Metastasis in Cancer

The term metastasis describes the process by which cells within a tumor develop the ability to invade in to surrounding tissues and pass through tissue boundaries to form new growths at distant parts of the body relative to the original tumor site. Metastasis is the leading cause of death in many cancers, although the final outcome of this process for an individual tumor cell is dependent on complex interactions between the cell and its environment, and varies widely depending on the tumor cell type and the metastatic site.

The metastatic process involves complex and diverse molecular mechanisms, but has historically been formalised in to a number of discrete stages. These consist of the separation of cells from the primary tumor, invasion in to the surrounding tissues, intravasation in to the lymphatic or blood vascular systems, arrest at a new site, and extravasation in to the tissue followed by the growth of a new tumor. It is not possible to study each of these stages in isolation due to the multiple interactions that are involved in the metastatic process, and while the role played by some molecules is better understood than that of others, many types of molecule are known to function at multiple stages of metastatic progression.

It would be impossible to provide a detailed insight to the entirety of molecules that have been implicated in metastasis, especially since many of these have yet to be identified; however below are some of the key protein classes and research areas that are of interest.

- Adhesion molecules enable cells to adhere to one another and to interact with the extracellular matrix, thereby playing a key role in controlling cellular responses to external stimuli. They are divided in to four classes based on structure – integrins, cadherins, selectins and immunoglobulin superfamily members (1). Integrins are heterodimeric glycoproteins, with eighteen alpha subunits and eight beta subunits currently known; the αVβ3 integrin has been reported to promote angiogenesis, the growth of new blood vessels from the existing vasculature which is a key process in tumor growth, (2) whereas the α2β1 integrin has been shown to suppress metastasis in a mouse model of breast cancer (3). The cadherin superfamily is involved in calcium-dependent cell-cell adhesion, and consists of more than 350 members with different isoforms localised to different tissues; E-cadherin (CDH1) has been shown to play a tumor suppressor role, with reduced expression reported in highly invasive tumors (4), while elevated N-cadherin (CDH2) levels are associated with increased cellular invasiveness (5). Selectins are carbohydrate-binding molecules that are involved in trafficking cells of the innate immune system. The selectin family consists of three members, E-, L- and P-selectin, all of which share a similar structure (6). E-selectin in particular has been implicated in the extravasation of tumor cells during metastasis, with its expression on endothelium shown to facilitate tumor cell seeding (7). The immunoglobulin superfamily consists of over 700 proteins that are characterised by the presence of immunoglobulin-like domains in their extracellular regions; Junctional Adhesion Molecule A (JAM-A) is a member of this family with roles in cell-cell adhesion, leukocyte migration and angiogenesis, and has been shown to have elevated expression levels in non-small cell lung

cancer (8).

- Proteases, categorised as metalloproteases, cysteine proteases, serine proteases, aspartic proteases or threonine proteases, catalyse the cleavage of peptide bonds during protein breakdown (9). They have the ability to digest the extracellular matrix and cellular adhesion contacts, allowing for tumor cell invasion, intravasation, extravasation and angiogenesis. Many different proteases have been implicated in cancer progression, including the matrix metalloproteinases (MMPs), the plasminogen receptor family, and the cathepsins. The MMPs are a family of 23 zinc-dependent endopeptidases, with roles in tissue remodelling and organ development; they are tightly regulated by physiological inhibitors, which include the tissue inhibitors of metalloproteinases (TIMPs) (10). The expression of MMPs and their inhibitors in the tumor micro-environment is diverse; MMP-9 has been shown to be induced in angiogenic lesions, where it increases the availability of the angiogenesis inducer VEGF (11), while MMP-2 can increase the motility of colon cancer cells via digestion of the extracellular matrix (12). The plasminogen receptor family consists of at least twelve members, all of which have the ability to interact with plasminogen and plasmin (13). Many of these receptors convert inactive plasminogen to plasmin, which degrades various components of the extracellular matrix; others members of the family protect bound plasmin from inactivation by inhibitors. Over-expression of the plasminogen receptor uPAR (urokinase-type plasminogen activator receptor), a serine protease, across a variety of tumors is associated with cancer invasion and metastasis; uPAR converts plasminogen to plasmin upon binding of its ligand urokinase-type plasminogen activator (uPA) (14). At least twelve different cathepsins have been identified, and many of these cysteine, serine and aspartic proteases have been implicated in different stages of the metastatic process. Over-expression of the cysteine protease cathepsin B has been observed in brain, lung, breast, prostate and colorectal cancer (15), while another cysteine protease, cathepsin K, has been shown to degrade native collagen I and facilitate tumor expansion in the bone (16).

- Apoptosis is used as a defensive mechanism to eliminate harmful or defective cells; apoptosis resistance is known to be a critical component of the metastatic process. Under normal conditions apoptosis can be initiated through ligand binding to cell surface receptors, or as a result of intracellular signalling. The dysregulation of several receptor-mediated apoptotic pathways has been implicated in metastatic tumor cells, with affected ligand and death receptor pairs known to include Fas ligand/Fas receptor, Tumor Necrosis Factor (TNF) α/TNF receptor 1, Apo-3 ligand/death receptor (DR) 3, TNF-Related Apoptosis-Inducing Ligand (TRAIL)/DR4 and TRAIL/DR5 (17). Multiple molecular mechanisms underlie the loss of function of these apoptotic pathways in cancer; for example soluble Fas lacking a transmembrane anchor may be synthesised (18), or expression of the TRAIL receptors DR4 and DR5 may be down-regulated (19).

- The tumor micro-environment plays an important role in tumor progression, with hypoxia being of particular interest since it has been found to alter the expression of many different genes, enhancing the ability of tumor cells to form metastases as a result. Many tumors contain regions of hypoxia, and two proteins that have been extensively studied within the hypoxia field are Hypoxia-Inducible Factor (HIF) and Vascular Endothelial Growth Factor

(VEGF). These proteins can mediate angiogenesis, metabolism and cell proliferation, all of which are processes that are critical to tumor cell growth and metastasis. HIF is a heterodimer, consisting of an oxygen-regulated HIF-1α or HIF-2α subunit and a constitutively-expressed HIF-1β subunit; HIF-1α is found in all cell types, while HIF-2α shows a more limited distribution (20). Under normoxic conditions the alpha subunits are continuously transcribed and translated, and are hydroxylated by HIF prolyl hydroxylases, allowing them to bind to the von Hippel-Lindau (VHL) protein and resulting in ubiquitination and proteasomal degradation. Under hypoxic conditions hydroxylation does not occur, and the HIF heterodimers translocate to the nucleus where they regulate the expression of over a thousand different genes (21). Clinical data indicate that the HIF isoforms have differing cellular functions in cancer, and the role played by these proteins varies depending on cell type (22). The VEGF genes, all of which play a pivotal role in angiogenesis, are among the many genes that are regulated by HIF (23). Five distinct VEGF genes exist - VEGF-A (also known as VEGF), placenta growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D (24). All of these exert their effects through receptor tyrosine kinases, with tumor angiogenesis primarily relying on the interaction between VEGF-A and its receptor, VEGFR-2 (25).

Recently, it has been postulated that the epithelial-mesenchymal transition (EMT) plays a key role in the invasion and metastasis of cancer cells from the original tumor. Under normal conditions the EMT plays a pivotal role in embryonic development, and is characterised by the loss of epithelial cell apical-basal polarity, enhanced proteolytic activity and acquisition of the ability to invade the extracellular matrix (ECM). Apical-basal polarity, which is defined as the asymmetric distribution of proteins and lipids to distinct membrane domains, provides a tumor-suppressor function and is critical to ensuring that the communication between a cell and its environment is correctly interpreted (26). During the EMT cells lose epithelial markers such as E-cadherin, ZO-1 and cytokeratins, and develop a mesenchymal phenotype with markers such as N-cadherin, vimentin, fibronectin and alpha smooth muscle actin (27). Mesenchymal cells are self-renewing multipotent progenitor cells with the ability to differentiate into a variety of cell types; carcinoma cells can recruit mesenchymal cells to the tumor stroma, where they aid tumor progression (28).

In addition to the processes discussed above, metastasis may also be facilitated mechanically, for example through surgery during the process of carrying out a tumor biopsy. Since cancer cells are much easier to dislodge than healthy cells due to the disruption of normal cell-cell adhesion, they can accidentally be seeded in to the interstitial tissue fluid, from where they are carried to lymph nodes or veins (29). Metastasis is an extremely complex process and will continue to be the subject of a great deal more research; increasing our understanding of metastasis will be hugely beneficial in the ongoing fight against cancer.

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