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The role of osteopontin isoforms in cholangiocarcinoma

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Introduction: Osteopontin (OPN) is upregulated in liver cancer The OPN gene generates 3 isoforms (a, b and c) by alternative splicing Each isoform induces a different cellular response during tumorigenesis Studies of OPN isoforms in cholangio- carcinoma (CCA) have been limited to OPN-a (total OPN) We hypothesized that all OPN isoforms are overexpressed in CCA, and the pattern of isoform overexpression dictates CCA phenotype (i e more or less aggressive) Because both TGF-b and OPN are key regulators of cancer development and progression, we also evaluated the impact of OPN isoform expression on TGF-b signaling

Methods: HuCCT1, SG231 and CCLP1, human CCA cell lines were used Plasmids for each OPN isoform were used for overexpression Expression for 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 cells OPN isoform overexpression and their effects on the components of TGF-b pathway were evaluated by WB Functional assays to evaluate cell motility and metalloprotease activity were performed, and the biological significance of OPN isoforms determined in a xenograft model OPN isoform expression was also assessed in tumor and normal tissues obtained from patients undergoing liver resection for CCA

Results: 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), and the forced overexpression of OPN-c resulted in the greatest increase in mesenchymal gene expression, enhanced Smad2/3 phosphorylation, and augmented metalloprotease activities, leading to increased cell migration Microarray analysis confirmed that overexpression of OPN-c

induced the largest alteration in gene expression consistent with the mesenchymal phenotype, and the xenograft recapitulated in vitro findings, with higher tumour growth observed across all OPN isoforms compared with control, and highest growth rate detected in tumors expressing OPN-c In patients, the level of OPN isoform expression correlated with CCA grade

Conclusions: High levels of OPN were associated with a more aggressive CCA in mice and humans The specific over- expression of OPN-c directly promoted a more mesenchymal tumor phenotype, with enhanced migratory and metalloprotease activities, and increased Smad2/3 phosphorylation Future studies should evaluate utility of targeting OPN in CCA

Disclosures: The following people have nothing to disclose: Marco A Briones, Jason D Coombes, Massimiliano Mellone, R Rispoli, Ezequiel Tolosa, Paul P Manka, Naoto Kitamura, Alberto Quaglia, Ali Canbay, Gianfranco Alpini, Shannon S Glaser, Roger Williams, Salvatore Papa, Martin E Fernandez-Zapico, Wing-Kin Syn