Introduction and outline of thesis
Tumors of the colon and rectum, colorectal cancers (CRC), are the third most frequently diagnosed type of cancer in men and the second in women worldwide.\(^1\) Circa 60% of the CRC cases occur in developed countries and account for 8% of all cancer-related deaths, which makes CRC, with >600,000 patients, the fourth most common cause of death from cancer.\(^1,3\) Currently, surgical resection of the primary colorectal carcinoma is the preferred and the only treatment that provides long-term disease free survival. Unfortunately, post-surgical development of metastases is a frequent complication. Upon diagnosis of CRC, metastatic tumors are apparent in approximately 20% to 25% of all patients.\(^3\) Another ~10-25% of patients without visible evidence of metastases at the time of diagnosis and who are therefore eligible for surgery with curative intent, will manifest local and/or distant metastases within 5 years.\(^4,5\) Metastasis of CRC often occurs through the portal circulation and therefore favor the development of liver metastases that accounts for 70% of colorectal-related deaths.\(^6\) Occasionally, lung, bone, or brain metastases are observed as well.\(^7\) Alternatively, metastases develop through spread of tumor cells via the lymphatic system or intra-peritoneal dissemination. Thus, metastases to the mesenteric lymph nodes or the abdominal cavity are observed regularly.\(^2\) Tumor cells must undergo a complex cascade of events to form metastases in targets organs.\(^4\) Detachment of individual tumor cells from the primary tumor and invasion into the blood circulation or lymphatic system are the initial steps. To fulfill these steps, expression of specific adhesion molecules, such as cadherins and catenins, needs to be down regulated (Figure 1).\(^8\) Furthermore, production and secretion of proteolytic enzymes like metalloproteases is essential for degradation of the basement membrane and subsequent intravasation.\(^9\) However, mechanical forces and immune defense systems counteract to remove most of the disseminated tumor cells. In the target organs, adhesion molecules such as intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), and selectins expressed on both tumor cells and local host cells mediate tumor cells arrest. Because of the limited life span of tumor cells in circulation, these events must take place rapidly.\(^4\) Thus, classical metastasis is a complex and inefficient process because of the multiplicity of events that are required. However, an alternative route of metastasis that is initiated after surgery and which short-cuts several of these steps was proposed (Figure 1).\(^10\) This introduction provides an overview of surgery-induced liver metastases development and proposes novel treatment methods to prevent metastases development from primary CRC.

**Surgery-Induced metastases development**

Previously, it was shown in animal models that surgical trauma enhances loco-regional metastases with preferential outgrowth at injured sites.\(^11\) Moreover, severity of trauma correlated with the amount of tumor load, as an alternative route of metastasis (surgery in the abdominal cavity trough a small incision with the aid of a camera)
with less severe trauma induced lower tumor load compared with laparotomy (opening of the peritoneal cavity).\textsuperscript{12, 13} Interestingly, the influence of surgery on tumor development was not restricted to local sites. Several reports described that surgical trauma resulted in systemic alterations that stimulated tumor development in distant organs.\textsuperscript{14-16} A previous study reported enhanced tumor development in the peritoneal cavity when thoracotomy (surgery of the chest) was performed.\textsuperscript{17} Furthermore, increased liver metastases outgrowth as consequence of abdominal surgery was observed in an animal model.\textsuperscript{10} Thus, surgery induces both local and systemic changes that facilitate metastases development.

Unfortunately, clinical research investigating the effects of surgical resection on local and distant metastases development has limitations. As such, the influence of surgery on metastases development is still subject of debate and not yet generally accepted despite the overwhelming evidence from experimental studies. Clearly, omitting surgery in patients with CRC in an objective randomized clinical trial is unethical. Therefore, evaluation of limited and anecdotal clinical evidence to understand this phenomenon is necessary. Analysis of a database of breast cancer patients demonstrated that patients who did not have surgery showed one peak for death around the fourth and fifth year.\textsuperscript{18} In contrast, 2 peaks around 3 to 4 years after surgery, and around 8 years after surgery were observed in patients who underwent mastectomy.\textsuperscript{19} These findings strongly support that surgery alters the natural course of the disease by elongating life expectancy in the greater part of the patient population, but also by shortening survival in a smaller subset of patients. Thus, surgery, although greatly reducing tumor mass and is potentially curative, paradoxically can also augment metastases development in some patients.

**Tumor cell dissemination**

Circulating tumor cells can be detected in peripheral or portal blood of CRC patients before surgery.\textsuperscript{20, 21} However, the numbers of disseminated tumor cells in the circulation, peritoneal cavity or the liver after surgery were increased.\textsuperscript{19, 22-24} Thus, loss of cell-cell adhesion and mechanical handling of tumors during surgical removal may permit tumor cells spillage. Moreover, analysis of peri- and post-operatively obtained blood demonstrated that tumor cell presence was a strong predictor of CRC recurrence.\textsuperscript{20, 25} These studies not only indicate that examination of disseminated tumor cells in blood after surgery can serve as a prognostic tool, but also provide evidence that surgical resection may result in an increase of exfoliated tumor cells, which can develop into metastases. However, because most circulating tumor cells are rapidly removed by the immune system, implantation of circulating tumor cells is a highly inefficient process.\textsuperscript{26} Therefore, spillage of tumor cells during surgery cannot fully explain the high incidence of metastases development. Surgery unavoidably leads to tissue injury, which initiates a systemic stress response that encompasses a wide range of endocrinologic, immunologic, and hematologic effects. It is now clear that the systemic inflammatory response after surgery may also contribute to successful tumor development via several mechanisms.
Figure 1: Classical vs surgery-induced liver metastases.

**Left panel:** metastasizing tumor cells detach from the primary tumor by down regulating the expression of certain cell adhesion molecules (here indicated as red molecules). Detached tumor cells arrive and adhere in the liver and potentially grow out into distant metastases.

**Right panel:** because of mechanical handling during surgery, tumor cells disseminate from the primary tumor without the need of down regulation of adhesion molecules. Furthermore, surgery leads to exposure of sub-endothelial ECM. Disseminated tumor cells adhere to the exposed ECM through integrin α2 and develop into liver metastases. Thus, classical and surgery-induced liver metastases have different underlying causes. These differences may serve the design of therapies for prevention of the latter phenomenon.
Post-surgical immune suppression

Directly after surgery, pro-inflammatory acute phase responses with cytokines as main mediators are initiated.\(^{27-29}\) To counterbalance the effects of the acute phase response compensatory anti-inflammatory mediators are released.\(^{30}\) An unbalanced systemic compensatory response to acute phase responses may result in immune suppression and thereby render the patient susceptible for post-operative infections and hampered anti-tumor immunity. Compensatory immune responses were observed immediately after surgery and can last for several days.\(^{31,32}\) Moreover, the severity of trauma, blood transfusion, anesthetics and psychological stress were demonstrated to play a role in the magnitude and duration of immune suppression.\(^{13,28,33}\)

Additionally, disregulated functions of cells of the innate and adaptive immune system with anti-tumoral actions were observed after surgery.\(^{34-38}\) In mice undergoing surgery accelerated tumor growth was demonstrated. This was accompanied by diminished cytotoxic ability of natural killer (NK) cells that have anti-tumoral properties. In patients undergoing abdominal surgery decreased cytokine production by monocytes was reported. Moreover, trauma-induced suppression of pro-inflammatory and enhancement of anti-inflammatory T cell functions in humans after major surgery was demonstrated.\(^{39}\) Thus, imbalance in pro- and anti-inflammatory immune responses after surgery may hamper antitumor cytotoxicity and was suggested to facilitate metastases outgrowth.

Enhanced tumor growth

Increased proliferation rate of tumor cells was suggested to induce rapid tumor recurrence after CRC resection as well. It has been shown that both recurrent tumors and distant metastases had reduced apoptosis and increased proliferation rate compared to the primary tumor.\(^{40,41}\) Experimental and clinical data demonstrated that the primary tumor may regulate the growth of dormant metastases.\(^{42}\) Elimination of the primary tumor by surgery therefore might stimulate the outgrowth of either local and/or distant metastases. In patients with primary CRC high levels of the anti-angiogenic factor endostatin was measured.\(^{43}\) It was proposed that endostatin may suppress angiogenesis at distant sites and therefore prevent metastases development. In line with this, resection of primary CRC resulted in drop of endostatin levels. Additionally, vascular density in metastases of patients in which the primary tumor was removed, was higher than in the primary tumor of patients.\(^{44}\) Thus, surgery may induce metastases due to loss of suppressive capacity by removal of the primary tumor.

Tumor cell adhesion

A recent study reported that tumor cell derived factors up-regulated the synthesis of cytokine release by immune cells.\(^{45}\) These cytokines were shown to enhance the expression of E-selectin on the surface of endothelial cells -including the liver sinusoidal endothelium-, which was suggested to facilitate the tumor cells adhesion and enable metastases outgrowth. Moreover, enhanced tumor cell adhesion by
surgery-released cytokines was demonstrated both in vivo and in vitro.\textsuperscript{46-49} Surgical trauma, which is inevitable during resection of primary tumor, furthermore initiates systemic inflammation and leads to rapid activation of innate immune cells. These cells are potent producers of inflammatory mediators like reactive oxygen species (ROS).\textsuperscript{26} It was demonstrated that incubation of mesothelial cells with ROS enhanced expression of adhesion molecules ICAM-1 and VCAM-1.\textsuperscript{50} This consequently increased tumor cell adherence to mesothelial cells. Additionally, impairment of the mesothelial monolayer of the peritoneal wall or liver microvasculature after abdominal surgery was demonstrated.\textsuperscript{10, 51, 52} Moreover, in post-surgical samples, retraction and detachment of mesothelial cells or sinusoidal endothelial lining were demonstrated as well.\textsuperscript{10, 52} This resulted in formation of intercellular gaps and exposure of extracellular matrix (ECM) proteins, which served as preferable adhesion sites for tumor cells. Incubation of tumor cells with antibodies against integrin subunits prevented surgery-induced tumor cell adhesion and tumor outgrowth in the peritoneum or the liver.\textsuperscript{10, 52} This suggested that interactions between integrins on the tumor cell surface and exposed ECM played a major role in metastases development. Integrins are a family of cell adhesion molecules consisting of an $\alpha$ and a $\beta$ subunits heterodimer.\textsuperscript{53} Integrins bind ECM proteins, such as collagens and fibronectin, as ligands. Interaction between integrin heterodimers and ECM proteins of the target organs facilitates tumor cell attachment and eventually formation of metastases.

All these experimental and clinical data support a novel model of surgery-induced metastasis. During surgery, handling of the primary tumor results in tumor cells spillage, which overcomes the need of complex cellular changes, such as loss of E-cadherin and $\beta$-catenin expression. Alternatively to classic metastasis, surgery leads to exposure of sub-endothelial ECM in the liver vasculature. Next, circulating malignant cells adhere in the target organs through commonly expressed adhesion molecules such as integrins (Figure 1). Moreover, enhanced expression of adhesion molecules like ICAM-1 and VCAM-1, which are increased in post-surgical livers after $\sim$3 hours, may also contribute to enhanced tumor cell adhesion at later stage. Thus, surgery creates permissive circumstances for tumor cells to adhere in target organs and thereby increase chances of metastases development.

Surgery-induced systemic immune response

\textbf{Cytokines}

Performing surgery induce damage of the local tissue and release of danger signals. These signals are recognized by immune cells, which respond by release of cytokines with the intend to suppress further cell death and promote epithelial proliferation.\textsuperscript{54} Cytokines are soluble low molecular messengers of both local and systemic inflammatory responses. The major cytokines which are released as a
INTRODUCTION

direct response to trauma are tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6, which levels were increased in post-surgical plasma of patients. Importantly, laparoscopic surgery was shown to induce lower levels of cytokines, compared to open surgery with higher degree of trauma. This suggested that laparoscopy has advantageous effects on post-surgical inflammation. Because laparoscopy is minimally invasive and thus induces less tissue injury, it may be consequently less immunosuppressive. Patients undergoing laparoscopy show faster recovery compared to open surgery. Additionally, surgery-mediated release of cytokines in the periphery was suggested to regulate tumor metastases development in different ways. Cytokines such as IL-6, TNF, and tumor growth factor (TGF) ß, which are released in high concentrations after surgery, have been shown to accelerate tumor cell proliferation.

**Reactive oxygen species**

Previous studies furthermore suggested that surgical injury-induced responses result in activation of immune cells with ROS production capacities. Enhanced release and activation of the ROS producing enzyme xanthine oxidase (XO) and its substrate were observed during surgical trauma. Moreover, incubation with ROS caused damage of endothelial monolayers, leading to formation of intercellular gaps. This subsequently resulted in the exposure of subendothelial ECM proteins, which served as favorable adhesion sites for tumor cells. Moreover, in post-surgical liver samples of humans or rats reduced expressions of tight junction proteins were observed. This effect was reversed by perioperative injection of an anti-oxidant in a rat model. More importantly, surgery-stimulated tumor cell adhesion was significantly inhibited by using an anti-oxidant, supporting that surgery-induced ROS production enabled tumor cell adhesion. Alternatively, tumor cells may also produce ROS. The enzymes nicotinamide adenine dinucleotide phosphate oxidase (Nox), which generate ROS superoxide, were shown to be expressed highly in human tumors, especially in colon cancers. Members of Nox family are activated by inflammatory mediators such as cytokines, which are released directly after surgery. Therefore, it was suggested that tumor cells potentially regulate their own adhesion and grow out in local or distant metastases.

**Bacterial translocation**

Previous studies demonstrated surgery-mediated bacterial translocation from the gut lumen into the peripheral blood circulation. The bacterial component lipopolysaccharide (LPS), which is an important component of the outer membrane of Gram-negative bacteria, was detected in post-surgical plasma of patients. LPS concentration in peripheral blood was enhanced 1 hour after surgery and normalized after 24 hours. Elevation of the LPS concentration in blood was accompanied by intestinal permeability, which suggested that the epithelial barrier was impaired after surgery. Translocation of viable bacteria from the gut lumen to local mesenteric lymph nodes was demonstrated in CRC patients.
undergoing colectomy. Importantly, patients with positive bacterial translocation after surgical removal of the tumor had significantly shorter disease-free survival. This again emphasized that surgery can influence metastases development.

Previous studies demonstrated that LPS had stimulatory effects on tumor cells adherence and tumor formation in the lungs and the liver. Bacterial components are recognized by Toll-like receptors (TLRs). LPS is the main ligand for TLR4, which is expressed by a wide variety of immune cells. Interaction of TLR4 with LPS triggers potent immune responses including release of cytokines and ROS. Moreover, previous studies suggested that LPS elevated the metastatic potential of tumor cells both by enhancing the tumor cells or host cells adhesiveness. Furthermore, it was demonstrated that LPS can also induce apoptosis resistance in tumor cells and increase the chance of metastases formation. Thus, surgery-induced translocation of viable bacteria or bacterial products potentially may stimulate metastases formation.

**Surgery-induced activation of immune cells**

Abdominal surgery was shown to result in significant attraction of PMNs to the peritoneal cavity. Depletion of PMNs diminished abdominal surgery-induced tumor recurrence, supporting an essential role for PMNs in tumor formation. This suggested that PMNs are involved in surgery-induced tumor cell adherence as activation of these cells may cause local tissue damage by oxidative burst that facilitate tumor cell attachment. Furthermore, bacterial products that translocate to the periphery during surgery provoke systemic immune responses and stimulate the accumulation and activation of PMNs in the organs like liver and the lungs. It was demonstrated that administration of LPS led to increased tumor cell arrest in the liver sinusoids of mice. Importantly, the majority of adhered tumor cells co-localized with adherent PMNs. This suggested that PMNs might facilitate tumor cells adherence in the liver sinusoids. In patients with major surgery or trauma significantly delayed spontaneous apoptosis of PMNs and enhanced ROS production were observed. This implicated that surgery-released inflammatory mediators extended the life expectancy of PMNs.

Previous studies furthermore suggested that macrophages are involved by development of metastases. Activation of peritoneal macrophages and blood monocytes, which are the source of tissue macrophages, after abdominal surgery was reported. These cells were shown to release high levels of the cytokines TNF or IL-6 after surgery. Furthermore, the liver contains the major portion of the body’s tissue macrophages, referred to as the Kupffer cells (KCs). KCs are adhered to sinusoidal endothelial lining and reside in the luminal site of the sinusoids. This is an ideal strategic position for KCs to scan the blood flowing through the sinusoidal lumen. Therefore, KCs are the immune cells that encounter the gut-derived bacteria or tumor cells. As bacterial components are released during surgery and KCs express TLRs that recognize the bacterial products, KCs may become activated during surgery. Recently, activation of KCs by LPS was demonstrated, as indicated by release of high levels of cytokines such as IL-6, TNF, and TGFβ.
Moreover, LPS stimulation of KCs was also suggested to initiate the release of ROS by these cells. Because the KCs are in close proximity of sinusoidal endothelial lining, inflammatory mediators that are released by KCs may also affect the hepatic endothelial cells. Eventually, this may facilitate tumor cell adhesion and augment metastases outgrowth.

**Therapeutic options for prevention of metastases development**

**Blocking tumor cell adhesion**

Adhesion molecules mediate tumor cell arrest in the target organs and as such play a important role in metastases formation. In animal models, development of metastases was significantly diminished when tumor cells were incubated with antibodies directed against adhesion molecules such as carcinoembryonic antigen (CEA) before injection in animals. This suggested that inhibiting tumor cell adhesion might be a promising approach to prevent surgery-induced liver metastases. Furthermore, impairment of liver microvasculature integrity following abdominal surgery was demonstrated. This resulted in exposure of ECM proteins, which served as preferable adhesion sites for tumor cells. A previous study demonstrated that liver metastases outgrowth was prevented by an antibody against integrin α2, indicating a pivotal role for integrins in tumor development. This suggested that blockade of tumor cell integrins could potentially prevent recurrent or metastatic disease. Many integrins recognize the amino acid sequence Arg-Gly-Asp (RGD) in their ligands. Thus, peptides that specifically bind to RGD sequence may prevent tumor cell adhesion.

However, surgery is inevitably associated with tissue injury. Immediately after surgery wound healing processes are initiated, in which the interaction between integrins and ECM proteins play an essential role. Moreover, non-malignant cells like keratinocytes, which are involved in wound healing processes, also express integrins. A previous study demonstrated that interference with integrin β1 affected correct wound healing. In absence of integrin β1 adhesion, migration, spreading or proliferation of cells were impaired, which resulted in abnormalities of epithelial structure. Taken together, integrins have disadvantageous effects on tumor development through mediating tumor cell adherence. However, integrins also play an essential role in the healing of surgical wounds. Therefore, blocking integrins after surgery may be a risk strategy to prevent surgery-induced tumor cell adhesion.

**Reactive oxygen species scavengers**

Surgical trauma was shown to result in enhanced production of ROS. We previously demonstrated that ROS facilitated tumor cell adherence by disrupting vascular integrity and exposing the ECM to which tumor cells preferably bind. Therefore, prevention of tumor cell adherence by treatment with ROS scavengers
may be an option for prevention of surgery-induced metastases. In our rat model, pre-operative injection of a ROS scavenger reduced tumor cell adhesion significantly. Unfortunately, we did however not succeed in inhibiting liver metastases formation by using antioxidants. This was probably due to interference with tumor cell killing by macrophages, which is also a process in which ROS play a major role. The antioxidant which was used in the previous study has a half-life of 5.6 hours. It may be that this ROS scavenger was not suitable for pre-operative intervention, because it probably interrupted tumor cell killing by macrophages. Thus, an antioxidant, which does not perturb the macrophage-mediated tumor cell killing, may have therapeutic potential. Therefore, we speculate that developing novel anti-oxidants with short half-life may interrupt early ROS production, hereby leading to less damaged liver vasculature, while preserving long-term macrophage function.

**Inhibition of TLR signaling**

As described above, surgical procedures for resection of CRC result in bacterial translocation from the gastrointestinal tract into the systemic circulation. Moreover, the concentration of LPS was increased after surgery. Importantly, bacterial translocation had disadvantageous effects on metastases development in patients. Previous studies found that LPS enhanced metastatic capacity of tumor cells and induced apoptosis resistance. Moreover, it has been shown that treatment of animals with LPS gave rise to tumors in the liver and the lungs. The receptor for LPS, TLR4, is furthermore expressed by wide variety of immune cells. Therefore, contamination with LPS may lead to activation of strong inflammatory responses, which may enable tumor cell adhesion. Pharmacologically blockade of LPS-TRL4 interaction would prevent TLR4 triggering on immune cells, which may abrogate inflammatory responses after surgery. Alternatively, therapies targeting the intestinal micro-flora by selectively decontaminating the intestines with antibiotics before surgery may minimize the risk of bacterial translocation and subsequent spread of LPS. However, detailed studies must be conducted to confirm the advantageous effects of the suggested therapies on patients’ outcome.

**Antibody therapy**

A therapeutic approach to stimulate antitumor immune responses is the use of monoclonal antibodies (mAb). Therapeutic mAbs have been developed to specifically target proteins that are (over-) expressed on tumor cells. Treatment of established solid tumors with mAbs has been disappointing. In contrast, previous animal studies demonstrated that use of mAbs successfully prevented the formation of surgery-induced liver metastases that originated from circulating tumor cells. Therapeutic effects of mAbs are achieved by direct or indirect effects on tumor cells. Binding of mAb to its antigens may prevent the activation of cell signaling pathways. Subsequently, this can lead to cell cycle arrest and reduced tumor cell proliferation inhibiting tumor progress. However, mutational changes in these signaling pathways abrogate the therapeutic actions of mAbs, which is a pitfall for
INTRODUCTION

treatment of established solid tumors. Alternatively, mAbs may be used to stimulate
the recognition of tumor cells by immune cells as targets. Pre- or post-operative
administration of mAb can opsonize free accessible circulating tumor cells. Then,
recognition of the Fc tail of mAb by immune cells through Fc receptors, may lead to
clearance of tumor cell via antibody-dependent cell-mediated cytotoxicity (ADCC)
or antibody dependent phagocytosis (ADPh). It was demonstrated that when KCs
were depleted, mAbs treatment did not prevent tumor formation, suggesting that
KCs play an important role in mAbs-mediated anti-tumoral actions.112, 113

One potential target for mAb therapy is epithelial cell adhesion molecule (Ep-CAM).
This protein is over-expressed in most carcinomas including coloncarcinoma.116
Moreover, high expression of Ep-CAM was associated with decreased survival of
patients with different types of cancers.117 Previously, peri-operative treatment
with an antibody directed against Ep-CAM was reported to reduce tumor-related
mortality after surgery.118 Patients that were treated with this mAb developed
significantly fewer distant metastases.119 However, recent randomized clinical trials
did not confirm the previous promising results.120-122 Thus, whether mAbs that
target Ep-CAM will provide clinical benefits is under intense debate. An alternative
target for mAbs may be the epithelial growth factor receptor (EGFR) that is up-
regulated in 80% of colorectal tumors.123

Scope of this thesis

The scope of this thesis was to investigate how surgery-induced liver metastases
develop and design potential experimental therapies to prevent this phenomenon
(see figure 2 for an overview of chapters). A previous study demonstrated that
blockade of integrin α2 successfully prevented liver metastases outgrowth after
surgery in a rat model. This suggested that integrins may be involved in surgery-
induced metastases formation. Therefore, the relation between the expression of
integrins in human primary colorectal tumors and patients’ survival after removal
of the tumor was studied. We observed that high expression levels of integrin α2 in
the primary colorectal tumor was associated with poor overall survival of patients
and enhanced risk for liver metastases development (chapter 2). Thus, expression
of integrin α2 may be used as a prognostic marker in patients and is potentially a
target for novel therapies. In chapter 3 we further investigated the mechanisms
of surgery-induced liver metastases. We found that abdominal surgery led to
impairment of liver vasculature via release of ROS. This subsequently caused the
exposure of sub-endothelial ECM proteins to which tumor cells adhere through
their integrin molecules.
Figure 2: scope of thesis. Relation between the expression of integrins on primary colorectal tumors and patients’ clinical outcome was investigated in chapter 2. Mechanisms of surgery-induced liver metastases were studied in more detail in chapter 3. Furthermore, effects of colorectal resection on metastases development were investigated in chapter 4. Role of PMNs and KCs in metastases formation were evaluated in chapters 5 and 6. Potential therapies for prevention of surgery-induced liver metastases were studied in chapters 7 and 8.
INTRODUCTION

Clinical and experimental data furthermore suggested that bacterial components, which translocate during surgical resection of primary colorectal tumor, might play an important role in metastases development. Because the intestines were not resected in our laparotomy model, we developed a colectomy model in which a small part of the colon was removed (chapter 4). Culture of smear samples that were taken from the peritoneal site of the colon of rats undergoing colectomy resulted in significantly enhanced bacterial outgrowth, compared to laparotomy. Importantly, rats that underwent colectomy developed significantly more liver metastases than control rats or rats that underwent laparotomy. We investigated the role of bacterial products in metastases formation in more detail. Bacterial products such as LPS are potent activators of cells of innate immunity like PMNs and KCs. Therefore, the effects of bacterial products on PMNs (chapter 5) and KCs (chapter 6) activation and tumor cell adherence were evaluated.

Use of mAbs has been proposed as a potential therapy for prevention of surgery-induced liver metastases. Therefore, we investigated the mechanisms of mAbs therapy in vitro and in vivo. In chapter 7 we used intravital microscopy and showed in real-time that KCs are the main effector cells in mAbs-induced tumor prevention. Treatment with a tumor specific mAb resulted in effective ADP by KCs. Additionally, in chapter 8 we identified EGFR as a therapeutic target for treatment of metastatic colorectal cancers in humans. mAb-induced phagocytes of tumor cells is EGFR expression dependent. Importantly, we demonstrate that mutational changes in cell signaling pathways do not affect phagocytes of tumor cells by macrophages. Finally, chapter 9 is a general discussion implementing our new findings in the current knowledge of how surgery-induced liver metastases begin and future recommendations how to end it.
CHAPTER 1

Reference list

INTRODUCTION

Reference list

44. van Grevenstein, W.M. et al. Surgery-derived reactive oxygen species produced by polymorphonuclear...
CHAPTER 1

Reference list


INTRODUCTION

Reference list

96. Dancygier,H. Microscopic Anatomy 2010).
CHAPTER 1

Reference list


112. van der Bij,G.J. et al. Experimentally induced liver metastases from colorectal cancer can be prevented by mononuclear phagocyte-mediated monoclonal antibody therapy. J. Hepatol. 53, 677-685 (2010).


