Tumor development of colon cancer in rat liver
Mook, O.R.F.

Link to publication

Citation for published version (APA):

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Summary

The major issues that are addressed in this thesis are introduced in Chapter 1. Colorectal cancer patients rather die because of the consequences of metastasis than due to the primary tumor. Metastasis to the liver is most commonly found in colorectal cancer patients. Options for curative treatment are limited and most of the therapies are palliative. There are several ways to explain the high incidence of colorectal cancer metastasis in the liver. In this thesis, a rat model has been used to establish what mechanisms are involved that rule colorectal cancer metastasis in the liver.

Chapter 2 describes a study on the visualization of tumor growth noninvasively in live rats using magnetic resonance (MR) imaging. Tumors in livers of rats were visualized relatively easily without transfection or other modifications necessary to visualize tumors noninvasively. It was shown that MR imaging, performed multiple times on the same rat, allows determination of kinetics of tumor development. It is shown that tumor growth kinetics provide additional information over "classical" end-point assays to evaluate of tumor development and that end-point assays may lead to misconceptions of what happens in tumors in time. However, spatial resolution at the single cell level is still an illusion.

In chapter 3, early events in colon cancer metastasis in the liver were investigated. In order to do so, colon cancer cells were stably transfected with green fluorescent protein (GFP) so that the process could be visualized at the single cell level. The use of GFP-positive cancer cells enabled us to establish that cancer cell arrest in liver sinusoids is due to size restriction. All cancer cells targeted to the liver were retained in the sinusoids. Interactions between cancer cells and hepatocytes instead of endothelial cells were observed because endothelium between cancer cells and hepatocytes retracted rapidly. Extravasation of cancer cells into the liver parenchyma was not observed either and micrometastases appeared to develop intravascularly up to 4 days after administration. It is concluded that general assumptions that initial steps in the metastatic cascade, adhesion to endothelium and subsequent extravasation, are not essential for tumor formation in the liver. The letter-to-the-Editor of Vekemans et al. (chapter 4A) describes a possible explanation of disruption of endothelium on the basis of their in vitro findings. However, in our reply (chapter 4B) we argue that the mechanism proposed by them was not shown in vivo.
Chapter 4 describes the expression of different adhesion molecules that potentially are involved in the interactions between cancer cells and hepatocytes as described in chapter 4. The expression of adhesion molecules of the integrin family and the CD44 family were assessed on scraped cultured cancer cells. Several integrin subunits were expressed whereas others were not. Cancer cells were also found to be positive for CD44 and its splice variant CD44v6. Trypsinization of the cancer cells that is routinely used prior to administration of cancer cells to animals removes CD44 and its splice variant but not integrins. Apparently, members of the integrin family are more resistant than CD44 and its splice variant which were completely lost after trypsinization. These adhesion molecules were localized immunohistochemically in livers of rats 0-72 h after administration of cancer cells. Adhesion molecules that are potentially involved in interactions between cancer cells and hepatocytes were identified, but precise localization of these molecules should be obtained with electron microscopy. These studies are presently performed.

Chapter 5 is an overview of methods to detect proteolytic activity in situ. Small synthetic peptides with MNA or AFC as leaving group have been used to assess proteolytic activity of a wide range of proteases in situ. In situ zymography methods to detect activity of proteinases such as MMPs are discussed in particular. To obtain additional information on the (patho)physiological role of active gelatinases, localization of activity in situ is of great help. Various methods that have been developed to detect gelatinolytic activity in situ are discussed in conjunction with their biological applications. It appears that the introduction of dye-quenched (DQ)-gelatin as substrate greatly improved the resolution of these in situ assays for the detection of gelatinolytic activity and the applications of this substrate is discussed. Proteins other than gelatin with quenched fluorescence are potentially applicable to perform in situ activity assays.

In chapter 6 the development of the method to detect gelatinolytic activity with in situ zymography using DQ-gelatin as substrate is described applied to liver tumors of colon cancer. Gelatinolytic activity was localized on extracellular matrix components of intratumoral stroma. Combination of immunohistochemistry and in situ zymography showed colocalization of MMP-2 protein and gelatinolytic activity as well as colocalization of collagen type IV and gelatinolytic activity, indicating that MMP-2 was responsible for the gelatinolytic activity. Activity in extracellular matrix suggests a role of MMP-2 in tissue remodelling and /or angiogenesis but conclusions on the pathological role could not be drawn.
Chapter 7 is an overview of the role of gelatinases in colorectal cancer progression and metastasis in humans and animal models. In humans, a relation has been found between increased expression of MMP-2 and/or MMP-9 and cancer progression and metastasis. However, MMP-2 and/or MMP-9 levels in plasma or serum of cancer patients are not applicable as a prognostic tool. Gelatinases are expressed in cancer cells but also in non-cancer cells in tumors. In fact, interactions between cancer cells and host tissues have been shown to modulate gelatinase expression in host cells and can contribute to cancer progression. The in vivo use of broad-range and selective MMP inhibitors has shown a functional role of gelatinases in tumor growth in animal models, but therapeutic use of MMP inhibitors in cancer patients has been a disappointment so far. To understand this discrepancy, regulation of gelatinase expression in both cancer cells and non-cancer cells and the physiological effects of gelatinase activity has been reviewed. It is concluded that the multifunctionality of gelatinases makes it unpredictable in what stages of cancer development and in which cell biological processes gelatinase activity is involved. It is concluded that the use of MMP inhibitors to treat cancer should be considered carefully.

In chapter 8, the pathological role of gelatinolytic activity in relation with tumor growth was assessed by daily administration of a selective MMP inhibitor. It was found that daily administration of a high dose of MMP inhibitor resulted in a marginal inhibition of tumor size (30%). Reduction in tumor size can be explained by reduced tissue remodelling/angiogenesis that hampers tumor development as proposed in chapter 7. However, daily administration of either a low dose or a high dose of the inhibitor resulted in a two-fold increase in the number of tumors. Therefore, the effects of MMP inhibition on the host immune response was investigated. It was found that daily administration of the selective MMP inhibitor had an effect on the recruitment of neutrophils in rats with tumors but not in control rats. It is concluded that the outcome of treatment of cancer patients with MMP inhibitors is unpredictable because the inhibitor may have various counteracting effects.

Chapter 9 is a general discussion that puts the findings of this thesis on mechanisms of colorectal cancer metastasis and in particular the role of gelatinases in the progression of colorectal cancer and metastasis into perspective.