Mechanisms of tumour metastasis: anatomical mimicry?

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Abstract
Introduction
Tumour metastasis occurs when cancerous cells detach from the primary tumour, travel to a distant site via the circulatory system and establish a secondary tumour. This process requires the cancerous cells to acquire certain characteristics that are not expressed by normal somatic cells and which afford them migratory capabilities. This review analyses mechanisms through which metastatic cancer cells conquer normal body structure and function for them to move from their primary site to a secondary location.

Conclusion
For cancer metastasis to occur, the migrating cancer cells take advantage of and/or modify normal anatomical structures and functions.

Introduction
Metastasis of tumours, a leading cause of death among cancer patients, occurs when cancer cells from the primary site detach and travel to a distant site where they establish a secondary lesion. It entails interaction of the tumour cells with each other; other non-tumorigenic cells, and with the extracellular matrix (ECM) components. The key mechanisms required for metastasis include degradation of mesenchymal cadherins such as N-cadherin, integrin-αvβ6, vimentin and matrix metalloproteinase-9. Further, the cancer cells acquire mesenchymal-like properties via epithelial–mesenchymal transition (EMT) (Figure 1). Such cells have the ability to contribute to the ECM by synthesizing and organizing new components and by remodelling the ECM through the production of matrix-degrading metalloproteinases (MMPs).

Discussion
Initiation of tumour metastasis
Initiation of metastasis starts with the release of a malignant cell from its adhesive attachments to other cells and the ECM. This occurs following the dissolution of tight junctions that attach cells to each other on their lateral aspects and is mediated by cadherin molecule switching. This switching involves downregulation of E-cadherin and upregulation of mesenchymal cadherins such as N-cadherin, integrin-αvβ6, vimentin and matrix metalloproteinase-9. Further, the cancer cells acquire mesenchymal-like properties via a process called epithelial mesenchymal transition (EMT) (Figure 1). Such cells have the ability to contribute to the ECM by synthesizing and organizing new components and by remodelling the ECM through the production of matrix-degrading metalloproteinases (MMPs).

Different factors mediate cadherin switching in various tumours and via different mechanisms. For instance, in oral squamous cell carcinoma, downregulation of E-cadherin is induced by extensive methylation of the 5’ CpG Island in the E-cadherin promoter. This is accompanied by a concurrent upregulation of the N-cadherin expressed by these cells. On the other hand, cadherin switching in mammary epithelial cells is mediated by transforming growth factor β1 (TGF-β1). In ovarian cancer, over-expression of P-cadherin increases migration and invasion and is mediated by gonadotropin-releasing hormone (GnRH).

For the tumour cells to detach from the primary lesion, they also need to lose their polarity. Cell polarity, which is the generation of cellular asymmetries, is necessary for diverse processes in animal cells including cell migration and the spatial organization of the cells within a tissue. It has been noted that the molecular mechanisms that regulate apical–basal cell polarity also regulate cellular proliferation. This loss of cellular polarity is initiated by dissolution of cell–cell tight junctions, which then leads to increased migration. Polarity regulators include PAR-6, a cell polarity protein, and atypical protein kinase C (aPKC), which are involved in the formation of tight junctions. In contrast, phosphatase and tensin homolog (PTEN) control polarity by regulating the local concentration of the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2), which recruits the PAR-aPKC complex to the plasma membrane. Mutations involving genes coding for these molecules confer affected cells with the ability to move virtually in any direction, and hence enhancing tumour cell migration.

With reduced expression of E-cadherin, the more stable intercellular junctions are lost and, in their place, relatively dynamic links between affected cells are established. Such transient interactions are mediated by integrins and immunoglobulin superfamily cell adhesion molecules (CAMs). On their part, integrins...
provide physical links between actin cytoskeleton and ECM, and are also involved in the regulation of signal pathways that control actin dynamics and cell movements. They further allow transfer of signals from the ECM to the interior of the cell and vice versa and thus mediate the influence of the ECM on cell growth and differentiation. Due to these properties, integrins are involved in cell migration, invasion, intra- and extravasation and platelet interaction.

For the successful initiation and progression of migration by cancer cells, the manipulation of the ECM in their path of migration is paramount. This occurs not only during invasion but also during extravasation, extravasation and colonization at distant sites. Several enzymes are involved in this process and include the matrix metalloproteinases (MMPs), tissue serine proteinases, adamalysin-related membrane proteases (ADAM), bone morphogenetic protein (BMP)-1-type metalloproteinases, heparanase and cathepsins.

MMPs are produced by host stromal cells and non-invading cancerous cells. Their expression is however regulated by tumour cells through chemokines, cytokines and ECM metalloprotease inducers, thus forming a conspiracy between invading tumour cells and host cells. The MMPs exert their effects in cancer progression by releasing growth factors tethered to the ECM and by activating latent forms of growth factors and proenzymes through proteolytic cleavage. These growth factors act mainly on cancer cells leading to their proliferation and remodelling of ECM, besides inducing angiogenesis and cell migration.

Another important enzyme group is the adamalysin-related membrane protease family, formed of transmembrane proteins with disintegrin and metalloprotease domains. These enzymes are important in cleaving the extracellular domains of many membrane anchored cytokines, growth factors, receptors, adhesion molecules and enzymes. They thus regulate growth signalling and tumour cell adhesion.

Tissue serine proteinases include urokinase plasminogen activator; thrombin and plasmin. Urokinase plasminogen activator’s proteolytic cleavage of plasminogen, an inactive serine protease, activates it into plasmin. Plasmin then degrades extracellular proteins like fibronecin, vitronecin, laminin and fibrin. It also activates protocollagenases. These released factors stimulate tumour growth by activating latent forms of growth factors. Urokinase plasminogen activator activity is regulated by the plasminogen activator inhibitors PAI-1 and PAI-2 and mutations involving these inhibitors are known to advance tumour metastasis. Cancer types with enhanced expression of components of the urokinase plasminogen activator system include carcinomas of the lung, colon, stomach, breast and prostate as well as melanomas and gliomas.

On their part, heparanases, like heparan sulphate proteoglycans (HSPGs), play a key role in self-assembly, insolubility and barrier properties of basement membranes and extracellular matrices. They are also known to help in the sequestration and stabilization of bioactive molecules. HSPGs are prominent components of both the ECM and blood vessels, where they bind proteins like growth factors, CAMs, chemokines and enzymes ensuring that these molecules cling to the cell surface and the ECM. As a result, cleavage of heparan sulphate by heparanases affects the integrity and functional state of tissues and thus cellular response to changes in the extracellular microenvironment as well as cell migration. Heparanase expression is prevalent in cancers involving the prostate, bladder, pancreas, colon, breast, ovary, liver, stomach and the oral mucosa.

**Migration**

During migration, tumour cells interact with each other, other cells and the surrounding ECM. These interactions involve alternate transient attachment and release from the neighbouring cells and the matrix around them. CAMs different to those...
found in epithelial cells are expressed by migrating cancer cells. Such adhesive molecules include mesenchymal cadherins and various integrin molecules. They permit weak transient interactions between cancer cells, the surrounding matrix and endothelial cells. For instance, N-cadherin and integrin molecules expressed by cancer cells facilitate their interaction with endothelial cells during their lymphatic and haematogenous spread. Besides, the expressed CAMs also aid in directional orientation of migrating cancer cells by inducing pseudopod formation, which is an important characteristic also exhibited by migrating mesenchymal cells.

Another important event that aids in the migration of cancer cells is the release of growth factors from the surrounding ECM by proteolytic enzymes. These factors induce increased transcription of gene coding for surface receptors in host cells found in the vicinity of the tumour cells. Uprogulation of such receptors favours metastasis of cancer cells by facilitating their interaction with host cells and ECM.

It has been shown that as the tumour cells migrate, they alter the surrounding ECM by releasing proteolytic enzymes. These enzymes are aggregated in pseudopodia and facilitate sensing, protrusion, burrowing and traction of cancer cells through the surrounding matrix. They do so by activating latent growth and chemotactic factors held in the ECM. This ECM modification, therefore, affords the ability of the migrating cells to move in a three-dimensional extracellular environment.

For distant metastasis to occur, the cancer cells have to enter either the lymphatic or blood vessels (Figure 2). This requires mechanisms which help the metastatic cells in processes like intravasation into the vessels, evasion of immune responses while in circulation and extravasation from blood vessels at secondary sites. For intravasation and extravasation to occur, the cancer cells have to attach to the endothelial wall forming the vasculature. They then separate two of the endothelial cells to create enough space to go into or out of the vascular network. This process is called transendothelial migration (TEM) (Figure 3) and is similar to the one employed by the body’s immune system cells when they travel throughout the body. The greatest challenge encountered by cancer cells during TEM is breaking down the vascular endothelial (VE) cadherin bonds that bind endothelial cells together. Studies have shown that tumour cells overcome this limitation by attaching to the endothelial cells via N-cadherin proteins.

Metastatic cells unable to express N-cadherin proteins usually show delayed TEM.

Once the bonds between the N-cadherin molecules of cancer and endothelial cells have been formed, they need to be broken down to release the cancer cells and facilitate further migration. This is achieved by the upregulation of Src enzymes at the zone of heterotypic contacts between cancer and endothelial cells. These enzymes attach to intracellular binding domains of cadherins and disrupt the bonds after phosphorylating the catenins. This process helps not only to release the cancer cell–endothelial linkages but also endothelial–endothelial cell linkages, thereby helping in TEM.

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Lymphatic channels play a crucial role in the metastasis of certain cancers. Since lymphatic vessels are designed for entry and exit of immune cells, they become easier channels for cancer cells to transit. Besides, slow flow of lymph within the lymphatics favours metastatic cancerous cells since there is little stress to harm them. However, specific interactions between the cancer cells and lymphatics are vital for metastasis to occur. Moreover, certain factors which attract cancer cells towards lymph nodes need to be released. In the case of oesophageal cancer, for instance, expression of the chemokine receptor CXCR4 by lymphatics leads to metastasis of cancer cells to regional lymph nodes. Alternatively, increased expression of the chemokine receptor CCR7 on lymphatic endothelial lining increases cellular motility by enhancing interaction between cancerous cells and endothelial lining.

Haematogenous spread of cancer cells is of also of paramount significance. Cancer cells not only invade existing blood vessels but also induce formation of new ones and especially in response to hypoxia. For instance, under hypoxic conditions, cancer cells release insulin-like growth factor 2 (IGF2). This ligand then binds on insulin-like growth factor receptor 2 receptor (IGF2R) on endothelial cells resulting in homing of endothelial progenitor cells, eventually leading to neoangiogenesis. Thus, not only is blood supply to the tumour increased but also new routes of metastasis are created.

Homing

The successful initiation of a secondary tumour is called homing. Metastatic cells proliferate and get established only at sites in which there is a similar environment as the one found in the primary tumour: Breast cancer, for example, spreads to the bone, brain, lungs and the liver, while the distant metastasis of prostate cancer is mainly to bones. Thus, for successful 'settling' of a metastatic tumour cell, random genetic and epigenetic alterations in the cell must be supplemented by a plastic and responsive microenvironment.

Specific molecules need to be released for a tumour cell to migrate towards a particular destination. This is as evidenced by the expression of the chemokine CCR7, which promotes homing of tumour cells to lymph nodes in an autocrine fashion. This ligand, when expressed in lymphatic endothelial lining under static conditions, induces metastasis of cancer cells. The rate of metastasis increases in high interstitial flow, meaning that a transcellular gradient of CCR7 helps the metastatic process. In another scenario, transient ectopic expression of α4 integrin (CD49d) on mesenchymal stem cells greatly increases bone homing. Cancer cells which have increased expression of this ligand have a greater propensity of bone metastasis. It has similarly been observed that the expression of the chemokine receptor CXCR4 in oesophageal cancer results in an increase in lymph node and bone homing.

Cancer cells that have metastasized to a secondary site need to proliferate to survive in their new environment. This is mediated by growth factors that are released by proteolytic activity on the ECM and which promote the proliferation of cancer cells. The growth factors bind to both surface and cytoplasmic receptors to mediate the cellular proliferation. Downstream activities of such receptor activation increase transcription of genes involved in cell proliferation. Factors that promote cancer proliferation include insulin-like growth factor, parathyroid hormone related peptide (PTHrP), interleukins 6, 8 and 11, bone morphogenetic proteins (BMPs), platelet derived growth factor (PDGF) and endothelin-1. Such an activity serves to maintain a vicious cycle for the growth of malignant cancers.

Figure 3: A diagram showing transepithelial migration by a neutrophil, a process similarly employed by cancer cells. (Adapted from www.sciencedirect.com)
For the successful initiation and establishment of a secondary tumour, there is need for continued proliferation of cancer cells, which necessitates a conducive host environment. The host environment thus has to express conditions similar to those found in the primary tumour to favour this process. This explains why certain tumours have a tendency of homing to particular sites. It has been reported that cancer cells manipulate the host environment by extracting factors from it and, by this, inducing the proliferation of not only cancer cells but other cells that may promote the growth of the secondary tumour. Following this phenomenon, cancer cells from different tumours have been known to affect the secondary site in distinct ways. For instance, prostate cancer metastasis to bone causes osteolytic lesions while breast cancer metastasis to bone causes osteolytic lesions.

Conclusion

From the current review of the ways via which various tumours metastasize, it is observed that the cancerous cells take advantage of, and/or modify normal anatomical structures and functions for them to metastasize from a primary to a secondary location.

Abbreviations list

ADAM, adamalysin-related membrane proteases; aPKC, atypical protein kinase C; BMP, bone morphogenetic protein; CAMs, cell adhesion molecules; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; GnRH, Gonadotropin-releasing hormone; HSPGs, heparan sulphate proteoglycans; IGFBP, insulin-like growth factor 2; IGFZR, insulin-like growth factor 2 receptor; MMPs, matrix metalloproteinases; PDGF, platelet-derived growth factor; PIP2, phosphatidylinositol 4,5-biphosphate; PTEN, phosphatase and tensin homolog; PTHrP, parathyroid hormone related peptide; TEM, transendothelial migration; VE, vascular endothelial.

References

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