

Review

The Multifaceted Role of the Microenvironment in Liver Metastasis: Biology and Clinical Implications

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Abstract

The liver is host to many metastatic cancers, particularly colorectal cancer, for which the last 2 decades have seen major advances in diagnosis and treatment. The liver is a vital organ, and the extent of its involvement with metastatic disease is a major determinant of survival. Metastatic cells arriving in the liver via the bloodstream encounter the microenvironment of the hepatic sinusoid. The interactions of the tumor cells with hepatic sinusoidal and extrasinusoidal cells (endothelial, Kupffer, stellate, and inflammatory cells) determine their fate. The sinusoidal cells can have a dual role, sometimes fatal to the tumor cells but also facilitatory to their survival and growth. Adhesion molecules participate in these interactions and may affect their outcome. Bone marrow-derived cells and chemokines also play a part in the early battle for survival of the metastases. Once the tumor cells have arrested and survived the initial onslaught, tumors can grow within the liver in 3 distinct patterns, reflecting differing host responses, mechanisms of vascularization, and proteolytic activity. This review aims to present current knowledge of the interactions between the host liver cells and the invading metastases that has implications for the clinical course of the disease and the response to treatment. *Cancer Res*; 73(7); 2031–43. ©2013 AACR.

Introduction

The liver is the main site of metastatic disease for many gastrointestinal and extragastrointestinal cancers, including melanoma, breast, pancreatic, and renal cancer (see Supplementary Table S1). Liver involvement is a major determinant of survival from cancer because it is a vital organ. The liver is the largest organ of the body, with a unique architecture suited for its diverse functions such as protein biosynthesis and detoxification of portal blood. The 2 sources of its unique dual blood supply (80% deoxygenated blood via the portal vein and 20% oxygen-rich blood via the hepatic artery) anastomose at the

point of entry into the sinusoids, and the mixed blood perfuses the liver parenchymal cells before draining via the centrilobular veins. It is believed that the flow rate within the sinusoids is controlled by the contractile properties of hepatic stellate cells (HSC; see later; ref. 1). Please see the following interactive website (2) for a 3-dimensional depiction of the liver and its vasculature.

Circulating metastatic cells can enter the liver through both vascular entry ports. Once in the liver, they encounter a diverse population of host cells that are specialized to carry out the multiplicity of hepatic functions, namely, the hepatic sinusoidal endothelial cells (HSEC), Kupffer cells (KC), HSC, Pit cells, and hepatocytes. Four major phases have been identified in the progression of liver metastasis: (i) the microvascular phase, which involves tumor cell arrest in the sinusoidal vessels and can lead to tumor cell death or extravasation; (ii) the extravascular, preangiogenic phase, during which host stromal cells are recruited into avascular micrometastases; (iii) the angiogenic phase, during which endothelial cells are recruited and the tumors become vascularized through several possible interactions with the microenvironment; and (iv) the growth phase, which leads to establishment of "clinical" metastases (3). Tumor cell fate can be determined early during the microvascular phase by the interactions that occur within the liver sinusoid. The cellular components of the hepatic sinusoids and their functions have been reviewed (4, 5). The aim of this review is to describe the current state of knowledge on the interactions between metastatic tumor cells and the liver microenvironment, with a particular focus on the sinusoidal cells that determine tumor cell fate. The evidence reviewed shows

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that these interactions can lead to tumor cell death or promote the intravascular arrest, extravasation, establishment, and growth of metastatic cells (summarized in Fig. 1). The potential influence of these interactions on the histologic growth patterns of liver metastases is also discussed. Finally, current gaps in our understanding of the metastatic process in the liver and strategies that could be used to fill these gaps are outlined. Because much of the available clinical data are based on the analysis of resectable colorectal carcinoma (CRC) metastases, a bias toward the biology of these metastases was unavoidable. However, data based on animal models and cell lines of non-CRC malignancies were also included, where appropriate. The differences in growth patterns of CRC and breast carcinoma metastases are specifically discussed in sections entitled "Histologic growth patterns of liver metastases" and "Vascularization of liver metastases." The reader is referred to refs. (6, 7) for reviews on liver metastasis with a nongastrointestinal origin.

Cells of the Hepatic Sinusoid Can Kill Tumor Cells

Although mechanical arrest undoubtedly plays a role in metastasis initiation, experimental, intravital microscopy studies have shown that the diameter of the sinusoidal vessels was usually larger than that of the colon carcinoma cells remaining in the vessel lumen and arrest could therefore occur under continued blood flow and does not exclusively depend on vessel occlusion (8). Tumor cells did not arrest in the periportal capillaries or venules, and no reduction in cell velocity before adhesion was seen, unlike leukocyte arrest at the site of inflammation. These data suggest that successful seeding of metastases to the liver is not merely a mechanical process but depends on specific cross-talk events between the tumor cells and the microenvironment (9).

The sinusoids of the liver are lined by HSEC that contain fenestrations of approximately 100 nm in diameter organized into structures known as sieve plates. These cells represent more than 70% of the sinusoidal cell population and are likely to be the first cells that blood-borne tumor cells encounter in the liver. Tumor cell entry and subsequent entrapment in the sinusoidal vessels can be followed by massive tumor cell destruction caused by mechanical stress and deformation-associated trauma. Obstruction of the sinusoidal vessels by larger tumor cells (or clusters thereof) can also result in blockade of the blood flow and a transient ischemia/reperfusion that triggers an inflammatory response. This can lead to further tumor cell damage and death due to the local release of nitric oxide (NO) and reactive oxygen species by HSEC and Kupffer cells (10, 11). The release of NO and IFN- γ by HSEC upon contact with metastasizing tumor cells can result in upregulation of FasL and subsequent apoptosis of an estimated 95% of tumor cells entering the sinusoids (12). The release of TNF- α in response to tumor infiltration can also cause tumor cells in the sinusoidal vessels to undergo apoptosis (13, 14). In addition, the tumor cells can also be eliminated by local, tumoricidal Kupffer cells. These bone marrow-derived resident macrophages of the liver represent approximately 80% of the total tissue macrophage population of the body. Under physiologic conditions, they play a role in erythrocyte phago-

cytosis and clearance and orchestrate the inflammatory response to hepatotoxins and pathogens. In response to an inflammatory trigger such as lipopolysaccharide (LPS), Kupffer cells can release multiple cytokines [interleukin (IL)-1, IL-6, IL-8, TNF- α , IFN- γ] and chemokines (MIP-2, IP-10, KC/GRO, MIP-1 α , MCP-1, RANTES; ref. 15) that can activate other innate immune response cells such as natural killer (NK) cells and neutrophils, adding to the local tumoricidal arsenal (16, 17). NK cells can mediate antitumor cytotoxicity by secreting perforin/granzyme or through death receptor-mediated mechanisms including the Fas/Fas ligand pathway (reviewed in ref. 18). Recent studies confirmed that a reduction or depletion of hepatic NK cells could enhance tumor growth in the liver (19), whereas enhanced NK activity contributed to reduced liver metastases in several tumor models (20, 21). Moreover, indirect evidence suggests that susceptibility of human cancer cells to NK-mediated immune attack can affect their ability to generate liver metastases (22).

Neutrophils can release reactive oxygen species, proteases, membrane-perforating agents, and soluble mediators such as TNF- α that could lead to tumor cell killing (23). The neutrophil-derived cytokines and chemokines can activate the tumoricidal potential of resident macrophages, as well as recruit host immune cells with antitumorigenic activities (24). Neutrophils can also mediate tumor cell kill via antibody-dependent cell-mediated cytotoxicity (ADCC) mechanisms (25) or by releasing cytolytic defensins that are abundant in neutrophil granules (reviewed in refs. 24, 26).

Cells of the Hepatic Sinusoid Can Also Facilitate Tumor Cell Arrest and Growth

Endothelial cell activation facilitates tumor cell adhesion and transmigration

Tumor cell interaction with the hepatic innate immune response is a double-edged sword. Whereas an efficient first-line defense can eliminate some infiltrating tumor cells, an inflammatory response can also promote tumor cell adhesion to vascular endothelial cells and in this way enhance transendothelial migration and tumor cell escape from the cytotoxic effects of resident Kupffer cells and NK cells. Although controversy still exists over whether mechanical trapping alone or specific interactions with the endothelium are required for metastases formation, the evidence is strong that the presence of tumor cells in the sinusoids causes an increase in the expression (and secretion) of specific cell surface adhesion molecules (CAM). A number of candidate CAM have been identified, but E-selectin, VCAM-1, ICAM-1, and carcinoembryonic antigen (CEA) seem to play essential roles in tumor cell arrest and stabilization in the sinusoids (27, 28; reviewed in ref. 29). Several groups have shown that tumor cell adhesion to sinusoidal endothelial E-selectin is relevant to liver metastases. For example, Brodt and colleagues (28) and Bressalier and colleagues (30) have shown that the liver-metastasizing potential of human CRC and murine lung carcinoma cells correlated with adhesion to HSEC *in vitro* mediated by cytokine-inducible E-selectin on the HSEC and sialyl-rich peripheral mucin carbohydrate structures on the tumor cells. Upon entry into the liver, these cells

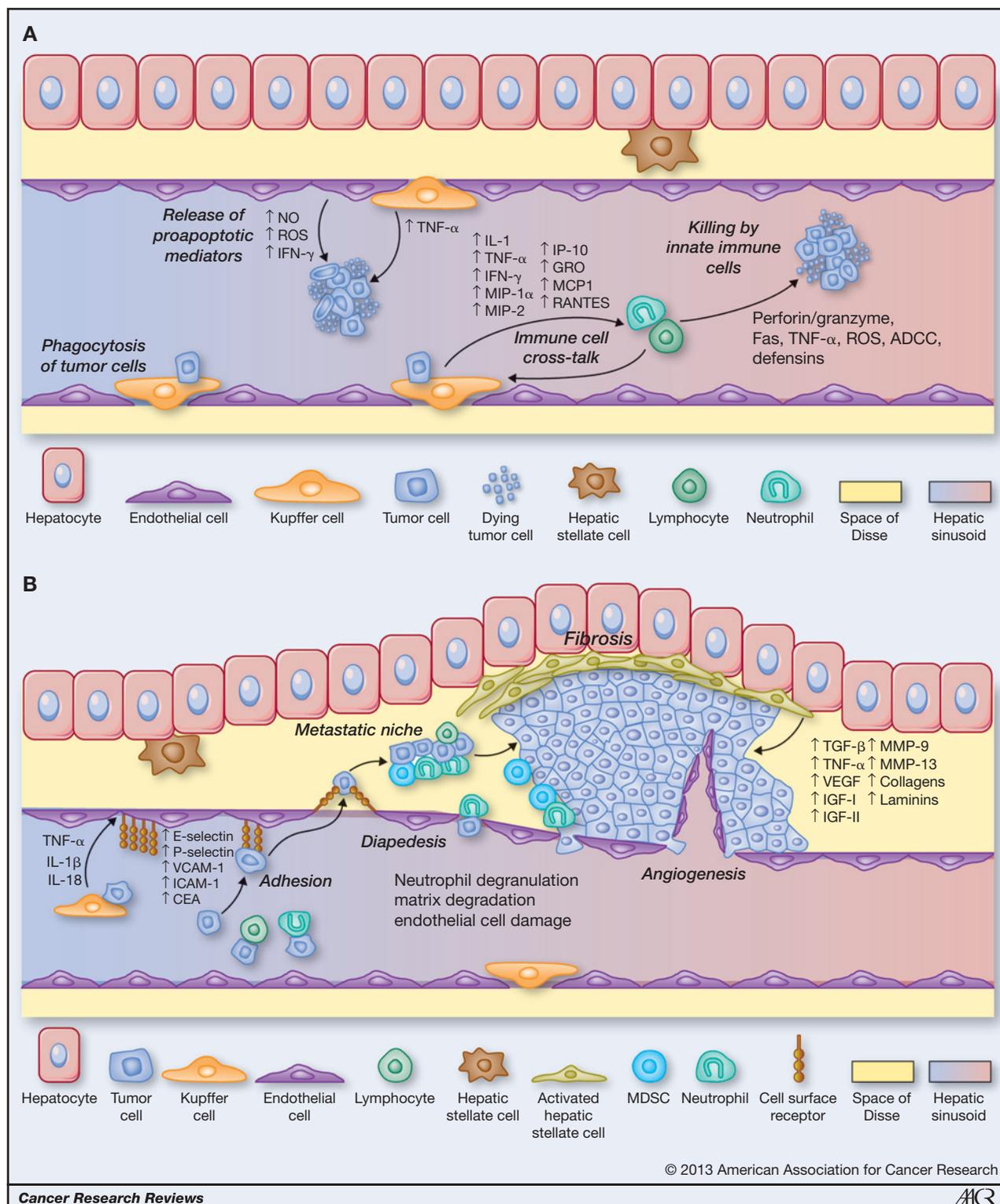


Figure 1. Tumor cell interactions with sinusoidal and extrasinusoidal cells of the liver during the early stages of hepatic metastasis. Shown are the major cell types of the hepatic sinusoids and the space of Disse and the major interactions during the sinusoidal (A) and extravascular (B) phases of liver colonization that influence tumor cell fate. Interactions that are detrimental to cancer cell survival are highlighted in A and those promoting survival and growth in B. MDSC, myeloid-derived suppressor cells.

activated a proinflammatory cascade involving Kupffer cell-mediated release of TNF- α and IL-1 that requires tumor-Kupffer cell association for some tumors (31). Subsequently, a sequential upregulation of vascular endothelial CAM such as E- and P-selectin (maximal expression at 6–12 hours postinjection), VCAM-1, and ICAM-1 (12–48 hours postinjection) occurred, increasing tumor cell arrest and extravasation into the hepatic parenchyma (32, 33). Inhibition of this cascade by antisense oligonucleotides or the anti-inflammatory secretory leukocyte protease inhibitor (SLPI) reduced liver metastases (34, 35), confirming the importance of these early proinflammatory events in the progression of metastasis. Kruskal and colleagues obtained similar results and observed by intravital microscopy that metastatic (CX-1) and nonmetastatic (MIP-101) CRC cells initially adhered to periportal Kupffer cells upon entry into the liver but only the metastatic cells could initiate a Kupffer cell/endothelial E-selectin activation cascade, whereas the poorly metastatic cells were targets of immune attack by recruited macrophages (36). The importance of TNF- α was also shown in TNFR1-deficient mice that developed fewer liver metastases due to significantly reduced VCAM-1 expression in these mice (37). However, in a study of 14 clinical specimens implanted orthotopically in the cecal wall of nude mice, TNF- α production by the cancer cells correlated with reduced liver metastases (38), highlighting the dual role of this cytokine that can act as an inhibitor or enhancer of liver metastases.

Cancer cells can attach to E-selectin via several counter receptors including oligosaccharide and sulfo-polysaccharide such as sialylated Lewis-a (sLew^a) and Lewis-x (sLew^x), glycoproteins such as PSGL-1, ESL-1, death receptor-3 (DR3), and MAdCAM-1, lysosomal membrane glycoproteins LAMP-1 and LAMP-2, and CD44 isoforms (reviewed in ref. 39). These counter-receptors have terminal fucosyl and sialyl groups that bind to the lectin domain of E-selectin. The attachment to endothelial E-selectin initiates signaling that facilitates diapedesis and subsequent invasion into the hepatic parenchyma. Tremblay and colleagues (40), using an *in vitro* system, identified 3 steps necessary for tumor cell diapedesis across an endothelial monolayer: (i) formation of a mosaic between cancer and endothelial cells; (ii) paracellular diapedesis at the junction of 3 endothelial cells; and (iii) transcellular diapedesis. Extravasation in this model was ICAM-1 and VCAM-1 independent and required extracellular signal-regulated kinase (ERK) activation downstream of E-selectin (40). Others have also shown that adhesion to E-selectin altered gene expression in the attached cells, and the changes were more pronounced in highly metastatic CRC cells than in poorly metastatic CRC cells, suggesting that metastatic cells may be more responsive to signals transmitted through this adhesion mechanism (41). Vidal-Vanaclocha and colleagues (42) have shown that within 24 hours of melanoma cell entry into the liver, VCAM-1 expression on HSEC increased significantly in an IL-1 β -, TNF- α -, and IL-18-dependent manner and that VCAM-1 blockade by antibodies decreased microvascular retention of the tumor cells and metastasis, suggesting that this cell adhesion mechanism may be broadly relevant across different tumor types.

These conclusions are supported by clinical data. For example, increased expression of E-selectin was noted in and around hepatic metastases of CRC (43). Elevated serum levels of soluble E-selectin, ICAM-1, and VCAM-1 were found in patients with primary CRC or locally recurring tumors, and these levels correlated with disease outcome (44). Moreover, increased expression of the E-selectin ligands sLew^x and sLew^a was noted on metastatic CRC and in CRC liver metastases (45), and their levels correlated with the metastatic phenotype (reviewed in ref. 46). Other, more indirect data have shown increased expression of inflammatory mediators such as COX-2 in CRC as compared with the normal mucosa and a still greater expression in liver metastases (47), suggesting that inflammation affects disease progression and may provide clinical biomarkers for this malignancy.

The dual role of neutrophils

Neutrophils can also promote metastasis in general and liver metastases in particular by several mechanisms that may partially depend on their ability to bind to the tumor cells. Indeed, dynamic interactions between neutrophils and tumor cells that increase tumor cell invasiveness have been documented *in vitro* (reviewed in ref. 26). *In vivo*, neutrophils colocalize with cancer cells within the liver sinusoids, and their depletion causes a significant reduction in cancer cell arrest (48). Neutrophils may participate in the formation of premetastatic niches in the liver. Using an orthotopic colon carcinoma implantation model, Yamamoto and colleagues found a significant increase in the number of neutrophils within the premetastatic liver sinusoids that was associated with increased production of the chemokine CXCL1. Inhibition of the CXCL1 receptor CXCR2 by a function-blocking antibody inhibited liver metastases (49), suggesting that neutrophil recruitment was essential for liver metastases formation. The ability of neutrophils to significantly damage the sinusoidal endothelium when they adhere and degranulate within microvessels, as well as their production of matrix-degrading proteinases, such as matrix metalloproteinase (MMP)-9 and elastase (50, 51), may contribute to metastasis by facilitating tumor cell transmigration and tumor expansion. Neutrophils seem, therefore, to play a dual role in the early stages of the metastatic process. Although they can cause tumor cell damage and death, these effects may be offset by direct and indirect metastasis-promoting mechanisms (24, 26).

Role of Kupffer cells

Kupffer cells can also contribute to the metastatic process. Several groups have shown that the release of inflammatory cytokines by Kupffer cells caused an increase in cellular adhesion molecules in the sinusoids, thus facilitating tumor cell adhesion (32). Kupffer cells also have a CEA receptor that can trigger release of inflammatory cytokines when activated, leading to increased expression of CAM and decreased NO production that favor tumor survival (52). Others have shown, however, that Kupffer cell depletion leads to increased numbers of metastases in a mouse model, suggesting that the

tumor-destructive potential of Kupffer cells may predominate in some models (53).

Chemokine/Chemokine Receptors Recruit Circulating Tumor and Host Cells into the Liver

The chemokines comprise a superfamily of at least 46 cytokines that bind and activate G protein-coupled receptors to induce directional migration (chemotaxis). First described as mediators of leukocyte migration to sites of tissue injury and inflammation, they have recently emerged as major drivers of tumor cell migration to sites of metastasis and the upregulated expression of their receptors is consequently associated with an aggressive tumor phenotype (reviewed in ref. 54). Their role in metastasis is not restricted to tumor cell attraction. Chemokines and their receptors shape the tumor microenvironment, and as such, they control tumor cell survival and proliferation, angiogenesis, and metastatic expansion.

Several chemokine/chemokine receptors pairs have been implicated in colon cancer metastasis to lymph nodes and liver. For example, high expression levels of CXCR4 in primary CRC tumors were found to be associated with an increased incidence of liver metastases and poor prognosis, implicating CXCR4/CXCL12 in CRC progression (55, 56). A concomitantly high expression of CXCR4 and VEGF was found to be a strong and independent predictor of early distant relapse in CRC and this was attributed to increased clonogenic growth, VEGF production, and ICAM-1 expression that were mediated by CXCR4 (57). Experimental studies implicated CXCR4 and CXCR3 in colon cancer liver metastases but their roles seem to be distinct; CXCR3/CXCL10 promote invasion-related properties and CXCR4 mediates primarily tumor expansion (58). The source of CXCL12 in the liver is still not entirely clear but recent studies identified HSEC, Kupffer cells (59), and activated HSC (60) as potential producers of this chemokine. Another CXCR4-interacting cytokine is the macrophage migration inhibitory factor (MIF) that has been implicated in various stages of cancer progression (61) and recently identified as a potential marker for CRC liver metastasis (62).

CCL20/CCR6 were also implicated in CRC liver metastases, based on a strong association between the CCR6 staining intensity in primary CRC specimens and the development of synchronous liver metastases (63). CCL20, the only known ligand for CCR6 is upregulated in the liver under inflammatory conditions (64) and could be involved in the attraction of CCR6⁺ tumor cells into this organ (65). For other chemokines, opposing effects on CRC liver metastases were reported. For example, high serum CXCL5 levels in patients with CRC were found to be associated with liver metastases and poor prognosis in one study (66), but disrupted expression of this chemokines was found to be associated with increased CRC growth rates and a worse prognosis, in another (67). CCL2—a potent recruiter of host immune cells—is involved in recruitment of tumor-associated macrophages (TAM) that promote tumor growth but can also activate an antitumor immune response (68). Analysis of colon cancer specimens by immunohistochemistry recently identified CCL2 as 1 of 2 immunohistochemical markers that together with other clinicopathologic features could predict, with high accuracy,

the development of hepatic metastases (69). CXCL16 and CCL17 can exert tumor suppressive effects by recruiting cytotoxic CD8⁺ T cells (70, 71; reviewed in ref. 72), whereas a group of chemokines involved in angiogenesis such as CXCL2, CXCL3, and CXCL8 may play an indirect role in metastasis by promoting tumor vascularization (61, 73).

Collectively, the data indicate that chemokines are a double-edged sword in CRC metastasis. While some chemokine/receptor pairs may promote CRC metastasis by enhancing tumor cell recruitment (CXCR6), invasion (CXCR3) and local tumor expansion (CXCR4), these tumor-promoting effects may be offset by the recruitment of host immune cells such as cytotoxic T cells and on balance, therefore, they may inhibit rather than promote metastasis.

Recruitment and Role of Bone Marrow-Derived Cells

In addition to interacting with various host cells already residing in the liver at the time of tumor invasion, tumor cell fate is also influenced by bone marrow-derived host cells that are recruited in response to mediators released by tumor and/or hepatic cells. Work by Kaplan and colleagues and others led to the insight that primary tumors can influence and prepare the microenvironment of secondary organs for future metastatic growth, even before tumor cells arrive at these sites, by forming so-called premetastatic niches (74, 75). These niches are populated by bone marrow-derived myeloid cells that are characteristically CD11b⁺/VEGFR1⁺ (76). Although the role of these niches in CRC liver metastases remains to be better defined, several elements suggest that they are involved. Namely, it has been observed that within VEGFR1⁺-containing niches, CXCL12 production is increased (77), and as discussed earlier, this chemokine could play a role in CRC recruitment to the liver. Recently, it was also reported that mice implanted orthotopically with metastatic human CRC cells and treated with the inhibitor TSU86 had significantly reduced liver metastases, attributable to a significant reduction in CXCR2-mediated neutrophil recruitment into premetastatic liver niches (49) and highlighting again the metastasis-promoting role of neutrophils. Another class of bone marrow-derived cells that have emerged as potential promoters of metastasis is the myeloid-derived suppressor cells (MDSC). This phenotypically heterogeneous cell population includes mature myeloid cells such as granulocytes, monocytes/macrophages, and dendritic cells, as well as immature myelomonocytic precursors that expand under pathologic conditions, such as cancer, and can enhance tumor growth by negative regulation of T-lymphocyte subsets, resulting in tumor cell escape from immune-attack. The MDSCs commonly express the surface marker CD11b and can be divided into 2 subsets with distinct functions: the granulocytic (Cd11b⁺Ly6G⁺Ly6C^{low}) and monocytic (CD11b⁺Ly6G⁻Ly6C^{hi}) cells (discussed and reviewed in refs. 78, 79). Several recent studies have implicated this family of bone marrow-derived cells in liver metastases. Connolly and colleagues (80) found that in the livers of mice with intra-abdominal malignancies, 2 CD11b⁺Gr1⁺ populations distinct from those found in other organs were recruited and could accelerate the development of metastases through modulation

of the T-cell response. The recruitment of bone marrow-derived myeloid cells into livers of mice inoculated with CRC cells has also been described by other groups, although the phenotypes identified by different studies differ. Kitamura and colleagues described chemokine-mediated recruitment of CD34⁺ Gr-1⁻ immature myeloid cells via the chemokine receptor CCR1 (81), whereas others show evidence for recruitment of CD11b⁺ Gr1⁺ myeloid cells to the metastatic site (82, 83). Some evidence for the involvement of these cells in human CRC metastasis has been provided (83). Commonly, in all of these studies, the bone marrow-derived myeloid cells seem to play a role in promoting the development of metastases.

Role of Hepatic Stellate Cells and the Fibrogenic Response of the Liver

During the early, avascular stage of the metastatic process, HSC play a major role and can contribute to CRC invasion and growth. These cells are found in the space of Disse and represent 5% to 8% of the liver cellular population. In their quiescent state they contain large amounts of vitamin A that is released upon activation and transdifferentiation. In their activated state, they acquire phenotypic characteristics of myofibroblasts, identifiable by the expression of α -smooth muscle actin (SMA) and deposition of increased amounts of extracellular matrix (ECM) proteins, particularly type I collagen. These activated HSC orchestrate the liver response to injury (84).

Tissue injury in the liver triggers a characteristic, well-defined wound healing (fibrotic) response that begins with the removal of damaged tissue by inflammatory cells, followed by recruitment and activation of HSC by Kupffer cell-derived cytokines (e.g., TGF- β), endothelial cell-derived fibronectin (a product of vessel injury) and apoptotic products released by hepatocytes. The activated HSC can release various factors, including the growth factors TGF- β , EGF, VEGF, and insulin-like growth factor (IGF)-I and metalloproteinases such as MMP-2, -9, and -13 (85, 86). This is followed by deposition of type I and IV collagens and laminin, recruitment of endothelial cells, neovascularization, and tissue repair. HSC activation and basement membrane assembly may be a crucial cue for cellular proliferation and renewal in the otherwise, nonpermissive liver microenvironment (84, 86).

Tumor cell invasion into the extrasinusoidal space seems to recapitulate this tissue repair process, triggering HSC, Kupffer cells, and HSEC activation. This was observed in experimental models (18) and is also evidenced by the increased production of collagen in and around hepatic metastases in clinical specimens (87). In an experimental metastasis study, using murine colon carcinoma CT-26 cells that produce the chemokine CCL2 *in vivo*, HSC and macrophage recruitment into the tumors was observed. In CCR2-deficient mice, a reduction in TAMs and HSC was observed, and this corresponded to reduced MMP-2 production, decreased neovascularization, and reduced metastases formation, suggesting that the recruitment of HSC and macrophages into the tumors was essential for tumor-induced angiogenesis, hence metastasis (88). HSC recruited

into the metastases as myofibroblasts release growth factors, cytokines, and MMPs that together contribute to recruitment of endothelial cells and angiogenesis, the assembly and turnover of ECM, tumor cell invasion, and proliferation (reviewed in ref. 3).

Recent evidence suggests, however, that the origin of the liver fibrogenic response to injury may be more complex. In addition to HSC, portal fibroblasts and extrahepatic cells may also be involved. An understanding of the process is further complicated by the fact that different cell types can contribute in distinct ways to mechanisms of hepatic fibrosis and bone marrow-derived cells can be pro- or antifibrogenic (89). When invading cancer cells are located in portal tracts and are unable to activate the HSC-dependent stromal response, the portal tract fibroblasts can initiate a stromal response through production of IL-8, a chemokine involved in invasion and angiogenesis, in response to TNF- α -induced NF- κ B signaling (90). Moreover, hepatocytes located at the periphery of the metastases can undergo epithelial-to-mesenchymal transition (EMT) in response to tumor and/or HSC-derived factors (3). These hepatocytes are characterized by an upregulated expression of vimentin, SNAIL, and nerve growth factor and a downregulated expression of the epithelial marker cadherin-H1 (3). Hepatocytes can also contribute to the process of fibrosis and neovascularization through production of IGF-I and IGF-II, factors that promote HSC recruitment and activation (91) and can also directly enhance tumor cell growth (92, 93).

Histologic Growth Patterns of Liver Metastases

Histologically, liver metastases are a heterogeneous disease. Different histologic patterns in the tumor-liver interface of solid tumors were described by Terayama and colleagues (94) and Vermeulen and colleagues (95), based on microscopic examination of hematoxylin and eosin and Gordon Sweet's silver (reticulin) staining. This assessment is reproducible (95, 96). Figure 2 summarizes the characteristics of each of the growth patterns. In the "desmoplastic" (95) or "encapsulated" (94) growth pattern, a band of desmoplastic tissue or a pseudocapsule separates tumor cells and the liver parenchyma. No contact takes place between epithelial tumor cells and hepatocytes, and the preexisting structure of the liver cell plates is not conserved within the metastasis. In the desmoplastic rim, a dense lympho-histiocytic infiltrate, (myo)fibroblasts, small capillaries, and bile ducts are present. Small nests of tumor cells infiltrating the desmoplastic stroma are often seen. In metastases with a "pushing" (95) or "expansive" (94) growth pattern, the liver cell plates are pushed aside by the metastases, again destroying the preexisting liver architecture. There is no desmoplastic reaction, and the tumor cells are separated from the hepatocytes by a thin layer of reticulin fibers. There is at the most, a mild inflammatory infiltrate at the interface. The third growth pattern is the "replacement" growth pattern (95), in which metastatic cells infiltrate the liver parenchyma without any disturbance of the preexisting liver structure at the interface. There is no fibrosis, minimal inflammation, and tumor cells and hepatocytes have intimate cell-cell contact. This growth pattern was divided by Terayama and

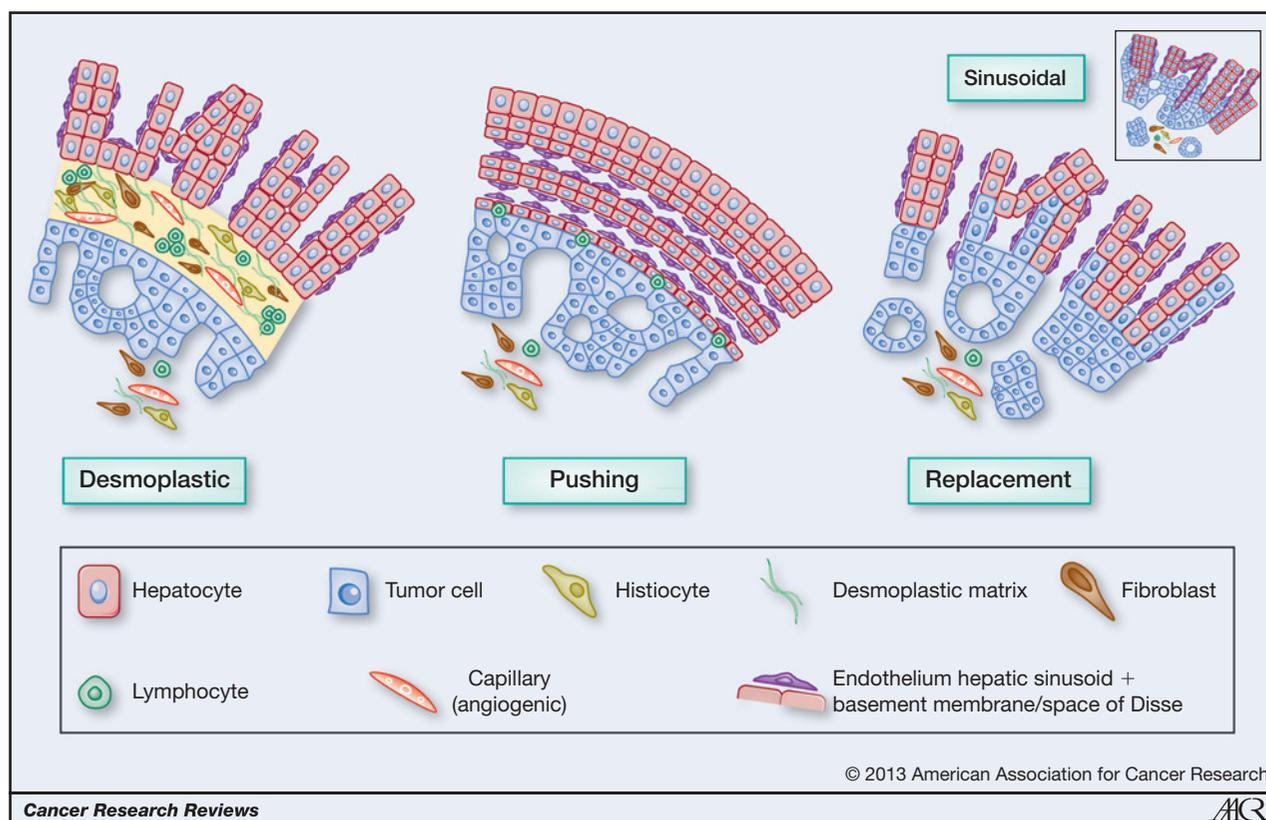


Figure 2. The 3 dominant growth patterns of liver metastases of solid tumors. Shown is a schematic representation of the characteristic histologic appearance of the 3 growth patterns. Mixed patterns consisting of characteristics of more than 1 growth pattern are occasionally observed. For a detailed description of the characteristics of each growth pattern, please see sections "Histologic growth patterns of liver metastases" and "Vascularization of liver metastases" in the text.

colleagues into "sinusoidal" and "replacement" growth patterns, based on the exact location of the tumor cells in the preexisting liver structure: in the sinusoids and within the liver cell plates, respectively (94). In some metastases with a "mixed" growth pattern a combination of 2 or more growth patterns is found (97).

Several observations suggest that these growth patterns are not random phenomena but must be the result of distinct interactions between metastatic tumor cells and other cells of the microenvironment. Namely, in patients with multiple liver metastases, one growth pattern generally predominates (95, 96, 98). Furthermore, the growth patterns depend on the tissue of origin of the primary tumor (94, 95, 99). In liver metastases of CRC, all 3 growth patterns are observed at different proportions, depending on the study population (95–97). On the other hand, liver metastases of breast cancer have a preponderance of replacement growth pattern (98), and this growth pattern is also prevalent in liver metastases of other adenocarcinoma histiotypes such as pancreatic and urothelial carcinoma (Vermeulen et al., unpublished observation; discussed in ref. 95).

These histologic growth patterns also seem to have clinical relevance. Encapsulated CRC liver metastases are associated with a better prognosis (94, 100, 101). In a recent study of 205 patients with CRC, liver metastases with a pushing growth

pattern or with a pushing component in mixed growth patterns were an independent prognostic factor of a worse rate of 2-year overall survival (97).

Vascularization of Liver Metastases

Establishment of a blood supply for tumors can be the result of neovascularization or tumors can co-opt the pre-existing host vasculature for growth. Histologic evidence suggests that liver metastases and hepatocellular carcinoma display persistent co-option, at least at the tumor–liver interface, independent of the size. Unique morphologic and physiologic features of the liver enable vascular cooption: The liver is a well-vascularized organ with high blood flow—27% of the cardiac output reaches the liver—and has a well-organized supportive tissue structure that, if not destroyed by tumor cells, can provide an efficient blood supply. The stromal reaction to tumor growth has been described as a nonhealing wound (102), with inflammation, development of blood vessels in newly formed fibrous tissue, and fibrin leakage from VEGF/VPF (vascular permeability factor)–stimulated capillaries all playing a role. As described in the previous section, this stromal reaction is largely absent in the replacement growth pattern of carcinoma liver metastases, at least at the tumor–liver interface (95, 99). In this growth pattern, carcinoma cells are arranged in "plates"

similar to the hepatocytes in the adjacent liver parenchyma. This pattern most likely entails *in situ* growth of the carcinoma cells on, and probably induced by, ECM deposited by activated HSC. There are several reasons why angiogenesis in the liver may differ substantially from angiogenesis in other organs: (i) the double blood supply of the liver; (ii) the presence of 2 types of endothelium—the fenestrated endothelium of the sinusoidal vessels lacking a true basement membrane and the continuous endothelial layer of the larger vessels in the portal tracts; (iii) the combined effect of the compact structure of the liver, where approximately 80% of the tissue volume is represented by hepatocytes, the lack of sufficient stromal tissue, and the high blood flow rate that together can prevent the formation of the growth factor gradients, normally required for angiogenesis; and (iv) the role of angiopoietin-like proteins, some expressed specifically in the liver (103).

The vascularization of liver metastases and its relationship with the histologic growth pattern have been studied in animal models and patient samples. Paku and colleagues studied the morphologic aspects of experimental liver metastases in several animal models (104–107). Using the murine LLC model, they found that in liver metastases with a "sinusoidal" growth pattern, intrametastatic vessels were continuous with the sinusoidal system of the liver and metastases grew without compression of the adjacent liver parenchyma. Tumor cells invaded between the HSEC and the matrix in the space of Disse (107). The bromodeoxyuridine (BrdUrd)-labeling index of co-opted HSEC within the metastases was 6-fold higher than in the perimetastatic zone, suggesting a mechanism of vascularization based on co-option of sinusoids. A second, "portal" growth pattern of liver metastases, more frequently seen when carcinoma cells were injected via the portal route and located near the portal tracts, was characterized by new vascular sprouts of small diameter and an intact basement membrane (106).

Terayama and colleagues (108) analyzed surgically resected liver metastases and reported that co-opted HSEC around liver metastases, in contrast with sinusoids at a distance of more than 1 mm, were frequently von Willebrand factor (vWF)⁺ and Ulex europaeus agglutinin (UEA)-1⁺ suggesting that phenotypic changes, described as "capillarization," occurred in the endothelium (108, 109). These endothelial cells first appeared within metastases of 200 μ m. Three-dimensional observations showed that these blood vessels were connected with the sinusoids in the surrounding liver. In these specimens, a replacement growth pattern was apparent (110). Immunohistochemical studies conducted by Döme and colleagues (105) revealed an accumulation of α -SMA-positive perisinusoidal cells in the vicinity of the pushing liver metastases together with an increase in components of basement membrane, evidence of capillarization associated with loss of characteristic features of HSEC. Basic fibroblast growth factor (bFGF) expression was upregulated in hepatocytes adjacent to, or entrapped in, the metastases and was higher than in the carcinoma cells. VEGF-expression was always present in liver tissue, independently of the distance to the metastases and at a level

comparable with the carcinoma cells. This suggests that bFGF is likely mediating the capillarization of sinusoids destined to be co-opted by the carcinoma metastases (105).

Vermeulen and colleagues (95) and Stessels and colleagues (99) reported on growth pattern–related differences in the properties of the vasculature in CRC and breast adenocarcinoma liver metastases. The endothelial cell proliferation fraction (ECP) was 3-fold higher in the pushing than in the desmoplastic and replacement growth patterns. The tumor cell proliferation (TCP) fraction was similar in the 3 growth patterns, resulting in the lowest TCP/ECP ratio in the pushing growth pattern, considered an indicator of angiogenesis-dependent growth. Eefsen and colleagues also recently found that ECP/TCP ratios were highest in the pushing liver metastases (96). The angiogenic parameters of the pushing growth pattern resembled those of primary CRC. In a comparison of the growth pattern of liver metastases of colorectal and breast cancer, nearly all metastases of breast cancer, but only a third of CRC metastases, had a replacement growth pattern. CA9-expression—an indicator of hypoxia-inducible factor (HIF)-dependent reaction to hypoxia—was present at the tumor–liver interface in only 16% of breast cancer liver metastases as compared with 54% of CRC metastases. This result coincided with more abundant fibrin deposition, regarded as a VEGF effect of leaky blood vessels, in the latter metastases (99). These differences are consistent with the proposed lack of angiogenic switch in the replacement growth pattern. Interestingly, endothelial cells of co-opted sinusoidal blood vessels at the periphery of metastases with a replacement growth pattern preserve their characteristic CD34[−]/LYVE-1⁺ phenotype (99).

Although most of the blood supply of the normal liver is derived from the portal vein, liver metastases are predominantly supplied by arterial blood. This was shown by Breedis and Young following injection of India ink into the hepatic circulation of rabbits with liver tumors (111). Paku and colleagues suggested a mechanism for this switch of blood supply based on studies of an experimental model of pushing-type CRC liver metastases. They proposed that sinusoids at the liver–tumor interface fuse, forming "blood lakes" that incorporate branches of the hepatic artery, and this is followed by ECP and vascular remodeling, leading to arterialization (104, 106).

Expansion of Metastases within the Liver: Contribution of ECM-Degrading Proteinases to Liver Metastases Growth Pattern

Degradation of proteins, such as fibrin, is pivotal to metastasis. Studies in mice deficient in components of the plasminogen activation system have highlighted the essential role that this system plays in fibrin surveillance in the liver (112). However, few studies have analyzed the role of the uPA/uPAR system in CRC liver metastasis. Illemann and colleagues (113) found that in metastases with a desmoplastic growth pattern, uPA receptor (uPAR) and uPA localization were identical to patterns in the corresponding primary tumors with uPAR expressed primarily in TAMs, and uPA

mainly in tumor-associated α -SMA⁺ myofibroblasts. uPAR and uPA were less abundant at the metastasis/parenchyma interface of liver metastases with the pushing growth pattern and confined to macrophages and neutrophils. Plasminogen activator inhibitor-1 (PAI-1) was expressed in all liver metastases but localized to myofibroblasts in the desmoplastic metastases and to hepatocytes in the pushing growth pattern (113). Eefsen and colleagues also recently found a significant uPAR upregulation in the desmoplastic growth pattern (96). Expression of MMP-9 in primary CRC and metastases was also distinct, with macrophages the main source in the primary tumors and neutrophils in the liver metastases (114). Studies in a small cohort of 6 primary CRC and matching liver metastases revealed MMP-2 mRNA mainly in the tumor stroma, as also reported by others (115), and this was characteristic of liver metastases of the desmoplastic but not the pushing growth pattern (M. Illemann et al.; unpublished observation).

These results suggest that ECM-proteolysis in the different growth patterns is mediated via distinct cells and mechanisms. Metastases with the desmoplastic growth pattern are characterized by a stroma rich in collagens (95) that may constitute a barrier to cancer cell spread, and ECM degradation may therefore be required for further tumor expansion. In metastases with the pushing growth pattern, this ECM barrier is absent and ECM-degrading proteases may not be as critical for tumor expansion. It is likely, therefore, that metastatic CRC cells use distinct mechanisms for spreading within the liver.

Challenges and Future Directions

As the information reviewed above indicates, much progress has already been made in our understanding of the complex biology of liver metastasis. However, a full characterization of the heterogeneity and multifaceted role of the tumor stroma in this process and the implication for prognosis and therapy are still lacking and will most likely be the main focus of future research. The following sections describe some of the most important remaining challenges.

Unraveling the underlying biology of the different growth patterns

Answers are still lacking to the following questions: Which tumor–stroma interactions and what mechanisms ultimately determine the outcome of the early disseminated stage of the disease (i.e., metastatic outgrowth or not)? What mechanisms underlie the different tumor–stroma interactions, as represented in the histologic growth pattern, once metastases have formed? To what extent are the growth patterns genetically, epigenetically, or microenvironmentally determined? What is the exact role of different subpopulations of immune cells? Is there plasticity in tumor–stroma interactions and growth patterns and can they be modulated by external interventions? Do genetic and physiologic differences or preexisting conditions of the liver microenvironment (e.g., fibrosis, steatosis) play a role? These questions can probably be best addressed through cell culture and animal model systems that better mimic the complexity and heterogeneity of the human disease

and are more easily accessible for data mining. Better intravital imaging techniques for visualizing tumor progression in the liver have recently been described (116), and these, together with improved techniques for separation of tumor and distinct stromal cell subsets for gene and protein profiling (such as those described in ref. 117), will provide valuable mechanistic and molecular data.

Elucidating the role of the growth patterns in clinical disease management

Several important questions remain unanswered: Does the liver metastasis growth pattern determine the response of metastatic disease to chemotherapy or targeted therapy, and can a change of growth pattern occur during treatment and predict resistance to response? To address these questions, high-quality and well-characterized tissue banks are essential, and noninvasive methods to assess tumor–stroma interactions in general, and the histologic growth pattern in particular, are needed. Although hepatic resections have become more common, tissue banks of hepatic metastases remain limited because tumors are often nonresectable and difficult to biopsy. Existing studies are therefore often based on autopsy material that is of inferior quality. Moreover, to gain prognostic value, the analysis of liver metastases should ideally be linked to information derived from the paired primary tumors, should be evaluated in the context of detailed clinical history (as patients are often subjected to multimodality pretreatments before surgery), and should contain information on stromal characteristics of the metastatic lesions. New powerful imaging techniques that include static and/or dynamic assessment of vascularization are needed to identify and even predict the growth pattern, thereby enabling new and large prognostic and predictive biomarker studies for a more personalized approach to treatment.

Although the questions outlined are complex and the challenges many, the latter are not insurmountable. The authors strongly believe that progress in this area will be expedited through (international) multicentric and multidisciplinary collaborations involving basic and clinician scientists, providing opportunities for exchange and sharing of samples, ideas, techniques, and personnel.

Summary and Conclusions

The response of the liver to invading cancer cells is multidimensional. Although some interactions with the liver microenvironment can be detrimental to these cells, others seem to aid and abet their growth. These interactions may depend on the unique characteristics of the tumor and/or the host and may therefore be patient specific. The balance of these opposing interactions seems to hold the key to the survival of the metastatic cells and ultimately, that of the patient. There are 3 growth patterns of CRC liver metastases, each with distinct biologic pathways and clinical outcomes. There is no doubt that surgical resection of CRC metastatic to the liver can now result in a cure for some patients. The present challenge is to identify those patients for whom the ultimate outcome of the various host–tumor

interactions is an adverse one. This process may select a subgroup of patients who could benefit from neoadjuvant therapy and provide an alternative to the current practice, in which many patients may be over- or undertreated. Thus, in the future, treatment strategies that are tailored to each patient based on knowledge of his or her individual host-tumor interactions and patterns of vascularization may offer the best chance for long-term survival.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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