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Opinion

Circulating extracellular vesicles and tumor cells: sticky partners in metastasis

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The intravascular behavior of tumor-derived extracellular vesicles (EVs) and circulating tumor cells (CTCs) lies at the heart of the metastatic cascade. Their capacity to disseminate and stop at specific vascular regions precedes and determines the formation of metastatic foci. We discuss in detail the central role of cellular adhesion molecules (CAMs) that are present on EV/CTC surface, as well as their endothelial ligands, in dictating their arrest site and their capacity to exit the vasculature. We focus on the differences and similarities between CAMs on CTCs and EVs, and speculate about their role in the organotropism of different cancer types. Better understanding of the binding mechanisms might pinpoint potential targets for novel therapies.

Adhesion is central to the vascular arrest of EVs and CTCs

Cell adhesion is instrumental not only in the development and physiology of multicellular organisms but also in pathological situations [1]. Cells exploit transmembrane CAMs to attach to neighboring cells and to surrounding material such as the extracellular matrix (ECM) [2]. In addition to mediating physical interactions between two juxtaposed structures, cell adhesion often leads to signal transduction that thus allows cells to adapt or react to the neighboring object. In so doing, cells sense and respond to their environment depending on its mechanical and biochemical properties [3]. Although cell adhesion contributes to homeostasis, it is also central to immune surveillance [4]. Dysfunction of cell adhesion can cause severe diseases such as inflammatory disorders, autoimmune diseases, and cancer. The latter exploits cell adhesion at each step of a cascade of events that favors the appearance of malignancy and that culminates in the formation of life-threatening tumors [5].

During cancer progression, tumors exploit the adhesive potential not only of tumor cell components but also that of non-malignant stromal cells to shape tumor growth, invasion, and colonization in distant organs. During metastatic progression, the adhesive properties of tumor cells are particularly important because tumor cells circulate in the vasculature and are subject to harsh hemodynamic forces [6]. In this context, CTCs use CAMs present at their surface to adhere to the endothelium, exit this harmful environment by extravasation, and develop metastatic foci at distant sites. The molecular mechanisms used by CTCs to bind to the vascular wall are similar to those used by immune cells, and determine the function and fate of these cells (Box 1).

Furthermore, although cells can communicate via cell adhesion, they can also communicate with distant cells through the specific release of EVs which, in the context of metastasis, shape premetastatic niches (PMNs) [7,8]. EVs are heterogeneous membranous and cell-derived nanoto micron-sized structures that carry important signaling molecules such as proteins, nucleic acids, metabolites, and lipids [9,10]. Interestingly, regardless of the cellular origin of the EVs, their lipid bilayer contains a spectrum of classical CAMs that mirrors their cell of origin, but in varying

Highlights

Cell adhesion is central to tumor metastasis and notably relies on the repertoire of cellular adhesion molecules (CAMs) at the surface of circulating tumor cells (CTCs) and extracellular vesicles (EVs).

Common CAMs are present at the surface of EVs and CTCs.

CTCs expose and use CAMs during metastasis to promote vascular adhesion to the endothelium, extravasation, and metastatic growth.

CAMs on tumor EVs contribute to cancer organotropism, the formation of the premetastatic niche, and ultimately to metastatic growth.

Targeting CAMs of EVs and CTCs is likely to impair metastasis.

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Box 1. Similarities between CTCs, EVs, and immune cells

The adhesion machineries engaged by CTC or EVs when targeting and exiting vascular beds closely resemble those that were first described for leukocytes extravasating at inflammation sites. As leukocytes are conveyed by the bloodstream, they approach the vessel wall in a process termed margination and tether to endothelial cells. This first adhesion is transient and weak, and enables the white blood cells to roll along the endothelium. Although rolling of CTCs is still under debate and might depend on the cancer types or the cell line [12,15,47,48], tumor EVs seem to roll along the endothelium before their uptake [49]. Early adhesion of leukocytes is mediated principally by glycoproteins such as CD44, selectins, and their counterreceptors. These weak but fast-to-engage adhesions to endothelial glycoproteins promote the activation of β_2 family integrins in leukocytes and the formation of stable adhesions to the endothelial wall. Integrin-mediated adhesions are stable and favor transendothelial migration [50]. Interestingly, CTCs display the same adhesion stages as leukocytes, first engaging metastable adhesion through glycoproteins such as CD44, followed by strong integrin-dependent adhesions. However, although CTCs share some adhesion receptors with leukocytes, they also express specific sets of surface glycoproteins. Among these, the tumor cell-specific CD24 glycoprotein has been implicated in CTC rolling. Strong attachments of CTCs to the endothelium are mediated through β_1 family and $\alpha 6\beta_4$ integrins. EVs possibly exploit the same mechanism because they also present multiple glycoproteins and integrins at their surface [12,22]. Among these, integrins are essential for targeting blood-borne tumor EVs to specific organs (e.g., $\alpha 6\beta_1$ and $\alpha 6\beta_4$ promote their uptake into lung).

Although leukocytes are recruited to inflammation-activated vessels, it appears that CTCs and tumor EVs can also adhere to normal vasculature [12,49]. These differences in vessel activation might lead to distinct luminal adhesomes and account for the differences observed between leukocytes, CTCs, and EVs.

proportions and compositions, giving them specific fingerprints. Although EVs can be internalized passively by cells with high phagocytic activity, they can also 'target' receiving cells, either locally or at a distance, to deliver specific contents or activate signaling pathways from the cell surface. For that purpose, molecules exposed at the surface of EVs, such as CAMs, favor such binding and promote their function. In this way, EVs can change the fate of receiving cells and even alter their own adhesive potential.

We and others have shown that tumor cells and EVs, in addition to exploiting similar vascular dissemination routes, share CAMs that appear to be instrumental for arresting CTCs at metastasisprone sites [11,12] as well as for delivering EVs to target cells, particularly while orchestrating PMN priming (Figure 1). We discuss the peculiarity of CAMs at the surface of CTCs and EVs, and emphasize their importance during the intravascular steps of tumor metastasis. We discuss their roles during the transit in the blood circulation and highlight their importance for the intravascular arrest of CTCs and EVs. We also discuss their shared fundamental functions during extravasation and the formation of tumor metastases.

CAMs are central to EV-mediated niche priming and CTC arrest and extravasation During metastasis, tumor-secreted components exploit multiple fluid routes such as the blood circulation to disseminate [6], prime metastatic niches [7], and seed secondary tumor foci. To reach the target destinations on their way to favoring and forming metastasis, respectively, EVs and CTCs expose at their surface CAMs from different protein families, including glycoproteins (CD44, CD146, ICAM1, MUC1, CD24) [11–18], integrins (α 3, α 4, α 5, α 6, α v, β 1, β 3, β 4, β 5) [12,19–23], tetraspanins (Tspan8, CD151, CD9, CD81, CD63) [13,20,23,24], and surface receptors (CD184, CD47, NRP2, DR3) [13,25–27]. These molecules determine the preferential vascular site to

Glycoproteins represent a large group of surface molecules on CTCs and EVs, and are important mediators of intravascular arrest. They possess specific sugar moieties that allow CTCs and EVs to interact with lectins leading to their tethering to the vessel wall, a step which is often important in the early stages of cell extravasation [28]. For example, the glycoprotein CD44 on CTCs forms transient interactions with endothelial cells [12]. In addition to mediating binding, CAMs can also modulate the levels and activities of other adhesion molecules at the CTC/EV surface, further

activate, bind to, and exit the vasculature, and they display a wide variety of adhesion characteristics.

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Figure 1. Circulating tumor cells (CTCs) and extracellular vesicles (EVs) use adhesion molecules to arrest and extravasate from the blood circulation during metastasis. In the metastatic cascade, the primary tumor ① sends CTCs and EVs into the bloodstream ②, through which they travel to the site of adhesion where EVs prime metastatic niches and CTCs extravasate and ③ form metastases in distal organs such as lung, liver, and brain. To facilitate adhesion, both EVs and CTCs present different cell adhesion molecules (CAMs) at their surface, which are listed in the blue and grey boxes, respectively, some of which are common between EVs and CTCs. Abbreviation: ECM, extracellular matrix.

enhancing their binding capacity. For example, CD44v6 on EVs regulates the expression of Tspan8, CD184, and to a lesser extent integrin α 6 β 4 on cancer cells [13]. Similarly, CD151 associates with α 3 β 1, α 5 β 1, and α 6 β 1 integrins to promote adhesion to the ECM [23]. Tetraspanins often act as orchestrators of cell-surface microdomains that are important in cell–cell adhesion and communication [29].

Various combinations of integrins typically bind to specific components of the ECM as well as to particular CAMs (ICAM, VCAM). Integrins come into play after the initial attachments are formed and further enforce CTC/EV vascular arrest. Integrins must undergo conformational changes before they enter an activated and adhesive state which favors metastasis. Indeed, constitutively activated mutant variants expressed in a small subpopulation of tumor cells are highly prone to form metastases [30]. Antibody-mediated inhibition of such activated integrin abolished extravasation and metastasis formation [31]. Furthermore, Talin-1 (a focal adhesion protein that is found in integrin-associated complexes) favors activation of integrin β 1, and promotes transendothelial migration of colon cancer cells and subsequent liver metastasis formation [21]. Whether similar



mechanisms are exploited by EV-located integrins is unknown (see Outstanding questions). CTC clustering promotes metastasis formation [32]. Interestingly, clustered breast cancer cells expressing ICAM1 are more efficient in transendothelial migration and have a 200-fold increase in lung metastasis [14]. Likewise, CTCs expressing CD44 and plakoglobin form clusters and have stronger metastatic abilities [32,33]. This suggests that the surface expression of CAMs might be relevant as prognosis markers for disease progression, and demonstrates that CAMs are key molecules driving the extravasation and subsequent outgrowth of tumor cells in distant organs.

CTCs and EVs share overlapping fingerprints, by nature and function

Despite their differences, CTCs and EVs generally share similar CAMs, of which the most abundant are glycoproteins and integrins (CD44 [12,13,17], CD151 [23,24], β1 [12,19,21] and β4 [20,22] integrin) as well as some other adhesion molecules (ICAM-1 [14,18]), raising the possibility that some common CTC and EV adhesion patterns might be elemental in targeting them to specific organs. Such similarity is shaped by the nature of CAMs, and thus their related adhesive potential, or their function during the priming and outgrowth of metastasis, in particular their organ-targeting potential. For example, the CD44 glycoprotein mediates CTC vascular arrest [12,33], and CD44deficient breast cancer cells lose the ability to produce lung metastases [33]. In addition, CD44 located at the surface of EVs controls their ability to form PMNs: CD44-deficient EVs do not promote metastasis [34]. Similarly, ICAM1 controls metastatic progression when located at the surface of EVs and CTCs. Indeed, surface expression of ICAM1 by breast cancer cells promotes their arrest and transendothelial migration (through homophilic binding to ICAM1 at the surface of endothelial cells), which can be inhibited by blocking antibody against ICAM1 [14]. In addition, recent work identified ICAM1 on EVs where it favors binding to T cells, thereby promoting immunosuppression and metastasis prevention [18]. Whether ICAM-loaded EVs favor their intravascular arrest remains to be investigated. Nevertheless, it is tempting to speculate that shared surface expression (EVs and CTCs) of CD44 and ICAM-1 is a key feature of metastatic progression. Another common feature of CTCs and EVs is surface protein glycosylation which generally negatively regulates metastasis formation [15,35,36]. However, under specific circumstances such as CD24-P-selectin interaction, such binding can be important for cancer cell adhesion to the endothelium [15]. Further work might thus identify shared surface expression of CAMs by EVs and CTCs that are likely to be hallmarks of metastasis.

CAMs in tumor organotropism and colonization of distant organs

The organotropism concept states that metastasis does not occur randomly and that each tumor type (breast, colon, etc.) targets specific organs [37]. Multiple parameters such as vascular anatomy, organ-specific niches, and tumor-secreted factors contribute to organotropism. To ensure vascular arrest and thereby the key subsequent steps of extravasation and metastasis formation, CAMs on CTCs and EVs must bind to their ligands at the surface of endothelial cells that constitute the vascular wall.

Only a few CAM-ligand interactions have been specifically studied in CTCs and EVs. Ligands are often components of the ECM that are present either within the perivascular niche (laminins [13,19,23] and collagens [13]) or on the luminal side of blood vessels (fibronectin [12,19,21]). Some cell-surface proteins have also been described, including ICAM1 [14,20,36], E- and P-selectins [15,16,22,27,38,39], and scavenger receptors [40]. Interestingly, the selective expression of CAMs on EVs seems to have a stronger effect on organotropism than their entry point in the circulation [19], strongly supporting a crucial role of CAMs in metastasis organotropism. Therefore, specific combinations of CAMs controlling CTC and EV organotropism must exist. Indeed, retention in the lung, bone marrow, and pancreas of CTCs/EVs injected into circulation [12,20,23,41] can be further regulated by modifications of surface glycoproteins such as



MUC1. Highly glycosylated surface proteins prevent EV retention in the lung and promote their delivery to brain [35]. Inhibition of *O*-glycosylation extension leads to increased CTC–MUC1 binding to endothelial cells [36] and EV uptake [35], suggesting that short *O*-glycosyl chains promote endothelial cell binding whereas longer chains have lower affinity and support EV/CTC delivery to more distal organs. Specific CAMs dictating organotropism have also been identified. Surface expression of integrins α 6, β 1, β 4, and Tspan8 lead to EV accumulation in lung (and in the pancreas in case of Tspan8) [12,19,20,23], whereas expression of integrin β 5 and β 3 resulted in liver and brain accumulation, respectively [19] thus promoting metastatic seeding of those organs. Integrin β 1-depleted cells do not reach the lungs in a classical experimental lung metastasis assay [12]. Furthermore, the adhesion protein CD146 promotes breast cancer EV targeting to the lung and subsequent metastasis growth [11]. Interestingly, CD146 is also found at the surface of CTCs [42], and its presence at the tumor cell surface promotes metastasis [43].

It is generally accepted that priming with EVs precedes the growth of secondary tumors and is an essential step in cancer organotropism [7]. Strikingly, pretreatment with lung-tropic exosomes can revert the metastatic properties of the primary tumor, leading to metastasis formation in the lung instead of the bone [19]. Furthermore, CD151/Tspan8 knockdown cells poorly metastasize, but they regain their metastatic capacity upon pretreatment with wild-type tumor EVs [23]. Experiments with a modified CAM repertoire at the EV surface show that the exact repertoire is likely to tune the target site of metastasis [19]. These results led us speculate that CAMs on EVs play more important roles in cancer organotropism than CAMs on CTCs. One possibility is that EVs use CAMs to prime metastatic niches by further stimulating the expression or deposition of pro-adhesive ligands or ECM molecules that later shape metastasis. Nevertheless, these recent discoveries suggest that the fate of EVs and CTCs is dependent on many factors, and the combined function of surface CAMs probably defines the outcome, especially because the expression of CAM ligands may differ between organs.

Indeed, there are notable differences between the vascular beds of different organs. For example, cells in brain capillaries are tightly connected and less permeable, whereas liver microvessels have a discontinuous layer of endothelial cells and are thus more permeable to CTCs and EVs [44]. Although this has not been properly described, it is likely that the vascular walls of different organs express different CAM ligands. This would explain how CAMs control the organ-specificity of EVs and CTCs dissemination in addition to mediating intravascular arrest at sites of metastasis.

Ligands and specificity of targeting: more to come beyond endothelial cells

During intravascular arrest, the consequences of CAM binding to their ligands differ between CTCs and EVs. After binding to the vascular wall, CTCs undergo extravasation whereas EVs are internalized by endothelial and other cells. Identifying the key ligands and the targeted cell types will be central to the understanding of the prometastatic behavior of CTCs and EVs.

EVs can either be targeted to late endosomal compartments, where they transmit their cargo to the receiving cells and are then degraded, or be redirected to the adjacent tissue by transcytosis [45]. CAM binding affects EV uptake beyond endothelial cells, and recent evidence demonstrates that the CAM repertoire is likely to influence EV uptake by specific cell types [20]. In addition, uptake of circulating EVs by monocytes is prevented by CD47 [25], and EV uptake by endothelial cells is promoted by CD146 [11]. However, whether CAMs affect the fate of internalized EVs, and in particular message delivery, is not known.

Regarding CTCs, ligand binding precedes their transendothelial migration, a step crucial for metastatic site formation. To facilitate binding, CTCs can modify the vessel environment



(deposition of glycocalyx along the endothelium [17]). For example, mechanical forces can stimulate the luminal deposition of fibronectin (integrin β 1 ligand) in zebrafish [12] and in mouse liver blood vessels [21]. Fibronectin deposits have also been found in human livers from patients with metastasis [21]. However, whether luminal fibronectin favors EV binding remains to be determined. Furthermore, selectins on endothelial cells are necessary for CTC adhesion, rolling, and diapedesis [39]. ICAM-1 present on the endothelial cells forms heterotypic interactions with glycoproteins (such as MUC1 expressed on the surface of breast cancer cells [36]) and homotypic interactions with ICAM-1 on the surface of CTCs [14] and EVs [20], thereby promoting their binding to the vessel. After slowing down in the blood flow, CTCs can directly bind to integrins on the endothelium. For instance, integrin α 5 interacts with NRP-2 receptor on CTCs and promotes extravasation (in pancreatic cancer patients, NRP-2 expression increases with tumor grade [26]). Upon binding to the vessel, the tumor cells transmigrate through the vascular wall where CAMs attach to the components of ECM. CD44v6 molecule adheres to collagens I and IV, as well as to laminin (LN332), and its knockdown impairs the invasive capacity of tumor cells [13]. Talin-1 knockdown decreased cancer cell binding to the ECM (collagen, fibronectin, laminin), potentially because of lack of integrin activation [21].

Although more work will be necessary to identify key, and perhaps common or organ-specific, ligands for EV and CTC binding to the vascular wall, such investigation is likely to expand the repertoire of inhibitory strategies with a potential to succeed in the frame of antimetastatic treatments.

Concluding remarks

Understanding the mechanisms of CTC/EV-specific adhesion molecules and ligands would allow the development of targeted therapies to prevent metastasis. By profiling the CAM signature of specific cancers, the CAM-ligand interaction might be specifically disrupted, thereby preventing the extravasation of CTCs or the niche priming of metastasis-targeted organs, and has high potential for personalized cancer protocols if combined with sensitive early detection of primary tumors. On the other hand, understanding the relationship between CAMs at the surface of EVs and their organotropism could be exploited in novel targeted therapies in which bioengineered EVs containing cargo of interest could be delivered to the diseased tissue. Pioneering studies have used EVs to mediate siRNA delivery to mouse brain [46] or to target oncogenic RAS in a mouse model of pancreatic cancer, resulting in increased lifespan of treated animals [25]. Alternatively, EVs might instruct cells to change their own adhesive potential. In so doing, they are likely to favor the surface presentation of ligands that support the dissemination of tumor cells. Our understanding of the dynamics of the adhesion machineries of CTCs and EVs is only beginning, and many issues remain to be resolved (see Outstanding questions), but this opens a promising area for future research.

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Declaration of interests

The authors declare no conflicts of interest.

Outstanding questions

What is the relative importance of each CAM on CTCs and EVs at each step of the metastatic cascade?

Do EV-loaded CAMs use activation/ conformational changes (i.e., integrins) for targeting the vascular wall?

What are the ligands used by CAMs at the surface of EVs when targeting specific vascular niches?

Are CAM ligands differentially present in the vasculature of different organs?

Do EVs trigger a change in the expression of surface molecules by endothelial cells at PMNs to further promote CTCs adhesion?

Does the CAM expression pattern affect CTC organotropism?

Can we use the identity of EV/CTC CAMs to predict the metastatic organ in patients?

Can we use specific CAM combinations to improve EV/CTC capture from liquid biopsies?

Would CAM-inhibiting strategies targeting EVs and CTCs synergize against metastasis?

Can we bioengineer EVs with specific CAMs to allow targeted compound delivery to metastatic sites?

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