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**Results:** Mitogen-activated protein kinase 13 (MAPK13) overexpression was observed in all our tumour specimens. Subgroup analysis showed that MAPK 13 overexpression correlated with shorter survival time, and survival gradually worsened with increasing MAPK13 scores. MAPK13 overexpression was found to correlate with tumour stage.

**Conclusion:** MAPK13 overexpression is a reliable prognostic marker for human cholangiocarcinoma and represents a potential target for targeted therapeutic interventions.

## **P-006 PTEN underexpression was associated with more aggressive tumor behaviour in hepatocellular carcinoma and PTEN suppressed cell invasion by downregulating NF- $\kappa$ B signaling pathway**

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**Background:** Hepatocellular carcinoma (HCC) is a major malignancy worldwide. The disease is often diagnosed at late stage and frequently associated with metastasis, when only limited options are then available for effective therapies. Phosphatase and Tensin Homolog (PTEN) is a tumor suppressor implicated in various cancers. However, there are relatively few reports delineating the role of PTEN in HCC development.

**Objectives:** This study aimed to characterize the role of PTEN in HCC.

**Methods:** We analyzed the expression of PTEN in human HCCs and correlated it with clinicopathological findings and patients' survivals. We also studied the cell migration and invasion abilities and metalloproteinases (MMPs) in HCC cells and PTEN-null mouse embryonic fibroblasts (MEFs) in relation to PTEN.

**Results:** In human HCCs, we found frequent (46%, N=41) underexpression of PTEN in the tumors as compared with their corresponding nontumorous livers. In addition, PTEN underexpression was significantly associated with larger tumor size ( $p = 0.024$ ) and presence of tumor microsatellite formation ( $p = 0.021$ ), the latter being a feature of intrahepatic metastasis in HCC. Significantly, it was also associated with shorter overall survival of patients ( $p = 0.021$ ). Stable knockdown of PTEN in SMMC7721 and BEL7402 HCC cells showed significant enhancement of cell migration and invasion, as demonstrated with transwell and Matrigel invasion assays, respectively, giving relevance of PTEN in HCC metastasis. We established PTEN stable knockdown HCC clones and PTEN-null MEFs. We found marked upregulation (by 3.5 - 10 folds) of MMP2 in these cell models. Furthermore, enzymatic cleavage of MMP2 was observed in the PTEN-null MEFs as demonstrated by gelatin zymography. Transient knockdown of PTEN resulted in activation of the NF- $\kappa$ B promoter activity. Furthermore, the PTEN-null MEFs had upregulation of NF- $\kappa$ B protein level. With bioinformatics analysis, we found two putative NF- $\kappa$ B binding motifs on MMP2 promoter.

**Conclusion:** Taken together, our data showed that PTEN was underexpressed in our human HCCs and its underexpression was associated with more aggressive tumor behavior. Our findings also suggested that PTEN suppressed cell migration and invasion by downregulating NF- $\kappa$ B signaling pathway.

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## **P-007 Association between pre-S, basal core promoter, precore mutations and risk of hepatocellular carcinoma in patients with HBV**

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**Background:** Mutations in hepatitis B virus (HBV) are known to be related with development of hepatocellular carcinoma (HCC). To date, however, association of mutations has been investigated mainly with single mutation.

**Objectives:** The aim of this study was to compare the frequency of known mutations of HBV in combination to analyze the association between HCC.

**Methods:** In this study, 135 patients with HBV-related HCC (HCC group) were compared with 135 patients with HBV but without HCC (non-HCC group), who were matched for age, sex and HBeAg status, and the pattern of mutations were analyzed. Amplification and direct sequencing of the pre-S, basal core promoter (BCP), and precore (PC) region was performed using nested PCR with specific primers after extraction of HBV DNA from serum.

**Results:** The baseline characteristics between HCC group and non-HCC group, respectively, were as follows: mean age (44.3 $\pm$ 7.8 vs. 44.3 $\pm$ 8.0 yrs, matched), proportion of male sex (83 vs. 83%, matched), HBeAg positivity (71.9 vs. 71.9%, matched), and mean HBV DNA (3.70 $\pm$ 3.42 vs. 3.45 $\pm$ 3.81 log<sub>10</sub> copies/ml,  $p=0.593$ ). In the HCC and non-HCC group, respectively, there were 25 (18.5%) vs. 6 (4.4%) patients with pre-S deletion mutants ( $p=0.001$ , OR=4.649, 95% CI=1.834-11.781), 82 (60.7%) vs 30 (22.2%) patients with BCP mutants ( $p<0.001$ , OR=5.415, 95% CI=3.178-9.226), and 35 (25.9%) vs. 34 (25.2%) patients with precore mutants ( $p=0.889$ ). When comparisons were made between patients with combined mutations, odds ratio was highest in patients with both pre-S deletion and BCP mutants (16 (11.8%) vs. 2 (1.5%),  $p<0.001$ , OR=8.941, 95% CI=2.014-39.698).

**Conclusion:** Our data demonstrate that HCC was associated with pre-S and BCP mutation, and combination of both mutation had a stronger association compared with single mutation.

**References:** Chen BF, et al. High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. *Gastroenterology*. 2006;130:1153-68.

## **P-008 JNK inhibition suppresses chemically induced rat HCCs and proliferation of human HCC cells via the switching of Smad3 signaling**

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**Background:** Among several factors implicated in hepatocarcinogenesis, recent reports highlight JNK activation and the phosphorylation of Smad3 as key steps in progression of HCC. In particular, Smad3 is converted into 2 distinctive phospho-isoforms: C-terminally phosphorylated Smad3 (pSmad3C) and linker-phosphorylated Smad3 (pSmad3L). Previous studies suggested that TGF- $\beta$  type I receptor (T $\beta$ RI)/pSmad3C pathway inhibits growth of epithelial cells including hepatocytes, whereas JNK/pSmad3L-mediated signaling promotes hepatic fibro-carcinogenesis in HCV-related chronic liver disorders.

**Objectives:** The aim of this study is to elucidate the role of JNK/pSmad3L and to evaluate the effect of JNK inhibition on both rat HCC carcinogenesis and human HCC cells.

**Methods:** (1) Chemical-induced rat HCC. Male Wistar rats were fed with 100ppm diethylnitrosamine (DEN) in drinking water for 8 weeks and kept for an additional 4 weeks without DEN. One week after DEN administration, rats were randomly assigned to either JNK inhibitor (SP600125) group or vehicle control group. Rats received subcutaneous injections 11 times weekly and were sacrificed for evaluation of HCC development one week after the last injection. (2) Human HCC cell line. Huh7 cells were infected with adenoviral vectors encoding dominant negative JNK1 (Ad-dnJNK1) and green fluorescent protein (Ad-GFP) as a control. Proliferation of cells was quantified 2 days after the Ad-dnJNK1 or Ad-GFP infection using cell counting kit (CCK) assay. (3) Human HCC samples. Phosphorylation of c-Jun was evaluated with Western blotting in both non-HCC and HCC tissue samples.

**Results:** (1) C-Jun was phosphorylated even 7 days after DEN administration, which was suppressed by single administration of SP600125. The number of tumor nodules greater than 3mm in diameter was significantly lower in JNK inhibitor group than that in vehicle control group (7.9 $\pm$ 3.1 vs. 18.0 $\pm$ 3.5;  $p<0.001$ ). The liver weight/body weight ratio was significantly lower in JNK inhibitor group than in vehicle control group (6.3 $\pm$ 1.2 vs. 7.1 $\pm$ 0.7;  $p<0.05$ ). Body weight and serum ALT were not different between the two groups. DEN induced pSmad3L expression and suppressed pSmad3C expression in non-HCC and HCC tissue as the tumors were enlarged and progressed. Although there were no differences in pSmad3C expression between two groups, SP600125 suppressed phosphorylation of Smad3L and c-Myc expression through down-regulation of phosphorylated c-Jun. JNK inhibition significantly prolonged median survival time in JNK inhibitor group (105 vs. 89 days;  $p<0.05$ ). (2) Inhibition of JNK activation by Ad-dnJnk1 significantly attenuated the proliferation of cultured Huh7 cells ( $p<0.05$ ). (3) C-Jun was clearly phosphorylated in human non-HCC tissue with HCV than in non-HCC tissue with HBV. Furthermore, c-jun phosphorylation was enhanced in both HBV and HCV related HCCs.

**Conclusion:** Inhibition of the JNK/pSmad3L pathway with SP600125 suppressed the progression of both rat HCC progression and human HCC cells. Thus, JNK targeting might be a promising approach in HCC treatment.

## **P-009 Tumor tissue response to ABT-869, a novel multi-targeted tyrosine kinase inhibitor, observed in an orthotopic hepatocellular carcinoma (HCC) model using MRI**

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