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<th>Pim1 is upregulated by hypoxia in hepatocellular carcinoma and promotes tumor progression</th>
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<td>Author(s)</td>
<td>Lo, R; Leung, CON; Kai, AKL; Wong, C; Ng, I</td>
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P-020  NF-KB MEDIATED INHIBITION OF HISTONE DEACETYLASES BY CURCUMIN SPECIFICALLY TARGETS STEM-LIKE HEPATOCellular CARCINOMA

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Introduction: Cancer stem cells (CSCs) have emerged to attract 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, NG2, 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. Furthermore, 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-EPIThelial TRANSITION IN A MOUSE XENOGRAFT MODEL OF HEPATOCellular CARCINOMA

M. Abe 1,2, H. Koga 1, T. Yoshida 1, H. Maeda 1, M. Sakuta 1, Y. Kozono 1, T. Nakamura 1, E. Taniguchi 1, T. Kawaiuchi 1, H. Yano 1, T. Toriumi 1

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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 phospholipase kinase. Indeed, the former five targets are expressed in HCC tissues and involved in cellular proliferation, invasion, and metastasis (Yoshii 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 spectrum of the targets may block escape pathways in HCC cells, that are resistant to conventional anticancer drugs, even sorafenib. The spectrum of the targets may block escape pathways in HCC cells, that are resistant to conventional anticancer drugs, even sorafenib. The spectrum of the targets may block escape pathways in HCC cells, that are resistant to conventional anticancer drugs, even sorafenib. The spectrum of the targets may block escape pathways in HCC cells, that are resistant to conventional anticancer drugs, even sorafenib.

Methods: The human HCC cell lines HAK-1A, HAK-1B (Yano et al., Hepatology 1993), KYN-2 (Yano et al., Patil P 1986), 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 EZH2, 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 analysis, the decrease in EZH2 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

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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 characterized 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%, P<0.01). 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 IF-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

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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, has been reported to date. However, the functional role of GNMT in HCC has not been fully understood.

Methods: Human HCC cell lines HAK-1A, HAK-1B (Yano et al., Hepatology 1993), KYN-2 (Yano et al., Patil P 1986), 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 EZH2, 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 analysis, the decrease in EZH2 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