Cancer metastases: challenges and opportunities

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Abstract  Cancer metastasis is the major cause of cancer morbidity and mortality, and accounts for about 90% of cancer deaths. Although cancer survival rate has been significantly improved over the years, the improvement is primarily due to early diagnosis and cancer growth inhibition. Limited progress has been made in the treatment of cancer metastasis due to various factors. Current treatments for cancer metastasis are mainly chemotherapy and radiotherapy, though the new generation anti-cancer drugs (predominantly neutralizing antibodies for growth factors and small molecule kinase inhibitors) do have the effects on cancer metastasis in addition to their effects on cancer growth. Cancer metastasis begins with detachment of metastatic cells from the primary tumor, travel of the cells to different sites through blood/lymphatic vessels, settlement and growth of the cells at a distal site. During the process, metastatic cells go through detachment, migration, invasion and adhesion. These four essential, metastatic steps are inter-related and affected by multi-biochemical events and parameters. Additionally, it is known that tumor microenvironment (such as extracellular matrix structure, growth factors, chemokines, matrix metalloproteinases) plays a significant role in cancer metastasis. The biochemical events and parameters involved in the metastatic process and tumor microenvironment have been targeted or can be potential targets for metastasis prevention and inhibition. This review provides an overview of these metastasis essential steps, related biochemical factors, and targets for intervention.
1. Introduction

Cancer metastasis is a process in which cancer cells disseminate from the primary tumor, settle and grow at a site other than the primary tumor site. Most cancer deaths are caused by cancer metastasis not the primary tumor. Cancer metastasis is the primary cause of morbidity and mortality and responsible for about 90% of cancer deaths\(^1\). It is now accepted that tumor distribution and secondary site growth is not a matter of chance, but rather it is determined by the dependence of the ‘seeds’ (the cancer cells) on the ‘congenial soil’ (the target organ for metastasis) as proposed by the English surgeon Stephen Paget in 1889\(^2\). Until recently, cancer research has primarily focused on the development of methods/agents that can detect tumor at the early stage, and on agents that inhibit tumor growth. Advances in early cancer detection and treatment have rendered that most solid tumors are now manageable or curable if they are diagnosed and treated before metastasis. However, once cancers spread beyond the initial primary site, they are usually highly incurable and fatal\(^3\). Due to a lack of understanding of the mechanisms that underlie the metastatic process, limited success has been made on prevention and inhibition of cancer metastasis.

Metastasis is a complicated event that involves multiple sequential and interrelated steps and multi-biochemical events with much to be elucidated. Metastasis is facilitated by four essential steps: detachment, migration, invasion and adhesion. Cancer cells first detach from the primary tumor, undergo migration, invasion, and travel to different sites through blood and lymphatic vessels, then settle (adhesion) and grow. Metastasis is regulated by various signaling pathways and is affected by the surrounding extracellular matrix (ECM). It is now known that metastasis genes are stress-response genes that physiologically contribute to inflammation, wound healing, and stress-induced angiogenesis\(^4\). This review is aimed to provide an overview of the metastasis process and targets for intervention with a focus on cancer cell detachment, migration, invasion and adhesion. It is not the intent of this review to provide an in-depth description of each parameters related to the four essential steps and relevant intervention targets since each topic itself can be a lengthy review. It is hoped that this review can serve as a lead for readers who are interested in cancer metastasis and intervention.

2. Cancer metastasis

Cancer metastasis is a process of dissemination of tumor cells from a primary tumor mass to a different site through blood vessels and lymphatic vessels (Fig. 1). It is a complex succession of a series of cell-biological events termed the “invasion–metastasis cascade”. The cascade involves the development of new blood vessels (angiogenesis), departure of metastatic cells from the primary tumor (detachment and migration), invasion through the basement membrane (BM) and ECM surrounding the tumor, invasion of the BM supporting the endothelium of local blood and lymphatic vessels, intravasation of the metastatic cells into the blood and/or lymphatic vessels, adhesion of the circulating metastatic cells to the endothelium of capillaries of the target organ site, invasion of the cells through the endothelial cell layer and the surrounding BM (extravasation), and finally the settling and growth of secondary tumors at the target organ site\(^5,6\). Fig. 1 provides a brief overview of the process.

Metastatic cell dissemination requires that cells first detach from the primary tumor\(^7,8\). Under normal circumstances, epithelial and endothelial cells will undergo apoptosis (programmed cell death) when detached, a phenomenon referred to as anoikis (induction of apoptosis caused by detachment from the ECM)\(^9\). During the process of anoikis, both death receptor pathways and mitochondrial pathway are activated\(^10\). This is a mechanism designed to protect multicellular organisms from cells establishing themselves outside...
their correct anatomical location. Normal epithelial cells require attachment to the ECM for survival. Metastatic cells must develop a mechanism to adapt and survive when detached from the ECM or in the absence of the ECM. In other words, they should develop a mechanism to resist anoikis11,12. The resistance to anoikis together with some other property changes of the tumor cells (such as changes in cell-to-cell or cell-to-matrix adhesion, cell polarity, and cell invasive and migratory property) are collectively known as the epithelial–mesenchymal transition (EMT). EMT is a characteristic feature of most metastatic cells11. Specifically, epithelial cells are transformed from highly differentiated, polarized, and organized cells into undifferentiated, isolated, and mesenchymal-like cells with migratory and invasive properties13. Additionally, many tumor cells also show changes in their plasticity via morphological and phenotypic conversions during cancer progression. These changes, in addition to EMT, include collective amoeboid transition (CAT) and mesenchymal to amoeboid transition (MAT)11. EMT enables cells to increase migratory and invasive capabilities through formation of invasive protrusions (invadopodia) while CAT and MAT enables cells to increase migratory capability through formation of non-invasive protrusions (lamellipodia and filopodia). Protrusions are the extended parts formed at the leading edge of motile cells. Lamellipodia and filopodia are also present in normal epithelial cells while invadopodia are mostly observed with metastatic cells (more discussion of protrusions in Section 2.1)11. Interestingly, EMT in tumor cells is transient. Before a metastatic cell settles down and grows, it needs to reverse its mesenchymal to a more epithelial phenotype, a conversion known as mesenchymal–epithelial transition (MET). The contribution of MET to cancer progression is still unclear12.

It is known that not all tumor cells are metastatic, nor are all cells within metastatic tumors capable of metastasizing7. The four essential steps of the cancer metastatic process (detachment, migration, invasion and adhesion) are distinct from each other but also interrelated. For example, cell migration involves cell detachment, adhesion and invasion, while invasion involves migration and adhesion. An understanding of these four steps and their role in cancer metastasis helps understand the metastatic process and also identify targets for intervention.

2.1. Cancer cell adhesion, detachment, migration and invasion

2.1.1. Cell adhesion

Cell adhesion basically refers to cell attachment among cells (cell–cell adhesion) and with cells' environment, mostly the ECM (cell–matrix adhesion). Physiologically, cells are held within their defined boundary through tight cell–cell adhesion and cell–matrix adhesion. Cell adhesion helps establish tight connections both between cells and between cells and the matrix. Since cellular motility is an essential part of cancer metastasis, and adhesion and de-adhesion (detachment) are prerequisites for cellular motility3, cell adhesion is critical for cancer metastasis. Adhesion is also involved in the settling of metastatic cancer cells at a distant site. Further, cell adhesion is not just a way to link cells or link cells with the ECM, but it also serves as a mechanism to activate cell proliferation and survival pathways through integrins’ interactions with downstream molecules that are essential for motile function and survival11.

Adhesion is primarily achieved by connecting intracellular cytoskeleton between cells (cell–cell adhesion) or connecting cellular cytoskeleton with ECM components such as collagen, fibronectin, fibrinogen, and laminin (cell–ECM adhesion) through a group of cell adhesion molecules (CAMs). CAMs are surface glycoproteins that are typically transmembrane receptors made up of three domains: intracellular domain, transmembrane domain, and extracellular domain. CAMs primarily include calcium-dependent CAMs (cadherins, integrins or selectins) and calcium-independent CAMs [the immunoglobulin superfamily (Ig-SF) and lymphocyte homing receptors (CD44)]12. Different types of CAMs are responsible for adhesion in different types of cells. For example, E-cadherins are responsible for epithelial cell–cell adhesion and R-cadherins are for retinal cell adhesion13,14. CAMs are critical for cell adhesion. A brief description of the structures and functions of CAMs is presented below.

2.1.1.1. Integrins. Integrins are responsible for cell–ECM adhesion. They are members of a glycoprotein family that form heterodimeric receptors for ECM molecules such as fibronectin (FN), laminin (LN), collagen (Col), fibrinogen, and vitronectin (VN). They are composed of α and β subunits with non-covalent bonds connected to each other. Both α and β subunit contains a large extracellular domain, a transmembrane domain, and a short intracellular domain. There are at least 19 α and 8 β subunits that dimerize to yield at least 24 different integrin heterodimers with distinct ligand binding and signaling properties15.

Cell adhesion to ECM is essentially achieved through integrin-mediated linkage to extracellular ECM molecules and intracellular cytoskeleton. The large extracellular domain of integrins bind to ECM molecules while the intracellular domain is linked to cytoskeleton through intracellular focal adhesions (FAs) as demonstrated in Fig. 2. FAs are supramolecular complexes formed by more than 150 different proteins, including kinases, scaffold, and adaptor proteins, as well as actin linking proteins14. FAs also mediate intracellular signaling pathways and are dynamic structures which assemble, disperse, and recycle during cell migration11,13. Binding of integrins to FAs and ECM molecules not only serve as a way for cell adhesion to ECM, but also relay the forces produced by the cytoskeleton onto ECM to generate the traction needed for cell adhesion and migration, and to transmit signals from the extracellular environment to the intracellular network, as well as signals from the intracellular network to extracellular environment. The transmission is mediated by integrin-activated signaling molecules, such as focal adhesion kinase (FAK), phosphatidylinositol 3-kinase (PI3K), and members of the extracellular signal-regulated kinase 1 and 2/mitogen activated protein (ERK1 and 2/MAP) kinase family to regulate cell proliferation, migration, and apoptosis of tumor and endothelial cells (Fig. 2)10.

Therefore, integrins are not only involved in adhesion but also in migration and invasion. They are important for cell motility due to their ability to modulate physical interactions with ECMs and to regulate signaling pathways that control actin cytoskeleton dynamics and cell movement11. They also play critical roles in regulating other biological processes, such as apoptosis, proliferation, survival, and differentiation through integrin-mediated downstream signaling pathways13.

During cancer differentiation and metastasis processes, up-regulation of integrins has been linked to cancer invasiveness15-17. The subunits α3, α5, α6, αv, β1, and β3 are expressed in metastatic cells and can be considered as indicators for metastasis18. Integrins mediate the synthesis of cyclins and inositol lipids as well as activation of FAK and mitogen-activated protein kinase (MAPK)11,19. Integrins also facilitate the metastatic process by proteolytically degrading the basement membrane through the activation of matrix metalloproteinases (MMPs)20,21. MMPs are the primary proteases responsible for
ECM degradation during cancer metastasis. In addition, integrins regulate tumor cell motility via Rho-A signaling cascade. Integrins promote invasion by activation of PI3K and Src which is a proto-oncogene encoding a tyrosine kinase. The various roles integrins play in cancer metastasis and proliferation make integrins an attractive target for cancer therapy.

2.1.1.2. Cadherins. Cadherins are a superfamily of transmembrane glycoproteins mediating homophilic (same type of cells) cell–cell adhesion. More than 20 members of the cadherin molecules have been reported with cell type-specific expression manner such as E-cadherins in epithelial cells, N-cadherins in mesenchymal cells (such as stromal cells), VE-, P-, and R-cadherins in vascular endothelial, placental, and retinal tissues, respectively. Two cadherins of the same type from adjacent cells interact in a non-covalent manner to hold two cells tightly together. E-cadherin with E-cadherin from the adjacent cells were tightly linked in a calcium-dependent manner. The intracellular domain of the cadherin is connected to cytoskeleton (α-actinin, vinculin, and actin cytoskeleton) through linker proteins (α-catenin, β-catenin, and p120 catenin). Cell adhesion among other cells is achieved in a similar way except different CAMs are employed.

ECM degradation during cancer metastasis. In addition, integrins regulate tumor cell motility via Rho-A signaling cascade. Further, integrins promote invasion by activation of PI3K and Src which is a proto-oncogene encoding a tyrosine kinase. The various roles integrins play in cancer metastasis and proliferation make integrins an attractive target for cancer therapy.

Figure 2  Illustration of cell detachment, cell–cell adhesion and cell–matrix adhesion of epithelial cells by E-cadherins and integrins respectively. Cell detachment: cell detachment from ECM can occur through breakage of adhesion proteins at both intracellular site and extracellular site. Cytosolic cleavage can be achieved through both mechanic forces and enzymatic cleavage while extracellular cleavage is primarily achieved through cleavage by proteases such as MMP. Cell–matrix adhesion by integrins: cell–matrix adhesion is achieved through interaction of integrins with intracellular cytoskeleton and extracellular ECM components. The large extracellular domain of integrin binds to ECM components such as FN, LN, Col, fibrinogen, and VN. The intracellular domain is connected to cytoskeleton through focal adhesions. Cell–cell adhesion by cadherins: cell A and cell B are tightly linked by E-cadherins at adherent junction. The extracellular domain of the same type of cadherin (homodimers) (e.g., E-cadherin with E-cadherin) from the adjacent cells were tightly linked in a calcium-dependent manner. The intracellular domain of the cadherin is connected to cytoskeleton (α-actinin, vinculin, and actin cytoskeleton) through linker proteins (α-catenin, β-catenin, and p120 catenin). Cell adhesion among other cells is achieved in a similar way except different CAMs are employed.
In contrast to E-cadherin, N-cadherin, which is not expressed in normal mammary epithelial cells but expressed in stromal cells, e.

g., fibroblasts, has been found to be increased in prostate cancer, breast cancer, and liver cancer. N-cadherin is one of the

mesenchymal cadherins, and involved in adhesion of cells to stroma. Down-regulation of E-cadherin with the concomitant up-

regulation of N-cadherin reduces cancer cell adhesion ability to epithelial cells, increases adhesion to stromal cells, and leads to

subsequent invasion of tumor cells into stroma. N-cadherin promotes cell migration and metastasis regardless of the expres-

sion and function of E-cadherin. The critical roles of N-

cadherin in tumor cell adhesion and migration make the protein an attractive target for cancer therapy.

2.1.1.3. Selectins. Selectins are vascular cell adhesion molecules (VCAMs) involved in adhesive interactions of leukocytes, plate-

lets, and endothelial cells that mediate leukocyte trafficking and hemostasis. Selectins are involved in processes of immune

response, wound repair, inflammation, and hemostasis. Reports showed that at least one selectin (P, L, or E) is capable of binding

to any human carcinoma, which demonstrates the potential of selectins to mediate contacts with tumor cells within vasculature.

The absence of L-selectin, constitutively expressed on cell surfaces of almost all leukocyte subtypes, leads to significant attenuation of

metastasis. Inhibition or downregulation of E-selectin expression results in attenuation of liver metastasis. On the other hand, its

upregulation redirects metastasis to the liver.

2.1.1.4. The immunoglobulin superfamily (IgSF). IgSF [Ig-cell adhesion molecules (Ig-CAM)] is a large group of cell surface proteins that are involved in the binding, recognition, and adhesion processes of cells, and are classified based on shared structural features with immunoglobulins. IgSF members mediate calcium-independent adhesion through their N-terminal Ig-like domains, which commonly bind other Ig-like domains of the same structure on an adjacent cell surface but may also interact with integrins and carbohydrates. Its C-terminal intracellular domains often interact with cytoskeletal or adaptor proteins through which their extracellular interactions can lead to signaling within the cell, enabling these proteins to function in a range of normal biological processes and/or tumor genesis. They play important roles in antigen recognition, leukocyte trafficking, and formation and maintenance of endothelial cell junctions. A number of IgSF molecules have been identified as biomarkers for cancer progression. For example, melanoma CAM (MCAM) has been implicated in the progression of melanoma, breast, and prostate cancer, neuronal cell adhe-

sion molecule, member of the L1 protein family CAM (L1CAM), neural CAM (NCAM), platelet endothelial CAM (PECAM-1), alypsia CAM (ALCAM), and intercellular CAM-1 (ICAM-1) have been related with metastatic cancers including melanoma, glioma, breast, ovarian, endometrial, prostate, and colon. NCAM led to susceptibility to apoptosis in murine lung tumor cells. Further, NCAM, MCAM, ALCAM, and L1CAM were found up-regulated in cells following the loss of E-cadherin expression and associated with an active, mobile state that retains enough cell–cell junctions to allow a group of cells to move as a unit in invading melanoma and colorectal carcinoma.

2.1.1.5. CD44 members. CD44 members have a single pass transmembrane glycoprotein involved in cell–cell, cell–matrix

adhesion, and cell signaling. CD44 are lymphocyte-homing receptors and play an important role in lymphocyte homing, inflammation, cell signaling, adhesion, migration, aggregation and hyaluronan (HA) decomposition, lymphocyte activation, myelo- and lymphopoiesis, angiogenesis, and clearance cytokines. CD44 proteins also regulate growth, differentiation, survival, and migration, which are all involved in tumor development and metastasis. They are expressed in different tissues including lung, liver, central nervous system, and pancreas. CD44 family differs in extracellular domain by addition of variable states through alternative splicing. The extracellular N-terminal of CD44 mediates binding to its primary physiologic ligand HA and to extracellular matrix proteins such as collagen, laminin, fibronectin, L-selectin, and osteopontin. The intracellular C-terminal is attached to actin cytoskeleton, ezrin, and ankyrin, which are vital not only in cell migration but also in signal transduction. All physiological functions of CD44 are related, in one way or another, to cell adhesion. The most important property of CD44 is its ability to bind HA, a vital factor for the metastatic process. Therefore, inhibition of HA binding to CD44 appears to interfere with events that are critical for tumor development like angiogenesis, apoptosis inhibition, and invasion.

2.1.2. Cell detachment

Cell detachment refers to a process by which cells detach mostly from the ECM. It is the first required step in the metastatic cascade. Detachment is not a simple reversal process of cell adhesion and remains poorly understood. Kirfel and colleagues describe a rear detachment during cell migration process in embryogenesis, tissue repair and regeneration as well as cancer and the inflammatory response. The paper provides some light on the cell detachment process. Cell detachment involves both mechanical forces and protease-mediated cleavage. As presented in Fig. 2, mechanical forces generated by actomyosin-driven contraction are believed to contribute to the dissociation of substrate adhesions at both the cytosolic site and the extracellular site. The cytosolic dissociation of cell–substrate adhesions can also be performed by the calpain cysteine proteases, by phosphorylation/dephosphoryla-
tion of cytosolic adapter proteins and by posttranslational modification of integrins or adapter proteins. Extracellular dissociation of cell–substrate adhesions can be achieved by proteolytic cleavage of matrix constituents mediated by matrix proteases or through shedding of matrix receptors such as integrins by specific sheddases leaving parts of the receptors on the substrate (Fig. 2).

Cell detachment from the ECM in normal epithelial cells and endothelial cells simultaneously triggers down-regulation of Bel-41, (an anti-apoptotic component of the mitochondrial pathway) and up-regulation of Fas ligand (FasL) (an activator of the death receptor pathway) within a minute resulting in anoikis. Metastatic cancer cells need to develop a mechanism to resist anoikis in order to survive. Although our knowledge on ECM-detachment and cell death has grown exponentially, how metastatic cancer cells adapt and adopt mechanism(s) that help evade anoikis is only beginning to be understood. This includes the alteration of enzyme systems in the signaling pathways that regulate anoikis, such as small GTPases and effectors, receptor tyrosine kinases and other kinases, NF-κB, and EMT factors. In addition to anoikis, it is also noted that there are multiple mechanisms (anoikis-independent) by which normal epithelial cells would die once detached from the ECM. Metastatic cancer cells must overcome these anoikis-dependent and anoikis-independent barriers in order to survive once they lose the attachment to the ECM. To effectively
eliminate metastatic cancer cells, it is suggested that both anoikis-independent and anoikis-independent pathways should be targeted. Fortunately, many of the signaling pathways are already the targets of current FDA-approved therapeutic drugs such as bevacizumab (Avastin) against VEGF, ramucirumab (Cyramza) against VEGF receptor, cetuximab (Erbitux) and panitumumab (Vectibix) against EGF receptor. An excellent review on current understanding of the signaling pathways that regulate anoikis (anti-anoikis and pro-anoikis) and how the pathways are altered to evade anoikis by cancerous cells is provided by Buchheit and colleagues. The review also provides an insight of anoikis-independent cell death.

2.1.3. Cell migration and invasion
The migratory and invasive abilities of cancer cells are two critical parameters of the metastatic cascade. Metastatic cells achieve penetration of the ECM through two different mechanisms: mesenchymal (fibroblastoid) cell migration and amoeboid cell migration. Mesenchymal cell migration depends on protease activities (protease-dependent) to degrade the ECM for cell passage. Inhibition of ECM degrading proteases, such as MMPs, is effective in inhibiting mesenchymal cell migration. It needs to be noted that mesenchymal cell migration is not only used by cancer cells; it is also seen with normal untransformed cells, such as fibroblasts, and endothelial and smooth muscle cells. Amoeboid cell migration is a protease-independent process where cells employ mechanical forces to open a path in the ECM instead of degrading them. The hallmarks for amoeboid cell invasion are ECM loose attachment, complete cell polarity loss, and chemotaxis capability.

Invasive migration of metastatic cancer cells. A metastatic cell reorganizes its actin cytoskeleton and concomitantly forms F-actin-rich membrane protrusions (lamellipodia, filopodia, podosomes, and invadopodia) at the leading edge of motile cells. The protrusions are critical for migration and invasion through the use of mechanic forces and protease activities. Fig. 3 demonstrates the process of an epithelial cancer cell migrating and penetrating BM. The protrusions extend forward adhesion to their surroundings followed by trailing end contraction.

The F-actin-rich protrusions include non-invasive protrusions (lamellipodia and filopodia) and invasive protrusions (invadopodia). Lamellipodia and filopodia are present in normal epithelial cells while invadopodia are mostly observed with metastatic cells. Protrusions are named based on their shapes, i.e., filopodia (needle shape), pseudopodia (round), lobopodia (cylindrical), and lamellipodia (flat veils) and invadopodia (similar to lamellipodia but with ECM proteolytic activity). Functionally, protrusions serve as the cells’ sensory organ (filopodia) for signals like chemotactants and nutrients, or the main organelle for cell locomotion (lamellipodia), or for both cell locomotion and degradation of the ECM through the use of proteases (invadopodia). Lamellipodia also interact and attach to their environment via different adhesion molecules, including integrins and cadherins. Filopodia and lamellipodia are highly interactive and interconvertible structures.

Invadopodia appear when cell adhesion junctions and matrix need to be concomitantly degraded. Invadopodia share an actin filament-rich core and a multicentric protein complex surrounding the actin core, including integrins and integrin-associated proteins like vinculin, talin, and paxillin. They mediate ECM proteolysis through different MMP expression. Formation of invadopodia involves initiation, assembly, and maturation and is initiated by growth factors such as EGF (epidermal growth factor), PGEF (platelet-derived growth factor), TGF-β (transforming growth factor β), and/or α6β1 integrin. Growth factor receptor signaling activates PI3K leading to Src activation, which in turn phosphorylates multiple proteins including Tks5 (tyrosine kinase substrate). Assembly involves actin polymerization. Proteins involved in this process include cortactin (key regulator of actin polymerization), MENA (regulator of actin polymerization), Tks, etc. Maturation involves Src kinase, integrins, and proteases recruiting (including MMPs). Src kinase is a major regulator of invadopodia formation and function.

Structurally, invadopodia can be divided into three parts: proteolytic domain, invasive domain, and adhesive domain (Fig. 3). The proteolytic domain contains primarily proteases (MMPs, ADAM (a disintegrin and metalloproteinase, also known as sheddases) and serine proteases). The invasive domain is localized inside invadopodial protrusions into the ECM and composed of actin and actin-associate proteins such as cortactin and MENA. The protein interactions within this domain lead to actin polymerization that provides mechanical forces to move the cell. The adhesive domain is localized at the edges and anchored to the ECM. It achieves adhesive function through integrin-mediated adhesion.

Invadopodia have been identified in numerous cancer cell lines, including malignant melanoma, breast cancer, glioma, and head...
and neck malignancies\textsuperscript{11}. It needs to be noted that invadopodia also appear in some normal cells such as immune cells when they have to cross tissue boundaries\textsuperscript{11}.

Metastatic cancer can invade and migrate either as single cells or as a collective group of cells\textsuperscript{11,62}. While cancer cells migrating as single cells can employ either the protease-dependent mesenchymal migration or protease-independent amoeboid-like migration, collective cell migration employs only mesenchymal cell migration.

Single cells that leave a primary tumor at its periphery involve the loss of epithelial polarity and the achievement of a mesenchymal morphology through EMT. The essential characteristics of EMT are the disruption of tight cell–cell contacts, and acquisition of a fibroblastoid spindle-shape morphology, with increased invasiveness and cell–stroma interactions, as well as slower rates of cell division; altogether, these can result in the release of single cells from a solid epithelial tumor\textsuperscript{62}.

In contrast to single cell migration, cells that migrate collectively retain their cell–cell junctions through continuous expression of adhesion molecules. They migrate as sheets, strands, tubes or clusters and can remain connected to the primary tumor (coordinated invasion) or move as detached cell groups or clusters (cohort migration). Collective cell migration requires force generation for pulling cells from the front or pushing them from the rear. This energy is provided by substrate binding integrins in leading cells. Therefore the leading edge expresses $\beta 1$ and $\beta 3$ integrins to mediate adhesion complexes in order to connect to ECM components such as fibronectin\textsuperscript{11}. ECM attachment activates cytoskeletal adaptor proteins such as cortactin, vinculin, paxillin, and talin. As with single invasive cells, collectively migrating cells form membrane protrusions and integrin-mediated focal adhesions that are connected to the actin cytoskeleton. To penetrate the ECM, the leading cells generate an invasion path by the use of $\beta 1$ integrin-mediated focal adhesions and local expression of MT1-MMP (MMP14) at their leading edges to cleave the collagen fibers and to orient them in tube-like structures in which the following cell mass can migrate\textsuperscript{65,66}. Collectively migrating cells do not retract their cellular tails, but instead exert mechanical forces, such as moving by pulling on adjacent cells that are connected by adhesion junctions\textsuperscript{67}. This clustered cohort-like cancer cell dissemination appears to be highly efficient in embolizing lymphatic or blood vessels and in cell survival in the circulation\textsuperscript{11,62}. Collective cell migration is mainly seen for basal cell carcinomas and squamous cell carcinomas of various origins\textsuperscript{62}.

2.2. Tumor microenvironment and cell motility\textsuperscript{3,11,68}

It is now well understood that tumor proliferation and metastasis are not an act-alone events of tumor cells, since tumor is required to interact with its microenvironment [tumor microenvironment (TME)]\textsuperscript{69–73}. As presented earlier, tumor interactions with ECM components are essential in EMT and acquisition of tumor invasive abilities (invadopodia formation and function, proteases secretion and function, actin polymerization, etc.). TME is closely involved with all four essential steps (adhesion, detachment, migration, and invasion) of the metastatic process.

Structurally, TME includes tumor’s surrounding and supportive stroma, different effectors of the immune system, blood platelets, fibroblasts, endothelial cells, proteases, cytokines, hormones and other humoral factors. Together, these components are involved in a complex crosstalk with tumor cells that affects growth, angiogenesis, and metastasis\textsuperscript{69,74}. The mechanisms of the interactions between tumor cells and the TME are complex and can fall into two main categories: contact-dependent mechanisms that involve cell–cell and cell–ECM adhesion molecules, and contact-independent mechanisms that involve soluble molecules such as growth factors, chemokines, and cytokines.

2.2.1. Chemokines

Chemokines are peptide signaling cytokines that act as a chemoattractants to guide the migration of cells. They are involved in a variety of physiological and pathological conditions including lymph node organogenesis, inflammation, infection, tissue repair, initiation, and progression of cancer\textsuperscript{11}. Chemokine receptors are expressed in a variety of cancers\textsuperscript{11}. Additionally, to shift the microenvironment to a metastasis-promoting state, cancer cells need to either transform the resident normal stroma cells to facilitate their growth/invasion or recruit other metastasis-promoting stromal cells to remodel the microenvironment. Some of chemokines are shown to have involvement in this process\textsuperscript{75}.

2.2.2. Growth factors

In addition to cell growth stimulation and cell proliferation, many growth factors, such as VEGF (vascular endothelial growth factor), FGF (fibroblast growth factor), PDGF, CTGF (connective tissue growth factor), HGF (hepatocyte growth factor), and EGF, are fully involved in cell migration, angiogenesis, lymphangiogenesis, EMT, and regulation of cell adhesion, etc\textsuperscript{76}. For example, activation of ErbBs ligands, a family member of EGF-related peptides, and overexpression of EGFR (EGF receptor) significantly increase tumor cell motility and intravasation\textsuperscript{77}. In addition, HGF/Met signaling was found to be involved in a variety of cellular processes including cell motility. HGF and Met have also been indicated in the modulation of actin cytoskeleton and MMPs secretion\textsuperscript{11,78}. CTGF/CCN2 family member 2 (CCN2) has been reported to be associated with extracellular matrix remodeling, angiogenesis, chemotaxis, cell adhesion and migration, and expression of MMPs. Overexpression of CCN2/CTGF was associated with invasive potential of lung and breast cancers. Neutralizing CCN2/CTGF with an antibody attenuated metastasis of pancreatic cancer\textsuperscript{79}. Inhibition of CCN2/CTGF expression could lead to inhibition of migration\textsuperscript{79}. Growth factor signaling pathways have been effective targets for cancer growth inhibition and metastasis inhibition\textsuperscript{79}.

2.2.3. Cancer-associated fibroblasts (CAFs)

CAFs are distinctive cell types recognized as constituting part of the carcinoma and increasingly implicated as functional participants in tumor formation and progression\textsuperscript{12}. CAFs have been shown to enhance primary tumor growth and promote malignancy via HGF secretion\textsuperscript{80}. Moreover, fibroblast HGF expression can be induced by tumor cells due to cytokine secretion such as IL-1\textsuperscript{81}. In a similar manner, CAFs have been found to express highly stromal cell-derived factor-1 (SDF-1), implicating the tumor growth-promoting role of fibroblasts, and to provide chemotactic signals for cancer cell invasion and migration\textsuperscript{82,83}. CAF signaling can be a target for cancer treatment\textsuperscript{11}.

2.2.4. Tumor-associated macrophages (TAMs)

The macrophages within tumor are referred to as tumor-associated macrophages. Upon activation by cancer cells, the TAMs can release a vast diversity of growth factors, proteolytic enzymes, cytokines, and inflammatory mediators. TAMs promote cancer metastasis through several mechanisms that include tumor angiogenesis, tumor growth, and tumor cell migration and invasion.
Highly motile TAMs can also control actin cytoskeleton remodeling pathways in metastasis\(^1\).

### 2.2.5. Proteases and MMPs

Proteases are enzymes that cleave protein peptide bonds. At least 500–600 proteases have been identified that cleave peptide bonds through different catalytic mechanisms\(^1\). Proteases employ serine, cysteine, and threonine residues or a water molecule as a nucleophile in the active site. They serve various different functions such as activation and inactivation of enzymes, activation of growth factors, gene expression, cell differentiation, cell cycle progression, cell proliferation, and cell death. The link between proteases and cancer was identified in 1946 by Fisher who proposed that tumor-associated proteolytic activity could be responsible for the degradation of the cell matrix and tumor cell invasion into the surrounding normal tissues. The major enzymes responsible for matrix degradation are matrix metalloproteases (MMPs). In addition to MMPs, serine proteases are also involved in matrix degradation\(^1,84\).

MMPs are a family of zinc-dependent endopeptidases capable of cleaving the basement membrane and all ECM constituents. MMPs are produced by different cells including endothelial cells, leukocytes, macrophages, fibroblasts, and tumor cells\(^1\). They are synthesized as inactive enzymes and activated outside the cell by other MMPs or serine proteinases\(^1\). MMPs consist of at least 26 proteases and are subdivided into four groups: collagenases, gelatinases, stromelysins, and matrilysins\(^1\).

MMPs' role in metastasis is complex. In addition to cleavage of ECM, their targets also involve growth factor receptors, cytokines, chemokines, CAMs, apoptotic ligands, and angiogenic factors that contribute to all stages of tumor progression such as proliferation, adhesion, migration,angiogenesis, apoptosis, and evasion of the immune system\(^1\).

The serine proteases (SPs) are one of the largest preserved multigene proteases with well-described roles in the different processes including blood coagulation, wound healing, digestion, immune response, tumor growth, invasion, and metastasis.\(^1,11\) uPA (urokinase plasminogen activator) was first demonstrated by Duffy et al.\(^87\) and is a cell surface serine protease that is involved in ECM degradation, cancer invasion, and metastasis\(^11\). It has been demonstrated that the uPA system is able to induce human cancer cell proliferation by the proteolytic activation of factors such as HGF, TGF-\(\beta\), and basic fibroblast growth factor (bFGF) or through uPA receptor interaction with integrins and following activation of the FAK and EGF tyrosine kinase receptors\(^11\).

### 3. Targets for intervention

Because metastatic cancer shares a number of common biochemical parameters and steps, targeting these parameters and steps has unique advantages in controlling metastasis as compared to targeting the parameters that control cancer growth; the latter is the base of most current chemotherapeutic agents. Various steps related to EMT, anoikis, cell motility, and tumor microenvironment have been targeted. The biochemical parameters and steps involved in motility are especially metastatic unique. The intervention methods employed include the use of miRNA, monoclonal antibodies, and small molecules\(^1\).

#### 3.1. Targeting EMT and anoikis

Blocking EMT and/or reversing anoikis-resistance are rational approaches to inhibit cancer proliferation and metastasis\(^10\). Sakamoto and Kyprianou\(^10\) provide an excellent review for the current understanding of molecular signaling networks involved in anoikis and the development of agents that reverse anoikis-resistance. The review also provides the current status of various classes of agents developed. These agents include quinazoline-based anoikis inducers as well as inhibitors of PPAR\(\gamma\), TrkB, and SRC (Fig. 4)\(^10\).
3.1.1. The quinazoline-based anoikis inducers

The quinazoline-based anoikis inducers started with the finding that two quinazoline α1-adrenoceptor antagonists [doxazosin and terazosin (Fig. 4)] exhibit significant anti-tumor activity through induction of receptor-mediated apoptosis involving death-inducing signaling complex (DISC) formation/caspase-8 activation and inhibition of Akt activation88–90. Structural modification of these two lead compounds led to more potent analogs DZ-3 and DZ-50 (Fig. 4) that showed effective induction of anoikis and inhibition of cell migration and adhesion of prostate cancer cells91.

3.1.2. PPARγ inhibitor

Peroxisome proliferator-activated receptor gamma (PPAR-γ) of epithelial cell tumors91. Inhibition of PPAR-γ recently become a putative therapeutic cancer target in a variety of epithelial cell tumors92. and Gandellini provide a good overview of miRNA-based therapy12. and Src98.

3.1.3. TrkB inhibitor

The Neurotrophic tyrosine kinase receptor (TrkB) has been found to be a potent anoikis suppressor. TrkB overexpression in nonmalignant cells promotes the formation of lung and heart metastases. Consistently, overexpression of TrkB is frequently found in many aggressive gastric and prostate carcinomas11,92–94. Trk inhibitor CEP-751 (Fig. 4) was demonstrated to exhibit antitumor activity95. Later, a soluble lysyl-β-alanyl ester of CEP-751 named CEP-2563 dihydrochloride (Fig. 4) was also reported and underwent clinical trials96.

3.1.4. SRC inhibitor

The Src kinases are currently being investigated as valuable therapeutic targets for cancer treatment. Several Src inhibitors are now in clinical development, including dasatinib, bosutinib, and saracatinib. Among these Src inhibitors, dasatinib (Fig. 4) is the most clinically studied SRC inhibitor97. In orthotopic nude mouse models, dasatinib treatment effectively inhibits both tumor growth and development of lymph node metastases in both androgen-sensitive and androgen-resistant prostate cancer98. Dasatinib suppresses cell adhesion, migration, and invasion of prostate cancer cells by blocking the kinase activities of the SFKs, Lyn, and Src98.

3.1.5. Metastatic-related endogenous miRNAs

Metastatic-related endogenous miRNAs have been found to play a significant role in various steps of the metastatic cascade. Depending on their roles, metastatic-related miRNAs are referred to as pro-metastatic or anti-metastatic. Potentially this can be a basis to develop therapeutic intervention for the prevention and cure of metastatic cancer through either inhibiting pro-metastatic or by over-expressing anti-metastatic miRNAs. Efforts have been made to develop anti-miRNA oligonucleotides with different chemical modifications to affect EMT and anoikis92. Profumo and Gandellini provide a good overview of miRNA-based therapy12.

3.2. Targeting cell motility

Cell motility plays an essential role in cancer cell detachment, migration, invasion, and adhesion. Interference of cell motility becomes an appealing approach in developing agents for the treatment of metastatic cancer3. The approaches primarily include inference of interactions of CAMs with their targets and related signaling pathways, and interference of the formation and function of invadopodia.

3.2.1. Interference of CAMs’ interaction with their targets and related signaling pathways

3.2.1.1. N-cadherin inhibitors. The critical roles of N-cadherin in tumor cell motility make N-cadherin an attractive target for the inhibition of cancer metastasis. The first synthetic N-cadherin antagonist, a linear decapeptide (N-Ac-LLRAHADVNGNH2), was described in 1990 by Blaschuk and co-workers99. Since then, several types of N-cadherin antagonists have been reported. They include synthetic linear peptides, synthetic cyclic peptides, and non-peptidyl peptidomimetics designed based on the cell adhesion recognition (CAR) sequence His-Ala-Val100. Another type of antagonist is a synthetic linear peptide that harbors a Trp residue in the second position from the N-terminus (similar to N-cadherin)101. One of these peptides is His-Ser-Trp- Thr-Pro-Ser-Gly-Gln-Ser-Lys-NH2. The peptide inhibits endothelial cell tube formation in vitro indicating that it has anti-angiogenic properties13. Additionally, two monoclonal antibodies against the extracellular domain of N-cadherin were reported to inhibit the invasiveness and proliferation of N-cadherin expressing PC3 human prostate carcinoma cells in vitro102. Among all these inhibitors, the most studied cyclic peptide is N-Ac-Cys-His-Ala-Val-NH2 (designated ADH-1) (Fig. 5). ADH1 (Exherin) was the first N-cadherin antagonist that entered clinical trials. ADH1 selectively and competitively binds to N-cadherin and blocks its function. ADH1 has been tested in a phase II clinical trial as a monotherapy and in various phase I combination trials with cytotoxic drugs such as docetaxel, carboplatin, cepacitabine, and melphalan103.

3.2.1.2. Integrin antagonists. Integrins play key roles in cell motility because of their ability to mediate physical interactions with the ECM, cytoskeleton, and signaling pathways that control actin cytoskeleton dynamics and cell movement11. In addition, integrins also play roles in EMT and anoikis through signaling pathways. The α5β1, αvβ3 and αvβ5 integrins are widely expressed in different cancers and recognize the tripeptide Arg-Gly-Asp (RGD) motif present in several ECM proteins104. Integrin antagonists have been found effective in controlling cell proliferation and metastasis11,13,102. The first small molecule integrin antagonist developed was cilengitide (EMD 121974) [Cyclo-(L-Arg-Gly-L-Asp-D-Phe-N(Me) l-Val)]103 which is a cyclic peptide belonging to the RGD-peptide family. Cilengitide binds to the integrin β chain and prevents the interaction of integrins with their endogenous ECM ligands. Cilengitide is effective in treating lung cancer, prostate cancer, melanoma, glioblastoma, leukemia, brain and CNS tumors, breast cancer, and squamous cell cancer and has undergone Phase I and II clinical trials11. 1α-RGD (Fig. 5) is an RGD-like integrin antagonist containing a bicyclic pseudopeptidpeptide that binds αvβ3, αvβ5 and α5β1 integrins with in vitro preferential affinity towards αvβ3. 1α-RGD was demonstrated to decrease cell migration and attachment, disassemble the actin cytoskeleton, reduce FAK phosphorylation, decrease the expression of target integrins at transcriptional level and induce anoikis in human U251 and U373 glioblastoma cell lines that express αvβ3 and αvβ5 and α5β1 integrins101.
ATN-161, an acetylated pentapeptide (Ac-Pro-His-Ser-Cys-Asn-NH₂), was designed based on a sequence of fibronectin (Pro-His-Ser-Arg-Asn) through replacement of Arg with Cys. ATN-161 interferes binding of α5β1 integrin with this region of fibronectin to inhibit cancer growth and metastasis104–106 and has undergone phase I trials11,13.

In addition to these small molecule antagonists, monoclonal antibodies against integrins have also been reported. They include CNT095, etaracizumab (MEDI-522), and volociximab. These antibodies have undergone various phases of clinical trials11,13.

3.2.1.3. Selectin inhibitors. Selectins have been implicated in mediating contacts with tumor cells within vasculature. Inhibition or downregulation of E-selectin expression results in attenuation of liver metastasis. In contrast, its upregulation redirects metastasis to the liver13. Heparin is shown to inhibit P-selectin-mediated interactions of platelets with cancer cell ligands in mice metastatic states107. Thus, compounds having both heparanase and selectin inhibition properties are promising for cancer therapy. Borsig et al.108 reported the novel semisynthetic sulfated trimannose CC-linked dimers (STMCs) (Fig. 5) with inhibitory activity for heparanase and selectin. This STMC hexa-saccharide is an in vivo effective inhibitor of P-selectin with antimetastasis activities in animal models11,108.

3.2.1.4. CD44 antagonists. The CD44 transmembrane glycoprotein family, a hyaluronan receptor, mediates cellular responses to the microenvironment through binding of hyaluronic acid (HA) and other proteins of the ECM. These interactions start intracellular signaling cascades that foster tumor growth, survival and spread109. CD 44 is associated with aggressive tumor growth, proliferation, and metastasis, and has been a target for metastasis treatment24,109. In normal physiology, this receptor has a crucial role in cell adhesion, inflammation, and repair processes109. Systemic use of antibodies against CD44v epitope decreased pancreatic adenocarcinoma metastasis6. Anti-CD44 antibody-targeting radiolabels or anticancer chemotherapeutics have been adopted in some patients6. Immunotoxin, a humanized antibody complexed with a cytotoxic drug mertansine against CD44v6, has entered into Phase 1 clinical trials and was reported to improve the conditions in 30 incurable squamous cell carcinoma patients6. Liu et al.12 showed that miRNA-34a was a negative CD44v prostate cancer cell regulator. It enforced expression of miRNA-34a in CD44+ prostate cancer cells and inhibited clonogenic expansion, tumor regeneration, and metastasis.

3.2.2. Interference with the formation and function of invadopodia

Unlike other actin-based protrusions such as lamellipodia and filopodia that are present in normal cells, invadopodia are uniquely present in invasive cancer cells and considered as the transformed version of podosomes which are present in highly invasive normal cells such as macrophages, osteoclasts and dendritic cells64. The main function of invadopodia in tumors is to promote matrix degradation and tumor invasion. Invadopodia play critical roles during three steps of the metastatic process: invasion into the surrounding stroma, intravasation into the vasculature and extravasation63. Invadopodia has emerged as an appealing target for metastasis prevention and inhibition64.

Intervention of invadopodia can be achieved by impacting the formation, structure and function of invadopodia. For example, since formation of invadopodia involves growth factors, growth factor inhibitors affect invadopodia formation. Suppressing invadopodia formation, structure, and function by inhibiting Src, Twist1 or Tks5 has been convincingly shown to inhibit tumor metastasis in various tumor models64. MMP inhibition also inhibits invadopodia formation112. Blocking MMP activity by inhibitors, antibodies, or siRNA impairs invadopodia function and matrix degradation64,112.

Since invadopodia are only involved in metastasis not cell proliferation, it is suggested that invadopodia inhibitors should be used in combination with an inhibitor of cell proliferation to prevent metastasis as well as to inhibit cancer growth.

3.3. Targeting tumor microenvironment (TME)

As described earlier, TME plays essential roles in the metastasis cascade. Extensive research efforts have been made to interfere with communication of tumors with TME to achieve the inhibition of cancer growth and metastasis. Various TME components and signaling pathways that impact cancer metastasis have been targeted. They include inhibition of proteases, interference with
inflammatory processes, inhibition of integrin signaling, interference with hypoxia processes, and remodeling of ECM.  

3.3.1. Inhibition of growth factor signaling

As presented earlier, growth factor signaling is not only involved in cancer proliferation, but also essential in EMT and acquisition of a tumor invasive abilities. Various inhibitors against VEGF, FGF, PDGF, and EGFR signaling have been developed. These inhibitors include small molecules (Fig. 6) and monoclonal antibodies such as bevacizumab against VEGF, ramucirumab against VEGF receptor, and cetuximab and panitumumab against EGF receptor.  

3.3.2. Inhibition of proteases

A hallmark of tumor cell invasion is upregulation of proteolytic enzymes, especially MMPs. Inhibition of MMPs was considered to be a very promising approach and was studied in a variety of clinical trials as therapy for various types of cancers. Unfortunately, those trials were largely unsuccessful. The disappointing outcomes are probably caused by various factors that include the development of drug resistance by the tumor cells, lack of sufficient specificity of the inhibitors, and changes in the cancer cell migration and invasion mechanism from proteolysis-dependent migration to proteolysis-independent migration (amoeboid cell migration through mesenchymal-amoeboid transition). It has been suggested that the combination of a MMP inhibitor with other chemotherapeutic agents would probably yield a better therapeutic outcome. Presented in Fig. 7 are representative small molecule MMP inhibitors, which are grouped chemically as hydroxamates, thiol-based analogs, pyrimidine-2,4,6-triones and others. Additionally, monoclonal antibodies against MMPs have also been reported.  

uPA is a serine proteases involved in ECM degradation, cancer growth and metastasis. A number of small molecule uPA inhibitors have been reported. They include A6 [a capped, eight L-amino acid peptide (Ac-Lys-Pro-Ser-Ser-Pro-Pro-Glu-Glu-NH₂) derived from the biologically active connecting peptide domain of the serine protease], Suramin, WX-UK1, and WX-671 (Fig. 8).  

3.3.3. Interference with inflammation

It is well accepted that inflammation is closely associated with cancer growth and metastasis. Inflammation can impact cancer by providing bioactive molecules from cells infiltrating the tumor microenvironment. These bioactive molecules include cytokines, growth factors, chemokines, cell survival signals to avoid apoptosis, proangiogenic factors, and ECM modifying enzymes. Interference with the function of these bioactive molecules has been demonstrated to be effective in the inhibition of cancer growth and/or metastasis. Fig. 9 presents representative small molecules that interfere with the inflammation process. In addition, neutralizing antibodies against CCL2 (C–C motif ligand 2), CSF-1 (chemokine, colony stimulating factor-1), IL-6 (Interleukin-6, cytokine), IL-6 receptor, TNF, TGF-β have also been reported. IL-6 plays a key role in promoting proliferation and inhibition of apoptosis by binding to its receptor (IL-6Rα) and co-receptor gp130 (glycoprotein 130). The binding activates the JAK/STAT signaling pathway of the Janus kinases (JAK) and signal transducers and activators of transcription (STATs) STAT1 and STAT3. STATs belong to a family of transcription factors closely associated with the tumorigenic processes. Several studies have highlighted the effect of the IL-6/JAK/STAT3 signaling pathway on cancer initiation and progression. Current attempts to target the IL-6/JAK-STAT3 pathway include the clinical use of IL-6 and IL-6 receptor blocking antibodies, specific STAT3 inhibitors and JAK inhibitors.  

The IkB kinase (IKK) complex is the key enzyme in activation of the NF-κB pathway. NF-κB is a transcription factor that controls the expression of a number of important genes for mediating immune and inflammatory responses. IKK inhibitor PS-1145 (Fig. 9) has been found effective in treating lymphoma.  

3.3.4. Inhibition of integrin signaling

Inhibition of integrin signaling has been presented in the earlier section and will not be discussed further.  

3.3.5. Interfering with hypoxia process

Hypoxia is a characteristic microenvironment in the majority of solid tumors. Hypoxia is a result of rapid tumor cell

![Representative compounds which inhibit growth factor signaling](image-url)
proliferation that exceeds the development of the tumor's blood supply. Hypoxia-inducible factors (HIFs) are transcription factors that respond to a decrease in oxygen in the cellular environment. Activation of HIF-1 (hypoxia-inducible factor 1) in cancer can increase the transcription of many genes involved in glucose metabolism, apoptosis resistance, invasion, metastasis and...
angiogenesis. HIF-1 has been considered to be an attractive target for the development of novel cancer therapeutics. Fig. 10 presents some selected inhibitors of HIF-1.

3.3.6. Remodeling of ECM
Remodeling of the ECM has also been explored for metastasis inhibition. Hyaluronan [hyaluronic acid (HA) or hyaluronate] is one of the chief components of the ECM and plays a critical role in tumor cell adhesion and migration. Hyaluronidases are a family of enzymes that degrade hyaluronan. There is compelling evidence that the administration of exogenous hyaluronidase can impose significant anticancer activity in HA-overexpressing tumors. Recombinant human hyaluronidase, halozyme (Hylenex) also known as rHuPH20, is an FDA-approved enzyme that can reversibly degrade HA, lower the viscosity of hyaluronan, increase tissue permeability, and hence enhance the absorption and dispersion of chemotherapeutic agents. Its PEGylated form (PEGPH20), which exhibits longer survival time in vivo, has recently been introduced into clinical trials.

Heparan sulfate proteoglycans (HSPGs) are an integral and dynamic part of normal tissue architecture at the cell surface and within the ECM. Sulfatases and heparanase are key enzymes for the degradation of HSPGs. Recently, these enzymes have been reported to be required in tumor initiation and progress. Inhibitors of heparanase, mostly heparan sulfate mimetics, have been found to be effective as anti-metastatic agents and some of them have undergone various stages of clinical trials. While heparanase inhibitors exhibit promising potential for cancer treatment, the effect on cancer growth and metastasis of sulfatase inhibitors is less certain.

Figure 9 Chemical structures of representative compounds that interfere with the inflammation process. CCL2: chemokine (C–C motif) ligand 2; CSF-1: chemokine colony stimulating factor-1; JAK: Janus kinases; IKK: The IκB kinase (IKK) complex; TGF-β: transforming Growth Factor β; CXCR2: chemokine receptor type 2; CCR3: chemokine receptor; COX2: cyclooxygenase 2.
3.3.7. Cancer-associated fibroblasts (CAFs)

CAFs are distinctive cell types recognized as constituting part of the carcinoma and increasingly implicated as functional participants in tumor formation and progression\(^1\). CAFs have shown to enhance primary tumor growth and promote malignancy via HGF secretion\(^1\). Moreover, fibroblast HGF expression can be induced by tumor cells due to cytokine secretion such as IL-11\(^1\). In a similar manner, CAFs have been found to highly express SDF-1, implicating the tumor growth-promoting role of fibroblasts, and provide chemotactic signals for cancer cell invasion and migration\(^1\). CAF signaling can be a target for cancer treatment\(^1\). Inhibitors of CAFs include those that inhibit growth factor signaling such as TGF-\(\beta\), PDGFR, VEGF, VEGFR, HGF/MET, and IGF-1R\(^1\). The also include monoclonal antibody (Avastin/bevacizumab), antisense oligo (AP12009/trabedersen), fusion protein (Afibercept/VEGF-TRAP) and small molecule inhibitor such as LY2157299 (Fig. 11), a receptor kinase inhibitor targeting cancer-associated fibroblasts\(^1\).

4. Summary

Despite extensive research efforts on cancer treatment, overall survival has not been improved significantly in metastasis cancer patients. This is primarily due to the reason that the predominant cancer treatment focuses on inhibition of cancer growth, with little emphasis on metastasis. Limited success has been made in terms of treating cancer metastasis, though the current new generation anti-cancer drugs (predominantly neutralizing antibodies for growth factors and small molecule kinase inhibitors) do have effects on cancer metastasis in addition to their effects on cancer growth\(^1\).

The rapid advance in our understanding of cancer metastasis at molecular and cellular levels as well as signaling pathways in the past 30 years provides numerous potential targets for the intervention of cancer metastasis. This is especially true in terms of intervention with biochemical processes and signaling pathways involved in cell detachment, migration, invasion, adhesion, and cancer cell communication with tumor microenvironment. In view of the complexity of cancer metastatic cascade such as anoikis-dependent vs. anoikis-independent pathways, mesenchymal cell migration (protease-dependent) vs. amoeboid cell migration (protease-independent), a combination that inhibits multiple elements in the cancer metastasis cascade might be needed to produce effective metastasis inhibition. Further, simultaneous inhibition of both cancer growth and metastasis will likely be required to produce clinically effective therapeutic outcomes.

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