0.5). The SNPs consisted of three exonic (rs8643, rs7764128 and rs1043784) and four intronic SNPs (rs1225944, rs1225943, rs1225945 and rs1225958). We selected and assessed these SNPs in 160 HCC patients and 178 controls. Genetic data were analyzed using SNPAnalyzer Pro, SNPStats, and Haploview programs. Two SNPs of the TXNDC5 gene were found to be associated with the risk of HCC development. The genotype frequency of rs1225944 was associated with HCC in the recessive model (CC/CT vs. TT, p=0.43, Fisher’s exact p=0.032, OR:0.54, 95% CI=0.11-2.71). The genotype frequency of rs1225943 was associated with HCC in the codominant 2 (AA vs. CC, p=0.07, Fisher’s exact p=0.001, OR:0.23, 95% CI=0.05-1.17), and log-additive models (p=0.08, Fisher’s exact p=0.002, OR:0.68, 95% CI=0.44-1.05). The haplotype CA and TC, consisting of rs1225944 and rs1225943, demonstrated a significant association with HCC. Our results suggest that TXNDC5 polymorphisms could be concerned with the development of HCC in the Korean male population.

Haplotype analysis of TXNDC5 polymorphisms

| Haplotype (block 1) | Control (+) | Control (-) | HCC (+) | HCC (-) | P value |
|--------------------|-------------|-------------|---------|---------|--------|
| CA                 | 260.9       | 91.1        | 241.7   | 50.3    | 0.008  |
| TC                 | 84.6        | 267.4       | 48.5    | 243.5   | 0.021  |

Disclosures:
The following people have nothing to disclose: Min Su Park, Youn-Hwan Nam, Sang-Mok Lee

TXNDC5 Gene Polymorphism Contributes to Increased Risk of Hepatocellular Carcinoma in the Korean Male Population

Min Su Park, Youn-Hwan Nam, Sang-Mok Lee; Surgery, Kyung Hee university, Seoul, Republic of Korea

Thioredoxin domain-containing protein 5 (TXNDC5) has been found to be associated with cancer development and growth. We investigated whether TXNDC5 gene polymorphisms are associated with hepatocellular carcinoma (HCC) in Korean male population. Seven SNPs were selected based on minor allele frequency (≥0.5). The SNPs consisted of three exonic (rs8643, rs7764128 and rs1043784) and four intronic SNPs (rs1225944, rs1225943, rs1225945 and rs1225958). We selected and assessed these SNPs in 160 HCC patients and 178 controls. Genetic data were analyzed using SNPAnalyzer Pro, SNPStats, and Haploview programs. Two SNPs of the TXNDC5 gene were found to be associated with the risk of HCC development. The genotype frequency of rs1225944 was associated with HCC in the recessive model (CC/CT vs. TT, p=0.43, Fisher’s exact p=0.032, OR:0.54, 95% CI=0.11-2.71). The genotype frequency of rs1225943 was associated with HCC in the codominant 2 (AA vs. CC, p=0.07, Fisher’s exact p=0.001, OR:0.23, 95% CI=0.05-1.17), and log-additive models (p=0.08, Fisher’s exact p=0.002, OR:0.68, 95% CI=0.44-1.05). The haplotype CA and TC, consisting of rs1225944 and rs1225943, demonstrated a significant association with HCC. Our results suggest that TXNDC5 polymorphisms could be concerned with the development of HCC in the Korean male population.

Haplotype analysis of TXNDC5 polymorphisms

| Haplotype (block 1) | Control (+) | Control (-) | HCC (+) | HCC (-) | P value |
|--------------------|-------------|-------------|---------|---------|--------|
| CA                 | 260.9       | 91.1        | 241.7   | 50.3    | 0.008  |
| TC                 | 84.6        | 267.4       | 48.5    | 243.5   | 0.021  |

Disclosures:
The following people have nothing to disclose: Min Su Park, Youn-Hwan Nam, Sang-Mok Lee
411 Effects of peripheral blood platelets on HCC cell growth, migration and invasion

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Background: Thrombocytopenia has been reported as a cirrhosis surrogate and as a predictor of HCC and was recently found to be mainly associated with small size HCCs, whereas thrombocytosis occurred with large HCCs (Carr, Guerra, Pancoska Oncology 2012; 83:339. Dig. Dis. Sci 2013; Jan 12 Epub). Aims: To investigate the effects of platelet factors on HCC cell growth. Methods: Extracts were made of time-expired pooled normal human donor platelets. Effects were examined on human HCC cell line growth and migration (PLC/PRF/5 cells) and by Matrigel assay for invasion (Huh7-GFP cells) in vitro. Results: Compared with 2% serum alone (controls), platelets increased HCC cell growth by 40-60%, measured by MTT assay at 72 hr. of treatment in culture. Cell stimulation required a minimum 24 hr. of exposure to the platelet extracts. Both cell migration and invasion were enhanced by 100 and 300%, respectively in the presence of platelet extracts. Sorafenib and Regorafenib caused a concentration-dependent suppression of cell growth, which was increased 3.3-fold and 2.1-fold respectively, by platelet extracts. Apoptosis was also antagonized, as measured using Annexin V. Percentages of apoptotic cells were: controls 7.6, Regorafenib 26, Regorafenib plus platelet extracts 10.5. Furthermore, platelet extracts decreased the levels of the apoptotic markers Bid, p-Bad and p-Bcl2 by WB and also increased AFP concentrations by 2-fold in the cell culture medium. Conclusions: Extracts of normal platelets enhance HCC cell growth, migration and invasion and antagonize apoptosis, as well as the growth inhibitory actions of both Sorafenib and Regorafenib. Platelets may thus have a role in HCC biology and may modulate HCC responses to therapy.

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The following people have nothing to disclose: Rosalba D’Allessandro, Maria G. Refolo, Catia Lippolis, Caterina Messa, Aldo Cavallini, Antonio Mazzocca, Brian I. Carr.

412 Molecular and clinical characterization of HBV-related hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is the most common form of liver cancer. Nearly 50% of HCC are caused by hepatitis B virus (HBV) infection. Aim: characterize a series of HBV-related HCC by studying the viral status, genetic alterations and the transcriptome, to better understand the role of HBV in hepatocellular carcinogenesis. Materials and methods: 86 HBV-related HCC was obtained by surgical resection. For each tumor, somatic mutations were investigated in nine genes. Expression of 37 genes involved in liver carcinogenesis including 16 genes to classify HCC into 6 groups (G1-G6) was studied. The HBs and HBx genes have been sequenced in all HCC and their non-tumor counterpart. Results: We identified inactivating mutations of HBx in 70% tumors and 32% non-tumor tissues (p<0.0001). TPS3 was the most mutated gene (41%, p=0.0002), including R249S mutations in 14 samples (16%, p<0.0001). This hot spot is associated with aflatoxin B1, developed in non-cirrhotic (p=0.01) tumors. IRF2 mutations were found exclusively in HBV-related HCC (p=0.03). Regarding the transcriptome groups (G1-G6), HBV-related HCC were more frequently classified in G1-G3 (57%, p=0.001). Overall, in the G1-G2, we observed a majority of young patients (age<60 years, p=0.003) and the presence of IFNε mutations (p=0.006). In the G2-G3, tumors were poorly differentiated (Edmondson III-IV, p=0.001) with a higher rate of early recurrence (<24 months, p=0.01). G2-G3 was strongly associated with TP53 mutations (p=0.0009), especially R249S (p=0.003). In the G1-G3 groups, tumors were larger (diameter>55 mm, p=0.006) with both Axin1(p=0.03) and HBx (p=0.001) inactivating mutations. G5-G6 constitutes a homogeneous group of HCC, composed by elderly patients (≥60 years, p=0.0007), strongly linked to CTNNB1 mutations (p<0.001). In general, G4-G6 was characterized by small tumors (<55 mm, p=0.006) and was associated with other cofactors (Alcohol/HCV/NASH, p=0.04). In addition, all the HCC classified in G1-G3 were characterized by overexpression of several genes involved in cell cycle and of genes encoding oncofoetal proteins such as EPCAM, KRT19, AFP and CCNB1 (p<0.001), while HCC in G5-G6 were characterised by the over expression of β-catenin-target genes: GLUL, TBX3, and RHBG(p<0.001). Conclusion: The TP53 pathway is the most altered in HBV-related HCC. Transcriptomic classification shows a predominant distribution in G1-G3. The cofactors are most frequently found in G4-G6. Inactivating mutations of HBx were associated with G1-G3.

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The following people have nothing to disclose: Qian Cao, Giuliana Amaddeo, Yannick Ladeiro, Sandrine Imbeaud.

413 miR-21 and miR-146a induce resistance to interferon-based therapy in hepatocellular carcinoma

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Purpose: The prognosis of patients with hepatocellular carcinoma (HCC) remains poor, particularly in patients with tumor thrombi in the major trunk of portal vein, even after curative resection of the tumor. We have reported clinical efficiency of interferon (IFN)-based therapy for advanced HCC. However, prediction of the response to the therapy remains unsatisfactory. Accordingly, it is necessary to find novel biological markers that can accurately predict the clinical response to the therapy. Recently, some investigators have reported a correlation between microRNAs (miRNAs) expression and chemoresistance in several types of cancers. In the present study, we identified miRNAs that govern the chemoresistance to the therapy in HCC. Methods: In the first experimental step, we focused on miRNAs that govern the chemoresistance to the therapy in parental cells (PLC-P) in order to identify the candidate miRNAs. Changes in the anti-tumor activity of parental cells (PLC-P) in response to the therapy was investigated in sur-
gically resected 30 HCC specimens. Results: miRNA microarray analysis showed that miR-146a expression level is significantly higher in PLC-Rs than in PLC-P. Based on this finding, miR-146a was selected as a candidate miRNA related to chemoresistance to IFN-α. HCC cells overexpressing miR-21 and miR-146a were significantly resistant to IFN-α through the suppression of apoptosis. Further experiments showed that the miR-21-related resistance to IFN-α is mediated through suppression of PTEN and PDCD4, and that the resistance to IFN-α induced by miR-146a is mediated through SMAD4 suppression. In clinical HCC specimens, miR-21 expression was significantly higher in non-responders to the IFN-based therapy than in the responders (P = 0.0109), and the overall survival rate of the miR-21 low-expression group was significantly better than that of the miR-21 high-expression group (P = 0.0250). Conclusions: The results indicated that miR-21 and miR-146a regulate the sensitivity of HCC cells to the cytotoxic effects of IFN-α, suggesting that these miRNAs could be potentially suitable markers for prediction of the clinical response and potential therapeutic targets in HCC patients on the IFN-based therapy.

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The following people have nothing to disclose: Yoshifumi Iwagami, Yoshito Tomi-maru, Hidetoshi Eguchi, Akira Tomakuni, Naoki Hama, Hiroshi Wada, Koichi Kawamoto, Shogo Kobayashi, Koji Umeshita, Yuichiro Doki, Masaki Mori, Hiroaki Nagano

414 Module-based analysis on Sorafenib-induced functional genomic changes of hepatocellular carcinoma reveals the optimal combination strategies of targeted drugs
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Sorafenib is the only approved targeted therapy for hepatocellular carcinoma (HCC) but its survival benefit on patients with advanced HCC is marginal as varying over a wide range depending on patho-genetic conditions. Thus, enhancing sorafenib sensitivity is essential for achieving efficient control of otherwise intractable HCCs. We employed a systems approach by combining biochemical experimentation and miRNA microarray analysis with in silico simulations to investigate the resistance mechanism and functional consequences of sorafenib. To this end, we analyzed sorafenib-induced miRNA changes in HCC cell lines by gene-module based analysis methods and found that, in the presence of sorafenib, metabolic response module, including glycolysis is activated. In addition, the effect of sorafenib on ATP cellular levels was also studied in human HCC cells and we found that sorafenib stimulated ATP production by up-regulated glycolysis, as indicated by higher amount of lactic acid formation in the presence of sorafenib. This sorafenib-stimulated ATP and lactic acid productions were significantly lowered in the presence of 3-bromopyruvate (3-BP), a hexokinase II inhibitor. Furthermore, in MTS assay, sorafenib cytotoxicity was significantly enhanced in the presence of 3-BP and this synergistic antitumor efficacy of sorafenib and 3-BP was confirmed in vivo mouse model. These results demonstrate that sorafenib sensitivity can be enhanced by adding more stress through a systems approach. Therefore, these combination strategies may efficiently be used in the management of otherwise intractable HCCs.

Disclosures:
The following people have nothing to disclose: Su Jong Yu, Jung-Hwan Yoon, Jae-Kyung Won, Yun Bin Lee, Yuri Cho, Dong Hyeon Lee, Joon Suk Kim, Jeong-Hoon Lee, Yoon Jun Kim, Hyo-Suk Lee, Chung Yong Kim

415 Quercetin suppresses human liver cancer cell growth through upregulating miR-34a expression caused by stabilizing p53
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Background and objective: Dietary polyphenols have been correlated with a reduced risk of developing cancer. Quercetin, an ubiquitous bioactive plant flavonoid, has been shown to inhibit cell proliferation in several cancer cell lines, including HepG2 cells, through modulating several signal transduction pathways. Recently, micro RNAs (miRNAs) have been identified as powerful posttranscriptional gene regulators. However, the effect of quercetin on miRNA regulation is largely unknown. The present study aims to determine whether quercetin could target miRNA, and the role of miRNA involved in anti-cancer effect of quercetin. Methods: HepG2 (p53 wild-type) and Huh7 (p53 mutant) cells were treated with quercetin for 24, 48 h and 72 h at various concentrations (1-100 μg/mL). Cell index calculation, Annexin V/PI, and cell cycle assay were used for determining the cellular growth inhibition, apoptosis, and growth arrest, respectively. MiR-34 inhibitor and p53 siRNA were used for down-regulating miR-34a and silencing p53, respectively. SQ-Real time-PCR was performed to analyze the expression of miR-34a and miR-34a target genes. And, western blotting was used to determine the expression level of p53 and phospho-p53. Results: We found HepG2 cells were more sensitive to quercetin than Huh7 cells, indicating that p53 get involved in the anti-cancer effect of quercetin. Quercetin suppressed the viability of HepG2 cells by inducing G2/M arrest and apoptosis. SQ-Real time-PCR data revealed that quercetin specifically up-regulated the expression of miR-34a, a major miRNA regulated by p53, in a dose- and time-dependent manner. Consistently, the up-regulation of miR-34a was found to be correlated with the stabilized p53 in HepG2 after quercetin treatment. Moreover, quercetin-induced up-regulation of miR-34a was significantly inhibited by p53 silencing. And miR-34a inhibitor abolished the down-regulation of miR-34a target genes, such as Cyclin E2, CDK4/6, bcl2, c-Myc, by quercetin treatment, and partially impaired the anti-proliferative effect of quercetin. These data further suggesting the involvement of p53/miR-34 axis in quercetin-induced apoptosis in HepG2 cells. Conclusions: Our results demonstrated, for the first time, the elevation of miR-34a by quercetin in liver cancer cell lines and this is mediated by the stabilization of p53. This event contributes to the anticancer activity of quercetin.

Disclosures:
The following people have nothing to disclose: Guohua Lou, Yanning Liu, Zhi Chen
416 Decreasing ALR expression in partially hepatectomized rats induces hepatocyte apoptosis and decreases cell proliferation
Antonio Francavilla, Barbara Pesetti, Angela Tafaro, Giussy Bianco, Francesco Russo, Michele Linsalata, Lorenzo Polimeno; IRCCS de Bellis medical center, Castellana Grotte, Italy

BACKGROUND: ALR is a sulfhydryl oxidase enzyme present in all mammalian tissues. We reported its anti-apoptotic and anti-oxidative activity both, in vivo (1) and, in vitro (3), in neoplastic glioma cells treated with specific ALR siRNA. In both experimental models, we demonstrated that ALR is a key factor for the maintenance of cell survival (apoptosis) cellular normoxic state control and for mitochondrial membrane integrity.

AIM: To evaluate the biological effects of ALR on hepatocyte apoptosis and proliferation by reducing AL expression in hepatic tissue of PH rats. MATERIALS AND METHODS: Seventy percent PH rats were administered, just after the surgery, with adenovirus-carrying AL shRNA (AD-shRNA) and followed for 48 hours. At the time of the sacrifice (12, 24, 48 hours after PH), polyamine levels, ALR expression (Western Blot analysis), cell proliferation (BrdU+ and mitotic index) and apoptosis (Bcl-2, Bax and activated caspase 3) and liver mass recovery were evaluated on hepatic tissue. Liver cell toxicity was determined by ALT serum levels and by histology. PH rats injected with adeno-LacZ were used as control. RESULTS: The AD-shRNA treatment did not show any cell toxicity. A statistically significant reduction of ALR expression was demonstrated at 12 and 24 hours after PH with a parallel decrease of hepatocyte proliferation and polyamine synthesis. In liver tissue of AD-shRNA treated rats, a decrease of anti-apoptotic factors (Bcl-2) and an increase of pro-apoptotic factors (activated caspase 3 and Bax) was observed. At 24 hr after partial hepatectomy, there were major expression differences in ALR protein and mitotic index, comparing rats treated and not treated with AD-shRNA: AD-shRNA treated: ALR:actin 4.7 mitotic index 0.8% Controls: ALR:actin 10.7 mitotic index 0.003 %; Controls: ALR:actin 10.7 mitotic index 0.8%. CONCLUSION: Our data confirm that abolishing ALR expression in vivo in 70% PH rats there is a complete change in the physiological state of hepatocytes with prevalence of apoptosis, increase of reactive oxygen species induced by surgery, decrease of polyamine synthesis, all parameters necessary for a correct liver mass regeneration after 70% PH confirming the physiological importance of ALR in the first phase of liver regeneration. References: (1) Francavilla A. et al. Hepatology 1994 (2) Polimeno L. et al. Free Rad. Res. 2011 (3) Polimeno L. et al. Cell Death Dis. 2012

Disclosures:

The following people have nothing to disclose: Antonio Francavilla, Barbara Pesetti, Angela Tafaro, Giussy Bianco, Francesco Russo, Michele Linsalata, Lorenzo Polimeno

417 INF-α siRNA increases Ad-GFP transduction and transgene expression in Huh7 cells
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Introduction: Recombinant adenoviruses (rAd) are the most common vectors used in clinical trials for gene therapy. Systemic administration of rAd show prominent tropism for liver. However, immune response of host against rAd which is orchestrated by Interferon type 1 (α and β) limits the therapeutic gene expression and prevents subsequent administrations. Aim: To evaluate the effect of IFNα inhibition by a small interfering RNA (siRNA) on rAd-GFP transduction and transgene expression in Huh7 cell line. Methods: Huh7 cells were cultured in DMEM, 5% FBS at 37 °C and 5% CO2 and then transfected with 70 nM of IFNα siRNA or Irrelevant-siRNA. Six hours later culture was exposed to 1 x 109 vp/ml of rAd-GFP for 24 hrs. Expression of IFNα and TNFα were determined by qRT-PCR. Cell transduction was analyzed by flow cytometry (FC) and qPCR. GFP protein was analyzed using western blot. Results: 70 nM of IFNα-siRNA inhibited 96% of IFNα gene expression (p < 0.001) and 65% of TNF-α(p < 0.05) compared to control irrelevant-siRNA. Ad-GFP transduction measured by FC and qPCR increased 39.2% and 27%, respectively in IFNα-siRNA treated cells compared to control. GFP protein also increased 50% when IFNα-siRNA was used compared to control. Conclusions: Inhibition of IFNα mRNA using an IFNα-siRNA permits a higher transgene expression (GFP) indicating the crucial role of IFNα on adenovirus elimination in transduced cells. This strategy could be useful in clinical trials conducted for liver diseases, where adenovirus is used as vector for therapeutic genes; allowing an increased transgene expression leading to better results in the resolution liver diseases.

Disclosures:

The following people have nothing to disclose: Ana A. Sobrevilla-Navarro, Ana Sandoval-Rodriguez, Jesus Garcia-Banuelos, Luis D. Hernandez-Ortega, Jose Macias-Barragan, Juan Armendáriz-Borunda, Adriana M. Salazar Montes

418 Interferon-Stimulated Gene Expression in Hepatitis C: Analysis Before and After Treatment with Interferon Post-Transplant
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Hepatic expression of interferon-stimulated genes (ISGs) is associated with HCV treatment response in nontransplant populations. Little is known about their expression in the post-transplant setting, where treatment response rates to interferon are lower. We examined hepatic ISG expression in patients before and after treatment with interferon and ribavirin (IFN+RV). Forty-one patients with recurrent HCV post-transplant treated with peg-IFN+RV were included in the study (genotype 1, n=32; 2, n=7; 3, n=2). All patients had fibrosis stage ≥2/6 or inflammation stage ≥8/18 before treatment; pre-treatment biopsies were collected within a year prior to treatment. Post-treatment biopsies were collected at an average of 350 days post-treatment with no difference in time between sustained viral response (SVR) and nonresponse (NR) groups. Patients with major complications other than recurrent HCV were excluded. ISG expression was studied by qPCR of hepatic mRNA. Nine predictive ISGs were analyzed. The population was divided into four groups for analysis based on pre- and post-treatment SVR and NR. Results: Pretreatment biopsies show no significant difference in the levels of hepatic mRNA of ISGs. In general, patients achieving SVR had slightly lower levels of ISGs than those who are eventual NR. However, in the post-treatment group, ISG levels are significantly higher in NR than SVR patients, by an average of 10-fold (figure, p<0.05***, p<0.01***). This data differs from studies in nontransplant populations where preactivation of ISGs is considered predictive of NR. In this post-transplant population, we do not see significant preactivation of ISGs. However, we see markedly higher levels in NRs in the post-treatment stage while SVRs remain low. It seems unlikely that per-
Gene expression analysis of recipient NK cells after Liver Transplantation demonstrates downregulation of STAT4

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Introduction The role of natural killer (NK) cell alloreactivity after liver transplantation (LT) remains undefined. We have previously demonstrated that NK cells from LT recipients are more difficult to activate than those from healthy controls, apart from those transplanted for hepatitis C virus (HCV). This suggests that the allograft can induce NK cell tolerance. In this study we investigated a mechanism for this through microarray analysis and subsequent quantitative RT-PCR. Methods Blood was collected from post LT patients attending out-patient clinics, including those infected with HCV (LT HCV), and uninfected patients (LT non HCV). RNA was extracted from purified NK cells and microarray analysis was performed on the Agilent Whole Genome Oligo Microarray platform. Gene expression was compared between LT HCV, LT non HCV and healthy controls (HC). Ingenuity Pathway Analysis (IPA) software was used to analyse differences in cellular processes and canonical pathways. Candidate genes were identified and expression of these in a further cohort of individuals was assessed using quantitative real-time RT PCR. Results Microarray analysis was performed on samples from 4 HCs, 4 LT non HCV and 4 LT HCV patients. Over 850 genes were differentially expressed, with the largest effects on cellular development, growth and differentiation and cell-to-cell signalling. JAK-STAT signalling was the most significant canonical pathway affected. Candidate genes were then selected for qRT-PCR in 13 HCs, 17 LT non HCV and 13 LT HCV patients. qRT-PCR confirmed the microarray data for STAT4, ZNF683 and KIR2DS3. STAT4 was significantly downregulated in both LT groups relative to HC (10.7, and 3.8 fold downregulation in LT non HCV (p=0.0004) and LT HCV (p=0.01) respectively). ZNF683 was upregulated (2-fold, p=0.06), and KIR2DS3 was downregulated (2-fold, p=0.05) in LT non HCV vs HC. Conclusions We have demonstrated that, after LT, there is altered gene expression in important differentiation and signalling pathways in recipient NK cells. Specifically, STAT4, which is highly downregulated regardless of HCV status, appears to be a key factor in this NK cell response to LT. These data suggest a derangement in IL-12 induced signalling of NK cells post liver transplantation.

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419 Serum MicroRNAs as differential markers and a molecular therapeutic target for HCV associated liver disease

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Aim: This study was conducted to assess the validity of using serum MicroRNAs (miRNAs) as novel noninvasive biomarkers for classifying liver disease and the candidate gene for molecular target therapy. Methods: MiScript miRNA PCR Arrays For SYBR Green-based, real-time PCR profiling of 13 microRNAs in 480 serum samples comprising; 192 HCC, 96 Liver Cirrhosis, 96 Chronic Hepatitis C and 96 Healthy Control subjects. Results: We have found significant fold changes in the expression levels of miRNA genes in patients’ sera of different HCV associated liver disease when compared to the control group. In chronic hepatitis C group, we observed a significant fold increasing in the expression level of miR-885-5p (p=0.003), miR-602 (p=0.01), miR-125a-5p (p=0.00006) and a significant fold decreasing in the expression levels of miR-29 (p=0.0008), miR-75 (p=0.00003). In the Cirrhotic group, we found a significant fold decreasing in the expression levels of miR-885-5p (p=0.01), miR-375 (p=0.000006), miR-22 (0.009) and miR-221 (p=0.001). In HCC group, a significant fold elevation in the expression level of miR-885-5p (p=0.03), miR-122 (p=0.021) and significant fold decreasing in miR-29 (p=0.007). In addition, we compared the groups with each other and there was significantly changes in expression level values of some miRNAs. In case of the cirrhotic versus the non-cirrhotic we found significant fold decreasing in the expression levels of 4 miRNAs “miR-885-5p (p=0.0002), miR-221 (p=0.02), miR-602 (p=0.04), miR-125a-5p (p=0.008),” while with HCC versus cirrhotic, a significant fold increasing was observed in 4 miRNAs “miR-885-5p (p=0.006), miR-221 (p=0.02), miR-122 (p=0.01), miR-181b (p=0.04).” Finally, on comparing HCC group versus non-cirrhotic group a significantly fold decreasing was reported in two miRNAs “miR-22 (p=0.026), miR-199a-3p (p=0.03).” Conclusion: The unique expression pattern of serum miRNAs can serve as fingerprint and noninvasive biomarkers for classifying liver disease. miR-885-5p is a potential differential marker between the infected HCV and the healthy control. In addition, miR-375 can serve as diagnostic marker for liver inflammation and cirrhosis and also may serve as a therapeutic target for HCV-positive HCC. As miR-375 targets yap protein and its mimics will inhibit hepatocarcinogenesis. miR-602 and miR-125a-5p can be used for early detection of hepatocellular carcinoma and targeted for molecular therapy. Downregulation of miR-221 and miR-22 can serve as potential diagnostic marker for liver cirrhosis.

Disclosures:
Gamal E. Esmat - Advisory Committees or Review Panels: MSD & BMS companies, MSD & BMS companies; Speaking and Teaching: Roche & GSK companies, Roche & GSK companies
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Hypoxic apoptosis of hepatocellular carcinoma triggers apoptosis of vascular endothelial cells

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Background/Aims: Hypoxia is deprivation of an adequate oxygen supply and induces hypoxic apoptosis. Hypoxia inducible factor-1α (HIF-1α) and interleukin (IL-8) activate tumor survival in different pathways. We evaluated whether adenovirus-mediated small hairpin RNAs for HIF-1α (shHIF-1α) and IL-8 (shIL-8) induced apoptosis in hepatocellular carcinoma (HCC) and endothelial cell lines. Methods: The HCC cell line was infected with an adenovirus expressing shRNA for HIF-1α and IL-8 and maintained under hypoxic conditions (1% O2, 24 h). The expression levels of HIF-1α and apoptotic and growth factors were examined by real-time quantitative polymerase chain reaction and Western blotting. We also investigated apoptosis by terminal deoxynucleotidyl transferase dUTP nick-end-labeling assay (flow assisted cytometry and immunofluorescence) and measured cytochrome c levels. Results: Inhibiting HIF-1α and IL-8 up-regulated the expression of apoptotic factors while simultaneously down-regulating anti-apoptotic factors. Knockdown of HIF-1α and IL-8 increased cytochrome c concentration and enhanced DNA fragmentation in the HCC cell line and human umbilical vein endothelial cells (HUVECs). Moreover, the culture supernatant collected from knockdown of HIF-1α and IL-8 in the HCC cell line induced apoptosis in HUVECs under hypoxia. Conclusions: These data suggest that adenovirus-mediated knockdown of HIF-1α and IL-8 induced apoptosis in HCC and triggered apoptosis in vascular endothelial cells.

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MicroRNA-29a regulates epithelial-mesenchymal transition via repression of E-cadherin expression by modulating CpG promoter methylation in hepatocellular carcinoma

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[Backgrounds] Transforming growth factor (TGF)-β induces epithelial-mesenchymal transition (EMT) which is a crucial step for invasion and metastasis in various types of cancer. Reduced expression of E-cadherin, a hallmark of EMT, is reported in hepatocellular carcinoma (HCC), however, involvement of microRNAs (miRNAs) in this process is poorly understood. CDH1, which encodes E-cadherin, has CpG islands in the promoter region. DNA methylation of CpG islands are regulated by DNA methyltransferases (DNMTs) which are the targets of miR-29a. We investigated the involvement and role of miR-29a in epigenetic regulation of E-cadherin expression during the process of EMT induced by TGF-β. [Methods] Human HCC cell lines, PLC/PRF/5 and HepG2 were treated with 1-10 ng/ml of TGF-β for ~72 hours to induce EMT. Expression of E-cadherin was examined by using real-time qPCR and immunoblotting. Methylation specific PCR (MSP) was performed to determine the methylation level of CpG islands in the E-cadherin promoter that contains E-boxes. To force the expression of miR-29a, cells were electroporated with synthetic precursor miR-29a. [Results] After treatment with TGF-β (1-5 ng/ml, 48 hours), HCC cells showed EMT feature with spindle-shape morphology, and a decrease of E-cadherin and an increase of vimentin were detected by immunoblotting. After treatment with TGF-β (10 ng/ml, 96 hours) the methylation of E-cadherin promoter was induced (35.5 ± 1.96% in PLC/PRF/5), which was abrogated by pre-treatment with a methyltransferase inhibitor 5-aza-2’-deoxycytidine (5-Aza) indicating the involvement of DNA methylation in this process. Treatment of HCC cells with TGF-β (10 ng/ml, 72 hours) increased protein expression of DNMT3B and DNMT1, which are reported as targets of miR-29a. After treatment of cells with TGF-β (10 ng/ml), expression of miR-29a decreased by 27.8% in PLC/RF/5 cells and by 26.7% in HepG2 cells by 72 hours. Transfection of precursor miR-29a in PLC/PRF/5 cells partially blocked the suppression of E-cadherin protein expression (control, 48%; miR-29a, 72%) and methylation level of the promoter CpG islands (control, 27.3 ± 9.15%; miR-29a, 13.7 ± 2.85%) induced by TGF-β. [Conclusion] We show that the involvement of miR-29a in TGF-β-induced EMT via epigenetic regulation of E-cadherin in HCC cells. These observations identify miR-29a as a unique mechanism of the regulation of EMT in HCC.

Disclosures:

The following people have nothing to disclose: Takayuki Kogure, Yasuteru Kondo, Eiji Kakazu, Masashi Ninomiya, Osamu Kimura, Tomaoki Iwata, Tatsuki Morosawa, Yusuyuki Fujisaka, Tooru Shimosegawa

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Targeting the Hippo pathway to improve the regenerative capacity of the liver

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Background: Protein kinases MST1 & MST2 are the core of the Hippo pathway, which inactivate the transcriptional co-activator YAP via LATS phosphorylation. Inhibition of MST1 & MST2 leads to the activation of YAP where it translocates to the nucleus and promotes the transcription of pro proliferative genes. We hypothesize that knockdown (KD) of MST1 & MST2 will push hepatocytes into cell cycle through activation of YAP.

Methods: We are exploring a gene therapy approach using siRNAs coupled with liposomes to target the Hippo pathway to promote hepatocyte proliferation in non-regenerating livers. Results: We identified siRNA sequences that lead to >92 and 89% KD of MST1 and MST2 in a mouse liver hepatoma cell line in vitro. siRNA:liposome complexes injected i.v. resulted in 80% KD of expression in the liver using FVII as a control gene target. Using siRNAs targeting MST1 & MST2 with liposomes reduced expression to 66 and 40%, respectively in liver after 72 hours. The KD was confirmed by RT-qPCR and immunoblot. KD of MST1 and MST2 in mouse liver resulted in an increase of nuclear Yap localization and hepatocyte proliferation measured by incorporation of EdU and Ki67 immunostaining. After
MST1 and MST2 KD there was a 3 and 5-fold increase of BIRC5/survivin and Foxm1, respectively -both YAP target genes normally up-regulated in a regenerating liver. Conclusion: The KD of MST1 & MST2 provokes nuclear YAP translocation and hepatocyte proliferation in wild-type mice. To determine if targeting MST1 & MST2 is clinically relevant, we will demonstrate that there is impairment of this pathways in diseased livers or small-for-size during regeneration.

424 Developing an RNAi Therapeutic for Liver Disease Associated with Alpha-1-Antitrypsin Deficiency

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AIM: Liver disease associated with Alpha-1-antitrypsin (AAT) deficiency is a common cause of both morbidity and mortality in patients. We propose that down regulating the mutant allele (Z-AAT) at the mRNA level will prevent the progression and development of liver disease. METHODS: A set of chemically modified siRNAs designed using bioinformatic algorithms was synthesized and screened by transfection in Hep3B cells for activity leading to selection of a highly potent siRNA for further studies. The siRNA was tested in transgenic mice expressing human Z-AAT. These mice develop liver disease and show protein accumulation similar to the human patients. RESULTS: Intravenous administration of theAAT siRNA, formulated in a lipid nanoparticle (LNP-AAT), led to dose-dependent inhibition of human AAT-Z mRNA in livers, with maximum inhibition of 95% observed at a dose of 1 mg/kg, and approximately 85% inhibition at a ten-fold lower dose. Similar reductions in circulating hAAT-Z protein levels were also detected (90% at 1 mg/kg, approximately 50% at 0.1 mg/kg). Additional disease-related endpoints were evaluated in hAAT-Z transgenic mice after seven bi-weekly doses of 0.3 mg/kg LNP-AAT siRNA. Immunoblot analysis of liver lysates demonstrated a 45% reduction in Z-AAT polymers; the precursors of the pathogenic aggregates. A decrease in the extent of liver injury as measured by markers of fibrosis (75% decrease in collagen 1a1 expression) and hepatocyte proliferation (91% reduction in BrdU incorporation) was also observed. Similar physiological benefit as measured by decreased proliferative index was obtained with long-term dosing in aged animals with advanced liver disease. In parallel to the LNP-AAT, we have also developed a N-acetylgalactosamine (GalNAc) -conjugated siRNA (ALN-AAT). This allows for subcutaneous administration and is taken up by the hepatocytes via asialoglycoprotein receptor. ALN-AAT was found to efficiently reduce the levels of mutant circulating protein in these mice in a dose-dependent manner. CONCLUSION: The efficacy achieved by administration of RNA therapeutics targeting AAT in a well-established mouse model of AAT-deficiency associated liver disease supports further development of this novel therapeutic strategy.

Disclosures:
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425 Mutation Analysis of 42 Chinese Patients with Wilson Disease

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Background: Mutation of the ATP7B gene is the cause of Wilson's disease(WD), which is an autosomal recessive disorder. The missense mutation Arg778Leu is the most common mutation found in patients ascertained from the southern part of China. However, data regarding the ATP7B mutation in the northern part, especially in Jilin Province, is unknown. Objective: To determine mutation frequency of ATP7B gene in WD patients from Jilin Province of China. Methods: 42 Chinese patients clinically diagnosed with WD from 40 unrelated families from Jilin Province were enrolled in this study. Family members from 19 families were available for the family screening study. 60 healthy Chinese people participated in the control group. DNA was extracted from peripheral blood and whole coding region of the 21 exons of the ATP7B gene were sequenced by Sanger method and analyzed by a commercial mutation software. Results: Mutation analysis revealed 26 identified pathogenic mutations. The frequency of Arg778Leu(c.2333G>T) and Pro992Leu(c.2975C>T) was 28.3% and 14.1% respectively. A