

## Inhibition of MAPK signaling promotes cell cycle arrest and sensitizes intrahepatic cholangiocarcinoma cells to chemotherapy.

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**Introduction and Aim:** Intrahepatic cholangiocarcinoma (ICC) is the second most common primary hepatic malignancy, accounting for approximately 15% of cases of primary liver cancer. Although new treatments have increased survival for many other cancers, including the more common primary hepatocellular carcinoma, treatment strategies and survival for patients with ICC have seen little improvement. Our previous studies suggest that the mitogen-activated protein kinase (MAPK) signaling plays a central role in the regulation of cell proliferation in human ICC. However the molecular mechanisms are poorly understood. In this study, we aim to explore whether chemical inhibition of the MAPK pathway and its downstream effectors enhances the sensitization of ICC cells to the chemotherapeutic agent cisplatin.

**Methods:** We used a combinatorial approach of immunohistochemical and gene expression analyses of microarray datasets to investigate the expression of MAPK-related genes in ICC tumors. Furthermore, by using *in-vitro* and *in-vivo* analyses we have characterized the function of a novel MAPK downstream effector in ICC cells.

**Results:** The expression of MAPK signaling was determined by immunohistochemical staining in tumor samples from a cohort of 14 ICC patients. High expression of phospho-activated MAPK was observed in 71.4% (10/14) of ICC cases as compared with surrounding nontumor tissue. Likewise, expression of JDP, a downstream effector of the MAPK signaling, was scored as high intensity in 64.3% (9/14). Strikingly, elevated expression of JDP transcripts was also observed in two independent cohorts of human ICC (n=149 and n=109 per group, respectively) compared to surrounding normal liver tissue. Consistent with the *in-vivo* analyses of human samples, immunoblotting analyses showed constitutive activation of MAPK and expression of JDP in ICC-derived cells (i.e. SG231, CCLP-1 and HuCCT1). Using loss-of-function analyses, we demonstrate that knockdown of JDP in ICC-derived cells resulted in cell cycle arrest and reduced expression of cell cycle regulators (i.e. cyclin D1, cyclin E), and had minimal effect on apoptosis. Chemical inhibition of JDP significantly sensitizes ICC-derived cells to cisplatin (\*\*P<0.0001).

**Conclusions:** These results demonstrate that enhanced activation of MAPK signaling is important for ICC cell proliferation and suggest that targeting its downstream effectors is a potential therapeutic strategy for ICC.