Metastasis of circulating tumor cells: Favorable soil or suitable biomechanics, or both?

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Metastasis is the end product of a multistep process where cancer cells disseminate and home themselves in distant organs. Tumor cell extravasation is a rare, inefficient and transient event in nature and makes its studies very difficult. Noteworthy, little is known about how cancer cells arrest, adhere and pass through the endothelium of capillaries. Moreover, the key events driving metastatic growth in specific organs are not well understood. Thus, although metastasis is the leading cause of cancer-related death, how cancer cells acquire their abilities to colonize distant organs and why they do so in specific locations remain central questions in the understanding of this deadly disease. In this review, we would like to confront 2 concepts explaining the efficiency and location of metastatic secondary tumors. While the “seed and soil” hypothesis states that metastasis occurs at sites where the local microenvironment is favorable, the “mechanical” concept argues that metastatic seeding occurs at sites of optimal flow patterns. In addition, recent evidence suggests that the primary event driving tumor cell arrest before extravasation is mostly controlled by blood circulation patterns as well as mechanical cues during the process of extravasation. In conclusion, the organ tropism displayed by cancer cells during metastatic colonization is a multi-step process, which is regulated by the delivery and survival of circulating tumor cells (CTCs) through blood circulation, the ability of these CTCs to adhere and cross the physical barrier imposed by the endothelium and finally by the suitability of the soil to favor growth of secondary tumors.

Introduction

Metastasis can be considered as the end product of a multi-step bio-mechano-chemical process where cancer cells disseminate to anatomically distant organs. They home and establish themselves in a new tissue microenvironment¹ (Fig. 1). Metastases are resistant to multiple therapies and are responsible for the large majority of cancer-related deaths.² It is now clear that the metastasis cascade is not only dependent on genetic and epigenetic alterations within cancer cells, but also involves non-neoplastic stromal cells that contribute to cancer progression.³ This emerging theme has shaped the way solid tumors are perceived: rather than a mass of tumor cells the behavior of which is dictated by its intrinsic cell-autonomous properties, primary tumors are now considered as a complex cellular and matricial ecosystem where the microenvironment plays a fundamental role. The extremely rare nature of cancer cells extravasation makes metastasis studies difficult.

Very little is known about how metastatic cancer cells arrest and adhere to the endothelium of small capillaries and leave the blood stream by breaching through the vascular wall and squeezing their nucleus. Thus, although metastasis is the leading cause of cancer-related death, the main mechanisms enabling this process remain to be elucidated. While the “seed and soil” hypothesis states that metastasis will occur at sites where the local microenvironment is favorable, the “mechanical” concept argues that adhesion/metastasis occurs at sites of optimal flow patterns.⁵ While growth of metastatic foci will depend on the compatibility and molecular interaction of tumor cells (TCs) (the ‘seed’) with the local environment (the ‘soil’), growing evidence suggests that the initial delivery and arrest of TCs is mainly driven by mechanical cues.⁶ The current dogma of tumor extravasation states that tumor cell must first bind a vessel wall to extravasate and that this active arrest occurs by either physical occlusion or cell adhesion, both of them are tightly linked to the vessel diameter.⁶,⁷ Cell adhesion is key during the attachment of Circulating Tumor Cells (CTCs) to Endothelial Cells (ECs) leading to secondary cancer growth. During transit into the blood circulation, tumor cells are subjected to hemodynamic forces and collisions with host cells such as blood and endothelial cells. Only CTCs that overcome or exploit the effects of those shear forces will eventually adhere to and exit the vasculature to form a secondary tumor. Although much information has been gathered in vitro and provide molecular explanation as to how cancer cells attach and extravasate through the endothelium, there is no information as how this mechanism occurs in vivo and what are the underlying mecha-molecular mechanisms.
In this review, we will first discuss these concepts from a molecular angle and describe the recent discoveries gathered through the study of CTCs, which is now possible thanks to advanced purification and molecular characterization methods. We will then quickly review the principal mechanisms driving the successful extravasation of CTCs and focus on discussing the multiple mechanical cues leading to seeding of specific locations within distant organs. These cues, in concert with favorable soil and endogenous tumor gene programs, lead to the growth of deadly secondary tumors.

**Circulating Tumor Cells: Current Knowledge**

Because tumor dissemination mostly occurs through the blood circulation, CTCs are of obvious interest and thus increasingly being recognized for their potential value in cancer monitoring and therapeutic targeting. CTCs represent an intermediate stage in metastatic dissemination and their analysis has tremendous potential for liquid biopsy of cancers, by a routine blood specimen. Clinical value of CTCs detection and enumeration, has been demonstrated by several studies where higher CTCs levels are associated with decreased progression-free survival and decreased overall survival in a variety of cancers. Interestingly, these cells can easily be isolated from human and murine blood samples, whose collection is simple and minimally invasive. The presence of CTCs in cancer patients was observed more than a century ago and their number before treatment is an independent predictor of progression-free survival and overall survival in patients with metastatic breast cancer. CTCs can potentially be used as a predictive marker for individualized cancer treatment. Further, serial CTCs testing can be used to assess patient prognosis and treatment efficacy. Importantly, CTCs have been shown to seed distant organs before malignancy could be observed. Although these CTCs are rare, a recent study shows their potential of initiating metastasis in a mouse xenograft model. The main technical challenge lies in the ability to isolate these rare cells from blood (1 CTC per 10⁶–10⁸ blood cells). There are currently more than 40 different techniques that have been developed to successfully isolate and characterize CTCs (elegantly reviewed recently). Historically, immuno-magnetic separation and thereby positive selection of CTCs using antibodies recognizing the tumor-expressed EpCAM (Epithelial Cell Adhesion Molecule) antigen has been widely used. It is the only technique so far that has been approved by the American Food and Drug Administration (FDA, USA) for diagnostic purposes, via the commercialized semi-automated CellSearch technology. This technology allows to provide reliable measurements of the number of CTCs within a sample of blood from cancer patients and has revealed that CTC counts mirror the progression of the disease toward metastatic cancer. EpCAM-positive, but also MUC-1 positive, CTCs can be captured by optimized microfluidic device platforms. An important caveat of these technologies rely on the heterogeneity of expression of reliable markers in CTCs. Recent work suggest that CTCs also undergo Epithelial-to-Mesenchymal Transition leading to decreased expression levels of EpCAM, among others, in addition to over-expression of mesenchymal...
markers such as EGFR and vimentin. Further work is thus required to allow successful detection of CTCs with variable marker levels. In addition, recent work suggest that cluster of CTCs have an increased metastatic potential. This can be potentially accomplished using a label-free method (Cluster-Chip) which physically captures clusters of CTCs from unprocessed blood, thanks to specialized microfluidic bifurcating traps under low-shear stress conditions. In order to circumvent the limitations imposed by variable expression of markers, negative selection methods have been developed for depleting CD45-positive leukocytes, in combination with red blood cell lysis or density gradient centrifugation. Size-based filtration methods, called CTC-iChip, can also successfully be used for removing non-CTCs such as red blood cells. While alternative methods exploit the size and deformability of CTCs in microfluidic chips in order to sort them in specific chambers, others use membraneous filter devices to isolate and sort CTCs by size. There is thus a plethora of technologies available and currently under development for successfully detecting, isolating and enriching CTCs. However, lack of detailed understanding of the factors affecting CTCs survival and tumorigenic potential, and their molecular and genetic makeup, limit the current use of this important diagnostic approach. Nevertheless, CTCs are a putative reservoir for metastasis-initiating cells holding great promise to be used to monitor tumor progression and aggressiveness. It is self-evident that CTCs offer a window to metastasis studies and molecular characterization is needed to confirm their therapeutic targeting potential. However, this field is still in its infancy and the next step is to apply the constantly improving genomic and transcriptomic techniques for in-depth characterization.

CTCs: Transcriptomic profiling

CTCs constitute the main source of metastasis-initiating cells; therefore, molecular analyses of these cells can potentially identify genes important for the processes of metastasis, dormancy and organ specificity. Global gene expression profiling of CTCs in colorectal, prostate and breast cancer patients, yielded CTCs-specific signatures identified by hierarchical clustering, which included genes related to cell movement, cell adhesion, death, proliferation, signaling and interaction. RT-PCR analysis of CTCs signature, enabling highly sensitive detection and is being widely used in clinical settings for many different types of cancer, given the ease of use. However, CTCs may have different progenitors within tumors, which are heterogeneous in nature, and experimental pooling could mask cell-to-cell variations in expression that are biologically interesting and important. Recently, several reports charting the molecular heterogeneity of the CTCs population, at single cell level, in breast, prostate and pancreatic cancer patients have been published. This has mainly been possible due to the rapid advances in Next-Generation Sequencing (NGS) and CTCs enrichment technologies. In most cases, clustered separately from the primary tumor showing the dynamic nature of the changes during metastasis, and also showed a gene profile associated with aggressive tumors. In spite of the considerable heterogeneity displayed by single CTC significant commonality was observed such as expression of stromal and extracellular-matrix genes like Klf4, Igfbp5, Dcn, and Sparc and both epithelial and mesenchymal markers, stem cell-associated gene like Aldh1a2 and Bmi1. Interestingly, several studies have shown the presence of stem cell-like markers in CTCs, leading to the hypothesis that tumor progression and metastatic spread can be traced to a small fraction of tumor cells with stem cell-like characteristics. Indeed, studies on CTCs from primary and metastatic breast and prostate cancers showed the presence of markers such as CD44+/CD24low, ALDH1 and Bmi1 which is correlated with advanced stage of the disease and therapy resistance. These studies have led to a paradigm shift in both CTCs capture techniques and therapeutic strategies for metastatic tumors. Several CTCs specific gene signatures have been elucidated which have important prognostic value and new treatment options are being developed, such as drugs targeting specific cancer stem cell factors (Hedgehog, Notch, and Wnt).

CTCs: From tissue invasion to survival within the blood circulation

Aggressive tumor cells have been observed to undergo extensive molecular changes, which enhance intravasation and survival in the blood circulation. Initially, epithelial cancer cells use EMT, Epithelial-to mesenchymal transition (EMT), to cause loss of intercellular adhesion and apico-basal polarity, and finally, gain the ability to migrate and invade the bloodstream. This involves widespread cellular reprogramming by the activation of transcription factors such as ETV5, NOTCH1, SNAIL1, TGB1, ZEB1, ZEB2, TWIST that maintain mesenchymal phenotype, such as up-regulation of vimentin and loss of E-cadherin and EpCAM expression. These mesenchymal cells actively migrate along while remodeling the surrounding tissue either as single or as clusters. Collective migration of cell clusters requires a combination of stable cell–cell adhesion and multicellular coordinated movement. Therefore, they possess cells of both epithelial and mesenchymal morphologies. Interestingly, in breast and pancreatic cancer patients, single CTC often possess mesenchymal phenotype while cell clusters consists of cell of both morphologies. CTCs have been observed to use a number of complementary strategies for survival in the harsh environment of blood, such as molecular changes leading to anoikis resistance, EMT, immune escape and cloaking by platelets. Cells without cell-matrix interactions undergo caspase-induced apoptosis, anoikis. Activation of receptor tyrosine kinases (RTK) such as tropomyosin-related kinase B (TrkB) and Wnt suppresses caspase related apoptotic pathways and improve survival in circulation. Similarly, CTCs clusters demonstrate increased anoikis resistance due to a combination of mesenchymal properties, persistent cell-cell junctions and stromal derived signals like formation of microemboli with stromal cells. EMT also seems to confer resistance to shear forces from the blood flow. This process is also influenced by several oncogenes. This is similar to the mechanotransduction of gene expression by blood flow in endothelial cells, platelets and leukocytes. Recently, platelets have emerged as an important ally for CTCs survival and extravasation. They can contribute to the progression of tumor
metastasis by several mechanisms. First, platelets can protect CTCs from shear stress and immune clearance (NK cells) in the bloodstream cells by a cloaking effect.\textsuperscript{75,76} CD47 upregulation has also been observed for CTCs in colon cancer patients.\textsuperscript{77} Second, growth factors secreted by activated platelets, like TGF-\textbeta, enhance the growth and motility of tumors by inducing EMT.\textsuperscript{78} Ablation of TGF-\textbeta\textsuperscript{1} expression solely in platelets protects against lung metastasis in vivo. Finally, CTCs coated by platelets become bulky and adhesive, increasing the rate of tumor embolization in the microvasculature.\textsuperscript{79,80} Metastatic tumor cells can express high levels of tissue factor (TF) and adhesion molecules, such as P-selectin ligands, through which they bind to and activate platelets,\textsuperscript{81} confirming mechanistic link between activation of the blood coagulation system and the spread of tumor metastases.

Extravasation of CTCs: Molecular and cellular mechanisms

While in vivo evidence is still lacking, in vitro studies show that, similarly to leukocytes, cancer cells roll on the endothelium under flow conditions before initiating a more stable attachment.\textsuperscript{82,83} Potential mediators of the cancer cell rolling on the endothelium are the endothelial selectin (E-selectin) and N-cadherin.\textsuperscript{82,84-86} E-selectin ligands such as HCELL, PSGL1, MUC1

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Box 1: Relevant models to study tumor cell extravasation and hemodynamics

Study of metastasis in vivo has been made possible through recent developments and the use of suitable model organisms amenable to intravital imaging. The main limiting factor for being able to track tumor extravasation in living organisms is the amenability of the tumor extravasation site to intravital imaging (depth of imaging) which itself is strictly dependent on the ability to find such an event which, by definition is very rare. Precise patterning of the zebrafish embryo\textsuperscript{157} (Box figure) is very useful in tracking tumor extravasation, upon direct intravascular injection of fluorescently-labeled tumor cells. Thanks to an immature immune system, a relative transparency at the embryonic stage, the establishment of tissue-specific fluorescent transgenic lines and a fully functional and perfused vascular system, the zebrafish embryo is a perfect model\textsuperscript{87,158} for tracking metastatic cells non-invasively in an in vivo context. This model has already provided useful information about tumor extravasation\textsuperscript{159,160} and similar experiments in juvenile individuals should be soon possible with the creation of a transparent zebrafish line\textsuperscript{161} (Casper) and the recent development of an immunocompromised strain\textsuperscript{162} (Rag2(E450fs) mutants). Another in vivo model with a fully functional vascular system and capable of providing useful insights into tumor extravasation is the ex ovo chicken embryo model\textsuperscript{163} (Box figure) where fluorescently-labeled cells are injected in veins within the chorioallantoic membrane and endothelium is labeled upon intravenous injection of lectins\textsuperscript{164}. The most relevant, yet invasive, approaches for studying tumor extravasation are offered by the mouse models. Several approaches permit to track tumor single arrested and extravasated cells in the brain\textsuperscript{132}, the liver\textsuperscript{136,165} and the lung\textsuperscript{127} of living mice. Despite the high relevance of those models, they often require surgical procedures and the creation of optical windows on living animals. The depth of imaging in the mentioned organs limits imaging resolution of those techniques. Development of correlative imaging strategies\textsuperscript{166-168} could fill this gap within the coming years. This resolution is within reach of recently developed in vitro microfluidic models\textsuperscript{154,155,169} capable of mimicking vessel lumens and extracellular spaces through the usage of hydrogels. If perfusion of relevant blood components can be achieved and in vivo flow patterns can be mimicked, these models could provide instrumental tools to decipher tumor extravasation at high resolution and in a reproducible manner.
and LGALS3BP are expressed at the surface of cancer cells and contribute to rolling in vitro. Importantly, it was also reported that the expression of E-selectin ligands in cancer cells is correlated with higher metastatic potential and poor prognosis.\(^83\),87,\(^88\) The initial cancer cell arrest is followed by the stable attachment of cancer cells to the endothelium and was suggested to involve receptors such as integrins, CD44 and MUC1.\(^89\)-\(^92\) In addition, the complementary expression of chemokines and their receptors in cancer cells and endothelium respectively contributes to extravasation and metastasis of tumor cells. The receptors involved in these processes will vary depending on the cancer type and the vascular bed. Upon stable attachment, it was shown using zebrafish and chick models that cancer cells perform intravascular migration before initiating extravasation which could allow them to find the optimal sites to undergo extravasation.\(^93\),94\) In the case of leucocytes, the endothelium plays an active role in the extravasation step by the delivery of certain localization signals that help to guide this process. Namely, it changes dynamically its architecture, receptor expression and cell-cell contacts at the extravasation sites.\(^83\) Inspired by the mechanism of leucocyte extravasation, different studies aimed to address whether tumor cells would use similar extravasation cues. Previous work in leucocytes has defined 2 major extravasation routes: paracellular and transcellular. The paracellular migration is defined as the migration occurring through the endothelium cell junctions while the transcellular route involves the migration through the endothelial cell. It remains unknown which is the route used by cancer cells in vivo. However, in vitro studies have shown that cancer cells use preferentially the paracellular route.\(^83\),89,90\) It was recently shown in vivo that T/Cs can infiltrate and circulate either as single cells or in clusters. When in clusters, CTCs are more rapidly cleared from the circulation and have a much higher metastatic potential when compared to the single CTC.24\) In addition, the aggregation of platelets with CTCs was reported to facilitate extravasation and increase metastasis.95-97\) Likewise, it was shown that immune cells, namely monocytes and macrophages interact with CTCs and act as important mediators of the adhesion and extravasation events.98,99\) Therefore, the interaction with other cells seems to play an important role during cancer cell dissemination since it increases the extravasation efficiency by improving the resistance to shear stress, protecting against the immune system or increasing the interactions with the blood vessel wall. Growing evidence suggests that the arrest of cancer cells is mainly driven by mechanical cues. It was shown that cancer cells reduce their speed and arrest in small capillaries by size restriction which is then followed by the formation of stable attachments.89,93,94,100,101\) However, arrest by adhesive interactions is also occurring in pre-capillary vessels (bigger than the cell diameter) when there is activation of the endothelium.101\) There is thus still a long way ahead in understanding the exact mechanistic details driving the extravasation of CTCs in vivo. Usage of recently developed in vivo models well suited for studying extravasation of CTCs in vivo (in addition to a fine evaluation of blood flow patterns) will undoubtedly provide further and unexpected insights into that mechanism (see box 1).

### Tumor Extravasation and Metastasis: A Phenomenon also Driven by Mechanical Factors?

Despite being an old question, when, where and how efficient CTCs will form metastasis is still poorly understood. It is, thus, still subjected to intensive biological study and much remains to be described to provide a fine understanding of the driving mechanisms.

The first efforts explaining the metastatic behavior of cancer cells and the homing of certain cancers to specific sites stands from Stephen Paget in 1889. Indeed, the « seed and soil » hypothesis was first proposed by Stephen Paget when he observed 735 breast cancer patients with non-random metastasis to bones and visceral organs.4\) He proposed that tumor cells (the « seeds ») colonize selectively distant organs (the « soil ») presenting a favorable environment that facilitates the survival and proliferation of the tumor cells. Thus, metastasis will form when the correct « seed » meets the correct « soil » (for further reading, see4,102-104\). This concept of metastasis organ tropism has been reinforced over the last decades. Indeed, growth of metastatic seeds into macrometastasis is driven by specific organ-dependent conditions as well as by their ability to survive in harsh organ microenvironment. The vast majority of CTCs infiltrating a distant organ will eventually die.105 Survival signals can be mediated by local macrophage binding to VCAM-1 expressed at the surface of disseminated tumor cells.106 While Src activity has been shown to favor the survival of disseminated breast cancer cells within bones,107 miR-31 expression impedes metastatic growth by inhibiting initial survival at distant sites.108 Metastatic growth can also be stimulated by the ability of tumor cells to exploit organ-specific stromal components. While cancer cells can exploit astrocytes for brain metastasis,109 they are also capable of inductrinating local osteoclasts and osteoclast progenitors during bone metastasis.110,111 Bone metastasis can also be driven by exosome-driven education of bone marrow progenitor cells to express the receptor tyrosine kinase MET. In addition, organ-specific metastasis signatures have also been identified. For example, subsets of tumor-expressed genes favor metastasis to the brain112 and to the lungs.113,114 Disseminating tumor cells can also tune secretion of extracellular matrix proteins and educate local stromal cells to favor during metastatic colonization.\(^115\),116\) Altogether, this demonstrates that a favorable microenvironment, in addition to activation of specific gene programs within CTCs, act as a major bottleneck in successful metastatic seeding.

Importantly, there are additional bottlenecks during the metastasis cascade as most of these traits mentioned above come into action only upon successful arrest and extravasation of metastatic tumor cells. They therefore do not fully explain the metastasis organ tropism, which can be driven by blood circulation patterns. Indeed, in 1929, James Ewing challenged Paget’s « seed and soil » hypothesis and proposed that metastatic dissemination occurs only by mechanical factors that are a result of the anatomical structure of the vascular system.117\) Later, in 1951, Coman and colleagues experimentally tested this hypothesis by injecting Brown Pearce rabbit tumor cells directly in the heart of the rabbit. They observed a positive correlation between the arrest of cells detected
in the capillary bed of certain organs and the formation of tumors in those organs. This led them to conclude that metastases fail to appear in certain organs simply because tumor cells fail to reach them. Moreover, some vascular beds are more permissive than others. For example, the fenestrated endothelium of the bone marrow or liver capillaries can potentially ease extravasation of tumor cells, while other beds covered by basal lamina, such as the blood brain barrier, would impede extravasation. Thus, while growth of metastatic foci will depend on the compatibility and molecular interaction of TCGs (the ‘seed’) with the local environment (the ‘soil’), growing evidence suggests that the initial delivery and arrest of TCGs is mainly driven by mechanical cues.

**Metastasis as a result of blood circulation patterns and origin of CTCs**

Aggressive tumors are capable of shedding thousands of tumor cells into the circulation each day. Once they reached the circulation, CTCs may reach distant capillary beds within seconds of leaving the primary tumor. Blood circulation patterns thus naturally act as the first bottleneck of the metastasis cascade. Interestingly, organ tropism of organ-specific primary tumors mirrors blood flow patterns. Therefore, it is intuitive to expect the initial delivery and arrest of cancer cells to specific organs to be primarily based on blood flow circulation and/or lymphatic drainage.

Blood circulation contributes to global homeostasis of the body by distributing oxygen to every important organ and allowing exchanges between them. In the systemic path, the flow can be subdivided into 2 different parts: the arterial and the venous circulation. These two streamlines differ in their biological functions (i.e. oxygenation and feeding for the arteries versus detoxification for the veins), but also in their physical properties. Most of arteries are highly elastic and deform when blood pulse arise, giving them the ability to regulate the blood flow, they carry less than 25% of the total blood volume. In contrast, the venous system is not affected by high pressure but acts as a blood reservoir. This compartment contains the 2/3 of blood, whereas the remaining volume is distributed in the heart, liver and the spleen. In the pulmonary path, blood flow exits the heart right ventricle through the pulmonary artery before entering the lungs exchanging O2 and CO2. Once blood is oxygenated, it flows back through the pulmonary vein to the left ventricle before being introduced in the systemic circulation. In the systemic path, the O2 enriched blood is directed to all organs. Organs usually show large vascular ramifications allowing fast, effective gas and nutrient exchange. Blood travels back to the lungs through the venous system. It is thus easy to understand that depending on the location of entry of CTCs into the circulation, they will naturally shuttle through specific organ compartments guided by blood circulation patterns. Moreover, the venous and the arterial circulation stream drastically differ in flow patterns, with arteries and veins carrying pulsatile and laminar flows respectively.

As a result, blood circulation patterns guide CTCs to specific organs. It is for example well accepted that the mesenteric circulation is capable of transporting colorectal tumor cells to the liver and is thus the primary cause or liver-specific metastasis of colorectal carcinoma. In addition, breast CTCs frequently metastasizes to bones, liver, brain and lungs. As for breast cancer, prostate cancer preferentially spreads to bone and neuroblastoma preferentially colonize the liver. Localization of preferred metastatic sites for squamous carcinomas of the head and neck nicely correlated with the lymphatic draining in the neck, which is tightly linked to the venous circulation. CTCs shed from primary tumors located in the breast tissue (but also from other organs) will follow the part of the systemic path before accessing the lungs, where blood will be naturally oxygenated. Lungs and its capillary-type circulation thus appears as the first natural host for disseminating tumor cells and it is not surprising to find lungs as the mostly-seeded organ during metastatic colonization. Cells that do not metastasize within the lungs are then shed to remote organs via arterial circulation leading to a wider distribution of the metastatic patterns. This very simple circulation pattern thus explains a wide majority of metastatic seeds. In contrast, as mentioned above, blood irrigating splanchnic organs first travel through the hepatic-portal circulatory system toward the liver. Thus, blood circulation patterns naturally select the primary organs that are seeded by CTCs and is very likely the first determinant driving metastasis organ tropism. This has been nicely documented several decades ago, when Leonard Weiss documented metastasis patterns from a series of autopsy studies. He concluded that roughly the 2/3 of the metastatic colonization sites is fully correlated with blood flow patterns. To complete his work, he introduced the ‘metastatic efficiency index’ (MEI). MEI represents the ratio of metastatic involvement to blood flow through an organ. Three types of organ pairs emerged: low MEI, where the soil–organ relationship is hostile; high MEI, where it is friendly and medium, where blood flow patterns to a large extent explain patterns of metastatic spread. Weiss however based his study on autopsies performed from the 1900th to the 40th. Only few clinical studies were performed to better tackle the problem of metastatic pattern, in particular for breast cancer. However, Pienta and collaborators showed a lack of correlation between blood flow patterns and metastasis in prostate cancer. Hagemeister et al. identified the principal causes of death due to breast cancer with help to autopsies performed during 1973–1977. Metastases were considered as the primary cause of death in 45% of the cases. The five leading sites of metastatic involvement of breast carcinoma were the lung, bone, lymph node, liver, and pleura. All five sites were reported to contain metastasis in more than 50% of the cases and are all irrigated by capillary-type vasculature. These conclusions confirm the original observations made by Weiss and reinforce the idea that hemodynamic patterns govern the distribution of metastatic sites. Recent studies also propose a new theoretical model. They take into account the flow and the organs filtering effect. This is of high importance for organs such as lungs, liver and kidney. All these organs possess a high capillary vasculature and there biological function is to purify and to filter blood. In conclusion, metastatic sites are not colonized randomly and a significant number of metastatic sites can simply be explained by the circulation patterns between the primary and secondary tumor site.
Metastasis as a result of physical trapping of CTCs

While circulation patterns can explain organ tropism, many solid tumors can send cells throughout many organs. There is a reasonable amount of evidence suggesting that the initial delivery and arrest of TCs is primarily regulated by physical parameters. As a best proof, lungs and liver, whose perfusion is driven mostly by small capillaries, are known as preferred organs for metastatic seeding. Because CTCs are bigger than CBCs and WBCs, capillary-type blood circulation will trap them by size restriction, allowing successful extravasation and growth of secondary foci. This method of arrest of CTCs arrest questions the importance of active adhesion between metastatic cells and the endothelium.

Metastasis as a result of optimal blood flow patterns

Even if CTCs adhesion is of highest importance in the metastatic cascade, only a poor number of mechanical or flow induced arrests site studies are available. After entering the circulatory system, the survival and metasamorhosis of the CTCs into metastasis depends on their physico-chemical interactions with the blood constituents, the vascular walls, as well as the broader vascular flow patterns. The combination of all these parameters leads the cell to arrest and later on to metastasis. Tumor cells are usually larger than CBCs and WBCs and therefore experience shear stress and flow velocity, which are strongly toxic for cells. Therefore, they developed different strategies to take advantage of the flow as well as the environment. The blood stream drives the components at least once in a cycle through organs whose function is filtering blood through small capillaries. Additionally, studies suggest that venous levels of shear stress are more favorable for optimizing the residence time of CTCs and active adhesion of CTCs to ECs that leads to stabilization of metastasizing cells prior to extravasation (Fig. 2). Although much information has been gathered in vitro and provide molecular explanation as to how cancer cells arrest, attach and extravasate through the endothelium, there is indeed little information as to what this mechanism occurs in vivo and as what are the underlying mechano-molecular mechanisms. Use of in vivo models amenable to live imaging of arrest, extravasation and hemodynamics will very likely resolve most of these unanswered questions (Box 1).
with the endothelium so that significant adhesion can be achieved,\textsuperscript{139,140} allowing eventually CTCs extravasation. As discussed above, CTCs preferentially arrest in the small blood vessels such as those found in the lungs, the liver, the brain, and the bones where the microvasculature appears to be more curved, branched, and stretched.\textsuperscript{141,142} These geometries lower the flow velocity therefore stresses, and shear rates.\textsuperscript{5,143,144} Tumor cells take advantage of their high deformability to move deeply through the capillaries and clog them. Another strategy used by the CTCs to lower the shear stress is to coagulate with platelets.\textsuperscript{145} Platelets surround the tumor cell and protect them from the immune system, they also lower the experienced shear stress\textsuperscript{2} while increasing the global size and thus the binding to vascular walls. This significantly alters the blood flow by generating larger pressure gradients and shear along the endothelial cells due to size separation in fluid.\textsuperscript{146,147} This size or phase separation brings the CTCs close to the vasculature walls. Because of shear forces, a torque appears on the agglomerate making it roll on the surface. This rolling on the endothelial surface favors interaction between carcinoma cells and endothelial cells through integrin-mediated adhesions or platelet-induced adhesion.\textsuperscript{139,148} Nevertheless CTCs do not necessarily co-opt platelets, they often remain alone in the flow. Adhesion depends not only on the velocity and shear profile,\textsuperscript{149} it also highly depends on cells deformability. This deformability brings the tumor cell to be more sensitive to drag forces even if they are bigger than the common blood cells. The drag force brings the cells to the center of the streamline and, in the case of bifurcation, the probability these cells encounter and stops at the endothelial wall becomes high. Vasculature is composed of a high number of branching (arterial or venous). These branching alters locally the flow pattern and depending on specific angle and size in the branching shows areas where the flow is highly dropped and cells could be trap a time, long enough, to allow arrest and extravasation. It becomes, thus, more and more important to dissect as precisely as possible local flow stream especially in bifurcations where shear and velocity can drop drastically locally favoring cells arrest due to quasi no flow or local rounding flows.\textsuperscript{142} The usage of animal models often fail to reconstitute the complete metastasis cascade and are thus not flexible enough to allow the study of these processes, with high-throughput. Increasing the throughput by mimicking realistic in vivo situations could thus provide useful solutions for dissecting this complex phenomenon. 3D microfluidic models replicating many aspects of the in vivo microenvironment have thus been developed, allowing flow and concentration gradients manipulation in addition to high-resolution real-time imaging of single cell behaviors.\textsuperscript{150} Microlithography techniques coupled to hydrogels or polymers such as PDMS provide flexible tools for mimicking organ architecture.\textsuperscript{151,152} Sophisticated 3D microfluidic flow chambers wrapped with endothelial cells are perfectly suited for flow tuning and tumor extravasation assays (See box 1). Combining these 3D models with high-resolution imaging allows to accurately reproducing the biophysical and biochemical context encountered by cells in vivo. Recently, an elegant microfluidic platform was designed to study tumor cell extravasation from in vitro microvascular networks formed via vasculogenesis.\textsuperscript{153-155} Endothelial cells can be cultured within a 3D matrix, allowing the formation of microtubes where MDA-MB-231 metastatic cells can be perfused and imaged. This allowed the fine visualization of the extravasation process. These very recent developments confirm the trend of moving from traditional transmigration models to more sophisticated microfluidic devices, and are compatible with high-throughput screening of molecules or drugs potentially inhibiting tumor cell extravasation,\textsuperscript{156} which is highly relevant for developing targeted therapies.

**Conclusion**

Metastatic growth, as well as organ tropism, is driven by sequential mechano-molecular events. The initial steps of cancer cell dissemination and micrometastasis formation are mostly driven by mechanical cues such as blood circulation patterns between the primary and the secondary site. These circulations patterns, as well as optimal blood flow profiles, will carry CTCs to capillary beds with high physical constraints leading to a stable arrest of disseminating cells within the vasculature. Cells that successfully survive and exploit the mechanical permissiveness of the endothelial barrier will eventually seed the distant organ. Then, survival, stemness and growth of the extravasated tumor cells will mostly rely on the suitability and the fertility of the host niche parenchyma. All in all, this shows that the metastasis cascade is driven by a sequence of mechanical and molecular bottlenecks, which makes it a highly inefficient process. Identification and deeper understanding of each of these bottlenecks could pave the way for the development of new and targeted therapeutic strategies to either prevent or impede the metastasis process.

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No potential conflicts of interest were disclosed.

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