Cell-cell interactions in the gastrointestinal tumour-microenvironment

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Chapter 2

Outline and aim of the thesis

In normal tissue homeostasis as well as in pathological conditions cells are influenced by surrounding cells both via direct cell-cell contact and soluble factors. Interactions of malignant cells with the tumour-microenvironment have been shown to be very important in the progression of carcinomas. However, the tumour-microenvironment is very complex and although animal models provide the complexity of the different cell types in a living animal, they do not necessarily reflect of the human tumour-microenvironment due to interspecies differences in for example growth factors and their receptors. Therefore, when studying cancer cells one should preferentially take their human tumour-microenvironment into account. This thesis evaluates cell-cell interactions by using various *in vitro* 3-dimensional cell culture models. After analysis of the human tissue by determination of protein expression levels and cellular localisation by immunohistochemistry, *in vitro* human cell models, closely resembling the *in vivo* situation, are developed. The aim is to elucidate the interaction between colon cancer cells with two prominent cell types in the tumour-microenvironment: angiogenic endothelial cells and myofibroblasts.

Chapter 3 describes the expression and cellular localization of TGF-β1 in gastric cancer, focusing on the active TGF-β1 molecule, as this is presumably the key mediator of myofibroblast differentiation. In **chapter 4** these observations are confirmed in a larger series colorectal cancer samples and a new method to quantify the myofibroblast content in these samples is described. These chapters reveal that enhanced active TGF-β levels are clinically important and correlate to the presence of myofibroblasts. Subsequent studies described in **chapter 5** evaluate the activation mechanism of TGF-β and illustrates that the interaction between tumour cells and fibroblasts leads to myofibroblast differentiation and subsequent upregulation of MMPs in both tumour cells and myofibroblasts, reflecting a double paracrine tumour-promoting mechanism.

The contribution of myofibroblast and neutrophil derived MMPs to the initiation of the angiogenic switch by liberation of VEGF from colon cancer extracellular matrix, is described in **chapter 6**, showing a key role for neutrophil-derived MMP-9 in the initiation of the angiogenic switch. Besides MMP-9 also endothelial MMP-7 contributes the angiogenesis as described in **chapter 7**. To be able to quantify MMP-7 activity levels, **chapter 8** describes the development of a MMP-7 bioactivity assay. Furthermore the contribution of cathepsin S to the angiogenic process, by liberating pro-angiogenic molecules for colon cancer extracellular matrix and a new method for the identification of specific inhibiting peptides using phage display is described in **chapter 9**. Finally, **chapter 10** describes the role of the TGF-β co-receptor Endoglin as an additional factor in colorectal cancer angiogenesis. This receptor is

mainly expressed by angiogenic endothelial cells and has been implicated in the pro-angiogenic effects of TGF-β. The role of MMPs in the cleavage of this membrane receptor into soluble Endoglin is also investigated. The different studies in this thesis are summarized and discussed in **chapter 11.**