Chapter 9

Summary and Conclusions

SUMMARY AND CONCLUSIONS

In haematogenous metastasis, circulating tumour cells have to overcome many defence mechanisms before adhering to the endothelium of distant organs. Next, the cells invade this layer followed by adhesion to and invasion through the extracellular matrix to enter a distant organ and grow out to form a metastasis. Implantation of circulating tumour cells appears to be highly inefficient and most tumour cells are rapidly destroyed. Nevertheless, recurrences to distant sites after intentionally curative surgery remain a major problem for most gastro-intestinal tumours. Resection handling of the tumour may increase the amount of circulating tumour cells, but that will not entirely explain the high recurrence rate. In previous *in vivo* and *in vitro* studies it was found that surgical trauma itself influences the development of loco-regional recurrences by the inflammatory reaction it provokes. This thesis aimed at the influence of surgical trauma on the development of distant tumour recurrence.

Surgical trauma and tumour recurrence at distant sites: *in vivo*

Although surgical resection remains the most effective therapy for metastatic colorectal cancer to the liver, the recurrence rate is high. It is thought that tumour growth promoting factors, released during liver regeneration, are responsible for this high recurrence rate. This hypothesis was evaluated in **chapter 2**. An *in vivo* rat model was developed in which the effect of partial liver resection on tumour recurrence in the lung was investigated. Performing a partial liver resection followed by peripheral injection of CC531 rat colon carcinoma cells resulted in significant increased tumour nodules in the lung compared to a sham operation. However, the addition of serum obtained from rats undergoing partial liver resection to *in vitro* cultures of CC531 cells did not enhance the growth of these cells. Furthermore, only when the resection is carried out shortly before tumour cell injection, but not when the resection is performed a day before or a day after tumour cell injection, increased tumour nodules were observed. Therefore, partial liver resection exerts its tumour promoting effect merely during a short time frame peri-operatively. Additionally, partial ileum resection gave enhanced tumour nodules as well, indicating that surgical trauma in general and not only partial liver resection causes enhanced tumour recurrence. These results exclude a role for tumour growth promoting factors produced by the regenerating liver in the enhanced tumour recurrence seen after partial liver resection. An effect of surgical trauma on adhesive interactions between circulating tumour cells and the endothelium of the lung seems to be the responsible mechanism.

Surgical trauma and tumour recurrence at distant sites: *in vitro*

The previous experiments performed in animal models demonstrated a relation between surgical trauma and distant tumour recurrence. Surgical trauma provokes a local as well as a systemic inflammatory reaction in which leukocytes are activated with the release of inflammatory mediators like pro-inflammatory cytokines and reactive oxygen species. Our hypothesis is that these factors modulate the adhesive interactions between tumour cells and the microvascular endothelium.

To make the changeover from animal models to a human model, human *in vitro* assays were developed. In these assays, adhesive interactions between tumour cells and microvascular endothelial cells, which are of pivotal importance in the development of distant tumour recurrence, can be investigated without interference of unknown in vivo factors. In this way a better insight in the role of specific parts of the inflammatory reaction caused by surgical trauma in these adhesive interactions can be obtained.

During inflammation, like in surgical trauma, leukocytes are activated with the release of pro-inflammatory cytokines. In **chapter 3**, the influence of the main pro-inflammatory cytokines IL-1β, TNF-α and IL-6 on adhesion of the human HT29 colon carcinoma cell line to the endothelium was investigated. A distinction was made between adhesion to human umbilical vein endothelial cells (HUVEC), which are macrovascular endothelial cells mostly used for *in vitro* assays because of their readily availability, and adhesion to human microvascular endothelial cells of the lung, reflecting a more real model, since adhesion occurs in the microcirculation.

Pre-incubation of microvascular endothelial cells with IL-1β and TNF-α, but not with IL-6, resulted in concentration- and time-dependent enhancement in tumour cell adhesion of at least 150%. Comparable results were found for adhesion to the macrovascular endothelial cells. Pre-incubation of the tumour cells did not modulate adhesive interactions. Exposure of endothelial cells to IL-1β and TNF-α caused an upregulation of the adhesion molecules E-Selectin, ICAM-1 and VCAM-1, showing a time course comparable to tumour cell adhesion. Their counter partners, sialyl Lewis^{a} and ^{x}, LFa-1 and VLA-4 are expressed on HT29 cells. Although the binding to activated macrovascular endothelium was E-Selectin dependent, the binding to activated microvascular endothelium was not dependent on E-Selectin and is probably formed by a complex of adhesion molecules.

The role of these pro-inflammatory cytokines in the adhesion of pancreatic carcinoma cells to microvascular endothelium was studied in **chapter 4**. Again led pre-incubation of endothelial cells with IL-1β and TNF-α but not with IL-6 to a significant increased adhesion of all three pancreatic carcinoma cell lines PanC1, BxPC3 and MiaPaCa. Exposure of the pancreas carcinoma cells to these cytokines did not affect adhesion, comparable to the results obtained with the colon cancer cells. Contrary to adhesion of pancreas carinoma cells to macrovascular endothelium, which is E-Selectin-dependent according to the

literature, is the adhesion to microvascular endothelium E-Selectin-dependent nor ICAM-1-dependent.

During surgical trauma polymorphonuclear leukocytes (PMN) are activated with the release of reactive oxygen species (ROS). Previous animal models proved that these ROS play an important role in enhanced local tumour recurrence after abdominal surgical trauma. In **chapter 5** it was found that exposure of the endothelium to activated PMN significantly increased adhesion of both colon and pancreatic carcinoma cells to this endothelium. The ROS superoxide anions, the main ROS produced by PMN, and hydrogen peroxide are mostly responsible for this enhancement, since addition of the anti-oxidant enzyme superoxide dismutase and/or catalase gave a considerable reduction in this enhancement.

To study the influence of ROS on tumour cell – endothelial cell interactions in more detail, the superoxide anion producing complex xanthine/xanthine-oxidase was used (**chapter 6**). This complex affected the adhesive interactions comparably to PMN. Again, these interactions could be reduced by the addition of the anti-oxidant enzymes superoxide dismutase and/or catalase. Exposure of the endothelium to ROS brought the cells in apoptosis with accompanying increase in expression of the cell adhesion molecules E-Selectin, ICAM-1 and VCAM-1. The enhanced expression of these adhesion molecules may explain the mechanism of the observed enhancement in adhesion.

Tumour cell interactions with the extracellular matrix

After adhesion to the microvascular endothelium the next step in successful metastasis formation is the invasion through this layer and subsequent adhesion to the underlying extracellular matrix (ECM). Therefore, **chapter 7** describes the development of a reproducible human in vitro model to study the adhesion of 2 colon carcinoma cell lines, HT29 and Caco2, to the major components of the ECM. These major components comprise of collagen type I, type IV, fibronectin and laminin. Both colon cancer cell lines displayed a strong adhesion to the ECM, although the adhesion pattern to the various components differed between the 2 cell lines. For HT29, highest adhesion was to collagen type I (60.9%) and lowest to fibronectin (20.6%), whereas for Caco2 highest adhesion was to collagen type IV (51.4%) and lowest to laminin (21.9%). To explore how the binding between these tumour cells and components of the ECM is accomplished, inhibition assays using functional blocking antibodies were performed. Antibodies against the adhesion molecules ICAM-1, VCAM-1 and the VLA-integrins were studied. These inhibition assays clearly display that the α2- and α6-unit play a major role in the adhesive interactions between the different tumour cells and components of the extracellular matrix, although both tumour cell lines display different adhesion patterns to the components of this matrix. Still, the complexity of the

matrix and the heterogeneity between tumour cells retains the exact identity of adhesion molecules in this adhesion indistinct.

CONCLUSIONS

- The enhanced development of distant tumour recurrence after partial liver resection is not caused by stimulation of tumour growth but seems to be caused by increased adhesive interactions between tumour cells and the microvascular endothelium. (chapter 2)
- Not only partial liver resection but surgery in general stimulates the development of distant tumour recurrence. (chapter 2)
- Exposure of microvascular endothelium to the pro-inflammatory cytokines IL-1β and TNF-α, but not IL-6, resulted in a significant enhancement in the adhesion of both colon and pancreatic carcinoma cells to this endothelium. (chapter 3 and 4)
- The adhesion of tumour cells to microvascular endothelial cells is not dependent of E-Selectin, whereas the binding to macrovascular endothelial cells is E-Selectin dependent. (chapter 3 and 4)
- Activated polymorphonuclear leukocytes increase the adhesion of colon and pancreatic carcinoma cells to microvascular endothelium. (chapter 5)
- Reactive oxygen species are the main perpetrators in the enhanced tumour cell adhesion caused by the activated polymorphonuclear leukocytes. (chapter 5)
- Exposure of microvascular endothelium to superoxide anions enhances tumour cell – endothelial cell interactions. (chapter 6)
- Superoxide anions cause apoptosis in the microvascular endothelial cells leading to enhanced expression of adhesion molecules on these cells. (chapter 6)
- Adhesive interactions between tumour cells and microvascular endothelium are not affected by exposure of tumour cells to pro-inflammatory cytokines or superoxide anions. (chapter 3,4 and 6)
- Colon cancer cell lines display a strong adhesion to the ECM, although the adhesion pattern to the various components differed between the cell lines. (chapter 7)

- **The inflammatory reaction, provoked by surgical trauma, enhances the development of distant metastasis most probably by promoting the adhesion of circulating tumour cells to microvascular endothelium.**