Liver metastases: Microenvironments and *ex-vivo* models

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Abstract

The liver is a highly metastasis-permissive organ, tumor seeding of which usually portends mortality. Its unique and diverse architectural and cellular composition enable the liver to undertake numerous specialized functions, however, this distinctive biology, notably its hemodynamic features and unique microenvironment, renders the liver intrinsically hospitable to disseminated tumor cells. The particular focus for this perspective is the bidirectional interactions between the disseminated tumor cells and the unique resident cell populations of the liver; notably, parenchymal hepatocytes and non-parenchymal liver sinusoidal endothelial, Kupffer, and hepatic stellate cells. Understanding the early steps in the metastatic seeding, including the decision to undergo dormancy versus outgrowth, has been difficult to study in 2D culture systems and animals due to numerous limitations. In response, tissue-engineered biomimetic systems have emerged. At the cutting-edge of these developments are *ex vivo* 'micro-physiological systems' (MPS) which are cellular constructs designed to faithfully recapitulate the structure and function of a human organ or organ regions on a milli- to micro-scale level and can be made all human to maintain species-specific interactions. Hepatic MPSs are particularly attractive for studying metastases as in addition to the liver being a main site of metastatic seeding, it is also the principal site of drug metabolism and therapy-limiting toxicities. Thus, using these hepatic MPSs will enable not only an enhanced understanding of the fundamental aspects of metastasis but also allow for therapeutic agents to be fully studied for efficacy while also monitoring pharmacologic aspects and predicting toxicities. The review discusses some of the hepatic MPS models currently available and although only one MPS has been validated to relevantly modeling metastasis, it is anticipated that the adaptation of the other hepatic models to include tumors will not be long in coming.

Keywords: Liver metastasis, metastatic models, tumor microenvironment, hepatic niche, microphysiological

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Introduction

The liver is a highly metastasis-permissive organ. It is the most frequently afflicted organ by metastasis, second to lymph nodes, for the majority of prevalent malignancies, namely gastrointestinal cancers, breast and prostate carcinomas, uveal melanoma, neuroendocrine tumors, and sarcomas.[1–4] Moreover, liver metastases are much more common than primary hepatic tumors.[5] Yet, liver involvement in metastasis is frequently overlooked and under-investigated as lesions are often symptomless—even extensive infiltration by metastatic tumors may not alter the function or homeostasis until very late stages of the disease.[6] Comparatively, metastases to other organs are more readily identified due to their more symptomatic location, e.g. difficulties breathing for lung metastases or pain in the case of bone metastases. Presently, the true prevalence of liver metastasis is unknown, but between 30% and 70% of patients dying of cancer have liver metastases[7] and most patients with liver metastases will die of their disease.[8]

Biology of the liver

The liver is a complex organ responsible for biosynthesis, metabolism, clearance, and host defense. The unique and diverse architectural and cellular composition of the liver enables it to undertake these specialized functions. Architecturally, the liver is the only organ endowed with a dual blood supply, receiving visceral blood via the portal vein (80%, deoxygenated blood) and arterial blood via the

hepatic artery (20%, oxygen-rich blood). At the cellular level, 70% of the liver is composed of parenchymal cells, namely hepatocytes and cholangiocytes. Together these two cells are responsible for the glandular, metabolic, and detoxifying functions. Non-parenchymal cells (NPCs) comprise the remaining 30% and include liver-specific endothelial, immune, and stromal cell populations. All have systemic and local significance; systemically these specialized cells are responsible for blood filtration, molecular scavenging as well as inflammatory and immune responses, and locally they control the liver microcirculation, extracellular matrix (ECM) composition as well as liver tissue renewal and regeneration.[9]

Unfortunately, the distinctive biology of the liver is such that it renders it intrinsically susceptible to metastases.[10] Some of the key aspects include:

1. Architectural and hemodynamic features—the liver's significant role in the circulatory system, and the dual, slow, and tortuous liver-specific microcirculation provides increased access of disseminated tumor cells carried in the blood. Moreover, the NPCs that line the hepatic capillaries present a copious number of surface molecules that facilitate attachment and intrahepatic retention of circulating tumor cells. These liver sinusoidal endothelial cells (LSECs) have fenestrations that allow for direct access of the tumor cells to the basement membrane.
2. Regenerative capabilities—the cellular tissue-reconstruction machinery involved in self-renewal and reconstruction can be coopted to create a favorable environment for survival and growth by signals produced by tumor cells that promote the formation of intratumoral stroma and blood vessels.
3. Regional immune suppression—the general foreign body reaction is diminished to limit potential damage to the liver, due to its constant exposure to inflammatory stimuli from the gut. This results in a relatively tolerant microenvironment permissive to foreign tumor cell survival and growth.

Metastasizing to the liver

Tumor dissemination and the formation of metastases involve a complex set of biological processes (Figure 1). First, cells within the primary tumor undergo an epithelial-to-mesenchymal transition (EMT).[11–13] EMT enables detachment from surrounding cells and motility to intravasate into the circulation followed by extravasation into the parenchyma of distant organs (e.g. liver), and finally colonization. Colonization initially requires a partial reversion back to a more epithelial phenotype (mesenchymal-to-epithelial reverting transition [MErT]).[14] At this point tumor cells may enter a dormant state and reside as single cells (potentially emerging to proliferate years to decades later) or undergo yet another transition to a more mesenchymal phenotype to enable proliferation and the formation of a clinically detectable macrometastases.[15–17] The mechanisms governing extravasation into and colonization of the parenchyma of distant organs can be quite distinct not only for each organ but also for each type of invading tumor cell. Given the liver's unique architectural and functional aspects, it is no exception to this rule—notable focused reviews include colorectal (CRC),[18] breast,[19] and pancreatic carcinomas.[20]

In general, upon entering the liver via either the portal vein or hepatic artery, disseminated tumor cells first encounter the sinusoid (i.e. the hepatic-specific capillary network). This step represents the beginning of the unique metastatic process that occurs within the liver, which involves a series of four interrelated phases: (i) the tumor-infiltrating microvascular phase, which involves tumor cell arrest in the sinusoidal vessels, leading to either tumor cell death or extravasation, (ii) the interlobular pre-angiogenic micrometastasis phase, during which host stromal cells are recruited into avascular micrometastases; (iii) the angiogenic micrometastasis phase, in which tumors become vascularized through several possible interactions with the microenvironment, and (iv) the growth phase that leads to the establishment of a ''clinical'' macrometastasis.[21] The first two steps 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 due to unknown stimuli to emergent masses as noted to in the latter two phases.

Notably, different cancers are predisposed to selectively establish metastases in particular organs though there is considerable overlap with the liver being nearly universally seeded by all disseminated solid tumor cell types; this phenomenon is known as organotropism and has been reviewed comprehensively by Joyce and Pollard.[22] However, it remains unsettled whether this organotropism is related to active attraction of the tumor cell to a site or a permissive microenvironment that supports the survival and subsequent outgrowth in that organ. A strong argument for the latter can be constructed as follows. First, tumor cells that reach circulation whether by lymphatic or hematogenous routes are distributed by flow forces. These tumor cells are significantly larger than capillary tubes and are physically arrested prior to extravasation.[23,24] The organs most commonly seeded either have 'open' capillary networks (bone marrow or liver sinusoids) or are the first major capillary bed faced by these tumor cells (lung). Importantly, studies have shown that survival in an ectopic site is the most rate-limiting step.[23,24] Thus, we focus on the unique combination of attributes of the liver that make it such a fertile soil for seeding.[10,21,25]

Hepatic metastatic tumor microenvironment

The hepatic metastatic tumor microenvironment is multifaceted and highly dynamic, the regulation of which depends on the interaction between cellular (i.e. tumor cells, resident hepatic cell populations) and non-cellular components (i.e. ECM, hypoxia, signaling molecules).[26] The tissue architecture and microenvironments encountered by the infiltrating disseminated tumor cells during each phase in the liver as well as the ensuing interactions are predictably distinct. Of particular focus for this review

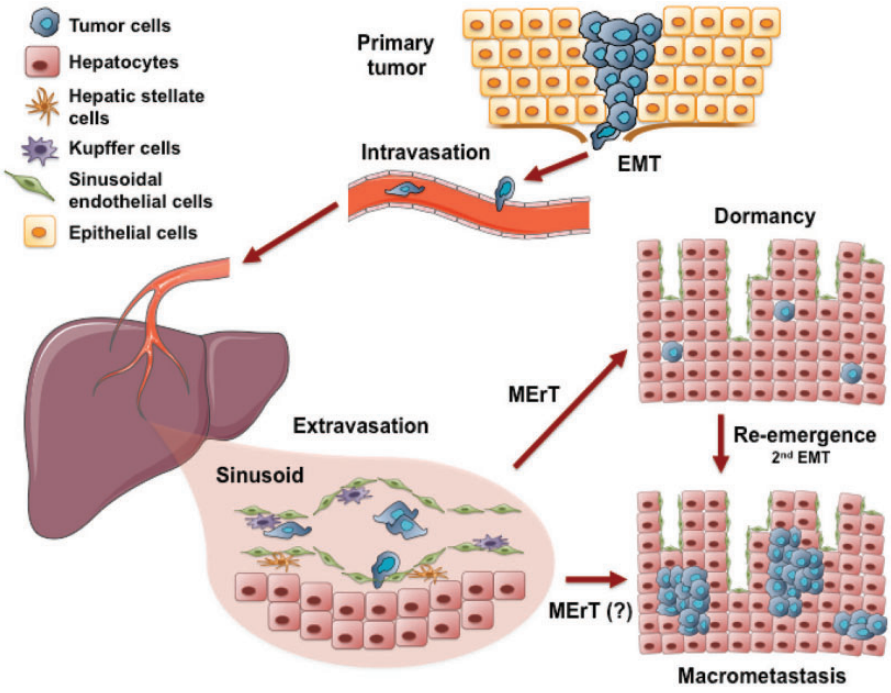

Figure 1 Schematic of the metastatic cascade to the liver. After a subset of cells in the primary tumor undergo an epithelial-to-mesenchymal transition (EMT) to enable escape, the tumor cells transit through the vasculature where they may then lodge in the hepatic sinusoids. This allows for extravasation after which they either undergo a mesenchymal-to-epithelial reverting transition (MErT) allowing for survival in a dormant state or rapidly progress to emergent outgrowth via unknown mechanisms. The dormant cells can be stimulated to undergo a second EMT and then outgrow as mortal macrometastasis. (A color version of this figure is available in the online journal.)

are the bidirectional interactions between the disseminated tumor cells and the unique resident cell populations of the liver; notably, parenchymal hepatocytes and nonparenchymal LSEC, Kupffer (KC), and hepatic stellate cells (HSCs). Each hepatic type is involved in different phase(s) and capable of playing tumoricidal and/or tumor progression-promoting roles. As depicted in Figure 2, communication occurs through: (i) soluble signaling factors (cytokines, chemokines, growth factors), (ii) receptor-mediated cell–cell and cell–ECM contacts, and (iii) proteolytic enzymes (metalloproteases; MMPs).[27] The specific molecules involved in each type of communication vary by cell type and liver status.

Liver sinusoidal endothelial cells

Upon entering the hepatic microcirculation, LSECs are the first cells encountered by disseminated tumor cells. The tumor–endothelial cell (EC) interaction is an important step in the metastatic cascade that determines if a tumor cell is eliminated or proceeds to extravasate and eventually form an overt metastasis. LSECs exhibit both tumoricidal and tumor progression-promoting activities in the metastatic hepatic microenvironment. Their role is determined indirectly through cytokines produced by KCs or directly by interactions with the invading tumor cells.

Regarding their tumoricidal activities, obstruction of the sinusoids by tumor cells can cause transient ischemia, triggering an inflammatory response and the release of cytotoxic nitric oxide (NO), reactive oxygen species (ROS), tumor necrosis factor (TNF)-α, and interferon (IFN)-γ by LSECs.[28–32] Via their innate endocytic activity, LSECs have also been found to quickly remove and degrade enzymes (e.g. autotaxin, a phosphodiesterase) from the circulation that promote angiogenesis and metastasis.[33] These events, in part, explain the high rate of tumor cell failure during dissemination; however, a portion of tumor cells are able to avoid these dangers and extravasate into the tissue.[24]

The tumor promoting role of LSECs is a little more complex and involves a protracted cascade of events. They contribute to metastatic progression primarily by fostering tumor cell arrest and extravasation into the extrasinusoidal space through physical and signaling mechanisms. The process begins with tumor cells activating KCs to secrete proinflammatory cytokines (namely, TNF-α and interleukin [IL]-1) which in turn stimulate LSECs to express high levels of adhesion molecules (E-selectin, P-selectin, vascular cell adhesion protein 1 [VCAM-1], intercellular adhesion molecule 1 [ICAM-1], platelet endothelial cell adhesion molecule [PECAM]) that enable attachment of tumor cells to the LSECs, leading to activation of a signaling cascade that enables diapedis and extravasation into the hepatic parenchyma.[34–38] Furthermore, in the early metastatic microenvironment, LSECs can also inhibit the antitumor immune response. In a murine model of CRC, interaction of lymphocytes with tumor-activated ECs decreased their antitumor cytotoxic. The mechanisms involved IL-1 induced upregulation of mannose receptor-mediated endocytosis of the lymphocytes.[39]

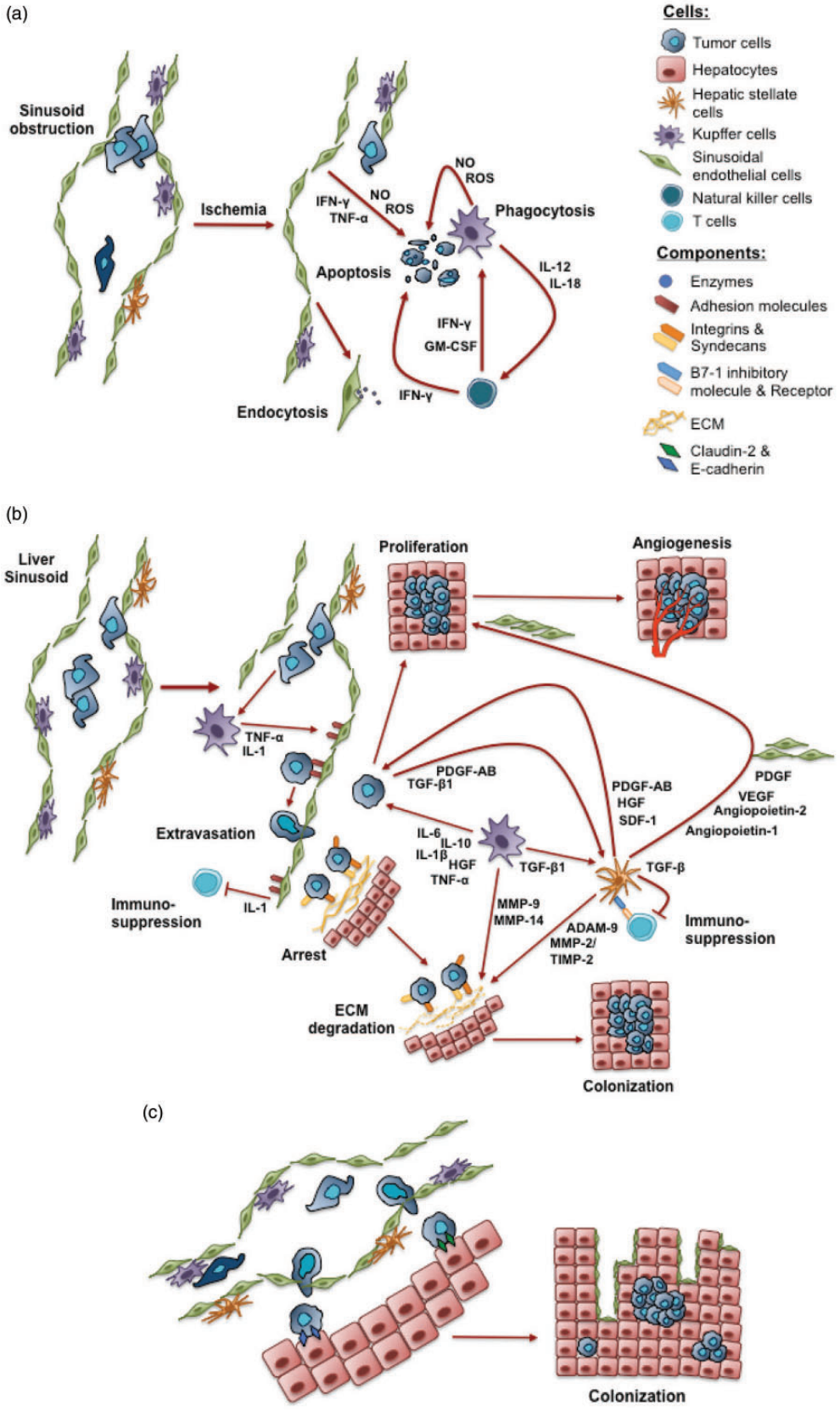

Figure 2 Bidirectional interactions between disseminated tumor cells and resident hepatic cell populations that govern liver metastasis. The resident cell populations of the liver (hepatocytes, LSECs, KCs, and HSCs) exhibit (a) tumoricidal (apoptosis, phagocytosis, and endocytosis) and/or (b,c) tumor progression-promoting activities (extravasation, arrest, colonization, proliferation, angiogenesis, and immunosuppression) in the hepatic metastatic microenvironment. Activities are mediated via soluble signally factors, direct receptor-mediated cell–cell or cell–ECM contacts, and proteolytic enzymes. (A color version of this figure is available in the online journal.)

Not to be overlooked is the fact that LSECs are fenestrated, thus allowing the arrested tumor cells direct access to the basement membrane proteins.[40] This barrier matrix supports the tumor cells by presentation of adhesion sites for integrins and syndecans, as well as cryptic matrikines that promote both cell survival and migration.[41,42] Chief among these are the laminin vascular endothelial growth factor (VEGF)-like repeats that will bind to the endothelial growth factor (EGF) receptor[43,44] as low affinity/high avidity ligands.[45] The first process promoted by these signals is tumor cell invasion through the matrix and entry into the parenchyma, after which the same signals likely act as survival signals to protect the tumor cells against both starvation as well as the aforementioned cytotoxic cytokines and oxygen metabolites.[46–48] As large numbers of such fenestrated LSECs are largely limited to the liver, this direct access of arrested tumor cells to a supportive matrix could explain the high level of liver metastases.

Kupffer cells

KCs, residing in the sinusoids, are involved in the microvascular and intralobular micrometastasis phases. Similar to LSECs, they play a bimodal role during the metastatic process. Their crucial physiologic role in the tumor surveillance system leads them to discriminate and remove neoplastic cells that reach the liver. Numerous mechanisms exist through which KCs exert cytotoxic activity towards disseminated tumor cells, including the production of oxygen metabolites, recruitment of other inflammatory cells, phagocytic release of cytotoxic cytokines, and the secretion of proteases.[31,32,49–55] The release of cytotoxic NO and ROS is triggered by the transient ischemia that follows obstruction of the sinusoids by tumor cells.[31,32] In line with this, CRC cells become vulnerable to macrophage tumoricidal activity during LSEC adhesion and extravasation.[50,56] Recruit of other inflammatory cells, such as liver-associated natural killer (NK) cells promotes the tumoricidal activity of KCs. Studies indicate that activated NK cells secrete pro-inflammatory cytokines, such as granulocyte macrophage-colony stimulating factor (GM-CSF) and IFN-γ, which in turn activate KCs or sensitize tumor cells to cytotoxic effects. Alternatively, NKs may induce CRC cell apoptosis, causing exposure of phosphatidylserine and enhancing phagocytosis by KCs.[54] The opposite interaction has also been reported in which activated KCs may produce IL-12 and/or IL-18, which enhance IFN-γ release by NKs that exhibit high tumoricidal activity, resulting in inhibition of CRC hematogenous metastasis in murine livers.[52,53] Thus, it appears that in the metastatic process, KCs and NKs act in close cooperation against the invading tumor cells. Both produce cytokines and interact to stimulate one another, eliminating tumor cells directly or mediating tumor cell death by their counterparts.

The tumoricidal activity occurs during the early events of metastasis, but KCs can exert tumor promoting activity during the later phases. The switch is determined predominantly by tumor cell burden. KCs exhibit a capacity for immuno-surveillance when tumor cells numbers are low. However, KCs switch to promote liver colonization and metastatic progression when their phagocytic capacity is overwhelmed due to excessive numbers invading the liver.[56] *In vitro* experiments also indicate that only the highly malignant cells are capable of reducing the phagocytic capacity of KCs.[57] This dual role was further elucidated by Wen *et al*.,[58] who demonstrated that depleting KCs early prior to tumor introduction was accompanied by increased liver metastatic burden, while depletion at the late stage of tumor growth decreased liver metastatic burden compared to untreated controls.

The outgrowth of the tumor metastases appears to be linked to inflammation,[59–61] and KCs likely play a role in this. Activated KCs are capable of directly stimulating metastatic proliferation through the release of growth factors (e.g. hepatocyte growth factor [HGF]) as well as cytokines (e.g. TNF-α, IL-1β, IL-6 and IL-10)[36,62] and indirectly via ECM modifications.[63] Secreted MMPs (notably MMP-9 and MMP-14) possibly enhance angiogenesis and tumor invasion, by altering the ECs,[63] conceivably by uncovering the cryptic matrikines.[44] MMP-9 is primarily derived from KCs, independently of its expression by tumor cells and MMP-9-deficient mice present considerably fewer liver metastatic lesions when CRC cells are injected intrasplenically.[64] In addition, even limited degradation of matrices induces a wound healing response that engenders the local generation of an immature matrix that is supportive of cell survival and proliferation.

Hepatic stellate cells

HSCs play a crucial role in organizing and accelerating the progression of metastasis by generating a pro-metastatic liver microenvironment.[65] Integral to their involvement is a bidirectional interaction with tumor cells as well as their early recruitment to the extravasated tumor cells.

Following the development of micrometastases, quiescent HSCs are triggered to transdifferentiate into myofibroblasts, highly proliferative and mobile cells. Transdifferentiation is induced in response to paracrine factors released by both tumor cells (e.g. transforming growth factor [TGF]-β1, platelet derived growth factor (PDGF)-AB) and KCs (TGF-β1).[66] In turn, activated HSCs promote the pathogenesis of hepatic metastasis by affecting tumor cell adhesion, invasion, proliferation, migration, survival, and eventually promote angiogenesis, the pivotal transition point necessary for the growing metastasis (comprehensively reviewed in Kang *et al*.[65]). Thus, promotion of metastatic progression by activated HSCs occurs via multiple mechanisms.

Growth factors and cytokines. Conditioned medium from activated HSCs has been repeatedly shown to promote proliferation, migration, and invasion of a variety of tumor cells.[67–71] These findings are corroborated by *in vivo* mouse models that involved the co-implantation of quiescent- or activated-HSCs with tumor cells in which the latter resulted in larger tumors.[67,69,70] Notable signaling molecules included PDGF-AB, HGF, and stromal cell derived factor (SDF)-1.[67,70–72]

ECM degradation. HSCs create a reactive tumor stroma by producing proteolytic MMP enzymes involved in ECM turnover, namely MMP-2/TIMP-2 and A disintegrin and metalloprotease domain-9 (ADAM-9). This generates an immature matrix that further enhances invasion and survival of tumor cells by the molecular mechanism noted above in ''Kupffer cells'' section.[66,68,73,74] Tumor-activated HSCs are also responsible for the re-modeling and deposition of this progression supporting tumor-associated ECM.[75–77]

Angiogenesis. HSCs play a crucial role in organizing and accelerating the progression of liver metastasis by initiating angiogenesis. Eveno *et al*.,[78] recently demonstrated that activated HSCs are already operating in the avascular growth stage of developing hepatic metastasis prior to angiogenic recruitment and organization of ECs into a neovessel network within metastases. Under tissue repair conditions, activated HSCs produce multiple angiogenic factors, including VEGF, PDGF, angiopoietin-1 and -2, which facilitate ECs recruitment to the hepatic metastatic microenvironment and stimulate their function.[70,78–82] *In vitro*, VEGF has been shown to increase EC migration, reduce apoptosis, and promote proliferation, behaviors all required for new vessel generation.[83] Additionally, both *in vitro* and *in vivo* experiments have reported that VEGF production is further potentiated by hypoxic conditions,[79,84] a common aspect of the tumor microenvironment. Furthermore, activated HSCs were observed to induce vascular tube formation by LSECs and vascular ECs.[85,86] They also strongly deposited matrix laminin, which not only supports tumor survival and progression via EMT, but also forms part of the basement membrane of the new vessels.[78]

Immunosuppression. There is also evidence to suggest that activated HSCs may play a role in suppressing the antitumor immune response. In tumor free *in vitro* experiments, activated HSCs were found to exert immuno-inhibitory activity by inducing T cell apoptosis.[87] Subsequent *in vivo* experiments identified the interaction to be mediated via the B7-homolog 1 inhibitory molecule.[83] Furthermore, numerous studies have shown activated HSCs produce TGF-β, a potent immune-suppressor.[55,71] While this aspect of HSC function is still being deciphered, should the role bear out; it may become more important in devising treatment strategies.

Hepatocytes

Hepatocytes, the main cells of the liver, appear to be primarily involved in the intralobular phase, and play a critical role in metastatic seeding, colonization, and survival. Upon seeding in the liver, breast cancer cells directly interact with hepatocytes by extending cellular projects through the fenestrated endothelium into the space of Disse[88] and form tight-junction-like complexes.[89] Direct cancer cell–hepatocyte interactions during liver metastasis have also been observed with CRC cells.[90,91] Interesting, liver metastatic breast cancer cells exhibit a lower adherence preference for the LSECs compared to weakly liver metastatic breast cancer cells, thus the former preferentially interact with hepatocytes over LSECs. Molecules typically used for homotypic cell–cell interactions, in part, mediate this interaction. One candidate is claudin-2,[92] which is specifically expressed in liver metastatic breast cancer cells compared to populations derived from bone or lung metastases. During breast cancer liver metastasis, claudin-2 shifts from acting within tight-junctional complexes to functioning as an adhesion molecule between breast cancer cells and hepatocytes.[92]

E-cadherin, the main marker of the epithelial phenotype and the initiating molecule for cell–cell adhesion junctions, plays a critical role not just for interactions with hepatocytes but as a master regulator of metastatic ability and tumor cell dormancy. Entry into the liver microenvironment has been shown to induce E-cadherin re-expression in infiltrating breast, prostate, lung, and melanoma cancer cells. E-cadherin is usually downregulated or silenced during EMT, which enables escape from the primary tumor, however re-expression or upregulation of E-cadherin is noted in human micrometastases.[17,93–95] This phenotypic reversion, or MErT, is driven by the hepatocytes as co-culturing mesenchymal breast, prostate, and lung carcinoma cells with hepatocytes can recreate this.[95–97] This upregulation of E-cadherin is critical for metastatic survival as prostate carcinoma cells that are prevented from re-expressing E-cadherin form fewer and less robust spontaneous metastases in mouse models, even when the primary tumors are equally if not more malignant. This is due to heterotypic ligation with hepatocytes promoting cell survival and thus protecting against detachment-induced cell death, or anoikis, in a caspase-independent manner.[98] The ligated E-cadherin leads to sustained activation of ERK MAP kinase, which further facilitates a functional survival advantage by increasing the resistance of breast and prostate cancer cells to cytokine and chemotherapy-induced cell death in the liver microenvironment.[96]

Our knowledge of the intricate networks and interactions governing metastasis in the liver is expanding, however much still remains to be elucidated as the initial cell biological aspects cannot be discerned in current models. A clearer understanding is required in order to identify new targets and processes and the cognate therapies and approaches with which to treat metastasis, the tumor stage that remains largely incurable. Efforts have been hampered, in part, due the absence of adequate model systems as discussed in the section below.

Model systems

The liver metastatic tumor microenvironment is extremely complex, consisting of the cells enumerated above along with their secreted factors (cytokines, chemokines, growth factors, proteases), exosomes, and ECM. Understanding the early steps in metastatic seeding, and the decision to undergo dormancy versus outgrowth, has been difficult to ascertain using animals due to numerous limitations. Firstly, animal models are not entirely representative of the human situation due to interspecies differences in cytokines and metabolism,[99,100] and such studies typically use

immune-compromised murine models,[101–103] yet it is well established that the immune system is crucial to the micrometastatic microenvironment.[104–106] Further, given the stochastic and inefficient nature of spontaneous metastases, there is an inability to define the initiation of seeding and study it.

In response to these limitations, bioengineers and cell biologists have joined forces to develop a new generation of tissue-engineered biomimetic systems.[106–110] These systems are quickly becoming an attractive alternative and/or complement to the traditional 2D and murine models. Although they are still very much in their infancy, significant successes in recapitulating key features of human disease have already been achieved (reviewed in Benam *et al*.[111]). These biomimetic systems utilize human cells in a tailored microenvironment and have the potential to recapitulate *in vivo* conditions and address the drawbacks of current tissue culture dish 2D models.

At the cutting-edge of these developments are *ex vivo* 'microphysiological systems' (MPS) which have the potential to recapitulate *in vivo* conditions.[106] MPSs are all-human cellular constructs designed to faithfully recapitulate the structure and function of a human organ or organ regions on a milli- to micro-scale level. In creating these systems, particular attention is paid to recreating the complex cellular microenvironment and heterogeneity by the incorporation of essential organ-specific cell types, including immune cells, with the latter being a mandatory component of the tumor microenvironment. Thus, in doing so, these devices permit analysis of more complex interactions between of tumor cells with parenchymal cells, stromal cells, immune cells, and ECM. Primary human tissue is typically used to populate MPSs, however, induced pluripotent stem cells and immortalized cell lines can also be incorporated. Additional advantages over traditional 2D cultures and murine models include the control of multiple physiological environment parameters (e.g. automated drug-dosing, sampling, medium exchange, diurnal variations, and oxygen levels) via microfluidics and drip-pumps, and the use of novel biomaterials (e.g. hydrogels) enables the mechanical and topographic rheology of an organ to be mimicked. Real-time monitoring of a variety of physiological events (e.g. post-translational modifications, production of metabolites, changes in various ion concentrations, and the dynamic interaction of proteins with defined macromolecules in time and space within cells) can be achieved via fluorescent protein biosensors (FPBs).[112] The incorporation of which is easily tracked using a wide range of fluorescence optical imagine methods.[112] Thus, MPSs offer an opportunity to accurately recapitulate the physiology of whole organs or specific organ regions within 3D with tissue-like perfusion, stiffness, and proper dynamic mechanical, chemical, and electrical cues.

Liver MPS

In engineering a biomimetic, physiologically relevant human liver platform, many aspects must be considered.[107] Desirable features include: (i) inclusion of all resident hepatic cells necessary for liver functioning (hepatocytes and the various NPCs), (ii) controllable flow to provide the physiologically relevant perfusion shear stimulation, oxygenation, nutrients replenishment, waste product removal, and extended culture periods, (iii) scaffolding or matrices that support a 3D multicellular microenvironment by encouraging cell self-organization and generation of macroscopic tissue morphology, and (iv) the ability to be assayed for a variety of real-time mechanistic readouts (e.g. genomic, phenotypic, mass spectroscopy, cell biochemical, and media secretion-based assays).[106–108,110,113,114] The technologies and fabrication techniques are developing fast,[107,115] and although each system described below captures many of the features of liver (Table 1), it is unlikely that a single *ex-vivo* model of liver will meet all the requirements for all applications of liver biology in research and industry. Preferences usually fall in favor of either high throughput, or high information content. In the case of recapitulating metastatic tumor biology, a definitive constraint is the scale of the tissue—i.e. the relative mass of liver versus small tumor capacity of the system to host growth of the tumor.

LiverChip (CNBio Innovations Ltd.). The LiverChip MPS was devised to recreate a liver microenvironment in terms of cellular composition, fluid flow, oxygen gradient, and shear stress. It was originally developed by the Griffith lab,[116,117] and has since been commercialized by CNBio Innovations Ltd. The LiverChip is a perfuseable bioreactor that uses a scaffold to recreate the architecture of the liver sinusoid. Unlike other systems, the LiverChip is an all-human cellular system that incorporates a full complement of donor matched primary human hepatocytes and NPCs, a critical feature given the importance of species-specific cytokine signaling and metabolism.[99,100] It comprises a 12-unit platform made of polystyrene with two connected chambers: (1) a media reservoir and (2) a reactor chamber fitted with high impact polystyrene scaffolds for cell culture. Continuous perfusion (1 μL/s) is achieved via a pneumatic controlled underlay and oxygen concentrations are similar to that observed in the sinusoid (145 μmol/L to 50 μmol/L at a flow of 0.25 mL/min).[117] Resident hepatic cells (hepatocytes and NPCs) are seeded onto the scaffolds within the reactor chamber and maintained for up to 15 days with high viability, functionality, and phenotype retention. The system is designed with the primary aim to mimic a functional liver microenvironment, drug metabolism, and real-time high volume sampling for elucidation of communication networks and drug metabolism.[116] The system is limited in terms of imaging capabilities as the optical windows of earlier interactions[118] were traded for enhanced throughput, with only endpoint analyses presently permitted. Importantly for this discussion, this MPS is the only one fully validated for the study of metastatic behavior.[110,116,119]

Sequentially layered, self-assembly liver model (SQL-SAL) and Platform (Nortis, Inc). The first generation SQL-SAL utilizes a commercially available TEMS single channel, microfluidic device manufactured by Nortis, Inc.[108] The model is constructed using cryopreserved

Table 1 *Ex vivo* liver model systems

Device	Cell types	Time (day)	Cell No.	Flow rate	Vol.	Features	Metastatic model potential	Refs.
LiverChip (CNBio Innovations Ltd.)	Co-culture and monoculture Freshly isolated primary human hepatocytes Freshly isolated, donor matched primary human NPCs Cryopreserved human hepatocytes	15–29	6.0 x 10^5 per unit	60 μL/min Controlled	1.6 mL	Units/device—12 Throughput—high Assembly—easy Material—plastic, polycarbonate, polystyrene Environment—physiological shear stress, oxygen gradient, scalable Readouts—endpoint fluorescent imaging of cancer cell growth and proliferation, microenvironmental signaling networks, oxygen levels, biochemical Pharmacological—hepatotoxicity, clearance, metabolism, drug dosage response	Spontaneous recapitulation of both growing and dormancy metastatic cells; easy to seed in metastatic cells; end point quantification of metastatic burden; elucidate communication networks; evaluate new therapies	110,116–119
SQL-SAL and Platform (Nortis, Inc.)	Co-culture Cryopreserved human hepatocytes Immortalized NPC lines	28	1.25 x 10^5 per unit	0.083 μL/min Controlled	0.36 mL	Units/device—1 Throughput—low Assembly—easy Material—PDMS Environment—low shear stress Readouts—high-content real-time fluorescent imaging physiological events via FPBs (e.g. cell movement, biochemical, and oxygen) Pharmacological—hepatotoxicity, metabolism, acute and subchronic liver injury	Easy to seed in cancer cells; real-time monitoring of metastatic cell migration	108
PEARL Perfusion Liver System (CellASIC)	Monoculture Freshly isolated primary human hepatocytes Freshly isolated primary	7–28	0.2–0.5 x 10^5 per unit	0.069 μL/min Uncontrolled	0.075 mL	Units/device—32 Throughput—high Assembly—easy Material—plastic, PDMS	Untested; lacks many features necessary (e.g. NPCs) to recreate a reflective metastatic	120–122

(continued)

Table 1 Continued

Device	Cell types	Time (day)	Cell No.	Flow rate	Vol.	Features	Metastatic model potential	Refs.
	rat hepatocytes Cryopreserved human hepatocytes Cryopreserved rat hepatocytes					Environment—low shear stress, high density culture, mimics acinus Readouts—high magnification imaging, biochemical Pharmacological—hepatotoxicity, metabolism, drug dosage response	microenvironment, may be possible to seed in cancer cells	
HμREL®'s hepatic model	Multiple and monoculture Cryopreserved human hepatocytes NPCs (source unspecified)	6–14	0.3×10^5 per unit	4.5 μL/min Controlled	0.1 mL	Units/device—4 Throughput—moderate Assembly—difficult Material—polycarbonate, polystyrene Environment—shear stress Readouts—endpoint, genetic, oxygen sensing Pharmacological—hepatotoxicity, clearance, metabolism	Untested; lacks necessary 3D architecture for metastatic microenvironment, unable to seed cancer cells post-hepatic tissue formation	[123,124]

human hepatocytes and three human NPC cell lines (LSECs [EA.hy926], macrophages [U937] and HSCs [LX-2]) at physiological ratios. Cells are sequentially layered in the devices and allowed to further organize via 'self-assembly' to form a layered tissue architecture that is a biomimetic of the hepatic sinusoid. The hepatic tissue maintains viability and functionality out to one month and permits the biochemical and mass spectroscopy measurement of secretions and metabolites in the efflux media, as well as real-time functional read-outs, such as ROS production, apoptosis, cell movements and cell division via FPBs, and a confocal high content imaging system. The SQL-SAL demonstrates acute and sub-chronic liver injury including direct induction of apoptosis, induction of apoptosis by immune-mediated toxicity, and an early indication of the activation of a fibrosis response. A microphysiology database is part of the platform to manage metadata and experimental data, analyze the multiparameter data, bring in external database information and ultimately to create models that are available for any investigator. The SQL-SAL is currently being used to study the temporal-spatial dynamics of subpopulations of dormant and actively growing cancer cells as part of a collaboration combining the strengths of the LiverChip and SQL-SAL to explore liver metastasis.

PEARL perfusion liver system (CellASIC). The PEARL perfusion liver system, manufactured by CellASIC, is a biomimetic of the porous liver sinusoid.[120,121] It refabricates an artificial endothelial fenestrae-like architecture by constructing a structural barrier (with posts), which subsequently eliminates the need for LSECs. The barriers shield hepatocytes from media-associated mechanical stress, while simultaneously facilitating nutrient exchange via diffusion. The device is designed as three-layer sandwich formatted to a standard 96-well plate, and each system contains 32 independent perfusion units that support hepatocytes under a gravity-based flow rate of approximately 100 μL/day. The base of the system is fitted with a glass bottom that allows for high quality microscopy, fluorescence, luminescence, and biochemical analysis, while the middle contains the micro-fabricated flow/membrane barriers features.[120,121] Similar to the LiverChip, cultures of hepatocytes can be maintained for over 28 days with phase I and II metabolic activity, morphological properties, induction/inhibition potential, and drug dosage response.[122] However, the artificial 'vessel' wall introduces many confounding variables including foreign body. The lack of tissue plasticity, the absence of the complex cell admixture, the small scale, and the lack of control over flow rates limit its applicability for metastasis studies.

HμREL®'s hepatic model. The HμREL® is a physiologically based pharmacokinetic model, which is designed to favor the investigation of hepatic clearance and metabolite generation, and is currently listed as being available for beta testing through collaboration with HμREL. The system is manufactured as two housing sets; each encloses four biochips, a fluid reservoir, and a peristaltic pump interconnected through tubing.[123] The device is seeded with hepatocytes and NPCs,

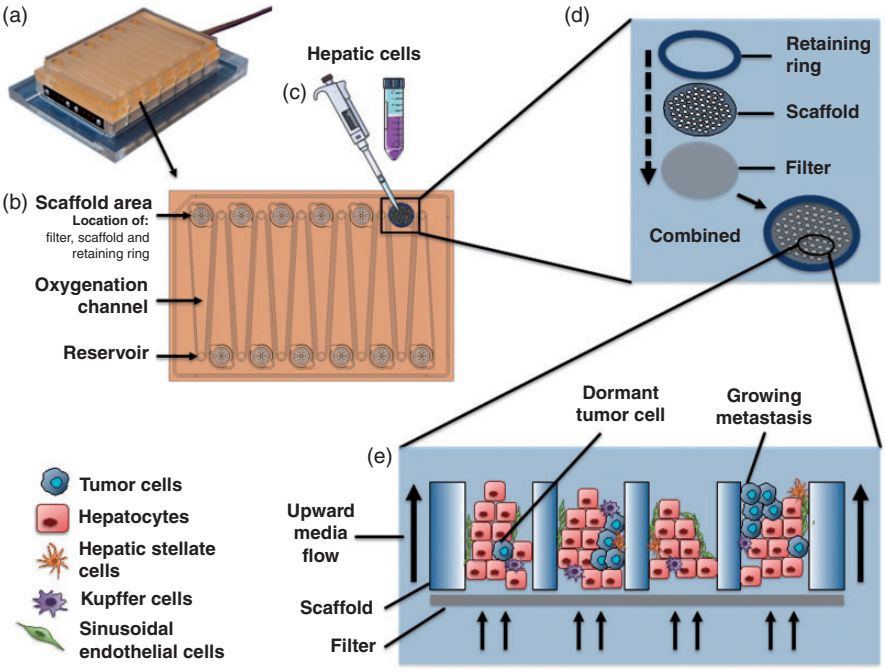

Figure 3 Modeling metastatic breast cancer using the LiverChip MPS. (a) The fully constructed LiverChip depicted within its docking station. (b) Aerial view of the top plate showing the 12 individual liver-units, the location where hepatic cells are seeded and micro-tissue forms. (c) Cells are directly seeded onto an imprinted scaffolding unit, which (d) comprises a layering of a scaffold on top of a 0.22 μm filter, secured in place by a retaining ring. (e) The actual micro-tissue resides in multiple channels that approach the size of a liver lobule. Within these tissues, tumor cells may intravasate and a subset outgrow immediately, while another spontaneously undergo dormancy. Fluid flow up through the micro-tissue supports hepatic tissue development and provides a constant supply of re-oxygenated medium enabling prolonged culture (up to 29 days; unpublished).[119] (A color version of this figure is available in the online journal.)

and demonstrates high-viability after seeding and enzymatic functionality out to six days[124] and recent unpublished data by HμREL report out to 14 days. The utility of this system for metastasis work or its availability to the general community remains to be determined, and the lack of 3D architecture greatly limits its potential.

Modeling metastasis in a hepatic MPS

Hepatic MPSs are attractive for studying metastases, and novel approaches to this disease. The liver is not only a major site of metastatic seeding, but also the principle site of drug metabolism and therapy-limiting toxicities. Thus, a hepatic-based metastasis model can not only be used to understand the fundamental biology of metastasis but also to fully evaluate established and new agents for efficacy while also monitoring pharmacologic aspects and predicting toxicities.[125]

The Wells and Griffiths groups are addressing the aforementioned gap in experimental models of metastasis by utilizing the validated capabilities of the LiverChip[110,116] and the Taylor group has begun complementing this effort by applying the SQL-SAL to investigate temporal-spatial dynamics (biochemical and cellular). Due its biomimetic characteristics, the LiverChip effectively recapitulates liver metastasis of breast cancer *ex vivo*; mimicking both rapid outgrowth and quiescent dormancy (Figure 3). This latter aspect is a distinctive feature being the first report of attaining such without genetic or chemical interventions.[119] This *ex-vivo* model also reflects molecular level changes observed in patients with metastatic disease. The MErT, as determined by re-expression of surface E-cadherin, observed clinically in small dormant micrometastases also occurs in the breast and prostate cancer cell lines cultured in the liver MPS.[117,118] Early iterations of the device were also able to support the growth of primary breast cancer explants and provided insights into the phenotypic plasticity of not only breast cancer cells, but prostate and lung as well.[118] Thus, this system is not limited to only metastatic breast cancer cells, but can be utilized in the future to investigate other solid tumor metastasis such as CRC, prostate and melanoma.

Commentary

The use of MPS to investigate the early stages of metastasis holds the promise of gaining novel insights and developing new approaches to this mortal development in cancer progression. However, the relevance of the findings in these *ex vivo* systems will depend on their being sufficiently representative of the human condition. This requires a complex multicellular tissue that includes not just parenchymal and support cells but immunologic effectors and an appropriate matrix. The physical attributes of the system will be critical as it is known that tumors behave differently based on the surrounding stiffness,[126] even the peripheral housing of the tissues can exert non-physiological effects. It is reasonable to assume that oxygen and nutrient gradients will be similarly impactful and are under investigation. Furthermore, the materials will need to be inert to any tested agents, making the commonly used polydimethylsiloxane

(PDMS)-based housings unsuitable for many studies without modification or replacement.

These single MPSs will be the first step in linking together multiple organs to recreate the physiological signaling of a human. It is known that liver functioning is attuned to effluent from the gut, including products of the microbiome; thus these inputs would be beneficial to create a more complete picture. Likewise, immune function is educated by such inputs. Sexual dimorphism similarly impacts normal physiology, and thus also likely alters the metastatic microenvironment; these signals cannot be truly recreated simply by adding sex hormones but would need hormone-producing tissues. Lastly, by linking together tissues in which a tumor arises, such as skin for melanoma or mammary gland for breast, one could hope to recapitulate the entire metastatic cascade from *in situ* growth and escape through ''vascular conduit transit'' to liver seeding, dormancy, and then outgrowth.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: All authors declare that they have no competing interests except the following authors A Wells: Patent on LiverChip now being commercialized by CNBio Innovations Ltd. L Griffith: Patent on LiverChip now being commercialized by CNBio Innovations Ltd.; consulting fees paid by CNBio Innovations Ltd. in 2012 but no current relationship.

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