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Liver Tropism in Cancer: The Hepatic Metastatic Niche

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The liver is the largest organ in the human body and is prone for cancer metastasis. Although the metastatic pattern can differ depending on the cancer type, the liver is the organ to which cancer cells most frequently metastasize for the majority of prevalent malignancies. The liver is unique in several aspects: the vascular structure is highly permeable and has unparalleled dual blood connectivity, and the hepatic tissue microenvironment presents a natural soil for the seeding of disseminated tumor cells. Although 70% of the liver is composed of the parenchymal hepatocytes, the remaining 30% is composed of nonparenchymal cells including Kupffer cells, liver sinusoidal endothelial cells, and hepatic stellate cells. Recent discoveries show that both the parenchymal and the nonparenchymal cells can modulate each step of the hepatic metastatic cascade, including the initial seeding and colonization as well as the decision to undergo dormancy versus outgrowth. Thus, a better understanding of the molecular mechanisms orchestrating the formation of a hospitable hepatic metastatic niche and the identification of the drivers supporting this process is critical for the development of better therapies to stop or at least decrease liver metastasis. The focus of this perspective is on the bidirectional interactions between the disseminated cancer cells and the unique hepatic metastatic niche.

Metastasis is the spreading of cancer cells from the primary tumor site to secondary distant sites in the human body, and it is estimated that metastasis accounts for about 90% of cancer related deaths (Chaffer and Weinberg 2011). The liver is a highly metastasis-permissive organ and the majority of the most common solid cancers, namely lung, pancreas, breast, colorectal, prostate, gastric, esophagus, cervix uteri, thyroid, and bladder cancer frequently metastases to the liver (Budczies et al. 2015). As a result, the liver represents the organ with the highest metastatic incidence (Hess et al. 2006). In fact, liver metastases are even more common than primary liver tumors (Bosch et al. 2004). It is estimated that 30%–70% of cancer patients die with liver metastasis (Pickren et al. 1982) and most patients with liver metastasis will die of their disease (Gilbert et al. 1982).

Clinical observations show that different cancer types display dramatic variations in their metastatic pattern. Some tumors mainly disseminate to only one organ (e.g., ocular melanoma to liver, prostate to bones), whereas others

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metastasize to multiple organs (e.g., skin melanoma, breast, and lung cancer) (Vanharanta and Massagué 2013). The cancers with high-hepatic metastatic prevalence include uveal melanoma (>90%) (Amaro et al. 2017) and gastrointestinal cancers, namely, pancreas (75%–80%) (Ryan et al. 2014) and colorectal (50%) (Chow and Chok 2019).

The concept of organ selectivity of metastases, or tissue tropism, was first introduced in 1889 by the English surgeon Stephen Paget (Paget 1989). He proposed that tumor cells have an affinity to certain organs, where they seed into a friendly "soil," which facilitates their initial survival and their later outgrowth. Because this original work, significant advances have been made in our understanding of both the cell-autonomous mechanisms that drive metastasis, and alterations in the "soil" at the secondary site that allow efficient metastatic colonization and outgrowth leading to clinically relevant metastatic lesions (Minn et al. 2005; Lu et al. 2011; Qian et al. 2011; Sevenich et al. 2014; Kitamura et al. 2015; Nielsen et al. 2016; Roe et al. 2017; Quaranta et al. 2018). Systemic effects from the primary tumor that occur before metastasis have also been shown to affect the tropism and efficiency of disseminated cancer cells to colonize the secondary site (Kaplan et al. 2005; Hoshino et al. 2015). This precondition of the future metastatic niche, known as the premetastatic niche, has also been described for liver and enhances the engraftment, survival, and outgrowth of arrested disseminated tumor cells (DTCs) (Costa-Silva et al. 2015; Lee et al. 2019).

In general, the metastatic colonization of the liver is divided into five phases: (1) adhesion and arrest phase within the sinusoidal lumen, (2) extravasation phase into the space of Disse, (3) hepatic niche activation phase, (4) latency and resistance, and (5) outgrowth phase (expansion of metastasis). The first four phases do not require angiogenesis and likely are the entirety of the process for dormant metastases; these could remain as such for years to decades or proceed because of unknown stimuli to emergent masses as noted in the last phase. In addition, the premetastatic phase could set the stage for efficient liver colonization by DTCs.

This perspective aims to discuss our emerging understanding of the bidirectional interactions between the disseminated tumor cells and the hepatic metastatic niche, and to highlight potential therapeutic opportunities to develop better treatments for liver metastasis.

LIVER ARCHITECTURE AND HEMODYNAMIC FLOW

The liver is the largest organ in the human body and is composed of smaller functional units called lobules. The main components of each lobule are the parenchymal hepatocytes and they are aligned in a sheet-like structure. These structures are surrounded by branches of the hepatic artery, portal vein, and bile duct, which build together the portal triad. The liver is the only organ that has a dual blood connectivity receiving blood from both the hepatic artery and the portal vein. The hepatic artery provides the oxygenated blood and contributes to ∼25% of the blood influx. The portal vein brings nutrient-rich blood from visceral circulation, which is connected to the intestine, pancreas, and spleen and contributes to ∼75% of the blood supply to the liver (Fig. 1A). At the cellular level, the liver is composed of 70% parenchymal hepatocytes and cholangiocytes, and 30% nonparenchymal cells. Parenchymal cells are responsible for the metabolic, detoxification, and glandular functions. The nonparenchymal cells are represented by a mixture of highly specialized cells, including Kupffer cells (KC), liver sinusoidal endothelial cells (LSEC), and hepatic stellate cells (HSC) (Kmiec 2001; Heymann and Tacke 2016). Finally, the liver can be functionally further classified into zones (I, II, III) depending on the level of oxygen, with zone I being proximate to the portal triad and thus the most oxygenated and zone III being located close to the central vein and thus the most hypoxic (Fig. 1B; Kmiec 2001).

The liver has several unique features which are necessary for its normal physiological functions, but make the liver intrinsically susceptible for blood borne metastasis: (1) the liver is a highly vascularized organ, (2) has an exceptional low-blood flow rate, and (3) the LSEC are

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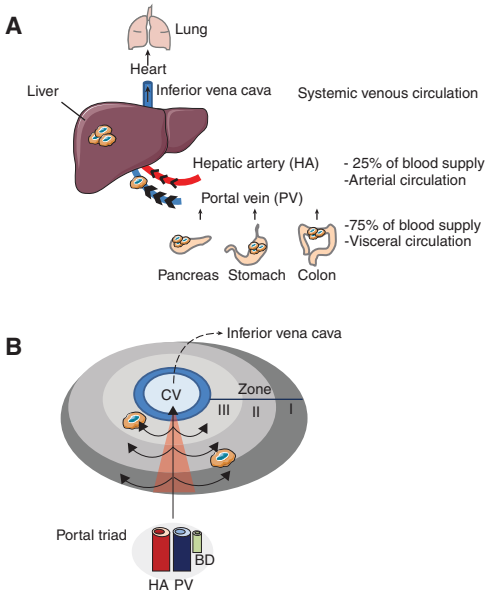

Figure 1. Anatomy of the liver and hemodynamic flow. (*A*) The liver is the largest organ of the human body and is the only organ which is connected to two blood circulation systems. Disseminated tumor cells (DTCs) in the arterial and visceral circulation are drained to the liver by the hepatic artery and the portal vein, respectively. Gastrointestinal cancers (pancreas, colon, stomach) are directly connected to the visceral circulation and show high-liver tropism for metastases. (*B*) General microanatomy of the liver showing the location of the portal triads [consisting of the hepatic artery (HA), portal vein (PV), bile duct (BD)], the central vein (CV), and the direction of the blood flow across the three different zones (I, II, III). Owing to extensive branching of portal vessels into liver sinusoids, and the accompanying increase of vascularization, the hepatic microcirculation is characterized by low pressure and slow blood flow.

highly fenestrated and lack a subendothelial basement membrane making them the most permeable endothelial cells of the mammalian body (Poisson et al. 2017). These organ-specific features not only facilitate the exchange of larger molecules necessary for the blood detoxification, as part of the liver's homeostatic function, but also allow the extravasation of DTC, as shown in quantitative cell-tracking studies in mice (MacDonald et al. 2002).

Whereas the close proximity and direct connection of the gastrointestinal track to the liver might in part explain the high-hepatic metastatic prevalence of gastrointestinal carcinomas, including pancreas and colorectal cancer (Ryan et al. 2014; Chow and Chok 2019), it does not explain the high hepatic metastatic rate for other cancer types such as breast, lung, and uveal melanoma. Thus, there is an emerging interest to better understand the cellular and molecular processes responsible for the high hepatic metastatic rate.

METASTATIC STEPS TO THE LIVER

The final step of cancer progression is the development of distant metastatic tumors, also known as secondary tumor sites. During the metastatic cascade, neoplastic cells need to undergo a series of steps before they are able to generate clinically detectable metastases. To start the metastatic cascade, at the primary site, cancer cells must invade from the confined primary tumor into the adjacent parenchyma and must intravasate into the circulation. Once tumor cells are in the circulation they must survive until they reach a potential secondary site. Survival of tumor cells in the circulating blood depends on their interaction with platelets and platelet-derived factors such as transforming growth factor β (TGFβ) and fibrin that promote a mesenchymal phenotype in the DTCs and protect them from natural killer (NK) cell-mediated elimination (Palumbo et al. 2005; Labelle et al. 2011). In breast cancer, DTCs have also been found within the blood stream in association with neutrophils, which enhanced cell cycle progression of DTCs and increased their metastasis to the lungs (Szczerba et al. 2019). Whether a similar survival mechanism exists for DTCs targeting the liver is currently unknown.

The main rate-limiting step for metastasis formation occurs during the colonization of distant organs (Vanharanta and Massagué 2013). DTC reaching the new microenvironment of the distant organ are vulnerable to immune surveillance and host-tissue defense. In the liver, initial immune surveillance is mediated by tissue resident KCs and NK cells (Heymann and Tacke 2016). In general, on entering the liver via either the hepatic artery or the portal vein, DTCs

become arrested and trapped in the sinusoidal capillaries of the liver whereby LSEC play a key function (phase 1). At this stage, DTCs are either able to extravasate or they die. On arrest, DTCs access the perisinusoidal space (space of Disse) by endothelial transmigration (phase 2). The up-regulation of cell adhesion molecules by LSEC promotes the arrest, retention, and transendothelial migration of DTCs. Even after successful extravasation, the vast majority of cancer cells die, but a minority of these cells may remain in the preangiogenic phases in dormancy (phase 3 and 4) or start their metastatic expansion (phase 5) (Fig. 2; Massagué and Obenauf 2016). Noteworthy, the colonization of the hostage environment represents a bottleneck in the metastatic cascade and can be critically facilitated by a fine-tuned bidirectional interaction between cancer cells and the hepatic microenvironment. The hepatic metastatic niche can facilitate the metastatic colonization in many different ways, including protecting of tumor cells from immune surveillance, providing growth and survival signals, and promoting the formation of intratumoral stroma and blood vessels as described in more detail in the following sections.

THE PREMETASTATIC NICHE

A growing body of research has shown that organs of future metastasis are selectively and actively modified by the primary tumor before metastatic spread occurs. Thus, tumors can induce the formation of a susceptible metastatic microenvironment before their arrival at these sites. These microenvironments are termed premetastatic niches (PMN) (Kaplan et al. 2005; Hoshino et al. 2015). Although the dependency of metastasis on these PMN formation remains controversial and difficult to verify in patients, numerous preclinical studies have identified various molecular and cellular changes that occur in the PMN, including the liver, to support future metastatic tumor growth (Psaila and Lyden 2009). Tumor-secrete factors and tumor-shed extracellular vehicles, called exosomes, have been identified to orchestrate step by step the formation of the PMN. Enhanced vascular leakage is the earliest event in this sequence, followed by the recruitment of bone marrow-derived cells and the local activation of resident stroma cells, such as fibroblasts, which all aim to better attract, arrest, and retain DTCs (Joyce and Pollard 2009; Becker et al. 2016). Tumor-derived tissue metallopeptidase 1 (TIMP1) has been linked to PMN formation in the liver in colorectal cancer (CRC). CRC patients showed increased TIMP1 levels, which correlated with liver metastasis. TIMP-1 led to increased stromal-derived factor (SDF) 1α levels, which in turn promoted recruitment of neutrophils to the liver (Seubert et al. 2015). VEGF-A has been identified as another CRC-derived factor linked to hepatic PMN formation. VEGF-A expressed by CRC induces the secretion of CXCL-1 in macrophages, which lead to the accumulation of $CXCR2^{+}$ MDSC in the liver and the formation of a hepatic PMN (Wang et al. 2017). S100 family proteins A8, A9, and P were also identified to drive liver PMN formation in preclinical CRC models (Zhang et al. 2013; Weidle et al. 2015). More recently, the role of exosomes in hepatic PMN formation attracted major attention. Macrophage migration inhibitory factor-1 (MIF-1) was identified as main cargo of PDAC-derived exosomes, able to induce a hepatic PMN. MIF containing exosomes taken up by KCs, up-regulated their expression of TGFβ which led to the activation of resident HSC. Activated HST formed a fibronectin-rich niche, thereby facilitating the adhesion of DTCs and the infiltration of bone marrow-derived cells via their fibronectin-binding surface receptors α4β7 and α4β1, respectively (Costa-Silva et al. 2015). Interestingly, high plasma exosomal MIF-1 levels were also detected in early stage PDAC patients, suggesting that a PMN could be formed at very early stages of PDAC development. Indeed, an early metastatic spreading during tumor progression has been reported in a genetically engineered mouse model of pancreatic cancer (Rhim et al. 2012). The molecular characterization of pancreatic cancer-derived exosomes found in the circulation of PDAC patients and tumor-bearing mice also provides an explanation of how cancers determine organ tropism. Integrin αvβ5 expres-

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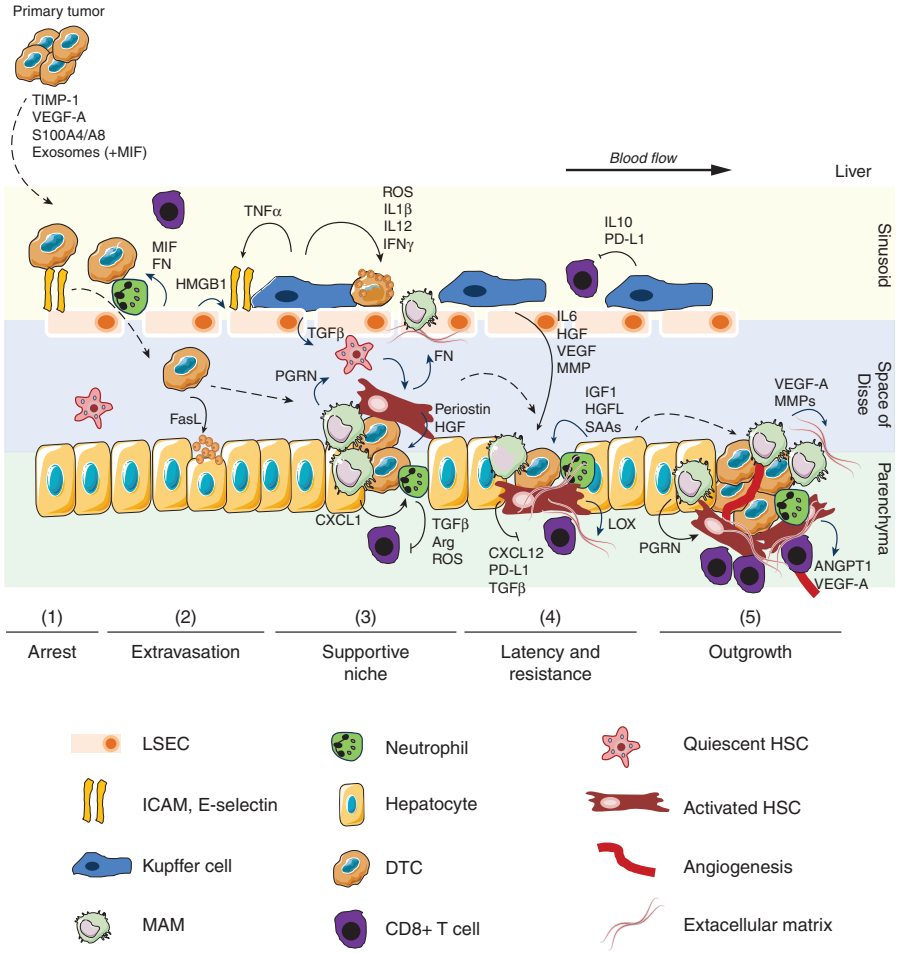

Figure 2. Tumor cell interactions with parenchymal and nonparenchymal cells during liver colonization by disseminated tumor cells. The colonization of the liver is a multistep process including arrest (1), extravasation (2), supportive niche formation (3), latency and resistance (4), and finally outgrowth (5). Major intercellular interactions and factors involved in this process are depicted. Primary tumors release factors involved in the generation of a premetastatic niche. On entry of disseminated tumor cells (DTC) into the sinusoidal vessels, DTCs first interact with LSEC and KC. Arrest of DTC is increased by cell adhesion molecules expressed by inflamed LSEC and via neutrophil interaction. Depending on the activation state, KCs release tumoricidal factors, suppress cytotoxic CD8+ T cells, or secrete growth and survival factors for DTCs. On extravasation, tumor secreted FasL induces apoptosis of hepatocytes, which facilitates colonization of the parenchyma. Metastasis-associated macrophages (MAMs), mainly monocyte-derived, rapidly accumulate in high numbers and MAM-released factors, including PGRN, CXCL1, MMPs, TGFβ, and VEGF-A promote the generation of a hospitable hepatic niche. Key events are activation of hepatic stellate cells (HSC), recruitment of immunosuppressive neutrophils, and remodeling of extracellular matrix (ECM). Hepatocyte-derived factors such as IGF1, HGFL, and SAAs contribute to the generation of a supportive niche. The supportive niche also protects neoplastic cells during potential latency and against anticancer therapies. Metastatic expansion and outgrowth requires the formation of new blood vessels (angiogenesis), sustained suppression of an antitumoral immune response, and continuous ECM remodeling. (ARG) arginase, (ANGPT1) angiopoietin 1, (FN) fibronectin, (HGF) hepatocyte growth factor, (HMGB1) high mobility group box 1, (IGF1) insulin-like growth factor 1, (IL) interleukin, (LOX) lysil oxidase, (MIF) macrophage migration inhibitory factor, (MMP) matrix metalloproteinase, (PD-L1) programmed death ligand 1, (PGRN) progranulin, (ROS) reactive oxygen species, (SAA) serum amyloid A1/2, (TGFβ) transforming growth factor β, (TNFα) tumor necrosis factor α, (VEGF-A) vascular endothelial growth factor A.

sion on PDAC-derived exosomes was identified as a key adhesion receptor that specifically binds to KC and is necessary for the update of exosomes into KC, leading to the subsequent formation of the hepatic PMN (Hoshino et al. 2015). In colorectal cancer, microRNA-21-5p-rich exosomes released by tumor cells induce the formation of a proinflammatory, premetastatic niche in the liver by binding to TLR7 on macrophages leading to the release of interleukin 6 (IL-6) (Shao et al. 2018). Although proteoglycan Glypican-1-positive exosomes have been described to specifically accumulate in PDAC patients, their role in hepatic PMN formation has not been reported (Melo et al. 2015). Likewise, the contribution of circulating tumor cells, present early in tumor development and after resection of the primary tumor, to hepatic PMN formation remains unknown (Bork et al. 2015; Tsai et al. 2016).

THE HEPATIC METASTATIC NICHE

Role of Tissue Resident Cells

Liver Sinusoidal Endothelial Cells

DTCs entering through the blood circulation first encounter the liver sinusoidal endothelial cells (LSEC), which cover the luminal side of the sinusoids. LSEC are a heterogeneous cell population (Strauss et al. 2017) that, similarly to the KC, function as scavengers, clearing macromolecular waste molecules from the circulation. LSEC express different scavenger receptors, including stabilin 1 and 2 allowing a high endocytic capacity (Sorensen et al. 2012). LSEC can regulate the arrest and adhesion of DTCs by expressing of cell adhesion molecules, including E-selectin, vascular cell adhesion molecule 1 (VCAM-1), and intercellular cell adhesion molecule 1 (ICAM-1). The induction of cell adhesion molecules on LSECs can be triggered by an inflammatory response mediated by KC and NK cells in response to the arriving DTCs. Although this immune response was initially thought to be a tumoricidal response, the inflammatory response and the activation of LSEC facilitates cancer cells adhesion and endothelial transmigration into the presinusoidal space, where cancer cells are protected from KC and NK cells (Glinskii et al. 2005). In pancreatic cancer, IL-35 is highly expressed by cancer cells and induces ICAM-1 expression on LSEC, thereby increasing adhesion of DTC to the endothelial wall and enhancing liver metastasis (Huang et al. 2017). Inhibition of integrin β2 expression, a ligand of ICAM-1, on the C26 CRC cell line led to reduced retention of DTCs in the liver in a preclinical mouse model of colon cancer (Benedicto et al. 2017), whereas blockade of adhesion molecules or inhibition of the inflammatory TNFα/TNFR2 signaling axis reduced CRC liver metastasis (Khatib et al. 2002, 2005; Yoshimoto et al. 2012; Ham et al. 2015). LSEC-mediated activation of Notch signaling also increased metastasis of melanoma and colorectal cancer cells to the liver. In this study, reduced liver metastasis was linked to a reduction of the cell adhesion molecule ICAM-1 expressed by LSEC, resulting in impaired adhesion and retention of tumor cells in sinusoids. Similarly, anti-ICAM-1 antibody treatment significantly reduced tumor cell adhesion to LSEC under normal Notch expression levels suggesting that Notch signaling controls liver metastasis via modulation of adhesion molecule expression on LSEC (Wohlfeil et al. 2019).

The interaction of DTCs with LSECs not only facilitates the extravasation of DTCs into a more protected environment, but also triggers the activation of signaling pathways in cancer cells enhancing their survival and growth potential. Namely, the interaction of ligands sLewA, sLewX, and CD44 isoforms expressed on cancer cells with E-selectin expressed on inflamed LSEC enhances liver metastasis in CRC (Witz 2008; Elliott et al. 2014) and induces the release of the pro-inflammatory signal factor high-mobility group box 1 (HMBG1), thereby further fueling the expression of adhesion molecules on LSEC (Aychek et al. 2008).

LSEC also secrete factors that enhance the metastatic potential of cancer cells. LSEC-derived fibronectin and macrophage migration inhibitory factor (MIF) induced EMT in CRC cells resulting in increased invasion and migration of CRC cells into the liver parenchyma (Ou et al. 2014; Hu et al. 2015).

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LSEC are a key component of tumor angiogenesis, which is a critical step in tumor expansion. Although LSEC under homeostatic conditions are fenestrated, fibrosis and the growth of neoplastic cells in the liver can lead to LSEC *trans*-differentiation with loss of LSEC markers and sinusoidal fenestrae, a process known as capillarization, during which LSECs lose their protective properties and promote angiogenesis and vasoconstriction (Ding et al. 2014; DeLeve 2015). In contrast, non-angiogenic-dependent expansion of metastatic lesions in the liver has also been described (Vermeulen et al. 2001; Stessels et al. 2004). In the liver, tumors cells have been shown to hijack the existing dense vascular network by migrating along LSECs instead of inducing angiogenesis. This mechanism is called vessel cooption and in patients with colorectal cancer liver metastases, vessel cooption is associated with poor response to the antiangiogenic agent bevacizumab (Frentzas et al. 2016)

Taken together, LSEC can play multiple roles in liver metastasis. Although they present a natural barrier for DTCs to access the liver parenchyma, in response to local inflammation, activated LSEC can help DTCs enter the liver. The increased expression of adhesion molecules on LSEC not only helps the DTCs to arrest in the sinusoids, but can also trigger prometastatic functions in cancer cells on ligation of cancer cell-specific receptors. Cooption of the dense LSEC network in the liver further promotes metastatic tumor growth by providing tumors with access to a preexisting blood supply system.

Kupffer Cells

The liver parenchyma is rich in cells of the innate immune system, potentially posing an obstacle to cancer cells. Particularly macrophages are highly abundant. In rodent livers, every 100 hepatocytes are accompanied by 10–20 macrophages (Lopez et al. 2011). These constitutively resident hepatic macrophages are known as Kupffer cells (KC) and are seeded along the LESC (Tacke and Zimmermann 2014). KC originate from fetal liver-derived erythromyeloid progenitors and they rely on self-renewal rather than on infiltrating monocytes for their maintenance (Gomez Perdiguero et al. 2015). Because in human and mouse the expression of KC surface markers largely overlaps with monocyte-derived macrophages and other phagocytes, a combination of several surface markers is needed to identify KCs. Murine KC stain positive for $F4/80^{+}$, $CD11b^{+/-low}$, $CD68^{+}$, and C-type lectin domain family 4-member F (CLEC4F), whereas they are negative for CX3CR1 because of their nonmonocytic origin (Heymann et al. 2015). Human KC are less well characterized and are commonly identified by $CD14^{+}$, $CD68^{+}$, $TLR4^{+}$, $CX3CR1^{neg}$ expression (Krenkel and Tacke 2017).

KCs are highly specialized macrophages and act as a prime sensor for immune surveillance during homeostasis and disease. The constant low-level exposure to bacterial components such as lipopolysaccharide (LPS) renders the cells refractory to LPS stimulation. The tolerogenic potential of KCs in homeostasis is reflected by their constitutive ability to secrete IL-10 on LPS stimulation, as well as by expression of the T-cell inhibitory molecule programmed cell death 1 ligand (PD-L1) and the induction of regulatory T (Treg) cells under homeostatic conditions in vivo (Heymann et al. 2015). In their interaction with invading cancer cells, KCs can play diverse and opposing roles that depend on several factors including the stage of the metastatic process, tumor antigen load, and interactions with other immune cells. KCs can exert cytotoxic activity toward DTCs by releasing oxygen metabolites, phagocyte tumor cells, release cytotoxic cytokines, and secrete proteases (Gardner et al. 1991; Wang et al. 2000; Seki et al. 2011). DTC adhesion to KCs can induce a rapid phagocytosis of tumor cells and their removal from the liver (Bayon et al. 1996). This antitumoral activity of KCs may require the recruitment of other inflammatory cells, such as NK cells, which promote the tumoricidal effect of KCs by secreting inflammatory factors such as granulocyte macrophage colony stimulating factor (GM-CSF) and interferon γ (IFNγ) (Timmers et al. 2004). KC can decrease hepatic metastatic tumor burden of CRC during early stages and this was associated

with increased TNFα and IL-1β levels (Khatib et al. 2005; Matsumura et al. 2014). Extensive phagocytosis and elimination of tumor cells is attributed to the first 24 h of tumor cell entry, suggesting that the tumoricidal activity of KC is limited to the early events of metastatic colonization of the liver (Matsumura et al. 2014). Cancer cells, which survive the initial insult of KC can later benefit from the KC tumor promoting functions. The switch is determined predominantly by tumor cell burden. KCs show a capacity for immune-surveillance when tumor cell numbers are low. However, KCs switch to promote liver colonization and metastatic progression when their phagocytic capacity is overwhelmed because of excessive number of DTCs invading the liver (Bayon et al. 1996).

KC can increase the adhesion of DTCs to the endothelium by inducing the expression of vascular endothelial cell adhesion molecules (Khatib et al. 1999, 2002). Moreover, KC can produce a plethora of factors including IL-6, hepatocyte growth factor (HGF), VEGF, and matrix metalloproteinases (MMPs) 9 and 14 that can accelerate tumor invasion into and within the parenchymal space as well as promote tumor cell proliferation and angiogenesis, thereby enhancing liver metastasis.

Hepatic Stellate Cells

In normal liver, hepatic stellate cells (HSC) maintain a quiescent, nonproliferative phenotype and are located in the peri-sinusoidal space (space of Disse), interposed between the basolateral surface of hepatocytes and LSEC. The storage of retinyl esters in cytoplasmic lipid droplets is the most distinctive feature of this cell type and enables their isolation by density gradient centrifugation (Friedman and Roll 1987). HSC can be further characterized by the expression of platelet-derived growth factors receptor (PDGFR)-β, enzyme lecithin retinol acyltransferase (LRAT), the cytoskeletal proteins desmin and glial fibrillary acidic protein (GFAP) (Mederacke et al. 2013, 2015; Tsuchida and Friedman 2017). In response to inflammatory stimuli, triggered by liver damage or the presence of neoplastic cells, quiescent HSC transdifferentiate from vitamin A-storing cells to myofibroblasts, which are proliferative, migratory, and contractile. In addition, activated HSC secrete a variety of chemokines and cytokines, which can shape the immune response and the tumor microenvironment. HSC are characterized by enhanced ECM production, which makes them a major driver for liver fibrosis and cancer-associated desmoplasia (Tsuchida and Friedman 2017).

HSC have been shown to promote the formation of the premetastatic hepatic niche as introduced before (Kaplan et al. 2006). Lyden and colleagues showed that in pancreatic cancer models, resident KC are initially activated by PDAC-derived exosome released by the primary tumor. These exosomes contain MIF, and are taken up by KCs, triggering their activation. Activated KCs release the HSC-activating factor TGFβ, which leads to the transactivation of HSC and increased deposition of fibronectin at the future metastatic site. Lyden and colleagues further showed that a FN-rich hepatic niche facilitates the recruitment of bone-marrow-derived myeloid cells. Most likely, extracellular deposited FN within the perisinusoidal space serves as a docking site for circulating myeloid immune cells, which characteristically express high levels of FN-binding integrins, including α4β1 and αvβ3 (Costa-Silva et al. 2015).

Tissue fibrosis can enhance cancer metastasis by creating a growth-permissive fibrotic microenvironment capable of supporting metastatic growth by enhancing tumor cell survival. Treatment of mice with the fibrosis inducing chemical dimethylnitrosamine (DMN) increased hepatic metastatic frequency of orthotopically implanted 4T1 breast cancer carcinoma cells. The enhanced hepatic spreading was linked to the presence of lysyl oxidase (LOX), secreted by activated (αSMA+) HSCs (Cox et al. 2013). LOX is an extracellular amine oxidase whose primary function is to posttranslationally modify collagens and elastins, which is a critical step in organ fibrosis and in the formation of a desmoplastic tumor stroma (Barker et al. 2012).

In pancreatic cancer metastasis, the transactivation of resident HSC by immune cells has been identified to be critical for efficient

hepatic metastatic outgrowth. On initial seeding of the liver by disseminated pancreatic cancer cells, bone marrow-derived inflammatory monocytes are rapidly recruited to the metastatic niche, where they differentiate into metastasis associated macrophages (MAMs). The accumulation of inflammatory monocytes and MAMs preceded the aberrant activation of HSC and the genetic and/or pharmacological targeting of MAMs abolished the formation of a desmoplastic hepatic niche (Nielsen et al. 2016). MAMs promote the activation of resident HSC by secreting progranulin, a glycoprotein and a potent activator of fibroblasts (He et al. 2003; Elkabets et al. 2011). Activated HSC secrete a plethora of ECM proteins, including periostin, which enhances the metastatic outgrowth of disseminated PDAC and CRC cells by increasing cell survival via the AKT pathway (Bao et al. 2004; Nielsen et al. 2016).

A proangiogenic role of HSC was also described. In a metastatic B16 melanoma model, activated HSC secrete VEGF-A and angiopoietin 1 to initiate angiogenesis (Olaso et al. 2003; Taura et al. 2008; Copple et al. 2011). In hepatic CRC metastasis, TIMP-1 is highly expressed in HSC which are in close proximity to $CD34^+$ endothelial cells, suggesting a vascular remodeling function of HSC (Illemann et al. 2016). Isolated HSC secrete laminin and show enhanced endothelial cell network formation in matrigel assays. Coinjection of HSC together with colorectal cancer cells enhanced the metastatic process to the liver by supporting angiogenesis (Eveno et al. 2015). Activated HSCs can also directly promote tumor growth by secreting HGF and TGFβ (Thompson et al. 2015). Less well understood is the function of HSCs in controlling the immune response in liver metastasis. Skin cancer associated fibroblasts regulate the recruitment of immune cells and their functions by releasing cytokines/chemokines (Erez et al. 2010). Activated HSC secrete a plethora of cytokines and chemokines with well-known effects on immune cell recruitment and functions (Nielsen et al. 2016), suggesting that HSC also shape the immune response during liver metastasis. In primary HCC, HSC-derived factors promote immune suppression by promoting the expansion of immunosuppressive regulatory T cells (Tregs) and the induction of myeloid-derived suppressor cells (Zhao et al. 2012; Xu et al. 2016). However, whether or how HSC influence immune cell recruitment during liver metastasis requires further investigation. Myofibroblasts have also been linked to the adaptive immune response, particularly regulating T cell infiltration and survival. In pancreatic cancer and melanoma models, cancer associated fibroblasts at the primary tumor site have been shown to impair $CD8^+$ T cell infiltration (Kraman et al. 2010; Feig et al. 2013) or induce their depletion (Lakins et al. 2018), although the exact mechanism(s) by which pancreatic tumors prevent $CD8^+$ T cell recruitment is not fully understood yet. In regard to the hepatic niche, in liver biopsies of metastatic PDAC patients, $CD8^+$ T cells were found in αSMA^+ myofibroblast-rich regions. In a metastatic mouse model of PDAC, activation of HSC led to $CD8^+$ T cell exclusion and resistance to immune checkpoint therapy (αPD-1), which could be reversed by reducing the fibrotic stroma (Quaranta et al. 2018). Taken together, these results suggest that liver fibrosis driven by HSC enhances hepatic metastasis growth and further showcases a bidirectional cross talk between immune cells and HSC in the hepatic metastatic niche.

Hepatocytes

The role of the parenchymal hepatocytes in liver metastasis is less well understood. In CRC, the interaction of tumor cells with hepatocytes increased their metastatic potential. Integrin αv, α6, and β1, desmosomes, as well as osteopontin enhanced the interaction of cancer cells with the hepatocyte ECM (Shimizu et al. 2000; Mook et al. 2008; Huang et al. 2012; Zvibel et al. 2013). The interaction of CRC with ECM induced the induction of gene signatures related to tumor cell survival and stemness, suggesting that tumor cells—hepatocyte ECM interaction promotes the adaptation of DTCs to the hepatic niche. Disseminated CRC expressing Fas ligand (FasL) induce apoptosis of Fas receptor bearing hepatocytes on arrival in the liver. The induction of hepatocyte apoptosis creates a niche in

the liver where blood borne DTC can settle and propagate (Li et al. 2009).

Hepatocytes release several growth factors, including insulin-like growth factor 1 (IGF-1) and inhibition of IGF-1 reduces liver metastasis of colon and lung carcinoma cells (Wang et al. 2015). Overexpression of the Ron receptor, a member of the Met family of surface receptor tyrosine kinases, has been reported in several human cancers including breast, pancreas, colon, liver and bladder. Ron is activated by the hepatocyte growth factor-like protein/macrophage stimulating-protein (HGFL) which is primarily secreted by hepatocytes. Activation of RON leads to the induction of signaling pathways related to cellular growth, motility, invasion, and metastasis (Wagh et al. 2008). Hepatocyte-derived heregulin (HRG) phosphorylates erbB3 and erbB2 in CRC cells, promoting integrin αvβ5 depended migration. Depletion of integrin αv or erbB3 reduced liver metastasis of CRC cells (Yoshioka et al. 2010).

Parenchymal hepatocytes promote hepatic metastasis for different cancer types, including pancreatic and colorectal carcinomas, by coordinating the formation of a pro-metastatic niche. In response to primary tumor-derived factors, particularly IL-6, hepatocytes up-regulate the release of serum amyloid A1 and A2 (SAAs), which increases myeloid cell recruitment and liver fibrosis. The secretion of SAAs by hepatocytes occurs in response to IL-6 stimulation and is STAT3 dependent. IL-6 is mainly produced by nonmalignant stroma cells at the distant primary tumor site, including αSMA+ myofibroblasts, but not at the metastatic site, thereby controlling hepatocyte activation in a systemic manner. The identified IL-6–STAT3-SAA signaling axis is responsible for the formation of a pro-metastatic niche in the liver, but not in the lung (Lee et al. 2019). Thus, this study provides an example of how parenchymal cells might steer metastatic organ tropism.

Role of Recruited Inflammatory Cells

Monocyte-Derived Macrophages

In addition to the presence of tissue resident KCs, liver metastasis is accompanied by the accumulation of blood-derived myeloid cells, including monocytes and neutrophils. Monocytes in the circulation can be differentiated into two subsets based on cell surface expression of different markers. Inflammatory monocytes are characterized by $Ly6C^{high}$ $CX3CR1^{mid}$ $CCR2^{+}$ $CD62L^{+}$ $CD43^{low}$ expression, whereas patrolling monocytes are characterized by $Ly6C^{low}$ $CX3CR1^{high}$ $CCR2^{-}$ $CD62L^{-}$ $CD43^{high}$ expression (Geissmann et al. 2010). The recruitment of inflammatory monocytes is necessary for efficient hepatic metastasis for several cancer types with liver tropism. In colorectal and Lewis lung carcinoma models, inflammatory monocyte accumulation is mediated by the CCL2/CCR2 axis. Tumor-secreted CCL2 attracts $CCR2^{+}$ myeloid cells to the liver and genetic and/or pharmacological ablation of CCL2/CCR2 signaling reduced myeloid cells recruitment and metastatic tumor burden. In pancreatic cancer, inhibition of CCR2 reduced primary tumor formation and metastatic spreading to the liver (Mitchem et al. 2013).

On infiltration of the hepatic niche, monocytes differentiate into monocyte-derived macrophages (Nielsen et al. 2016). Macrophages are highly plastic and depending on their activation status, macrophages can execute tumoricidal or protumorigenic functions (Biswas et al. 2013; Mantovani et al. 2017). On infiltration of the hepatic niche, monocytes differentiate into monocyte-derived macrophages (Nielsen et al. 2016). MAMs play a significant role in pancreatic cancer metastasis. During metastatic tumor growth in pancreatic cancer, MAMs rapidly accumulate at the metastatic site and represent the most abundant immune cell population. Bone marrow chimera studies revealed that MAMs mainly originated from bone marrow-derived monocytes (Nielsen et al. 2016). Genetic inhibition of MAM accumulation by depleting PI3Kγ reduced MAM accumulation and PDAC metastasis. Similarly, chemical depletion of macrophages by clodronate containing liposomes or pharmacological targeting of macrophages by anti-CSF-1/anti-CSF-1R ablated MAM numbers and impaired hepatic metastatic growth of disseminated PDAC cells (Nielsen et al. 2016; Quaranta et al. 2018). In regard of MAM acti-

vation, smaller, micrometastatic lesions were rich in macrophage expressing proinflammatory markers including COX, NOS2, and MHC class II, whereas established, large metastatic lesions were highly infiltrated by immunosuppressive macrophages, expressing elevated levels of *Arginase, Tgfb*, and *Il10*. The phenotype of macrophage polarization directly affected the activation state of metastasis infiltrating $CD8^+$ T cells. Increased abundance of immune-suppressive MAMs reduced $CD8^+$ T cell activation, whereas MAM-targeted therapies restored cytotoxic $CD8^+$ T cell functions (Quaranta et al. 2018). A clinical study showed that high numbers of circulating inflammatory monocytes correlates with shortened survival in pancreatic cancer, a disease which primarily metastasizes to the liver (Sanford et al. 2013). In a preclinical colorectal cancer model, the genetic depletion of the N-myc downstream-regulated gene 2 (NDRG2) reduced metastatic tumor burden, which correlated with an increased percentage of M1-like MAMs within the hepatic metastatic niche. Mechanistically, NDRG2-deficiency induced nuclear factor (NF)-κB pathway activation in macrophages, thereby promoting the expression of M1-like inflammatory cytokines including *Il12*, *Il1b*, and *Tnfa* (Li et al. 2018).

Neutrophils

Neutrophils are part of the innate immune response and are also rapidly recruited to site of tumor formation, including the liver. Similar to macrophages, neutrophils can acquire opposing roles in cancer depending on the environmental context (Fridlender et al. 2009; Shaul and Fridlender 2017). Neutrophils can release cytolytic factors and produce high levels of TNFα, FasL, reactive oxygen species (ROS), thereby executing tumorical activity. In contrast, it has been shown that, particularly in cancer, neutrophils can acquire an immunosuppressive phenotype and express high levels of arginase, MMP-9, and VEGF-A (Fridlender et al. 2009). In mice, independent of their phenotype, neutrophils are identified by their expression of CD11b and Ly6G. The same combination of markers is also used to identify granulocytic myeloid-derived suppressor cells (gMDSC), which are defined by their potent immunosuppressive activity (Talmadge and Gabrilovich 2013; Coffelt et al. 2016). It has recently become apparent that neutrophils represent a heterogeneous population of cells with significant functional plasticity and that immune suppressive functions associated to MDSC might have been performed by immunosuppressive neutrophils sharing the same surface markers and vice versa.

Several factors have been reported to promote the recruitment and accumulation of neutrophils/MDSC to metastatic liver tumors including CXCL1, CXCL2, CXCL5, and SDF-1α (also known as CXCL12), which bind to their cognate receptors CXCR2 or CXCR4 expressed on neutrophils (Zhao et al. 2013; Seubert et al. 2015; Steele et al. 2016). Immunosuppressive functions of neutrophils/MDSC promote liver metastasis. In a pancreatic cancer model, primary and hepatic metastatic tumors are infiltrated by Ly6G+ neutrophils. Neutrophil depletion resulted in increased T cell infiltrating into the pancreas and the liver. Inhibition of neutrophil recruitment by blocking CXCR2 ablated hepatic metastatic spreading (Steele et al. 2016). This study suggests that immunosuppressive neutrophils play an important role in metastatic spreading of pancreatic cancer to the liver, at least during the initial steps of the pre- and/or early metastatic niche formation. In CRC models, neoplastic cell-derived VEGF-A induced the secretion of CXCL1, a CXCR2 ligand for neutrophils/MDSC at the primary tumor site. Elevated CXCL1 levels promoted the accumulation of neutrophils/MDSC in the premetastatic niche that ultimately promoted liver metastasis (Wang et al. 2017). Increased circulating MDSC levels correlate with advanced clinical cancer stage and metastatic tumor burden. Although the analysis included a limited number ($n = 56$) of patients with different types of cancer (colon, pancreas, gastric, breast among others), patients with extensive metastatic involvement tended to have the highest number of circulating MDSC, suggesting their important role in promoting metastasis (Diaz-Montero et al. 2009).

Neutrophils promote hepatic metastasis independent of their immunosuppressive capaci-

ty. In a murine lung carcinoma model, neutrophils promote cancer cell adhesion within the liver sinusoids by providing a docking site for cancer cells to arrest. Intravital microscopy revealed that DTC adhere directly on top of neutrophils arrested on LSEC within the liver sinusoids. Cancer cell–neutrophil interaction was dependent on CD11b expression by neutrophils and ICAM by cancer cells (Spicer et al. 2012). Moreover, neutrophils can arrest DTC in the liver by neutrophil extracellular trap (NET) formation. NETs are extracellular structures composed of chromatin coated with histones, proteases, and granular and cytosolic proteins that help catch and kill microorganisms. In the presence of circulating tumor cells, NET formation in the liver sinusoids results in increased retention of DTC and enhanced tumor cells adhesion, proliferation, migration, and increased liver metastasis (Cools-Lartigue et al. 2013; Tohme et al. 2016).

Tumor promoting functions of neutrophils have been attributed to the release of extracellular matrix-degrading proteinases, including MMP-8, MMP-9, elastases, and cathepsin G, thereby increasing tumor invasion (Sionov et al. 2015), and by promoting angiogenesis through the release of VEGF and Bv8 (Coffelt et al. 2016). However, these studies focused on the TME at the primary tumor site and pulmonary metastatic site, and to understand whether these mechanisms also play a role in the formation of a hepatic metastatic niche requires further investigation (Jablonska et al. 2017).

Role of Liver Infiltrating Lymphocytes

$CD8^+$ T cells and NK cells are the main effector cells of the immune system that kill cancer cells. $CD8^+$ cytotoxic T cells are part of the adaptive immune system and recognize tumor antigens presented in the context of MHC class I and deliver cytolytic factors including granzymes, perforin, and FasL (Hadrup et al. 2013). $CD8^+$ T cells also release the proapoptotic cytokines IFNγ and TNFα to suppress tumor growth (Barth et al. 1991; Detjen et al. 2001). NK cells belong to the innate immune system and do not need MHC class I mediated antigen presentation to recognize and kill cancer cells. Similar to $CD8^+$ T cells, NK deliver cytotoxic hits to cancer cells through the release of perforin and granzymes (Lowry and Zehring 2017). The inhibition and evasion of a specific tumoricidal T cell and/or NK cell mediated immune response is critical for neoplastic cells to survive and grow in the liver. In contrast, the infiltration of other lymphocytes, namely regulatory T cells which suppress effector T cells, favors liver metastasis. Tregs are a subset of immunosuppressive $CD4^+$ T cells and are defined by their expression of the FoxP3 transcription factor. Although immunosuppressive Tregs have a critical role during tumor development and in response to therapy (Takeuchi and Nishikawa 2016; Oweida et al. 2018; Shabaneh et al. 2018), their role in the formation of a hepatic metastatic niche is less well understood. Efficient liver metastasis of colon and lung carcinoma correlates with not only the increase of MDSC, but also with the recruitment and accumulation of $CD4^+$ $FoxP3^+$ Tregs. In this report, the recruitment of Tregs was dependent on TNFR2 signaling, because TNFR2 depleted animals showed a marked decrease in Treg accumulation (Ham et al. 2015). In a retrospective analysis for $FoxP3^+$ Treg and $CD8^+$ cytotoxic T cell infiltration, using tissue sections from resected colorectal cancer liver metastases, a high ratio of $FoxP3^+$: $CD8^+$ cells correlated with shorter overall survival of patients after surgery (Katz et al. 2013), suggesting a potential pro-metastatic role for Tregs at the hepatic niche.

CANCER CELL INTRINSIC GENETIC PROGRAMS DRIVING LIVER METASTASIS

Unlike the deep understanding of mutational mechanisms that initiate cancer progression, the genetic basis for liver metastasis is less well understood. General metastasis promoting transcriptional programs regulate pathways including self-renewal and EMT (Kong et al. 2011; Neureiter et al. 2014; Krebs et al. 2017). A few studies have focused on cell intrinsic programs that specifically drive liver metastasis. Pro-metastatic programs driving liver metastasis have been identified in cancer from the gastrointestinal tract. In CRC, miR-551a and miR-483 sup-

press liver colonization and metastasis by inhibiting creatine kinase, brain-type (CKB) in CRC cells. The release of CKB by metastasized cancer cells generate phosphocreatine in the extracellular space, which was imported back into CRC cells and used for ATP generation and thereby enhanced metastatic survival (Loo et al. 2015). In pancreatic cancer, the expression of the pioneer factor FOX1 increases anchorage-independent growth of PDAC cells in vitro, and invasion and liver metastasis in vivo (Roe et al. 2017). Claudin-2 has been shown to mediate breast cancer liver metastasis and was identified to be specifically expressed by liver-metastatic breast cancer cells compared with populations derived from bone or lung metastasis. The extracellular loop of claudin-2 mediated tumor-hepatocyte interaction and thereby increased metastatic tropism to the liver (Tabaries et al. 2012).

CONCLUDING REMARKS

The papers discussed here, and many other reports that because of word limitation we could unfortunately not cite, provide evidence that the hepatic metastatic niche is critical for promoting liver metastases and that inhibition of key effector proteins and/or cells within the hepatic niche could open new avenues for better therapies against liver metastasis. Inhibition of the various prosurvival cues provided by the hepatic environment could improve the response to anticancer treatments. However, a major challenge that requires further investigation is that the type of interactions DTC establish with the different components of the hepatic niche constantly change during the metastatic steps. This might explain why targeting specific cellular interactions and effectors in the TME have markedly reduced, but not yet completely eradicated, metastatic disease progression.

Although it has been shown that stromal cells promote liver metastasis, an additional layer of complexity that also deserves further investigation is their potential heterogeneity and their distinct contribution to the formation (or inhibition) of a hepatic niche. However, the recent technical advances in single cell analysis now allows to deconvolute the complex structure of the hepatic niche to a single cell level and such studies will most likely shed light on the cellular heterogeneity of hepatic metastatic lesions.

The cellular and molecular structure of the hepatic metastatic niche is not only forged by DTC, but is also influenced by cues from the primary tumor site. The importance of the cross-talk between the primary and secondary tumor site has been clearly shown by the importance of the premetastatic niche formation in the metastatic process. However, whether and how the genomic profile of cancer cells orchestrates the formation of a cancer cell "personalized" hepatic niche is a question that remains to be addressed. Thus, to better understand how the hepatic niche supports metastasis, future studies should use preclinical (mouse) models that faithfully recapitulate the primary–secondary tumor interaction, and also provide the diversity of all stromal/immune partners. Based on the overall literature herein presented, we would like to reinforce the importance of further investigating the hepatic niche and help translate the exciting preclinical observations into the clinics.

COMPETING INTEREST STATEMENT

The authors declare no potential conflicts of interest.

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