Ruprecht-Karls-Universität Heidelberg
Medizinische Fakultät Mannheim
Dissertations-Kurzfassung

TGF-β Pathway Activity and the expression of SPARC and TSP-1 in Pancreatic Versus Biliary Tract Cancers

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Abstract:
Background:
It has been observed that pancreato-biliary cancers possess a prominent desmoplastic reaction, which plays an important role in migration and metastasis. Transforming Growth Factor β (TGF-β) is a key-player in tumor progression and metastasis contributes to this desmoplastic reaction. The histological characteristics of the desmoplastic reaction in Cholangio Cellular Carcinoma (CCC) seem to be comparable to those in Pancreatic Ductal Adeno Carcinoma (PDAC). The aim of the present study therefore was to analyze differences in TGF-β signaling between PDAC and CCC. Moreover, we investigated the expression of SPARC in BTC as well as its possible regulation by TGF-β.
Materials and Methods:
Using the Fluidigm's Biomark high-throughput qPCR chip platform we analyzed established cell lines of CCC and PDAC for expression of each of the 25 TGF-β pathway signaling genes. Eighteen PDAC and ten CCC cell lines served as samples. Furthermore, to validate the findings we either performed Western Blotting or immune histochemistry of Activin Receptor-Like Kinase 1 (Alk1) and Thrombospondin 1 (TSP-1). Moreover, we evaluated expression levels of Sparc, TGF-β1 and its receptor ALK5 by quantitative real-time PCR in 6 biliary tract cell lines as well as 1 immortalized cholangiocyte cell line (MMNK-1). Expression of Sparc, TGF-β type II receptor (Tb-RII) as well as Twist and ZO-1 was analyzed in tumor samples of 7 biliary tract cancer patients using quantitative real-time PCR. MMNK-1 cells were stimulated with TGF-β for 24 h, and Sparc, ZO-1 and E-Cadherin expressions were determined. SPARC protein expression in tumors from 10 BTC patients was analyzed by Immunohistochemistry.
Results:
The Fluidigm microfluidics dynamic array showed that an average expression levels of five genes were differently expressed in CCC versus PDAC cell lines with statistical significance. However, these findings could neither be confirmed by Western Blotting nor by immune histochemistry. In contrast, we found that basal Sparc transcript level was strongly downregulated in BTC cancer cell lines, but highly upregulated in MMNK-1 cells. Expression of Sparc in BTC patient samples showed a significant positive correlation with expression of the epithelial marker ZO-1. In contrast, the mesenchymal marker Twist and the TbRII showed a trend of negative correlation with expression of Sparc in these samples. TGF-β exposure significantly downregulated Sparc expression in MMNK-1 cholangiocytes in vitro in parallel to downregulation of epithelial markers (E-Cadherin and ZO-1). Finally, SPARC immunostaining was performed in 10 patient samples, and the correlation between absence of SPARC and survival times was analyzed.
Conclusion:
The majority of the identified members of the canonical TGF-β pathway seemed to be expressed homogenously in CCC and PDAC cell lines. Our findings imply that SPARC expression could correlate with differentiation level of BTC cell lines. In our patient samples we observed similar SPARC expression tendency with an epithelial marker, but inversely correlation with the mesenchymal marker, indicating SPARC involvement in EMT being possibly mediated by TGF-β. Collectively, SPARC could to be involved in EMT in pancreato-biliary cancers and might therefore be a promising prognostic marker in these cancer entities.