Role of the Microenvironment in Liver Metastasis: From Pre- to Prometastatic Niches

Pnina Brodt

Abstract

Liver metastases remain a major barrier to successful management of malignant disease, particularly for cancers of the gastrointestinal tract but also for other malignancies, such as breast carcinoma and melanoma. The ability of metastatic cells to survive and proliferate in the liver is determined by the outcome of complex, reciprocal interactions between tumor cells and different local resident subpopulations, including the sinusoidal endothelium, stellate, Kupffer, and inflammatory cells that are mediated through cell–cell and cell–extracellular matrix adhesion and the release of soluble factors. Cross-communication between different hepatic resident cells in response to local tissue damage and inflammation and the recruitment of bone marrow cells further enhance this intercellular communication network. Both resident and recruited cells can play opposing roles in the progression of metastasis, and the balance of these divergent effects determines whether the tumor cells will die, proliferate, and colonize the new site or enter a state of dormancy. Moreover, this delicate balance can be tilted in favor of metastasis, if factors produced by the primary tumor precondition the microenvironment to form niches of activated resident cells that promote tumor expansion. This review aims to summarize current knowledge on these diverse interactions and the impact they can have on the clinical management of hepatic metastases. Clin Cancer Res; 22(24); 5971–82. ©2016 AACR.

Introduction

Cancer metastasis remains the major challenge to successful management of malignant disease. The liver is the main site of metastatic disease and a major cause of death from gastrointestinal malignancies, such as colon, gastric, and pancreatic carcinomas as well as melanoma, breast cancer, and sarcomas (1).

Circulating metastatic cells that enter the liver encounter unique cellular populations. These include the parenchymal hepatocytes and the nonparenchymal hepatocytes, including liver sinusoidal endothelial cells (LSEC), hepatic stellate cells (HSC), Kupffer cells (KC), dendritic cells, liver-associated lymphocytes, and portal fibroblasts. Circulating and bone marrow–derived immune cells are also recruited to the liver in response to, and possibly in preparation for, invading tumor cells and can affect the final outcome (reviewed in refs. 2, 3; see Table 1).

This review summarizes our current understanding of the role of the liver microenvironment in the growth of hepatic metastases and the reciprocal interactions between metastatic tumor cells and different liver cell populations that regulate the process.

Pre- and Prometastatic Niches Drive the Progression of Liver Metastasis

The process of liver metastasis has previously been divided into four major phases (detailed in Table 1) that follow tumor cell entry into the liver (4, 5). Emerging evidence suggests that in addition, a premetastatic phase could set the stage for liver colonization by disseminating tumor cells.

Evidence for premetastatic niche formation in the liver

The term “premetastatic niche” was coined to describe a microenvironment in a secondary organ site that has been rendered permissive to metastatic outgrowth in advance of cancer cell entry through the activity of circulating factors released by the primary tumor (6–8). Although the dependency of metastasis on premetastatic niches remains controversial and difficult to verify in the clinical setting (9, 10), it appears that a consensus is emerging on two scores: (i) that their existence may have important implications for the clinical management of metastatic disease, and (ii) that much can be learned from their interrogation about the cellular/molecular changes required to facilitate tumor cell growth in a distant site, such as the liver. Two recent studies have confirmed their potential relevance to liver metastasis. In a mouse model of metastatic pancreatic ductal adenocarcinoma (PDAC), Costa-Silva and colleagues showed that PDAC-derived exosomes taken up by hepatic KCs, upregulate TGFβ production, leading to increased fibronectin production by HSCs and recruitment of bone marrow–derived macrophages. They identified macrophage migration inhibitory factor (MIF) as essential for premetastatic niche formation and metastasis. Moreover, in exosomes derived from patients with stage I PDAC who later developed liver metastasis, MIF levels were found to be higher than in patients whose tumors did not progress, thus identifying MIF as a potential predictor of PDAC liver metastasis (11). This group also showed that exosomes from human breast and pancreatic cancer cell lines that metastasize selectively to the lung (MDA-MB-231) or liver (BxPC-3 and HPAF-II) fused preferentially with fibroblasts and epithelial cells in the lung and KCs in the liver, and this was mediated by...
the exosomal integrin laminin receptors α6β1 and α6β4 and the fibronectin receptor α9β5, respectively. In exosome-fused KCs, the proinflammatory factors S100P and S100A8 were upregulated (Table 1; Fig. 1). Significantly, in PDAC patients, a correlation was documented between the levels of circulating, αα-bearing exosomes and disease stage, suggesting that exosomes may play a role clinically and their levels and integrin cargo may predict metastases in specific organs (12). Kowanez and colleagues reported that tumor-derived GM-CSF could mobilize Ly6G"Ly6G+" myeloid cells into metastases, enabling niches in the lung and liver, and also identified S100A8 as a driving factor (13). Tumor-derived TIMP-1 was identified as another potential inducer of increased liver susceptibility to metastasis, acting via hepatic SDF-1 and neutrophil recruitment (14). Interestingly, S100A8 was identified as a driver of liver premetastatic niche formation in several studies (12–15), suggesting that it may have clinical utility as a predictor of liver metastasis.

The stage of primary tumor development at which premetastatic niches can be formed is difficult to assess in the clinical setting. Costa-Silva and colleagues found exosomal MIF upregulation in mice with pretumoral pancreatic lesions, and high plasma exosomal MIF levels were also detected in patients with stage I PDAC (11), suggesting that they could be formed at very early stages of cancer development. The potential contribution of circulating cancer cells (CTC) that may be present early in tumor development and even after resection of the primary tumor (16, 17) is also unknown.

**Resident and Immune Cells Can Play Diverse and Opposing Roles in the Development of Liver Metastases**

Host innate resistance mechanism can destroy tumor cells prior to extravasation

Blood-borne cancer cells entering the liver first encounter the LSECs, KCs, and hepatic natural killer (NK) cells (pit cells) that together mount the first line of defense (18). Cancer cells entrapped in the sinusoids may undergo destruction due to mechanical stress and deformation-associated trauma or may die due to phagocytosis by KCs or cytolysis by NK cell–released perforin/granzyme (18; reviewed in ref. 4). NO, ROS, and toxic radicals released by LSECs in response to local ischemia/reperfusion can contribute to tumor cell death (19, 20). Release of NO and IFNγ by LSECs and KCs can result in upregulation of tumor cell Fas and apoptosis (18) that can be augmented by NK and LSEC-derived TNFα (21). TNFα is one of the numerous cytokines and chemokines unleashed by KCs in response to inflammation (Table 1; ref. 22). In addition to causing cell death directly, some of these factors can mobilize and activate additional innate immune cells, such as neutrophils, thereby adding to the local tumoricidal capacity (reviewed in ref. 23). In several animal tumor models, including colon cancer, loss of NK cells increased cancer cell growth in the liver, whereas enhanced NK activity reduced liver metastasis (24–26). Indirect evidence also suggests that susceptibility to NK-mediated immune attack can affect the ability of human cancer cells to generate liver metastases (27). Circulating neutrophils can also release various factors abundant in their granules to kill tumor cells (see Table 1), and their cytokines and chemokines can activate the tumoricidal potential of resident KCs and recruit host immune T cells with antitumorogenic activities (reviewed in ref. 28).

Cancer cells can escape these cytotoxic effects by forming clusters with blood or other cancer cells that shield them from the lethal effects of shear stress or NK-mediated cytotoxicity (reviewed in ref. 29). The release of inflammatory mediators, such as IL1β, TNFα, and IL18, can also initiate a cascade that facilitates their rapid exit from the vasculature to a less "toxic" microenvironment (see below and Fig. 2).

The sinusoidal endothelium actively participates in different phases of metastasis

Although the rapid inflammatory response initiated by cancer cell entry can lead to cell death, it may also have a tumor-protective effect by upregulating the expression of LSEC cell adhesion molecules (CAM) and in this way, enhance cancer cell adhesion and transendothelial migration into the space of Disse, where they can escape the cytotoxic effects of KCs and NK cells (30). Cancer cells can adhere to inflammation-induced E-selectin, VCAM-1, and ICAM-1, either as single cells or in association with host cells, such as KCs or neutrophils (reviewed in ref. 31). Blockade of this inflammatory cascade was shown to reduce liver metastasis (32–34), and TNFRI signaling was identified as a major driver of this process (35).

Ultimately, the outcome of these opposing effects on tumor cell survival and progression to the next phase depends on multiple factors, including the expression on the cancer cells of the respective counter receptors, namely the E-selectin ligands sLeα and sLeγ and CD44 isoforms (36; reviewed in refs. 29, 37), and VCAM-1 and ICAM-1 counter receptors integrins α4β1 and αLβ2 (38), respectively. E-selectin binding triggers a signaling cascade in both tumor cells and LSECs, leading to diapedesis and transendothelial migration (39) and altering gene expression in a tumor type–specific manner (40). Clinical studies have documented increased expression of E-selectin in and around colorectal cancer liver metastases and elevated soluble E-selectin, ICAM-1, and VCAM-1 levels that correlated with disease outcome in patients with colorectal cancer (reviewed in ref. 23).

LSECs may have other metastasis-promoting functions. LSEC-secreted fibronectin can induce epithelial–mesenchymal transition (EMT) in colorectal cancer cells via integrin α9β1, enhancing ERK signaling and tumor invasion (41). Human LSECs were shown to induce colorectal cancer migration and EMT via MIF (42), thereby increasing their metastatic potential. LSECs contribute to tumor-induced angiogenesis. A recent study identified Notch1 as a negative regulator of sinusoidal endothelial cells sprouting into micrometastases and thereby of angiogenesis and liver metastasis (43), raising questions about the advisability of Notch targeting for cancer therapy. Moreover, liver metastasis of different carcinomas, including colorectal cancer, can co-opt the sinusoidal endothelium at the tumor–liver interface, giving rise to a histologic growth pattern termed the “replacement” or “sinusoidal” growth pattern that seems to result from tumor cell invasion between LSECs and the matrix in the space of Disse and is characterized by the formation of intrametastatic vessels that appear continuous with the sinusoidal vessels (reviewed in ref. 23). The factors that drive this co-opting mechanism remain to be identified, but a recent study by Im and colleagues suggests that Ang2 may play a regulatory role (44).

Collectively, the evidence shows that LSECs are not a passive barrier to tumor cell extravasation but participate actively in the metastatic process. Cancer cell interaction with LSECs can reciprocally alter the phenotypes of both cell types, and this may lead to
intravascular tumor cell destruction but can also promote metastasis through enhanced tumor cell migration and increased angiogenesis (Table 1; Figs. 2 and 3).

### The role of macrophages is context dependent

KCs constitute approximately 10% of all liver cells. Recent studies suggest that they may be established prenatally and maintained into adulthood, independently of replenishment from blood monocytes but in an M-CSF and GM-CSF–dependent manner (45). They reside mainly in the hepatic sinusoids and are anchored to the endothelium by long cytoplasmic processes (46; reviewed in ref. 47). In their interaction with invading cancer cells, KCs can play diverse and opposing roles, depending on factors such as the stage of the metastatic process, tumor load, and interactions with other immune cells. As discussed, KCs can promote metastasis by orchestrating the premetastatic niche. However, tumor–KC interactions can have opposing effects. Several studies have documented a rapid adhesion of tumor cells to KCs in the sinusoidal lumen, resulting mainly in tumor phagocytosis or apoptosis, within hours of tumor cell entry (47). This tumoricidal effect may require cooperation with local or recruited NK cells (48), and its efficiency in removing cancer cells likely depends on the tumor load (49). An intravital microscopy study (50) recently revealed that 74% of intra-arterially injected rat colon cancer cells adhered to the KCs within 6 hours, and this was associated with increased liver TNFα and IL1β levels, consistent with our own findings (51, 52) and indicative of KC activation. Extensive phagocytosis of tumor cells was observed within 2 hours postinjection. Interestingly, although elimination of KCs 2 days prior to tumor injection increased liver metastasis, it had no significant effect up to 1 week after tumor injection, suggesting that KCs exerted their most potent antitumor effect within the first 24 hours of tumor cell entry into the liver (50). A bimodal effect of KC depletion was also documented in another study of murine colorectal cancer, and it documented in another study of murine colorectal cancer, and it

### Table 1. Cells and mediators involved in the different phases of liver metastasis

<table>
<thead>
<tr>
<th>Phase of the metastatic process</th>
<th>Cell type involved</th>
<th>Cell surface markers</th>
<th>Recruiting or activating</th>
<th>Produced</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Microvascular phase: Tumor cells trapped into the vasculature</td>
<td>LSECs</td>
<td>CD31, TNFR1, TNFR2, αvβ3, E-selectin, VCAM-1, ICAM-1</td>
<td>M-CSF, GM-CSF, IFNγ</td>
<td>ROS, NO, IFNγ, TNFα</td>
<td>(5, 18-20, 23, 31)</td>
</tr>
<tr>
<td></td>
<td>KCs</td>
<td>CD11b, F4/80</td>
<td>M-CSF, GM-CSF, IFNγ</td>
<td>TNFα, IL1β, IL1β</td>
<td>(5, 23, 31, 45-47, 51)</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
<td>CD11b, Ly6G</td>
<td>S100A8, S100A8, IL8, CCL2</td>
<td>TNFα, ROS, defensins, peroxin, elastase</td>
<td>(28, 31, 64)</td>
</tr>
<tr>
<td></td>
<td>Hepatic NK cells</td>
<td>NK1.1, NK1.2, FcyRIII</td>
<td>CXCL9, CXCL10, IL1, IL12, IL18</td>
<td></td>
<td>(5, 31, 48)</td>
</tr>
<tr>
<td>(2) Extravascular, preangiogenic phase: Tumor cells transmigrate into the space of Disse, activating a local stromal response</td>
<td>HSCs</td>
<td>α-SMA, desmin, GAFP</td>
<td>KC-derived TGFβ, PDGF, bFGF, SDF-1, IGF-1, CCL2</td>
<td>ECM deposition, MCP-1, CCL5, CCL21, TGFβ</td>
<td>(74-77, 84)</td>
</tr>
<tr>
<td></td>
<td>Neutrophils</td>
<td>CD11b, Ly6G; N1: ICAM-1; N2: CXCR4</td>
<td>S100A8, S100A8, CXCL1, CXCL2, CXCL5, TNFα, IL8, G-CSF, IFNγ, CCL2, CCL5, TGFβ</td>
<td>TNFα, ROS, ROS, MMP-8, MMP-9, elastase, cathespin G, VEGF</td>
<td>(28, 60, 64, 71)</td>
</tr>
<tr>
<td></td>
<td>KCs</td>
<td>CD11b, F4/80</td>
<td>M-CSF, GM-CSF, IFNγ</td>
<td>IL6, VEGF, HGF, MMP-9, MMP-14</td>
<td>(5, 50, 55)</td>
</tr>
<tr>
<td>(3) Angiogenic phase: Micrometastases are vascularized</td>
<td>Blood-derived monocytes</td>
<td>CD11b, F4/80, CD68, CD163, CD206</td>
<td>KC-derived TGFβ, PDGF, bFGF, SDF-1, IGF-1, CCL2</td>
<td>VEGF, MMPs, bFGF</td>
<td>(57, 60)</td>
</tr>
<tr>
<td></td>
<td>HSCs</td>
<td>α-SMA, desmin, GAFP</td>
<td>VEGF, angiopep2-inhibitor, MMP-2, -9, and -13</td>
<td>Fibronectin, MIF</td>
<td>(74, 79-82, 88)</td>
</tr>
<tr>
<td></td>
<td>LSECs</td>
<td>CD31, TNFR1, ICAM-1</td>
<td>VEGF, Ang1, inhibited by Notch1 and regulated by Ang2</td>
<td></td>
<td>(23, 41-44)</td>
</tr>
<tr>
<td></td>
<td>Macrophages (M2)</td>
<td>CD11b, F4/80, Ly6C, CD163</td>
<td>TGFβ, IL10</td>
<td>NO, TNFα, IFN, VEGF, EGF, bFGF, TGFβ, IL10</td>
<td>(57-61)</td>
</tr>
<tr>
<td>(4) Growth phase: Metastases expansion</td>
<td>Hepatocytes</td>
<td>Albumin, tyrosine aminotransferase</td>
<td>Adhesion to tight junction integrin proteins</td>
<td>AREG, EGF, HB-EGF, Erb2, IGF-1, HGF, Erb3, bFGF</td>
<td>VEGF, TGFβ, IL10</td>
</tr>
</tbody>
</table>

NOTE: Shown are the four phases of the metastatic process that follow tumor cell invasion into the liver. Listed are the hepatic cells known to be involved at each phase, the mediators regulating their recruitment and activation, and the factors they produce to block or promote the metastatic process. The process has been simplified and divided into distinct phases in the interest of clarity. However, it should be viewed as dynamic with overlapping cellular and molecular drivers at different phases. Moreover, as the metastatic process advances, the cancer and hepatic cells evolve and their phenotypes change as a consequence of their interactions. These newly acquired properties help propel the process forward (see also Figs. 1–3).

Abbreviations: ECM, extracellular matrix; MDSC, myeloid-derived suppressor cell; MMP, matrix metalloproteinase; NK, natural killer; Treg, regulatory T cell.
from their protumorigenic functions. Adhesion to KC can facilitate tumor cell extravasation, among others, by sequential activation of endothelial CAMs (29, 54). In addition, KCs can produce cytokines and growth factors, including IL6, hepatocyte growth factor (HGF), and VEGF, and matrix metalloproteinases (MMP), such as MMP-9 and MMP-14, that can accelerate tumor cell invasion into and within the parenchymal space as well as promote tumor cell proliferation and angiogenesis, thereby enhancing liver metastasis (see Table 1; Figs. 2 and 3).

Taken together, the evidence suggests that KC targeting could become an effective antimetastatic strategy only within a very narrow window. Limiting KC activity may be beneficial if it could prevent premetastatic niche formation, whereas enhancing the KC tumoricidal potential using agents such as IFNγ, GM-CSF, or muramyl dipeptide may be most effective if achieved before tumor cells enter the liver in large numbers (47, 55). The contribution of CTCs that may be present before liver micrometastases are established or after surgical resection of the primary tumor (16, 17) to the tumor load and KC activation is unknown. This multifaceted role of KCs in liver metastasis may explain the limited success to date of KC-targeting interventions (47).

Importantly, in addition to KCs, blood-derived monocytes can also be recruited to the tumor site in response to local injury and inflammation and differentiate locally into mature CD11b+Ly6G+ myeloid cells in a CCR2-dependent manner (56). These macrophages can also trigger HSC activation and fibrogenesis (57), a process important in the early stages of extravascular tumor expansion (see below).
Macrophages have inherent plasticity, and their phenotypes can change within a spectrum of activation states between the polar M1 and M2 phenotypes (58, 59). M1 macrophages are tumoricidal due to high NO and TNFα production levels and produce Th1, Th17, and the NK-attracting chemokines CXCL9 and CXCL10 (60). In contrast, M2 macrophages can promote tumor growth through production of growth factors, such as VEGF, EGF, bFGF, and TGFβ (61). In addition, M1 macrophages activate Th1-type immune responses that can further amplify M1/killer-type activity through the production of IFNγ, whereas M2 macrophages induce regulatory T cells (Treg) and thereby an immunotolerant microenvironment through release of TGFβ and IL10 (Table 1; ref. 62). Evidence regarding the role of macrophage polarization in liver metastasis is scant, because macrophages within or around hepatic metastases have, with few exceptions, not been subtyped. A recent clinical study identified the M2/M1 ratio in hepatic resections as a correlate of colorectal cancer metastasis (63), implicating M2 macrophages in the clinical disease. Furthermore, F4/80, the cell surface marker frequently used to identify KCs, is also expressed on recruited monocytes, and strategies used to eliminate macrophages in vivo are not KC specific. The source and identities of macrophages in many published studies remain therefore to be verified. As immune editing of the tumor microenvironment and the conversion of...
<table>
<thead>
<tr>
<th>Cell type</th>
<th>Antimetastatic effects</th>
<th>Prometastatic effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver sinusoid endothelial cells (LSEC)</td>
<td>Produce ROS, NO, IFNγ, TNFα</td>
<td>Express CAM, protect tumor cells from apoptosis, facilitate transmigration, participate in angiogenesis, induce EMT</td>
<td>(5, 18–20, 31, 33, 41)</td>
</tr>
<tr>
<td>Hepatic stellate cells (HSC)</td>
<td>Participate in premetastatic niche formation, drive fibrogenic response, ECM production, mediate angiogenesis, recruit inflammatory cells, produce MMPs</td>
<td>(11, 23, 77, 82–86)</td>
<td></td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>Mediate tumor adhesion, produce growth factors (e.g., IGF-I, HGF), alter tumor transcriptome, undergo EMT to provide stromal support, enhance angiogenesis via bFGF</td>
<td>(93–96, 101, 103, 105)</td>
<td></td>
</tr>
<tr>
<td>Kupffer cells (KC)</td>
<td>Phagocytose tumor cells, cause apoptosis (early in the process), produce TNFα, mobilize neutrophils and NK cells</td>
<td>Participate in premetastatic niche formation; activate LSEC CAM; produce VEGF, IL6, HGF, and MMP-9; activate HSC</td>
<td>(5, 11, 12, 47–53)</td>
</tr>
<tr>
<td>Macrophages</td>
<td>MI macrophages produce NO, TNFα, and T-cell– and NK-recruiting chemokines</td>
<td>M2 macrophages produce VEGF, EGF, and bFGF; induce Tregs via IL10 and TGFβ</td>
<td>(59–63)</td>
</tr>
<tr>
<td>Myeloid-derived suppressor cells (MDSC)</td>
<td>Induce Tregs, block T-cell expansion, suppress cytotoxic T-cell function, produce ROS, produce arginase</td>
<td>(113, 117, 123, 124)</td>
<td></td>
</tr>
<tr>
<td>Natural killer (NK) cells</td>
<td>Produce perforin and TNFα, induce Fas/FasL interaction, kill tumor cells</td>
<td></td>
<td>(4)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>N1 neutrophils produce ROS, NO, TNFα, and defensins; recruit CD8+ T cells</td>
<td>Participate in premetastatic niche formation, enhance transendothelial migration, entrap tumor cells; N2 neutrophils produce VEGF, MMP-9, and CCL5</td>
<td>(28, 64, 77)</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>Cytotoxic T cells kill tumor cells, release perforin and TNFα, recruit macrophages</td>
<td>Tregs inhibit effector T-cell expansion, eliminate cytotoxic T cells, subvert immune activation, produce VEGF</td>
<td>(109, 111)</td>
</tr>
</tbody>
</table>

**Figure 3.**
Parenchymal, nonparenchymal, and immune cells of the liver and their role in metastasis. Listed are the parenchymal and nonparenchymal cells of the liver and their anti- and prometastatic functions. Symbols shown for each cell type were used to depict intercellular communications in Figs. 1 and 2. EMT, epithelial-mesenchymal transition; Treg, regulatory T cell.
tumor-associated (TAM) M2 macrophages to M1 killer macrophages are emerging as potentially relevant anticancer strategies (62), further characterization of both the source and the status of macrophage populations involved in liver metastasis will become critical.

The neutrophils: A double-edged sword

Neutrophils are part of the innate immune response to pathogens and are also rapidly activated in response to invading cancer cells (28). Bone marrow–derived neutrophils are mobilized to sites of inflammation or cancer via their cell-surface receptor CXCR2 and in response to chemokines and cytokines (Table 1) secreted by activated macrophages, endothelial cells, or the tumor cells [reviewed in ref. 28]. At the tumor site, the neutrophils can exert opposing effects that depend on the inflammatory/immune context (reviewed in ref. 64). They can release cytolytic factors (detailed in Table 1) or inhibit tumor growth indirectly via recruitment of CD8+ cytotoxic T cells and macrophages. This may require direct contact with the tumor cells (64, such as can occur within the liver sinusoidal lumen (65, 66).

On the other hand, neutrophils can be mobilized into premetastatic niches in the liver in response to S100A8 and S100A9 (7) and were shown to contribute to niche formation in an orthotopic colon carcinoma model (67). Moreover, in the vascular lumen, the physical interaction with tumor cells that could lead to tumor cell kill may, alternatively, anchor circulating tumor cells to the vascular endothelium and enhance their migration into the extravascular space. Tumor-derived cytokines, such as IL8, induce expression of integrins, such as CD11b/CD18, on the neutrophils, increasing their adhesion to tumor cells via the counter receptor ICAM-1 and facilitating transendothelial migration, as shown in murine melanoma and lung carcinoma models (66, 68). Tumor cell entrapment in the sinusoids can also be mediated by neutrophil-expressed extracellular (DNA) traps, resulting in increased tumor retention in the sinusoids, enhanced tumor cell adhesion, proliferation, migration and invasion, and increased liver metastasis (69, 70). Neutrophils can also release extracellular matrix (ECM)–degrading proteinases, such as MMP-8, MMP-9, elastase, and cathepsin G, thereby increasing tumor invasion (64), and can promote angiogenesis through the release of VEGF (Table 1; Figs. 2 and 3).

Moreover, a TGFβ–mediated neutrophil polarization that alters the phenotype of tumor-associated neutrophils from tumor inhibitory (N1) to tumor promoting (N2) was recently described (71). TGFβ produced by M2 macrophages may contribute to this process (reviewed in ref. 59). However, the contribution of polarized neutrophils to liver metastasis remains to be confirmed.

Clinical studies implicate neutrophils in tumor promotion. With few exceptions, elevated circulating neutrophil counts or neutrophil-to-lymphocyte ratios were associated with poorer outcomes and distant metastases in patients with various epithelial malignancies (72), including carcinomas of the gastrointestinal tract. High neutrophil counts may also be associated with increased resistance to therapy (64, 72). The 5-year survival for patients with colorectal cancer undergoing hepatic resections that had neutrophil-to-lymphocyte ratios greater than 5 was worse than for those with normal ratios (73). However, high neutrophil infiltration into the tumor site may be a consequence of advanced tumor stage (and, therefore, poorer prognosis) rather than its cause.

HSCs orchestrate a prometastatic microenvironment that is required for transition from the avascular to the vascular stage of metastasis

HSCs orchestrate the characteristic fibrogenic response of the liver to injury. Normally quiescent in the space of Disse (74), they are activated (aHSC) in response to liver damage and inflammatory stimuli, acquire a myofibroblast-like phenotype (α-SMA+), and produce ECM rich in collagen I and IV (74, 75). Chemokines and cytokines released by aHSCs (Table 1) also recruit inflammatory/immune cells (76, 77), thereby shaping the immune microenvironment.

In addition to their role in premetastatic niche formation (above), HSCs can orchestrate a prometastatic niche following tumor extravasation. In response to growth factors and inflammatory mediators (detailed in Table 1), HSCs are activated (74) and can trigger a process akin to the early events in liver repair. This was observed in animal models (4) and is also evidenced by the increased production of collagen IV in and around hepatic metastases in clinical specimens (78). Macrophages, hepatocytes, and LSECs contribute to this process by releasing TGFβ and/or TNFα (57). In turn, aHSC-derived proangiogenic factors, such as VEGF and angiopep-1 (79, 80), initiate angiogenesis (3), and this is enhanced by HSC-derived MMPs that facilitate endothelial cell migration and tumor invasion (23, 74; reviewed in refs. 5, 81; Table 1; Fig. 2).

Several lines of evidence confirm the essential role of HSCs in liver metastasis. Olaso and colleagues showed that myofibroblasts that infiltrated avascular micrometastases of B16 melanoma cells induced a strong proangiogenic response to angiogenesis, which preceded endothelial cell recruitment and was followed by colocalization of myofibroblasts and endothelial cells within angiogenic structures (82, 83). HSC and macrophage recruitment into metastatic sites and subsequent angiogenesis were shown to be CCR2/CCL2 dependent (84). More recently, Eveno and colleagues (85), using immunofluorescence, showed that 9 days postinjection of colorectal cancer LS174 cells into SCID mice, the hepatic micrometastases consisted of proliferating cancer cells, a well-organized network of aHSC and laminin deposits, but no vascular network. As the liver metastases grew, an organized vascular network appeared and laminin colocalized with CD31+ endothelial cells. Co-injection of tumor cells and HSCs enhanced metastasis. Moreover, analysis of liver metastasis from patients with colorectal cancer revealed a surface-marker expression pattern similar to that observed in the coinjection studies.

Recently, suppression of TGFβ receptor II signaling by QGAP1 was shown to prevent HSC activation, and IQGAP1 downregulation was observed in myofibroblasts associated with human colorectal cancer liver metastases, consistent with their activation in this context (86). Furthermore, α-SMA+ cells, whose presence correlated with the degree of fibrous stroma, were identified in liver metastases of colon, gastric, and pancreatic adenocarcinomas (87).

The importance of HSCs to metastatic niche formation and their role in primary liver cancer (88) have raised interest in HSC targeting as an anticancer/antimetastatic strategy. There is little direct evidence that such an approach can succeed, due partially to the present lack of agents that specifically target HSCs without toxicity. Indirect evidence from a rat model of cholangiocarcinoma (89) and pancreatic stellate cell targeting in a PDAC model (81) suggests a potential therapeutic benefit,
but this remains to be verified. Of note, portal fibroblasts and bone marrow–derived fibrocytes (90) can also contribute to the stromal response. Liver-invasive cancer cells located in portal tracts and unable to activate HSCs may engage portal tract fibroblasts that produce IL8, a chemokine involved in invasion and angiogenesis (91). Specific targeting of HSCs may, therefore, not be sufficient to deprive metastatic cells of a growth-promoting stroma.

Hepatocytes can promote metastasis directly and indirectly

The role of the parenchymal hepatocytes in liver metastasis is not well understood. Tumor cell adhesion to hepatocytes was identified as one of the earliest events in liver metastasis formation and a correlate of the metastatic potential (92, 93). Desmosomes (94), integrins αv, 66, and β1 that bind to hepatocyte ECM (95), osteopontin binding via counter receptors CD44 and integrin αv (95), and claudins (96, 97) were all implicated. Adhesion of human colorectal cancer cells to hepatocyte-derived ECM was shown to upregulate the expression of genes involved in tumor cell survival, motility, and proliferation, in particular EGF family members implicated in liver metastasis (98) such as AREG, EGF, HBEGF, and erbB2 and stem cell markers CD133 (PROM1) and LGR5 (99), suggesting that colorectal cancer adhesion to hepatocyte ECM induces autocrine growth-promoting mechanisms for tumor expansion. Tumor cell adhesion to hepatocytes can have other consequences. The FasL/FLICE-like inhibitory protein on murine hepatocyte-derived ECM was shown to induce apoptosis in hepatocytes by activating Fas signaling, and this destructive process created a niche for tumor expansion (100). Furthermore, hepatocytes produce several growth factors, including IGF-I (101). As we have shown, blockade of IGF-I signaling in metastatic tumor cells could inhibit liver metastasis (102–104). Other factors include the HGF-like protein/macrophage-stimulating protein (HGFL) that can enhance tumor growth, motility, and invasion via the Ron receptor (105); heregulin, a ligand of ErbB3 shown to enhance integrin αvβ5-mediated cell migration and erbB3/erbB2 signaling in human colorectal cancer cells (106); and the proangiogenic factor bFGF (107).

The clinical relevance of tumor–hepatocyte interactions is not clear. Three different histologic growth patterns have been identified in liver metastases specimens, namely the desmoplastic, pushing, and replacement growth patterns. The latter two are characterized by close tumor–hepatocyte proximity (23). Whether or not direct adhesion between metastatic cancer cells and hepatocytes influences the eventual growth pattern of colorectal cancer liver metastases is not known. Blocking tumor–hepatocyte interactions could have beneficial antemetastatic effects, as recently demonstrated by Tabaries and colleagues (108).

An immunosuppressive microenvironment facilitates metastatic expansion

Tumor cells can activate specific T-cell–mediated immune responses that curtail tumor expansion through various mechanisms (reviewed in ref. 109). However, tumor cells can evade T-cell–mediated killing, among others, by giving rise to and/or recruiting immunosuppressive cells, such as myeloid-derived suppressor cells (MDSC) and regulatory T cells (Treg) that alter the immune landscape, inducing a state of immune tolerance that is permissive to tumor expansion (reviewed in ref. 110).

The role of Tregs. Treg cells may be thymically derived (natural, nTregs) or induced from naïve CD4+ T-cell precursors (iTreg) in response to IL10 and TGFβ. They exert an immunosuppressive effect by different mechanisms that result in the inhibition of an effective, antitumorogenic T-cell response (reviewed in ref. 111). Although high Treg levels were documented in the blood and local tumor sites of patients with various epithelial cancers (e.g., ref. 112), relatively little is known about their role in liver metastasis. Connolly and colleagues, using models of early, preinvasive pancreatic neoplasia and advanced colorectal cancer showed that Tregs accelerated the development of liver metastases (113). We recently documented the accumulation of Tregs around colon carcinoma MC38 liver micrometastases and showed that this was TNRF2 dependent (35).

Interestingly, in 57 colon cancer specimens from patients undergoing chemotherapy or chemoinmunotherapy, higher Treg infiltration scores were associated with a better prognosis and a better outcome (114). However, the relationship between Treg accumulation in the primary tumors and in the corresponding liver metastases has not been examined. Although Treg-targeting strategies are in development (115, 116; reviewed in ref. 110), their potential therapeutic benefit for liver metastases has not yet been examined.

The role of MDSCs. MDSCs are a heterogeneous population of myeloid cells that can be of the monocytic (Mo-MDSC, CD11b+Ly6C−) or granulocytic (G-MDSC, CD11b+Ly6G−) lineages (117). In humans, MDSCs are CD33+ and/or CD11b+ and HLA-DR− (118). Under normal physiologic conditions, bone marrow–derived immature myeloid cells differentiate into mature granulocytes or monocytes, able to mediate host innate immune responses in the target tissue. However, in the tumor microenvironment, these precursors do not mature and exert immunosuppressive and tumor-promoting effects instead (119). Although they express granulocyte and monocyte surface markers, these cells have been characterized on the basis of functional criteria, namely their immunosuppressive capabilities (117, 120). An increase in circulating MDSCs in cancer patients and their recruitment into tumor sites have been documented (117, 121, 122).

In the liver, MDSCs can be recruited to the metastases through chemokines, such as CXCL1 and CXCL2, produced by LSECs and KCs or by activated HSCs (77). There, they can promote tumor growth by inducing immunosuppression (123), producing arginase (117), and increasing Treg numbers (Table 1; Fig. 2; ref. 124). Clinical and experimental evidence confirms their role in metastasis (125). Recruitment of tumor-promoting myeloid cells into colorectal cancer liver metastases has recently been documented, and their depletion was shown to result in a marked reduction in liver metastasis (126). We recently reported that MDSCs accumulate in hepatic micrometastases of MC38 cells within days of tumor cell injection and documented a relative preponderance of Ly6Chigh cells (the more suppressive, arginase-high subtype; ref. 127). We also identified CD3+HLA-DR−TNRF2+ myeloid cells in the periphery of hepatic metastases from patients with colorectal cancer, implicating MDSCs in the clinical disease (35).

Several strategies are being developed to selectively eliminate MDSCs, including pharmacologic induction of MDSC differentiation, inhibition of MDSC expansion from bone marrow...
precursors, or blockade of their function [128]. However, some of the drugs developed are not MDSC specific, or their long-term administration is not possible due to toxicity [128]. Their translational potential remains therefore to be verified.

Conclusions

Cancer cells entering the liver encounter a unique and complex microenvironment. Parenchymal and nonparenchymal liver cells, as well as recruited inflammatory and immune cells, participate in the response to invading tumor cells and may inhibit or favor the progression of metastasis. The factors that determine the outcome of these opposing effects and the pattern of growth of the metastases are not yet well understood. This duality of function and the shifts in the types of cells and mediators involved at different stages of the metastatic process render the attempts to target specific cellular interactions in the microenvironment extremely challenging and may explain the slow progress to date in identifying agents that can successfully and specifically inhibit metastatic disease. With the advent of genomic profiling for personalized cancer treatment and the increased availability of surgically resected liver metastases for cellular/molecular interrogation, our understanding of the relationship between the genetic background and clinical history of the patient, the genomic/transcriptomic profile of the cancer cells, and the type of response mounted by the microenvironment may improve, opening new avenues for prevention and/or treatment of liver metastatic disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The author is indebted to Dr. Janusz Rak (McGill University Health Centre) for insightful editorial comments and to Simon Milette for the superb artistic rendering of the metastatic niches of the liver and for his help with the table.

Grant Support

This work was made possible by support from the Canadian Institute for Health Research and by a PSR-SSIRI-843 grant from the Québec Ministère de l’Économie, de l’Innovation et des Exportations.

Received February 21, 2016; revised September 19, 2016; accepted September 22, 2016, published OnlineFirst October 19, 2016.

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Pnina Brodt


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