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TGF-beta signaling inhibition boosts cholangiocarcinoma progression by promoting tumor cell growth

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Introduction and objectives: TGF-beta signalling is essential for tissue homeostasis and its dysregulation contributes to the development of diseases. During hepatocarcinogenesis, TGF-beta plays a dual role on the malignant cell, behaving as a suppressor factor at early stages, but contributing to tumor progression once cells escape from its cytostatic effects. Moreover, TGF-beta can modulate the response of stromal cells that may contribute to tumor progression and immune evasion. In this sense, the recent success of immunotherapies in different malignancies has raised interest in using TGF-beta inhibitors to boost the immune system and the effect of these therapies. In cholangiocarcinoma (CCA), a group of tumours that arise from biliary epithelial cells, combination of chemotherapy plus immune check point (ICP) inhibitors has recently shown very good responses, while clinical trials with chemotherapy plus a dual inhibitor targeting ICP and TGF-beta have been discontinued due to undesired responses. Since TGF-beta signalling is far understudied in CCA, we aim to determine the effects of TGF-beta on the CCA tumor cell.

Methods: Bioinformatic analyses were performed to determine the expression and mutational status of the members of the TGF-beta signalling pathway in CCA. In vivo, the SB1 syngeneic orthotopic and the hydrodynamic tail vein injection (HTVI) AKT-YAP and AKT-NICD models were used to induce CCA in mice. In vitro, SB1 cells and a panel of 7 human cell lines derived from intrahepatic CCA were used to characterize the response of the CCA tumor cell to TGF-beta and to the TGFBRI inhibitor galunisertib. Cell viability was determined by cell counting. The effect of galunisertib on cholangiocarcinoma growth was evaluated in 2D by performing colony formation assay and in 3D by using spheroids formed by CCA tumor cells alone or mixed with hepatic stellate cells (HSC) as surrogate of stromal cells. mRNA expression was analysed by RNAseq and RT-qPCR. Protein expression was analysed by WB, immunofluorescence and immunohistochemistry.

Results: All ligands (TGFB1, 2 and 3), receptors (TGFBR1, 2 and 3) of the TGF-beta pathway were strongly upregulated in CCA compared to non-tumoral liver (NTL). Mutation rate of TGF-beta signalling members was low (<6%), indicating functionality of the TGF-beta signalling, that was confirmed by correlation of TGFB1 and SMAD7. All ligands and TGFBR1 and TGFBR2 were also upregulated in the SB1, AKT-YAP and AKT-NICD murine CCA models compared to (NTL), indicating that TGF-beta signalling is highly active in CCA tumours across species. In vitro, 6 from 8 cell lines showed response to TGF-beta in terms of induction of morphological changes (reminiscent of EMT activation) and a very strong reduction of cell viability (?50%). The lack of TGF-beta effects in 2 of the 8 cell lines could be anticipated by alterations in members of the signalling pathway (SMAD3 loss and SMAD7 overexpression). Further analyses in responsive cells showed that TGF-beta suppressor effects run through a dual effect consisting in a potent arrest of cell growth, as ascertained by the reduction of CyclinD1 and Ki67 staining, and an induction of apoptosis, as indicated by the cleavage of PARP. GSEA analysis of RNAseq data showed that TGF-beta activates pathways related to production of extracellular matrix and cell migration while inhibited processes related to cell cycle progression and DNA synthesis. Surprisingly, galunisertib effects were concentrated

in upregulating pathways related to increase cell proliferation and DNA synthesis, while few processes related to ECM/migration were altered. Consistently, TGF-beta strongly reduced the number of colonies, while galunisertib increased colony formation ability of CCA cells. In 3D models, TGF-beta again reduced the size of CCA spheres, while galunisertib boost sphere growth, even when the spheres contained a mix of CCA tumor cells and HSC. In vivo, galunisertib increased tumor burden in the AKT-YAP HTVI CCA model compared to the vehicle.

Conclusion: Due to the strong suppressor effect of TGF-beta on the CCA tumor cell, the use of TGF-beta receptor inhibitors, such as galunisertib, may not be a good therapeutical approach in CCA patients. A deeper understanding of the effects of TGF-beta signalling on the tumor and stromal cells is necessary to identify biomarkers that allow selection of new therapeutic molecules that allow the specific inhibition of TGF-beta protumorigenic effects.

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