Discussion

Macrophages and tumour development

Solid tumours consist of a tumour stroma in which malignant cells interact with different non-malignant cell types and extracellular matrix components. This tumour stroma contains a plethora of different cell types. Fibroblasts produce extracellular matrix proteins, whereas endothelial cells form new blood vessels providing the growing tumour with necessary nutrients and oxygen. In addition, immune cells are also a major constituent of tumour stromas. In many tumour types, macrophages (mø) constitute a significant part of the tumour infiltrating immune cells, where they can play a complicated dual role in tumour development.

Macrophages are a major part of the innate immune system, and form a first line of defense against infiltrating pathogens in the body. Besides killing microorganisms, macrophages recruit other immune cells to the site of infection and activate both innate and adaptive immune responses. However, after the initial infection is cleared macrophages also play a pivotal role in tissue healing by scavenging apoptotic cells and cell debris, rearranging ECM components, producing growth factors and initiating angiogenesis. This led to the hypothesis that different subsets of macrophages exist, which can exert different functions in the body. First, during "classical" immune reactions, macrophages must clear infections in the body and therefore encounter microbial products like LPS. In addition to microbial products these macrophages are triggered by products from activated T helper (Th1) cells like interferon-γ (IFNγ) and become classically activated. Following Th1/2 nomenclature these "classically" activated macrophages are referred to as M1 macrophages. Through production of vast amounts of nitric oxide (NO), and reactive oxygen intermediates (ROI), M1 macrophages are profoundly efficient in killing of micro-organisms or virus-infected cells. Furthermore, by producing a wide array of pro-inflammatory cytokines like IL12, IL6 and TNFα as well as chemokines, M1 mø initiate an immune reaction capable of resolving infections. However, when naïve monocytes encounter Th2 associated cytokines like IL4 and IL13 or anti-inflammatory molecules such as IL10 and glucocorticoids they differentiate into so called "alternatively" activated or M2 mø. The function of M2 mø in the body is not yet entirely resolved. However, it has been demonstrated that they can produce different growth and angiogenesis stimulating factors as well as several anti-inflammatory cytokines. As such, they are thought to play an important role during wound healing and tissue rearrangements. It is however important to note that the qualification of macrophages into either M1 or M2 is oversimplified and M1 or M2 phenotypes should be considered as extremes in a continuum of different activational states.

Because macrophages have such diverse roles in the normal immune system of the body, the exact function of macrophages during tumour initiation and development is not fully understood. As explained in chapter 1 the presence of macrophages in either the tumour itself or in the surrounding tumour stroma often associated with prognosis of patients suffering from these malignancies. Because in several cancer types, including breast carcinoma and lymphomas, a clear association between increased macrophage presence and unfavorable patient survival is observed, macrophages are generally thought to play a role in tumour progression. These tumour associated macrophages or TAMs are thought to represent prototypic M2 macrophages that promote tumour growth, progression and metastasis by producing different growth - matrix remodeling - and
angiogenic factors as well as hampering immune reactions by producing anti-inflammatory cytokines. However, for other types of malignancy this association is much less clear, as presence of \(\text{M0}\) in tumours was not correlated with survival in many retrospective studies. Moreover, in the case of colorectal carcinoma or non-small cell lung cancer, a positive association with survival was observed.

It was earlier shown in a colon carcinoma metastasis rat model that total tumour load significantly increased if all resident macrophages were removed prior to metastasis initiation. This led to a major decrease in survival, indicating that macrophages play an essential role in inhibiting tumour growth in colon carcinoma. Interestingly, removal of macrophages not only led to enhanced tumour growth, but also to diminished microvessel density and better tumour differentiation. This suggests that during colon carcinoma development, macrophages are not solely confined to either M1 or M2 phenotype but that several subtypes of macrophages may coexist within the tumour. As such, we hypothesized in chapter 2 that the already present resident macrophages initially can kill tumour cells. During progression the tumour itself may however recruit new monocytes towards the tumour mass, e.g. via production of CCL2. These newly infiltrating macrophages will encounter a tumour microenvironment, which is immunosuppressive and will lead to immuno-editing of naive macrophages towards a tumour promoting M2 phenotype. However, we show that inhibiting recruitment of new macrophages with flavonoids into colon carcinoma metastasis led to a significant increase in tumour load, supporting that also infiltrating macrophages into colon carcinoma are necessary to reject tumour growth. By contrast, in a mouse model for mamma carcinoma influx of new macrophages by overexpression of CCL2 is associated with enhanced tumour progression. Thus, these data suggested opposing functions of newly recruited macrophages in colon versus mamma carcinoma, which was further investigated in chapter 3.

We demonstrated that colon carcinoma cells create a microenvironment, which enhances M1 characteristics of macrophages. Mamma carcinoma cells were shown to polarize macrophages towards an M2 phenotype, which was similar to previous experiments in which ovarian carcinoma cells had been used. Thus, carcinoma cells themselves produce and secrete factors into their surroundings, which make naive macrophages more prone to either M1 or M2 polarization. By analyzing the secretomes of different colon and mamma carcinoma cell lines we identified several factors that are uniquely expressed by colon carcinoma cells. One of these factors was Versican (VCAN), which is a member of the large aggregating chondroitin sulfate proteoglycan (SCPG) family. Deposition of VCAN by fibroblasts in the tumour stroma of different malignancies is often associated with poor patient prognosis, but data on the effect of VCAN production by malignant tumour cells is not so straightforward. In ovarian cancer high expression of VCAN by malignant epithelial cells is associated with enhanced overall patient survival, whereas an inverted correlation between cancer-cell expression of VCAN and survival is observed in endometrial and cervical cancers. We showed that the polarization of monocytes towards M1 macrophages by colon carcinoma supernatants could be diminished by blocking VCAN in the supernatant of colon carcinoma cells. Furthermore, led transduction of colon carcinoma cells with specific anti-VCAN shRNA constructs to decreased VCAN production by these cells and subsequently to downregulation of M1 characteristics of macrophages, which were incubated with...
these supernatants. VCAN is known to enhance e.g. TNFα production of macrophages by binding of TLR2 \(^{32}\) and subsequently did we show a downregulation of TNFα production by macrophages which were incubated with supernatants of anti-VCAN shRNA transduced colon carcinoma cells.

In patients with colorectal cancer stromal VCAN presence is not associated with survival. Interestingly however, VCAN expression by malignant epithelial cells in the periphery of colorectal tumours was correlated with a longer disease free survival in a cohort of stage II and stage III patients \(^{33}\). VCAN therefore is one of the factors secreted by tumour cells that induce a specific tumour microenvironment leading to polarization of macrophages, but probably other factors co-contribute in this polarization as well. Different carcinomas, commencing from different parts of the body, may be heterogeneous in the composition of their microenvironments. Colon or lung epithelial cells are constantly in contact with a pathogen rich environment, whereas e.g. epithelial cells of the mammary gland reside in a sterile environment, it’s reasonable that each cell type will influence the immune system differently \(^{34}\). As the the factors influencing mφ polarization in tumour micromilieus are produced by tumour cells are completely divergent from each other mφ polarization therefore differs between carcinoma types.

The role of macrophages in surgery induced metastasis

In addition to influencing growth and development of primary tumours, it was also demonstrated that macrophages play a pivotal role the induction of surgery-induced colorectal-cancer-metastasis formation (chapter 4). Colorectal cancer is the second most common malignancy and contributes to over 300.000 deaths in the western world annually. Surgical removal of the primary tumour is the preferred treatment and mandatory for successful 5 years survival. Unfortunately, 20-45% of patients, who are without detectable colorectal liver metastases (CLM) at time of diagnosis, will subsequently develop CLM after surgical removal of the primary tumour \(^{35}\). In earlier animal experiments we demonstrated that a simple operation like opening of the peritoneal cavity already inflicted trauma that leads to enhanced outgrowth of peritoneal and liver metastasis of colon carcinoma \(^{36, 37}\). It was demonstrated that tumour cells can disseminate from the primary colorectal tumour during surgery and are shed into the portal circulation leading directly towards the liver \(^{38-40}\). Increased numbers of circulating tumour cells in the portal system of colorectal cancer patients were furthermore shown to associate with decreased overall survival \(^{41}\). We previously unveiled that surgical trauma induces a systemic inflammatory response, which leads to endothelial damage and enhanced adhesion of circulating tumour to the microvasculature of the liver \(^{37}\). Adhesion of tumour cells after surgery can be inhibited by blocking α2 integrins on tumour cells, suggesting the involvement of sub-endothelial extracellular matrix proteins in this process \(^{37}\). We now show in chapter 5 that surgical trauma in both rats and human results in diminished expression of the tight junction protein claudin-5 in endothelium of the liver micro-vasculature, demonstrating endothelial damage \(^{42}\). This is in agreement with our earlier data that demonstrated decreased occludin expression between endothelial cells of liver vasculature already 45 minutes after surgical trauma to the peritoneal cavity \(^{37, 42}\). Thus, our data indicated that surgical trauma inflicted to the peritoneal cavity leads to systemic impairment of liver vessel integrity.
We now demonstrate in chapter 5 that downregulation of endothelial occludin in the liver after surgical trauma is dependent on macrophage activation, because depletion of liver macrophages (Kupffer Cells (KC)) prior to surgery abolishes this effect. Activated KC inflict endothelial damage probably by producing high concentrations of reactive oxygen species (ROS), and treating rats with a ROS scavenger inhibits surgery-induced down-regulation of the tight junction molecules ZO-1 and occluding, and rescues endothelial integrity in liver. Importantly, scavenging ROS leads to a significant diminished adhesion of circulating tumour cells after surgical trauma. Furthermore, we show that local production of ROS in vitro results in massive endothelial damage and exposure of underlying extracellular matrix proteins (ECM). We demonstrate that tumour cells exclusively and quickly adhere to exposed ECM, but do not exhibit enhanced binding to endothelial cells themselves. This is in agreement with our earlier studies with electron microscopy, in which we determine that surgical trauma leads to adhesion of tumour cells to ECM in the space of Disse, but not to the endothelial lining of the liver vasculature.

The mechanisms behind surgical trauma induced mØ activation are currently unknown. However, bacterial translocation due to increased intestinal permeability and fecal spillage during surgery may play an important role. It was demonstrated that anastomotic leakage in patients, who underwent surgery of primary colorectal tumours, is often associated with enhanced distant metastases outgrowth and poor disease free survival, supporting a role for bacterial products. Additionally, LPS, which is shed from Gram negative bacteria, enhances adhesion of colorectal tumour cells to the liver sinusoids. In chapter 2 we showed that macrophages are important in controlling colon carcinoma metastases growth and thus macrophages play a complicated dual role in both initiation and inhibition of liver metastases. As such, a fine balance exists between initial activation of mØ, which leads to local vascular damage as well as enhanced tumour cell adhesion and activation of mØ that is needed for efficient tumour cell killing (graphical overview in figure 1). Administering LPS led to retraction of endothelial cells in co-cultures of macrophages and endothelial monolayers and subsequently led to enhanced tumour cell adhesion in a mouse model after 1.5 hours after portal tumour cell injection. However in this same mouse model, LPS administration led to diminished metastases outgrowth in the liver, which was presumably due to a systemic activation of Kupffer cells and other mØ by LPS (N.Gül & M.Bögels, submitted).

Prevention of surgery-induced metastases

Although surgical trauma enhances the risk of liver metastases outgrowth, the initial effect of resection of primary colorectal carcinoma is removal of the bulk of the tumour mass. As such, only minimal residual disease, - consisting of single tumour cells or invisible micrometastases -, is still present. This makes the peri-operative period ideally suitable for e.g. adjuvant therapy. Preoperative systemic infusion with chemotherapy shows enhanced metastases free and overall survival in patients with colorectal stage III disease whereas no effects are seen in stage II patients. Another method to reduce surgery induced liver metastases formation is inhibiting adhesion of tumour cells to exposed ECM components in damaged liver vasculature. In earlier experiments we have shown that inhibiting β1 or α2
integrins on rat colon carcinoma tumour cells subsequently resulted in diminished adhesion of tumour cells to either damaged peritoneal surfaces or liver sinusoids, respectively.\textsuperscript{36, 37} Furthermore, blocking α2 integrins on rat colon carcinoma tumour cells, prior to injection into the portal circulation diminished outgrowth of liver metastases in rats that received surgical trauma.\textsuperscript{37} However, since integrins are also involved in wound repair\textsuperscript{53}, caution should be taken when treating patients with integrin blocking antibodies, not to interfere with healing of the anastomosis.

As such, a more elegant way to prevent surgery induced metastases outgrowth is to make the adhered tumour cells recognizable to the (already activated) immune cells of the liver. This can be achieved by opsonising tumour cells with specific mAb directed against membrane expressed tumour antigens. In chapter 6 we show that postsurgical administration of mouse IgG1 MG4 mAbs (MG4γ1), targeting an unknown membranous antigen on rat colon carcinoma tumour cells, counteracts the effect of surgical trauma on metastases outgrowth. Monoclonal antibody therapy completely prevented liver metastasis formation in both control rats and rats which received an operation prior to tumour cell injection. Opsonisation of rat colon carcinoma cells with MG4γ1 leads to increased phagocytosis of tumour cells by resident liver KC \textit{in vivo}, which are highly efficient in killing opsonised tumour cells as even treatment of rats with low mAb doses still results in inhibition of liver metastasis formation. Depletion of KC with clodronate liposomes, prior to injection of tumour cells makes the treatment with MG4γ1 ineffective. When KC depleted rats were however treated with a four timer higher dose, newly recruited ED1+ monocytes were partly able to take over the role of the KCs in the liver. So although newly recruited monocytes are less efficient in killing tumour cells, they still play an important role in preventing metastases formation. This is in accordance with data in chapter 2, in which inhibition of monocyte migration into colon carcinoma metastases enhanced tumour growth. When results with mAb therapy are translated into clinical applications, care should be taken with respect to the used mAb isotypes. Mouse IgG1 mAbs (like MG4γ1) are very effective in rats, but not in mice. By contrast, mouse IgG2a is suitable for targeting tumour cells in mice, but not in rats. For instance, administration of murine MG4γ2a antibodies has no effect on metastases development in our rat model, but mAb therapy with mouse IgG2a (TA99g2a) targeting melanoma cells was demonstrated to prevent liver metastases formation in mice\textsuperscript{54, 55}.

In chapter 7 we demonstrate that human IgG1 mAb Zalutumumab, directed against surface expressed EGFR, induces very efficient killing of human colon carcinoma tumour cells by both human and murine mο. By using time lapse microscopy we demonstrate that Zalutumumab opsonised whole tumour cells are rapidly phagocytosed by mο, which occurs within 60 minutes. After phagocytosis, tumour cells are killed through lysosomal fusion with phagosomes over a period of 10-24 hours, which is in accordance with our intravital microscopy data shown in chapter 8. To investigate the mechanisms of mAb therapy in more detail, we next performed intravital microscopy (chapter 8). Tumour cells are trapped quickly in the liver sinusoids after injection into the portal circulation. By treating mice with murine TA99y2a mAb prior to injection of tumour cells we were able to show with intravital microscopy that KCs are able to rapidly recognize and phagocytose whole opsonised tumour cells in vivo, which does not occur in the absence of TA99y2a. KC can
furthermore effectively degrade tumour cells, albeit more slowly, which ultimately prevents development of liver metastases. Murine IgG2a and human IgG1 show homology and can be used interchangeably in mice because of cross-reactivity, although hIgG1 is slightly less effective compared to mIgG2a.

It was previously demonstrated by us and others that for adequate mAb treatment of liver metastases both macrophages and the FcγRI are essential. Although the FcγRs on macrophages can react with both chimeric, humanized or fully human IgG1 and IgG2, human IgG3 is in theory the best ligand for all FcγR expressed on macrophages and other immune cells. Human IgG3 furthermore displays the strongest effector functions of all IgG subclasses. In chapter 9 we demonstrate that mAb therapy with either human IgG1 or IgG3 is equally efficient in abolishing the formation of liver and peritoneal metastases in a mouse model. In vitro human IgG1 initiates very efficient antibody dependent phagocytosis (ADPh) of tumour cells by macrophages, whereas human IgG3 is less adept in mediating ADPh. This suggests that human IgG1 and IgG3 differ in their mode of action as how they mediate tumour cell killing. Combining a matched set of specific anti-tumour IgG1 and IgG3 mAb may lead to a synergistic effect and to enhanced therapeutic potential in preventing metastases formation in colorectal patients. One objection against combining human IgG1 and IgG3 mAb in vivo is the fact that human IgG1 inhibits the recycling of human IgG3 via the neonatal FC receptor (FcRn), resulting in short half-life of IgG3 in vivo, making it less effective for mAb therapies. To overcome the inferior half-life of wild-type IgG3 a specific IgG3 was constructed containing a His435 in its Fc domain leading to enhanced rescue through FcRn and subsequently to enhanced half-life in vivo. Although other studies indicate that transferring parts of the IgG3 Fc domain into IgG1 enhances both complement binding, CDC and ADCC in anti-CD20 mAb therapy against lymphomas, further research is needed to confirm that mAb therapy which combines human IgG1 and human IgG3-R435H leads to enhanced therapeutic potential against colorectal metastases formation.
Future perspectives and recommendations

As demonstrated in this thesis, macrophages play important roles in development of primary tumours as well as liver metastases, but can also be targeted for mAb therapy of cancer (Figure 1). This knowledge can lead to several novel therapeutic strategies.

Macrophages and tumour development
The discovery that macrophages amplify the malignant potential of many types of cancer by secreting different cytokines, angiogenic- and growth factors, led to approaches to inhibit influx of macrophages into tumours. Although in some experimental models inhibition of macrophage migration resulted in diminished tumour growth we show that in colon carcinoma inhibition of mo influx has opposite effects, as infiltrating monocytes collaborate in preventing of tumour growth. Our data are consistent with other studies, which show reduced growth and suppressed metastasis after stimulating migration of macrophages into both colon and renal carcinomas. I therefore advise to be cautious with designing therapeutic approaches that interfere with influx of macrophages into tumours of different malignancies. As such, macrophage influx should be promoted. It was demonstrated that approaches, aimed to give a massive influx of macrophages into tumours lead to disruption of tumour growth in many types of malignancies.

It is however important to prevent development of infiltrated monocytes into M2 macrophages. For instance, in breast cancer, newly recruited monocytes may differentiate into M2 macrophages leading to enhanced tumour growth and decreased patient survival. Thus another promising approach is to re-educate the tumour associated macrophages to become more tumoricidal. In this thesis we discovered that colon carcinoma tumour cells create a micro-milieu which favors the differentiation of macrophages into more anti-tumourigenic M1 macrophages, whereas breast carcinoma cells enhance pro-tumourigenic or M2 characteristics (chapter 3). Analysis of secretomes of both colon and breast carcinoma cell lines demonstrated that tumour cells differentially secrete proteins, which influence macrophage development. Specific proteins secreted by colon carcinoma cells, (for example Versican), enhance TNFα and IL12 production by macrophages, which make these proteins ideal candidates for future therapeutic approaches. Enhancing production of M1 associated cytokines like TNFα in tumour infiltrated macrophages resulted in tumour regression in animal models for melanoma and ovarian cancer. Thus, analyzing secretomes from either colon or breast carcinoma cells will generate valuable insights into how tumour cells themselves create a micro-milieu, which favors either M1 or M2 differentiation of macrophages. This will also yield clues to develop therapies that can influence this process. It was already demonstrated that adjuvant therapies like LPS, muramyl peptides or INFγ treatment enhance tumoricidal activity of macrophages and decrease tumour growth in animal models. Factors like Versican, which favor differentiation of macrophages into a M1 phenotype, can hopefully be combined in the future with other adjuvant therapies to enhance the tumoricidal characteristics of tumour infiltrated macrophages.

Macrophages as target cells against surgery induced metastasis
Although resection of the primary colorectal carcinoma is mandatory to ensure short
time survival of colorectal patients, we and others have shown that trauma inflicted by this operation enhances the incident of distant metastases outgrowth 36, 37, 54, 72-78. We now demonstrate that surgical trauma causes a systemic effect leading to activation of macrophages and disruption of endothelial integrity, subsequently enhancing tumour cell adherence. Although we demonstrate that diminishing ROS production of macrophages by either ROS- or LPS scavengers (unpublished data), leads to diminished tumour cell adhesion during surgery, caution must be taken in extrapolating these data into therapeutic approaches for patients, as both treatments diminished tumouricidal activity of macrophages, which may result in enhanced metastases growth. In my opinion it would be better to promote tumouricidal capacity of macrophages.

We demonstrate that macrophages are beneficial in diminishing surgery induced metastasis by using them as effector cells for antibody targeted therapy. When tumour cells are opsonised with mAb directed against surface molecules, macrophages can recognise, interact with and kill individual tumour cells. Using specific rat and mouse models we demonstrated that peri-operative treatment with specific anti-tumour mAb abrogates surgery induced metastasis formation 54, 55. In the clinic anti-EGFR mAb have been used to treat colorectal cancer. These mAbs block interaction of EGFR with its endogenous ligands and inhibit EGFR downstream signaling on tumour cells. This leads to G1 cycle arrest and subsequent diminished proliferation and tumour regression in a small subgroup of colorectal carcinoma patients 79-81. Many patients, however have mutations in the EGFR downstream targets KRAS and BRAF, which make the tumour irresponsive for EGFR blocking therapies 82. We now show that targeting EGFR on human colon carcinoma tumour cells with Zalutumumab results in efficient tumour cell recognition and killing by macrophages, which is independent on KRAS or BRAF mutational status. As more than 80% of colorectal tumours show upregulation of EGFR on their surface, targeting EGFR will be an ideal candidate for prevention therapies against surgically induced metastases formation.

One single older study already hinted towards the feasibility of this approach by post-operatively treating colorectal patients with mouse IgG2a antibodies directed against surface expressed EpCAM 83. This study showed a slight decrease in distant recurrences and improved 7 year overall survival in a small cohort of patients. The fact that other studies could not reproduce these data indicates that designing a correct clinical study is extremely difficult. The use of a murine mAb likely influenced the poor effectiveness in this study. To effectively study the effects of mAb therapy against surgery induced metastasis formation a cohort of patients is needed who have EGFR positive tumours and are definitely free of distant recurrences prior to resection of their primary tumour. I anticipate that a well designed randomised clinical trial with human IgG1 mAb against surface expressed EGFR will demonstrate the strength of such a therapeutical approach. Combining anti-EGFR mAb (e.g. Zalutumumab) with other clinically used human mAbs against e.g. EpCAM (Adecatumumab), CEA (Labetuzumab) or Her2/neu (Trastuzumab) can probably further enhance the effectiveness of this therapy. Furthermore, combining mAb therapy against tumour cells with other adjuvant therapies, which enhance the tumouricidal activity of macrophages will in my opinion be most successful in inhibiting formation of (surgically induced) metastases in colorectal cancer patients.
Concluding remarks

It is clear that macrophages play a very complicated dualistic role in both tumour development and metastasizing. Macrophage infiltration into tumours can negatively influence patient prognosis and macrophage activation during operation predisposes distant vasculatures for tumour cell adhesion and subsequent metastases outgrowth. However in primary colorectal tumours macrophage infiltration is associated with enhanced patient survival and post-surgical mAb therapy enables macrophages to completely abrogate distant metastasis formation. I predict that enhancing tumouricidal properties of tumour infiltrated/infiltrating macrophages will improve strategies for mAb therapy. By focusing on the tumouricidal characteristics of macrophages they can play a decisive role in devising novel therapeutic strategies against and subsequently enhancing the live expectancy of cancer patients.

**Figure 1 Graphical abstract**

Macrophages play different roles in both tumour development and initiation and mAb therapy against surgery induced metastasis formation.

**Left side:** Solid tumours produce different chemokines like CCL2, which attract monocytes into the tumour. Tumour cells originating from different parts of the body produce different (unknown) factors which influence monocyte to macrophage development. Breast carcinoma cells for example produce factors, which skew monocyte polarization towards a more alternative or M2 like phenotype. Subsequently these monocytes will differentiate into M2 like macrophages (depicted as red M2 macrophages). Beside producing the anti-inflammatory cytokine IL10, these macrophages enhance angiogenesis by producing different angiogenesis factors. Also they can directly influence tumour growth by producing several growth factors and by producing different matrix degrading enzymes like MMPs, they enhance invasion of tumour cells into surrounding tissues and subsequently help tumour cell extravasation into the bloodstream. Extravasated tumour cells can adhere to the intact endothelial lining, where they subsequently grow out into metastases. Colon carcinoma cells however polarize infiltrated monocytes to gain more anti-tumour properties. These monocytes differentiate into macrophages which resemble more classical M1 macrophages (depicted as green M1 macrophages). These macrophages have cytotoxic capabilities and can either directly phagocytose tumour cells or induce tumour cell killing by producing ROS and NO.

**Right side:** Surgery induces tumour cell dissemination, which can directly go into the bloodstream. Furthermore, does surgery induce a systemic activation of macrophages, which will subsequently produce ROS. High concentrations of locally produced ROS will lead to damage and retraction of endothelial cells and subsequently to exposure of underlining ECM. Disseminated tumour cells adhere to exposed ECM and subsequently grow out into metastases. This surgery induced metastases formation can be abrogated by peri-operative treatment with mAb. This enables (activated) macrophages to recognise and kill adhered tumour cells by antibody mediated phagocytosis.
Monocytes migrate into tumour

VEGF TGFβ

CCL2 Chemokines

MMPs Invasion + extravasation

growth factors

Arginases

migration into tumour

M2 Macrophages

M1 Macrophages

endothelial damage (ROS)

ADPh

Tumour killing

Tumour development

Surgery induced metastasising

with mAb therapy

with out mAb therapy

Macrophages and

Macrophages and


54. 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.*(2010).


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