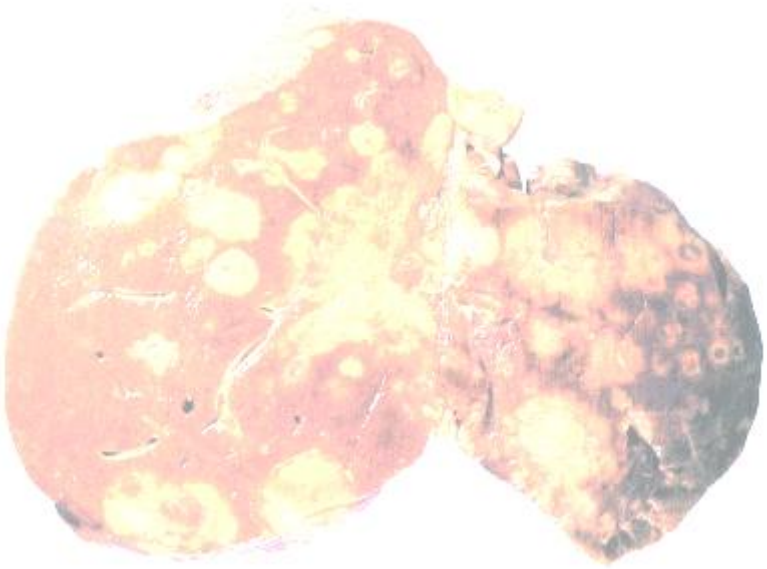
The role of tissue damage in hepatic cancer development and outgrowth



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Hepatocellular cancer (HCC) is the most common primary hepatic cancer and is overall the 5th most common cancer with 748,300 new liver cancer cases in 2008, and the 3rd most lethal cancer in the developed world with 695,900 fatalities in 2008.(Jemal et al. 2011) Chronic liver injury, inflammation, and fibrosis have been clearly linked to liver carcinogenesis through epidemiological studies.(El–Serag, Rudolph 2007, El-Serag 2002) Various etiologies are known to be able to induce this chronic state of liver injury, fibrosis and inflammation of the liver, including chronic drug abuse or alcohol consumption, autoimmune disorders, toxins, bacterial infections and the most important cause, chronic infection of the liver with viruses.(El–Serag, Rudolph 2007, El-Serag 2002, Blonski, Kotlyar & Forde 2010, Perz et al. 2006) It has been estimated that over 80% of HCC cases are caused by chronic liver infections with hepatitis B and hepatitis C viruses.(El–Serag, Rudolph 2007, El-Serag 2002, Perz et al. 2006)

Besides the clear causative relationship between chronic liver injury and carcinogenesis, liver injury has also been linked to increased outgrowth of preexisting primary and secondary liver tumors.(Harun et al. 2007, Mizutani et al. 1992, Nijkamp et al. 2009, Scheele, Stangl & Altendorf-Hofmann 1990, Schindel, Grosfeld 1997) Secondary liver tumors are even more common than primary liver tumors, which can be explained by the physiology of the organ.(Kasper et al. 2005) High volumes of blood pass the liver from a dual supply, the systemic circulation and the portal vein, increasing the chance of transporting tumor forming cells (TFCs) to the liver. Fenestrations in the hepatic sinusoidal epithelium then allow relatively easy penetration of TFCs, enabling TFCs to settle into the hepatic parenchyma, causing secondary tumors in the liver. Especially colorectal, lung, and breast tumors are known to have a high potency to metastasize to the liver.(Kasper et al. 2005)

Primary and secondary liver tumors are notoriously difficult to treat with chemotherapy. For the majority of hepatic malignancies surgical resection is considered the only real curative option.(Garrean et al. 2007) In many patients surgery is not possible due to the bad condition of the liver, tumor size, number of tumors and or location of the tumors. In these cases ablative techniques are used to destroy tumor tissue for palliative or curing purposes.(Garrean et al. 2007) These methods however, also cause damage to the liver that can lead to increased outgrowth of tumor cells that have survived the treatment, or probably cause development of new tumors.(Mizutani et al. 1992, Nijkamp et al. 2009, Scheele, Stangl & Altendorf-Hofmann 1990, Schindel, Grosfeld 1997, de Jong et al. 1998, Meredith et al. 2007, van der Bilt et al. 2005, Van Der Bilt et al. 2007)
In this review, the current knowledge of underlying mechanisms of liver tissue damage leading to carcinogenesis and cancer outgrowth will be discussed.

The regenerative response.

Primary liver tumors usually develop in a background of chronic fibrotic and inflammatory liver disease. However, scarring and inflammation of the liver are part of the normal wound healing process also seen in response to acute parenchymal cell death. Therefore the regenerative response of the liver will be briefly discussed.

The liver regenerative response can be divided in three phases, the priming phase, the proliferation phase, and the phase in which growth termination occurs (figure 1). (Pahlavan et al. 2006) Initiation of the priming phase is due to various damage signals released in the liver environment due to liver injury, including reactive oxygen species (ROS), IL-1α(Sakurai et al. 2008), damage associated molecular patterns (DAMPs)(Seki et al. 2007), and in case of liver injury due to microbial infection pathogen associated molecular patterns (PAMPs).(Seki et al. 2007) Furthermore changes in blood flow up regulate the vasodilators nitric oxide (NO) and prostaglandins, but also cause hypoxia in certain areas leading to up regulation of angiogenic cytokines like vascular endothelial growth factor (VEGF).(Breuhahn, Longerich & Schirmacher 2006)

Although multiple cell types can be activated by these signals, activation of hepatic stellate cells (HSCs) and also Kupffer cells seems pivotal for initiation of the wound healing reaction in the liver. DAMPs and PAMPs can activate these cells via toll like receptors (TLR4) (Seki et al. 2007) and through IL-1α binding to the IL-1 receptor.(Sakurai et al. 2008) ROS, also produced by activated Kupffer cells, can induce the ERK/MAPK in HSCs, activating HSCs, stimulating proliferation of HSCs, and stimulating production of platelet derived growth factor (PDGF) in stellate cells.(Svegliati-Baroni et al. 2001) PDGF binding to the beta-PDGF receptor present on HSCs provides a strong stimulus for HSC proliferation and activation. The importance of Kupffer cells (Abshagen et al. 2007) and HSCs (Shen et al. 2011) is emphazised by the observation that depletion of these cell types leads to impaired liver regeneration in experimental animal models.

Activated HSCs and Kupffer cells secrete IL-6 and necrosis factor-α (TNF-α) which both provide a stimulus for hepatocytes. IL-6 binding to the gp130 receptor and TNF-α to the TNF receptor activate the STAT3 and the NF-κB pathway in hepatocytes.(Pinzani, Marra 2001, Gao 2005) This triggers the transition of hepatocytes from G0 into the G1 phase and sensitizes them for hepatic growth factor (HGF), EGF (endothelial growth factor) receptor ligands, amphiregulin and insulin growth factor binding protein-1(IGFBP1) secreted by various cell types.(Gao 2005, Fausto 2000) Separate disruption of the IL-6 (Sakamoto et al. 1999, Cressman et al. 1996) and TNF-α (Yamada et al. 1997) signaling pathway (through IL-6 gene KO, and through hepatocytes specific IKKβ gene KO) resulted in significantly delayed regenerative responses and in the study of Cressman *et al.* even a significant increase in mortality(Cressman et al. 1996) after 70% hepatectomy in mice. This clearly illustrates their importance for the regenerative reaction in the liver. Besides priming hepatocytes for proliferation, IL-6(Taub 2003) and TNF-α(Maeda et al. 2005) are also important survival factors for hepatocytes in the regenerating liver environment. This double function of IL-6 and TNF-α, inhibiting hepatocyte death and stimulating hepatocyte proliferation, allows for efficient hepatocyte replenishment.

Furthermore TNF-α was found to enhance expression of metalloproteinase 9, important for the degradation of ECM.(Tarrats et al. 2011) Although chronic liver injury and chronic liver regeneration are marked by fibrosis of the liver, in early stages of the acute regenerative reaction an overall breakdown of ECM occurs. Increased secretion of matrix metalloproteinases is responsible for the ECM

breakdown.(Kim et al. 1997) The activity of matrix metalloproteinases appeared to be vital for hepatic regenerative reaction in rat after partial hepatectomy, although it is not known exactly why. It has been suggested that decreased degradation of ECM hampers the migration of endothelial cells and HSCs, or decreases release of pro-endothelial growth factors, compromising the regenerative response.(Alwayn et al. 2008, Isbert et al. 2002, Kallis et al. 2011) ECM production by HSCs is in this stage inhibited by paracrine signaling of vasodilators and hepatic growth factor to the HSCs. At this stage HSCs do excrete chemotaxic substances increasing inflammatory cell infiltration and interact with lymphocytes, coordinating the inflammatory reaction.

In the following phase, the proliferation phase, hepatocytes form clusters in which hepatocytes proliferate. Important pathways inducing hepatocyte proliferation are the ERK/MAPK pathway, activated through hepatocyte growth factor binding to the c-Met receptor(Pennisi et al. 2004), and pathways activated through binding of the EGFR (epidermal growth factor receptor) by EGF, HB-EGF (heparin binding EGF-like growth factor), TGF-α (transforming growth factor-α), and amphiregulin,(Michalopoulos, Khan 2005) and insulin growth factor binding protein-1 binding.(Leu et al. 2003) With a little delay also HSCs and endothelial cell clusters are formed that proliferate next to the proliferating hepatocyte clusters.

TGF-β, secreted by HSCs late in the proliferation phase and throughout the growth termination phase, stimulates production of ECM by a plethora of cell types including HSCs (auto-stimulation), portal fibroblast, fibroblasts derived from hepatocytes through epithelial-mesenchymal transition(Zeisberg et al. 2007), and possibly bone marrow derived cells. TGF-β also forms a signal for hepatocytes to stop proliferating.(Yasuda et al. 1993) At this time, hypoxic signaling from the hepatocyte clusters stimulates endothelial cells to migrate into the clusters of hepatocytes and form the vasculature of the hepatocyte cluster.(Grunewald et al. 2006) In the healthy liver this reaction ultimately leads to healing and full recovery of the liver.

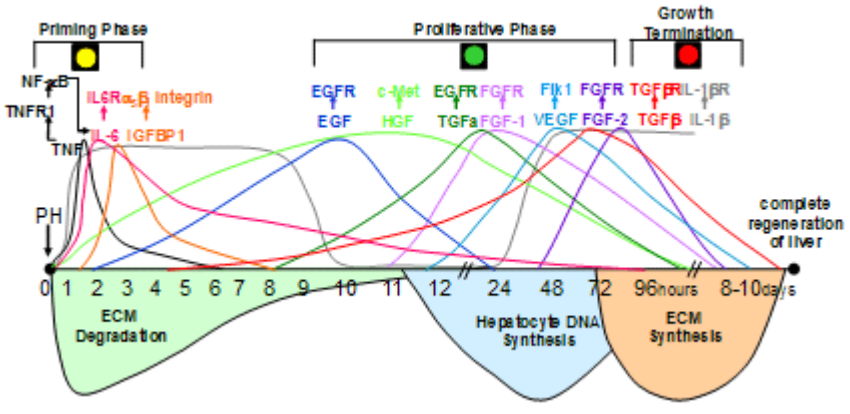

Fig.1 *Schematic representation of cytokine and growth factor levels in the three phases of liver regeneration after partial hepatectomy (PH.)* ***Adapted from Mohammed et al. 2005.***

Chronic liver injury in hepatic carcinogenesis

In chronic liver injury the catalyst of this reaction, liver injury, is not removed. So the regenerative response is ongoing, leading to a chronically inflamed liver environment in which accumulation of fibrotic tissue and continuous hepatocyte replacement occurs. Of course this is not a healthy situation, and multiple components of this chronic reaction enhance the development of HCC (figure 2).

First of all, chronic liver injury is accompanied by increased levels of oxidative stress. Oxidative stress is known to be able to induce DNA damage. Increased oxidative stress could in this way, increase chance of accumulating DNA damage needed to develop HCC.(Barash et al. 2010, Mohamed et al. 2011) Furthermore it was established that inflammation induces double strand DNA breaks, accelerating carcinogenesis.(Barash et al. 2010) Secondly, increased turnover of hepatocytes also leads to enhanced chance of accumulating mutations in the DNA. Through increased rounds of proliferation of adult hepatocytes or liver progenitor cells, required to replace dead hepatocytes, the chance increases that mutations vital for HCC development accumulate in the DNA of a cell during DNA replication. Inhibition of TNF-α signaling in rats, important for hepatocyte survival as previously mentioned, increased cell turnover after treatment with diethylnitrosamine (DEN, a carcinogen toxic to the liver through increased ROS production) and also was found to enhance the development of HCC compared to rats which only underwent DEN treatment.(Maeda et al. 2005) This clearly shows that increasing turnover of hepatocytes, and thereby increasing the need of compensatory proliferation increases chance of developing HCC. Although accumulation of mutations in the DNA plays a pivotal role in HCC development, HCC is a very heterogeneous cancer with respect to mutated genes. Pathways that are often affected include in HCC the mammalian target of rapamycin (MTOR) pathway, IGF1R, HGF and c-Met, Ras, EGFR and angiogenic pathways. P53 is mutated in about 20% of HCC cases in the western world.(Walther, Jain 2011)

While inflammatory mediators and growth factors are essential for induction of the regenerative response after liver injury, it was established that some inflammatory mediators also have pro-tumorigenic properties. TNF-α for instance, found to inhibit HCC development by lowering hepatocyte turnover, was found to also protect transformed cells from apoptosis.(Pikarsky et al. 2004) In line with this, inhibition of the NF-κB pathway was found to inhibit tumor progression at a later stage of HCC development.(Pikarsky et al. 2004) TNF-α mediated survival of cells with DNA mutations would probably not pose a problem in acute liver damage since after the healing process is finished, the TNF-α level drops and transformed cells would most likely die without the stimulus from TNF-α. In chronic liver injury and ongoing inflammation however, concentration of these inflammatory mediators do not drop and allow the transformed cells to grow, proliferate and form tumors. Also IL-6 appeared important for HCC development as was shown in IL-6 KO mice. Absence of IL-6 in these mice almost completely inhibited DEN induced HCC formation, showing its importance in tumor formation.(Naugler, Karin 2008) Lower IL-6 levels in females might therefore explain why HCC prevalence is higher in males.(Naugler, Karin 2008) EGFR signaling was found to enhance transformation of hepatocytes. Infiltrating and

activated macrophages play an important role in the development of HCC by secreting a plethora of growth factors and cytokines including TNF-α, TGF- β, andIL-6. (Zamarron, Chen 2011)

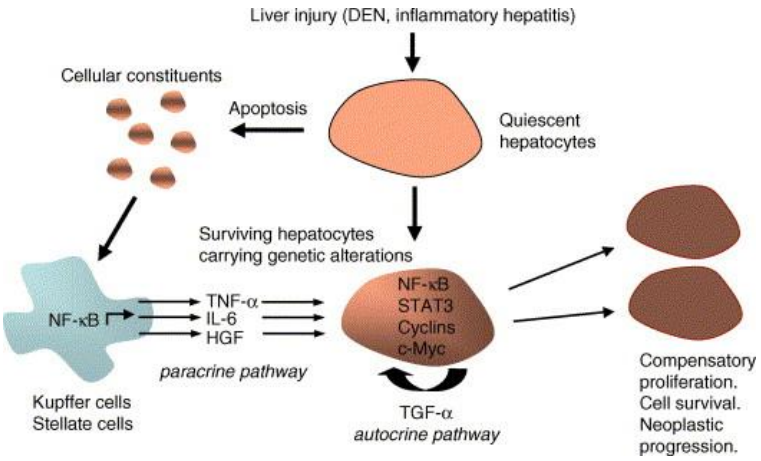

Fig.2 *Chronic liver injury induced neoplastic formation.* growth factor and cytokine mediated increased rounds of replication and genetically altered cell survival. ***Adapted from: Arsura et al. 2005***

Established solid tumors also often attract macrophages by which they benefit from the cytokines and growth factors secreted by these macrophages. The secretions of these so-called tumor associated macrophages can promote solid tumor growth, angiogenesis, metastases, ECM modification and immunosuppression(figure 3). (Zamarron, Chen 2011, Shirabe et al. 2012, Mantovani et al. 2006)(Breuhahn, Longerich & Schirmacher 2006) Higher levels of IL-6 (Naugler, Karin 2008) and TNF-α (Pikarsky et al. 2004), important for development of HCC, were also correlated with faster HCC progression. However also growth factors not necessarily/exclusively secreted by macrophages including HGF, VEGF and EGF can promote tumor cell proliferation and progression. (Breuhahn, Longerich & Schirmacher 2006) VEGF and HGF were also found to be involved in tumor angiogenesis. HGF mediated increased metallomatrix proteinase (MMPs) activity was reported to be involved in tumor basement membrane degradation, promoting metastasis.

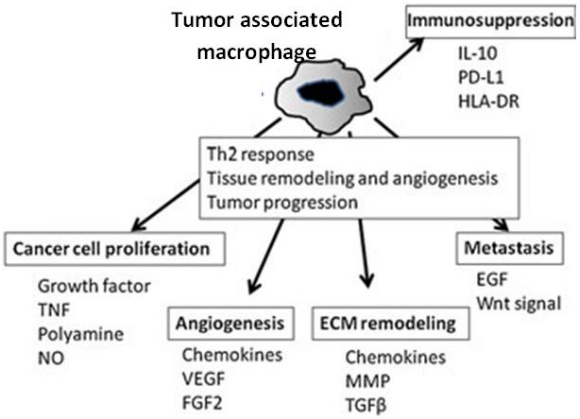

Fig.3 *Tumor associated macrophages play a central role in tissue remodeling, angiogenesis and tumor progression.* The processes are depicted with most important growth factors and cytokines for these processes. ***Edited from: Shirabe et al. 2012***

While there is agreement that these different factors are of importance for development of HCC, there is discussion which cells are the cell of origin for developing HCC. HCC could of course have adult hepatocytes as cell of origin but it is

also possible that liver progenitor cells transform into HCC cells.(Walther, Jain 2011, Wu, Chen 2006) Reasoning behind the theory that the liver progenitor cells are the cells of origin, is the fact that HCC tumors can contain a mix of mature cells and cells that contain stem cell markers and are phenotypically similar to liver progenitor cells.(Walther, Jain 2011, Wu, Chen 2006) These proliferating and differentiating liver progenitor cells would be more prone to accumulation of DNA damage than adult hepatocytes. However, it is also possible that adult hepatocytes dedifferentiate after transformation, and in this dedifferentiation process gain these stem cell markers. It has also been suggested that the different HCC types originate from different progenitor cells as depicted in figure 4. It is not yet clear which hepatic cell populations are the source of HCC. However, it is clear that increased replacement of hepatocytes, by proliferation of adult hepatocytes or progenitor cells, increases chance of developing HCC. (Walther, Jain 2011, Wu, Chen 2006)

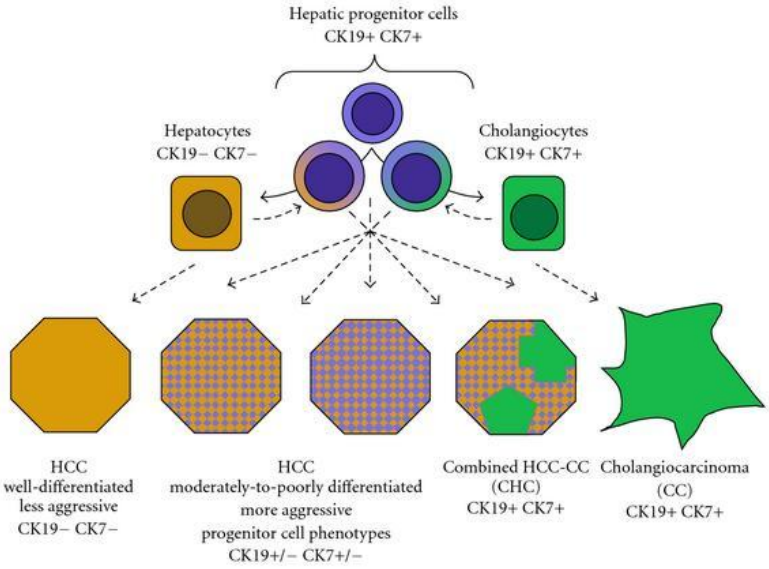

Fig.4 *The cell of origin for HCC development is not known*. Possibly multiple cell types are capable of forming hepatocellular tumors. Dotted lines depict the routes through which HCC is thought to be able to develop. It is possible that transformed hepatocytes and choliangiocytes could give rise to all depicted carcinoma's through dedifferentiation. ***Adapted from: Walther, Jain 2011***

Plenty of evidence is available showing that chronic liver injury and inflammation can lead to generation of malignancies in the liver by various mechanisms. Unlike chronic liver injury, acute liver damage has epidemiologically not been linked to the development of HCC. In line with this, acute liver injury, induced through RFA treatment in livers of wt mice, did not lead to HCC development. Even liver specific knockout of p53 did not lead to induction of HCC formation after RFA-induced liver injury. RFA on the livers of mice with liver specific p53/Rb double knockout however did lead to HCC development. This probably reflects that for the development of HCC to occur, multiple mutations in the DNA have to accumulate. Liver injury allows DNA mutations to accumulate through the previously described mechanisms. In wt mice the window of opportunity is probably too small to accumulate all of these mutations during the wound healing response induced by acute liver injury. The

double knockout mice already carry some of the mutations needed for HCC development and therefore the time of the acute response is probably enough to accumulate the other mutations needed to develop HCC. This experiment again shows the importance of liver injury to the development of HCC and could well be used to identify genes important for the development of HCC.(Unpublished results, de Bruijn *et al.*)

Acute liver injury affecting preexisting tumors

So, acute liver injury is not capable of inducing HCC in healthy wt livers of mice. In line with this acute liver injury in human acute liver injury has not been epidemiologically linked to HCC development. It has however been observed that acute liver injury can increase outgrowth of pre-existing malignancies and micro-metastases in the liver in animal models and also in human. Since the most commonly used treatments for hepatic malignancies cause acute damage to the liver, it is clinically relevant to study acute liver damage and their effects on preexisting tumors.

Curative resection, the golden standard in the treatment of hepatic malignancies, consists of removal of the part of the liver infested by the tumor. Failure to remove the entire tumor has been found to increase outgrowth of the remaining tumor cells. Blood from rats, which previously had endured partial hepatectomy, was observed to induce growth of cultured rat colorectal cancer cells.(de Jong et al. 1998) This indicates that regenerative mediators are secreted in the bloodstream and can induce tumor growth distant from the lesion. The tumor promoting capabilities of these mediators have been described in the previous section. In a rat model of HCC it was shown that the amount of liver tissue removed by partial hepatectomy, significantly correlated with residual tumor growth and invasiveness. Removal of 70% of the liver led to a measured tumor volume of roughly five times higher than seen in mice in which 30% of the liver was removed.(Sorin et al. 2009, Shi et al. 2011) This shows that also the magnitude, and most likely the amount of regenerative mediators released, increase tumor outgrowth and invasiveness.

In human it was confirmed that after partial hepatectomy, higher levels of regenerative mediators, like HGF, basal fibroblast growth factor (bFGF) and VEGF, are correlated to earlier recurrence of HCC.(Meredith et al. 2007, Osada et al. 2010, Poon et al. 2007) Curative resection is however only possible in a small portion of patients suffering of malignancies in the liver due to tumor size and number, and condition of the liver at time of discovery.(Bhardwaj et al. 2010) The most commonly used alternative for curative resection used to treat liver malignancies is radiofrequency ablation (RFA).(Bhardwaj et al. 2010) This method uses an alternating electrical current, running between a needle electrode, which is inserted in the tumor, and grounding pads.(Dobbins, Strickland & Maddern 2010, Nikfarjam, Muralidharan & Christophi 2005) This electrical current causes friction and increases temperatures in the tissue surrounding the tip of the needle electrode. When temperatures reach above 60°C necrotic coagulation of the tissue occurs destroying the tumor. The effect of RFA is greatly dependent on the size of the tumor that is treated. Tumors bigger than approximately three cm in diameter can't be ablated at

once.(Dobbins, Strickland & Maddern 2010, Nikfarjam, Muralidharan & Christophi 2005) Therefore in tumors bigger than three cm often tumor cells survive treatment leading to higher recurrence rates in these cases. Remaining tumor cells have been observed to have increased outgrowth.(Nijkamp et al. 2009) Clinical trials are currently running with VEGF blockers in unresectable HCC.(Ruers et al. 2010) First results of these trials show that RFA treatment in combination with VEGF blockers did lead to a positive trend in patient survival time, however this was not significant.(Ruers et al. 2010) If positive results are found in further trials it might be interesting to try VEGF blockers in patients with resectable HCC also, possibly preventing recurrence of the cancer after hepatectomy. The limited efficiency of VEGF inhibitory treatment might be due to the upregulation of other regenerative factors, like for instance HGF, in response to partial liver removal. Possibly a mix of multiple growth factor blockers would be more effective the prohibit tumor outgrowth and invasiveness in response to partial hepatectomy.

In a study that compared the effects of RFA therapy and liver resection on solid colorectal tumors (C26 cells) distant from the lesion, RFA was not found to induce increased growth of the residual tumor mass unlike liver resection. Hepatic resection was, like previously described, found to induce increased levels of numerous growth factors important for the liver regenerative response like HGF, bFGF and VEGF.(Meredith et al. 2007) No elevation of these growth factor levels was observed after RFA treatment. This shows that the observed enhanced outgrowth after RFA treatment does not act on a systemic level like hepatic resection. Since we have seen that the size of resection is important for the induction of outgrowth, lack of tumor growth induction by RFA probably reflects the low amount of hepatocytes lost in RFA compared to the massive loss of hepatocytes in hepatic resection. Because in response to RFA, enhanced outgrowth was observed these results suggest that RFA most likely acts locally to induce tumor outgrowth. (Meredith et al. 2007)

This was confirmed in by Nijkamp *et al.*. in a murine model of colorectal cancer micro-metastases. In this model colorectal cancer cells (C26 cells) are injected into the spleen, so that via the bloodstream these cells migrate and home in the liver allowing micro-metastases develop. After 10 minutes the spleen is removed to prevent tumor growth in the spleen. In this way a diffuse spread of micro-metastases throughout the liver is established. RFA treatment of the livers of these mice did not lead to outgrowth of micro-metastases distant to the site of liver injury, in line with the findings of Meredith *et al.*. Micro-metastases in the hypoxic rim, surrounding the lesion induced by RFA, did however show aggressive outgrowth.(Nijkamp et al. 2009)

An important trigger for the observed increased outgrowth was tissue hypoxia. Treatment with 17-Dimethylaminoethylamino-17-demethoxygeldanamycin (17DMAG), preventing hypoxia mediated HIF-1α and HIF-2α stabilization, reduced outgrowth of micro-metastases in the hypoxic rim with 40%.(Nijkamp et al. 2009) 17DMAG administration did not affect the growth of distant micro-metastases. Administration of tirapazamine, a pre-drug activated by hypoxia forming cytotoxic radicals, was found to reduce hypoxia mediated outgrowth induced by RFA.(Govaert et al. 2012) Addition of tirapazamine to RFA treatment could be an answer to

prevent outgrowth of hypoxia induced outgrowth of tumors in the liver. No effect was seen of tirapazamine on the growth of distant micro-metastases. Because hypoxia also occurs in hepatic resection through vascular clamping, needed to limit blood loss, hypoxia mediated enhanced outgrowth of tumor cells might also play a role in hepatic resection.

Also in HCC, hypoxia was found to increase growth via HIF-1α mediated transcription. HIF-1 activity was also found protect HCC cells from apoptosis via enhanced VEGF expression, which decreased the Bax/Bcl-2 ratio preventing apoptosis.(Montalvo-Jave et al. 2008) So also here, co-treatment with tirapazamine with RFA treatment and possibly hepatic resection could be beneficial to prevent outgrowth of tumor cells and inhibit recurrence.
RFA mediated liver injury also has positive side effects however. The ablation of large tumor masses increased presentation of tumor specific antigens and generated tumor specific $CD4^+$ T-cells.(den Brok et al. 2004) Inhibited growth of tumors was seen in rat that were rechallenged with the same tumor type (CC531 colon carcinoma cell line), after these mice had been treated with RFA to remove a large tumor mass.(Duijnhoven et al. 2005) In the murine model of colorectal cancer micro-metastases, RFA was found to increase the amount of neutrophils and $CD4^+$ T-cells in distant micro-metastases, but decreased amount of these cell types in the hypoxic rim surrounding the RFA induced lesion.(Nijkamp et al. 2010a) This suggests that this mechanism acts pro-tumorigenic at the lesion site, and anti-tumorigenic at distant lesions. So probably, reduced immunity in the hypoxic area surrounding the RFA induced lesion might be another reason for the increased outgrowth seen of tumor cells in this region. Reduced immunity in the hypoxic rim could be partly due to hypoxia mediated increase of CD95 and CD95L that was found.(Nijkamp et al. 2010b) CD95 functions in most cell types as a death receptor, promoting cell death via caspase-8, however not in colorectal cancer cells. Therefore increased levels of CD95L might have led to increased $CD4^+$ T-cells death, and lower $CD4^+$ T-cell levels while not killing colorectal cancer cells. Inhibition of CD95 and CD95L signaling appeared vital for the increased proliferation, progression and invasion of the colorectal tumors.(Nijkamp et al. 2010b)

Future perspectives
While knowledge about chronic liver injury induced HCC carcinogenesis, and acute liver injury induced outgrowth of pre-existing tumors in the liver has increased, treatment of these malignancies remains tough. Ablation and resection have as major drawback that recurrence rates are high and can increase outgrowth of residual tumors (partly) through hypoxic signaling.(Nijkamp et al. 2009, Meredith et al. 2007, van der Bilt et al. 2005, Van Der Bilt et al. 2007) Co-treatment with chemotherapeutics like tirapazamine, reducing the effect of hypoxia, might help to slow recurrent tumor formation at the lesion site in RFA treated patients. Since hypoxia is an important driving factor of the regenerative response of the liver, it would also be interesting to study whether co-treatment with tirapazamine could successfully reduce outgrowth and invasiveness of tumors after partial hepatectomy.(Osada et al. 2010, Poon et al. 2007) Because higher levels of VEGF and

HGF have been linked to shorter time until recurrence, mitigation of the regenerative response could lead to an improved prognosis for the patient.
In both, chronic liver injury induced HCC and acute liver injury induced increased outgrowth of liver malignancies, there is evidence that immune cells play an important role. Modulation of the immune response therefore could be another way to improve treatment of liver malignancies. Especially IL-6 seems an interesting target, since in mice IL-6 depletion led to a significant decrease in HCC development and tumor growth.(Naugler, Karin 2008) It would be very interesting to see if decreased IL-6 activity could also inhibit other liver malignancies and whether TAM activity could be altered through IL-6 inhibition.
Another promising target for HCC treatment is inhibition of TGF-β signaling, which was found to inhibit growth of hepatocytes but induced growth of HCC cells. Inhibition of TGF-β could allow healthy hepatocytes to proliferate while hepatocellular cancer cells would be inhibited. Also TGF-β was found to act as a survival factor for HCC cells and is one of the major promoters of liver fibrogenesis. Severity of liver fibrosis was found to be correlated with bad disease outcome.(Elliott, Blobe 2005) Therefore inhibition of TGF-β signaling could help improve patient health and disease outcome.
Because of the importance of the liver regenerative response in recurrence and outgrowth of liver malignancies, inhibition of regenerative factors are promising targets for the treatment of liver malignancies. More research is however needed to find a cure for hepatic malignancies.

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