



| | |
|--------------------|---|
| Title | Pim1 is upregulated by hypoxia in hepatocellular carcinoma and promotes tumor progression |
| Author(s) | Lo, R; Leung, CON; Kai, AKL; Wong, C; Ng, I |
| Citation | The 8th Annual Conference of the International Liver Cancer Association (ILCA 2014), Kyoto, Japan, 5-7 September 2014. In Book of Abstracts, 2014, p. 38, abstract P-022 |
| Issued Date | 2014 |
| URL | http://hdl.handle.net/10722/211332 |
| Rights | Creative Commons: Attribution 3.0 Hong Kong License |

P-020 NF-KB MEDIATED INHIBITION OF HISTONE DEACETYLASES BY CURCUMIN SPECIFICALLY TARGETS STEM-LIKE HEPATOCELLULAR CARCINOMA

J. U. Marquardt^{1,†}, L. Gomez-Quirez², L. O. Arreguin Camacho¹, F. Pinna³, M. Kitade⁴, J. B. Andersen⁴, K. Breuhahn³, P. R. Galle¹, V. M. Factor⁴, S. S. Thorgeirsson⁴

¹Department of Medicine I, University Hospital Of Mainz, Mainz, Germany, ²Departamento de Ciencias de la Salud, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, Mexico, Mexico, ³Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany, ⁴Laboratory of Experimental Carcinogenesis, CCR/NCI/NIH, Bethesda, United States

Introduction: Cancer stem cells (CSCs) have emerged to attractive cellular targets for the therapy of many solid tumors, including hepatocellular cancers. We have recently reported that activation of NF-κB signaling is consistently observed in human liver CSCs. Based on these data, we hypothesized that NF-κB may be a specific therapeutic target against CSCs.

Methods: Inhibition of NF-κB signaling was performed using (i) curcumin, an effective IKK inhibitor, (ii) siRNA against p65 and (iii) the specific inhibitory peptide SN50. Anti-proliferative and pro-apoptotic capacity was evaluated in different liver cancer cell lines. The effect on CSC was assessed by the Side Population (SP) approach, and expression levels of selected targets determined by RT-qPCR, gene expression microarray, EMSA, and Western blotting.

Results: Specific inhibition of NF-κB signaling by SN50 and siRNA caused a general suppression of cell growth accompanied by a drastic reduction in CSC properties. Curcumin treatment caused anti-proliferative and pro-apoptotic responses directly related to the extent of NF-κB inhibition. In curcumin-sensitive cell lines, the treatment selectively depleted CSCs and led to down-regulation of the CSC markers CD133, EpCAM, NANOG and c-Kit. Conversely, curcumin-resistant cells exhibited a paradoxical response. Mechanistically, CSC-depleting activity was exerted by NF-κB mediated HDAC inhibition leading to down-regulation of c-MYC and other key oncogenic targets. Co-administration of a class I and II HDAC inhibitor sensitized the curcumin-resistant cells to curcumin treatment. Further, integration of a predictive signature with our HCC database indicated that HCC patients with poor prognosis and progenitor cell features are most likely to benefit from NF-κB inhibition.

Conclusion: These data demonstrate that NF-κB inhibition by curcumin can specifically target CSC populations. Future investigations will determine the potential of combined inhibition of NF-κB signaling and HDAC for CSC-directed HCC therapy.

Disclosure of Interest: None Declared

P-021 THE MULTIKINASE INHIBITOR K252A INDUCES MESENCHYMAL-EPIHELIAL TRANSITION IN A MOUSE XENOGRAFT MODEL OF HEPATOCELLULAR CARCINOMA

M. Abe^{1,†}, H. Koga¹, T. Yoshida¹, H. Masuda¹, M. Sakata¹, Y. Ikezono¹, T. Nakamura¹, E. Taniguchi¹, T. Kawaguchi¹, H. Yano², T. Torimura¹

¹Division of Gastroenterology, Department of Medicine, ²Department of Pathology, Kurume University, Kurume, Japan

Introduction: There are several ongoing clinical trials of molecular-targeted therapy for hepatocellular carcinoma (HCC), but no clearly effective therapy has yet emerged. Under such conditions, we have been focusing on potentially powerful anticancer effects of the novel multikinase inhibitor K252a, since it targets unique kinases, including PKC, CaMK II, c-Met, TrkB, MLCK, and phosphorylase kinase. Indeed, the former five targets are expressed in HCC tissues and involved in cellular proliferation, invasion, and metastasis (Yoshiji et al., Cancer Res 1999, etc.). The broad spectrum of the targets may block escape pathways in HCC cells, that are resistant to conventional anticancer drugs and even sorafenib. The **Aim** of this study was to assess whether K252a altered malignant phenotypes such as epithelial-mesenchymal transition (EMT) both *in vitro* and *in vivo*.

Methods: The human HCC cell lines HAK-1A, HAK-1B (Yano et al., Hepatology 1993), KYN-2 (Yano et al., Pathol Int 1988), and Huh7 were used in this study. Protein expression and localization was analyzed by Western blot and immunocytochemistry, respectively. Cellular mRNA level was evaluated by real-time PCR using TaqMan probes. HAK-1B-based xenograft model in nude mice was used to evaluate *in-vivo* efficacy of K252a.

Results: K252a induced both polygonal transformation in cell shape and increase in cell size in several HCC cell lines having endogenous mesenchymal features. In Western blot analysis and immunocytochemistry, the expression levels of E2A, an EMT-regulating transcriptional factor, were universally decreased in the cells treated with K252a, in concert with increased expression levels of E-cadherin in some cell lines. In real-time PCR analyses, the decrease in E2A mRNA levels and the increase in E-cadherin mRNA levels were ubiquitously found. Of note, xenografted tumor tissues obtained from mice treated with K252a exhibited significant increase in E-cadherin protein expression, in comparison with those from control mice.

Conclusion: Our findings suggest that K252a has antitumor effects on HCC through, at least in

part, reversing EMT. This kind of multikinase inhibitor may help us establish the proof-of-concept for closing escape routes in drug-resistant HCC cells.

Disclosure of Interest: None Declared

P-022 PIM1 IS UPREGULATED BY HYPOXIA IN HEPATOCELLULAR CARCINOMA AND PROMOTES TUMOR PROGRESSION

R. Lo^{1,2,†}, C. Leung^{1,2}, A. Kai^{1,2}, I. Xu^{1,2}, C. Wong^{1,2}, I. Ng^{1,2}

¹State Key Laboratory for Liver Research, ²Pathology, The University of Hong Kong, Hong Kong, Hong Kong

Introduction: Hepatocellular carcinoma (HCC) is the second/third most common fatal cancer in Hong Kong and Southeast Asia associated with frequent tumor recurrence and metastasis. Apart from surgical intervention, tumor control at cellular and molecular levels can possibly improve clinical outcome. HCC is characteristically one of the most rapidly proliferating tumors which often outpace functional blood supply, leading to a regional oxygen deprivation. Therefore, molecular changes induced by hypoxia are attractive therapeutic targets. Overexpression of PIM1, a serine/threonine kinase, has been identified in recent years in solid cancers such as prostate cancer, gastric cancer, and pancreatic cancer. In the latter, PIM1 was upregulated by hypoxia. In this study, we aim at investigating the expression, functional role, and regulatory mechanism of PIM1 in HCC, which have not been reported to date.

Methods: Expression of PIM1 in clinical HCC samples and HCC cell lines was assessed by immunohistochemical method and Western blotting, respectively. Functional significance of PIM1 in HCC was examined by cell proliferation and Matrigel invasion assays.

Results: Immunohistochemical analysis in 20 paired primary and extra-hepatic metastatic HCC tissues showed that PIM1 was overexpressed in 10 (50%) primary HCC tissues. In the corresponding extra-hepatic metastatic HCC tissues, 17 cases (85%) expressed PIM1. These findings suggest that PIM1 overexpression may play a role in promoting HCC metastasis. By Western blotting, PIM1 expression was markedly upregulated in multiple HCC cell lines upon hypoxic condition (1% O₂) versus normoxia (20% O₂). By *in vitro* experiments with stable PIM1-knockdown clones, we observed a decrease in cell invasion as compared to non-target controls. This inhibitory effect became more pronounced under hypoxic condition. In addition, knockdown of PIM1 decreased cell proliferation rate *in vitro*. To explore the regulatory mechanism of PIM1 upregulation under hypoxia in HCC, we performed a time-point experiment, in which upregulation of PIM1 protein expression occurred at ~30 minutes upon hypoxic treatment and preceded that of HIF-1α, suggesting that the upregulation is HIF-1α independent and possibly related to post-translational events. By treating HCC cells with MG132 in normoxia, PIM1 protein level was stabilized and increased. The result suggested that hypoxia directly induced PIM1 by preventing the proteosomal degradation of PIM1 in HCC.

Conclusion: PIM1 is upregulated by hypoxia in HCC in an HIF-1α independent manner and promotes tumor growth and metastasis.

Disclosure of Interest: None Declared

P-023 ESTABLISHMENT AND CHARACTERIZATION OF LIVER CANCER CELL LINES FROM GNMT GENE KNOCKOUT MICE

C.-H. Yen^{1,†}, C.-C. Lai², Y.-M. Chen³

¹Graduate Institute of Natural Products, ²Kaohsiung Medical University, Kaohsiung, Taiwan, ³Center for Infectious Disease and Cancer Research, Kaohsiung Medical University, Kaohsiung, Taiwan

Introduction: Primary liver cancer is the fifth most common cancer worldwide and the third most common cause of cancer mortality. Hepatocellular carcinoma (HCC) accounts for more than 80% of primary liver cancers. The expression of Glycine N-methyltransferase (GNMT), a tumor suppressor gene for HCC, is down-regulated in more than 75% HCC patients. Previously, we reported that very high rate of *Gnmt* gene knockout (*Gnmt*^{-/-}) mice developed HCC at about fourteen to eighteen months. The objective of this study is to establish and characterize HCC cell line from this mouse model.

Methods: Liver tumor from *Gnmt*^{-/-} mice were cut into small pieces and digested by collagenase and trypsin. The resultant cells were seeded in culture dishes and subcultured for more than 60 passages. Allograft tumors were established both subcutaneously and intrahepatically and were harvested for histological examination. The expression profile of genes related to EMT and cancer stem cell like properties were determined by Q-PCR and western blot. Side population assay and sphere forming assay were used to evaluate the cancer stem cell like properties of our cell lines.

Results: We established three liver cancer cell lines, Ymac-1, Ymac-2, and Ymac-4. To investigate the tumorigenicity of these cell lines *in vivo*, they were injected subcutaneously into NOD/SCID mice. Ymac-1 and Ymac-4 can form tumor *in vivo*, but Ymac-2 cannot. The histopathological study of tumors from Ymac-1 and Ymac-4 revealed that Ymac-1 has spindle shape like sarcomatoid hepatocyte carcinoma (SHC) morphology and Ymac-4 has bile duct like Cholangiocarcinoma