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Osteopontin-c isoform promotes a mesenchymal phenotype in human cholangiocarcinoma cells

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Introduction: Osteopontin (OPN) is upregulated in tissue fibrosis and cancer. The OPN gene generates 3 isoforms (a, b and c) by alternative splicing, which can induce differing cellular responses in tumorigenesis. Although these isoforms have been investigated in breast, prostate, and ovarian cancers, studies in cholangiocarcinoma (CCA) have been limited to OPN-a (total OPN). We hypothesized that OPN isoforms are overexpressed in CCA, and the pattern of isoform overexpression is associated with either a more or less aggressive phenotype. Crosstalk between OPN and TGF-b signaling was evaluated as both are key modulators of cancer progression.

Methods: HuCCT1, SG231 and CCLP1, human intrahepatic cholangiocarcinoma cell lines were used. Plasmids for each OPN isoform were used for overexpression. Expression of OPN-a, b, c, and epithelial-mesenchymal transition (EMT) markers (vimentin, aSMA, E-cadherin) were evaluated by qRT-PCR. Global gene expression changes were examined by microarray using SG231. OPN isoform overexpression and their effects on the components of TGF-b pathway were evaluated by immunoblots. To evaluate the in vivo significance of the individual isoforms, a xenograft model was used. FFPE human cholangiocarcinoma and healthy liver sections were stained for total OPN.

Results: Total OPN was upregulated in cholangiocarcinoma. CCLP1 cells expressed the highest levels of total OPN and exhibited the most mesenchymal phenotype (high

vimentin and low E-cadherin). In contrast, HuCCT1 expressed the lowest amount of OPN and exhibited an epithelial phenotype (high E-cadherin and low vimentin). In all 3 cell lines, OPN-a, and b mRNA were more abundant than OPN-c (~10 fold). Overexpression of OPN-c led to the greatest increase in mesenchymal genes and proteins (vimentin and aSMA), and was associated with the highest level of TGF-β signalling as shown by Smad2/3 phosphorylation. Microarray confirmed that overexpression of OPN-c induced the largest alterations in gene expression profile, and an enrichment analysis revealed that OPN modulated genes which regulate phosphoprotein and acetylation. In the xenograft model, overexpression of all OPN isoforms was associated with greater rates of tumour growth than control, but OPN-c had the greatest rate.

Conclusions: OPN expression correlates with the degree of EMT (marker of aggressiveness) in human cholangiocarcinoma. OPN-c is associated with the most mesenchymal phenotype and TGF-β pathway activation. OPN-c also induces greater changes in the transcriptome and supports greater rates of tumor growth than OPN-a, and -b. High OPN-c expression may be a predictor of worse clinical outcomes.

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