35 Autocrine Wnt signaling regulates cell proliferation in bladder cancer

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INTRODUCTION & OBJECTIVES: Activation of embryonic signaling pathways such as autocrine Wnt signaling has been demonstrated to regulate tumour igenesis. The aim of this study is to examine activity and functional relevance of the Wnt/b-catenin pathway in bladder cancer.

MATERIAL & METHODS: RT-PCR analysis for the expression of Wnt ligands (2b, 4, 7a), LRP-5/6 receptors and the transcription factors TCF-1,-3, -4 and LEF-1 was performed in 9 bladder cancer cell lines. Wnt activity was characterized using a TCF-Luciferase reporter system (TOP/FOPflash). For gene silencing, siRNAs or lentiviral shRNAs against HSulf-2, beta-catenin and Wls were applied. The WLS gene was cloned from T24 bladder cancer cells and RT112 cells were established with stable Wls expression. BrdU incorporation in cell lines was analysed by FACS. Expression of Ki-67 in tumours was assessed by immunohistochemistry to quantify cell proliferation. Three dimensional tumour growth was characterized using the chicken chorioallantoic membrane (CAM) model.

RESULTS: Expression of ligands, receptors and intracellular molecular components of the Wnt signaling cascade was demonstrated in all cell lines examined. In addition, expression of activated b-catenin was detected. Canonical activation of Wnt signaling was demonstrated in all cell lines using the TOP/FOPflash assay. Manipulation of b-catenin, Wls, WIF1, DKK1, sFRP-4 and HSulf-2 expression level lead to regulation of Wnt activation. Ectopic expression of extracellular Wnt inhibitors (WIF1, DKK1, sFRP-4) reduced Wnt activity by 50-80% and BrdU incorporation by 40%. Also, modulating the 6-O sulfation modification in heparan sulfate proteoglycans, which can act as Wnt ligand co receptors, by silencing of HSulf-2 resulted in a 25-30% reduction. Silencing of b-catenin using siRNAs diminished Wnt activation by 60%. Furthermore, we could demonstrate a positive role for Wls in the regulation of Wnt signaling. Expression of the extracellular inhibitor WIF1 in the CAM model did result in a 50% reduction of tumour weight and 30% reduction in proliferating cells.

CONCLUSIONS: The results indicate an activated and responsive autocrine Wnt signaling pathway in bladder cancer and its involvement in cell proliferation and tumour growth. Although complete inhibition of Wnt signaling inhibition might be too detrimental, the modulation of individual players of Wnt signaling could be an interesting target in bladder cancer therapy.