INTRODUCTION

Metastasis, due to its complex spatial and temporal components, remains an enigma, despite all of our efforts to unravel its complexity. Tumors have been referred to as entities that constantly redefine themselves by their ever-changing nature. How these different potential mechanisms interact, intersect or share commonality is a major focus since a clearer understanding of metastatic disease is required to significantly reduce cancer morbidity and mortality. The fact that cancer patients might develop metastasis after years or even decades from diagnosis of the primary tumor makes the metastatic process even more complex and the disease more deadly. The promise of this article is to enhance the understanding on molecular mechanisms underlying metastasis and provide a better approach towards development of novel therapeutic treatment modalities.

Keywords: Cancer, metastatic process, primary tumor

Determining factors for metastasis are:
- Appropriate growth factors or extracellular matrix environment (ECM)
- Compatible adhesion sites on the endothelial luminal surface
- Selective chemotaxis at which the organ producing some soluble attraction factors to the tumor cells.

Metastasis requires a series of five crucial steps which are as follows:
1. Invasion and migration
2. Intravasation
3. Circulation
4. Extravasation
5. Proliferation and angiogenesis
INVASION AND MIGRATION

The initial steps of metastasis require proliferation of the primary tumor followed by invasion through adjacent tissues and basement membranes.\[^3\]

Normal cells are neatly glued to each other and their surroundings by a variety of adhesion molecules. For invasion to occur tumor cells often show a decrease in cell–cell and/or cell–matrix adhesion.\[^4\]

LOSS OF CELL–CELL ADHESION AND MIGRATION OF TUMOR CELLS

The cadherin family of transmembrane glycoproteins is of particular importance in cell adhesion. E-cadherins mediate homotypic adhesions in epithelial tissue, thus serving to keep the epithelial cells together and to relay signals between the cells. In several epithelial tumors, there is a downregulation of E-cadherin expression. Presumably, this downregulation reduces the ability of cells to adhere to each other and facilitates their detachment from the primary tumor and their advance into the surrounding tissues.\[^5\]

A large number of growth factors and their activated signal transduction pathways are known to provoke the loss of E-cadherin function and to induce cancer cell migration and invasion. These factors include transforming growth factor-\(\beta\) (TGF-\(\beta\)), hepatocyte growth factor (HGF), members of the epidermal growth factor (EGF) family, insulin-like growth factor (IGF), fibroblast growth factor (FGF) and Notch signaling. Among the many molecular alterations, these signaling pathways activate one or several transcriptional repressors of E-cadherin gene expression such as Snail1 (Snail), Snail2 (Slug), ZEB1 (\(\delta\)EF1), ZEB2 (Sip1), E47 and Twist.\[^6\]

IGF1 receptor interacts and phosphorylates E-cadherins and catenins leading to their subsequent internalization, ubiquitylation by the E3 ligase Hakai and proteasomal degradation. The HGF receptor c-Met assembles a complex consisting of CD44 and \(\alpha4\beta6\) integrin, which together facilitate signal transduction by c-Met. TGF-\(\beta\) stimulates Smad-mediated signaling by binding and activating its receptors TGF-\(\beta\)RI and TGF-\(\beta\)RII. All these receptor complexes synergize through downstream effector signaling pathways in inducing the expression of transcriptional repressors such as Snail1 and Snail2, ZEB1 and ZEB2 and Twist. E-cadherin can also be cleaved by proteases to generate a soluble form of the extracellular domain of E-cadherin. \(\gamma\)-Secretase cleavage of E-cadherin results in the formation of a COOH-terminal fragment that translocates to the nucleus and modulates Kaiso-mediated transcriptional repression\[^7\] [Figure 1].

Rho proteins help in maintaining the organization of the cell as well as cell motility. RhoA is responsible for maintaining the stress fiber assembly. As p120 is free, it has its effect on these rho proteins. The increased cytoplasmic p20 inhibits RhoA function, causing disruption of stress fibers (reduced contractility) and increased availability of G-actin, possibly through the actin severing action of cofilin. The subsequent activation of Rac1 and Cdc42 coupled with reduced contractility, promotes actin reorganization, formation of lamellipodia and filopodia and induces directed cell migration in collaboration with integrin signaling\[^8\] [Figure 2].

DEGRADATION OF EXTRACELLULAR MATRIX

In order for a tumor cell to intravasate and extravasate, the collagen-rich ECM and basement membrane must be degraded. This degradative ability can be through either enzymatic capacities of the tumor cell or through enzymatic activity of cellular components of the matrix such as fibroblasts. Likely, there is a cooperation between the two components enabling the tumor cell to reach its target organ and survive.\[^9\]

There are two main classes of enzymes that have been studied for their abilities to degrade and remodel the ECM: the plasminogen activator/plasmin system and matrix metalloproteinases (MMPs).\[^10\]

MMPs are a group of zinc-dependent proteases that are involved in the degradation and remodeling of the ECM in order for processes such as angiogenesis to occur. By degrading the matrix, MMPs not only provide physical space within the matrix for migration but also provide proliferation and differentiation signals to endothelial cells (ECs) by releasing cryptic sites on ECM proteins and soluble growth factors.\[^10\]

Figure 1: Flowchart depicting role of E-cadherin in loss of cell to cell adhesion
MMPs cleave ECM bound growth factors including proangiogenic factors. Various MMPs have been found to cleave heparin bound growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor, releasing soluble forms which then exert proangiogenic actions and stimulate the formation of new blood vessels. In particular, it was reported that MMP-9 stimulates the production of the proangiogenic growth factor VEGF.

Thus, an important role of MMPs is to generate from the ECM, factors that promote angiogenesis, tumor growth and tumor cell motility.

**INTRAVASATION**

The intrusion of cancer cells into the blood and lymphatic vessels is called intravasation. After the attachment on the ECs through adhesion molecules, the neoplastic cells secrete proteolytic enzymes which enable them to infiltrate the blood vessel.

The lymphatic system collects the interstitial fluid and conveys it through the lymph nodes and lymphatic vessels back again into the (blood) circulation. Thus, cancer cells can either directly or indirectly (through lymphatic vessels) reach the circulation provided that they do not persist in the next lymph node. The first metastases are often found in the lymph nodes which, therefore, are of great importance for tumor staging and prognosis. The penetration of lymphatic vessels seems to be easier due to the absence of continuous basement membrane, a coating layer of pericytes, and weak interendothelial connections.

Thus, all carcinomas except a few metastasize through the lymphatics as it provides path of least resistance. To penetrate the vessels, several proteases are secreted, which degrade the endothelium and enable the cells to enter the lumen of the vessel. The migration to the vessels is facilitated with the help of several chemokines as well as with the altered expression of several adhesion molecules. VEGF-C expression drives lymphangiogenesis and its expression is high several carcinomas.

**CIRCULATION**

Circulation in the blood is another critical step of metastasis due to the predominant conditions which can be toxic for tumor cells.

- Absence of exogenous growth factors
- High shear forces of the arterioles or capillaries
- High concentration of oxygen and lymphocytes

Normally, most cells cannot survive and proliferate without adhesion onto other cells or solid substrate. Cancer cells, however, have to evade this restriction to build secondary tumors. This stage is called “anchorage independence.” After a certain time, normal cells activate a special form of programmed cell death called anoikis in case they cannot adhere to solid substrate or other cells. If a cancer cell is still anchorage dependent at the time it enters circulation, anoikis is activated and the cell dies.

Technique displayed by neoplastic cells to antagonize toxic conditions is

- Formation of microemboli - Agglutinations of tumor cells with thrombocytes and erythrocytes
- Tissue factors which are special proteins in the plasma initiates clumping cascade which further activates thrombin leading to conversion of fibrinogen to fibrin.

More specifically, by forming relatively large emboli through interactions with blood platelets, a process that appears to be mediated by the expression of tissue factor and/or L- and P-selectins by the carcinoma cells, tumor cells are able to both shield themselves from shear forces and evade immune detection.

**EXTRAVASATION**

If cancer cells survive the adverse conditions in the vessels and reach the bigger venous blood vessels, they are carried further by the bloodstream and reach, after
the passage of the heart, the capillary network of the lung.\textsuperscript{[18]}

Cancer cells can get entangled in the capillary system of the lungs as the size of the cancer cells (∼20 μm diameter) is more in relation to the capillaries (∼3–8 μm diameter). Cancer cells, unlike erythrocytes, are not very elastic and often form microthrombi. Thus, they can become stuck in the arterioles.\textsuperscript{[19]}

However, not all cancer cells get stuck in the lung capillaries. It is postulated that the cells discard a great amount of their cytoplasm to form smaller, but still vital cells, which can passage the lung capillaries. Furthermore, the cancer cells can avoid the capillary network using “arterial-venous shunts” (direct connections which bypass the capillaries).

When the cancer cells leave the lung capillaries and reach the general arterial vessels, they can migrate into various kinds of body tissue. The now occurring extravasation can take place as the cells start to proliferate in the lumen of a vessel. Due to the growth of the tumor, the vessel wall is destroyed and thus the cancer cell’s way into the tissue of the organ is paved. They can also penetrate an organ by degradation of endothelium and basement membrane through proteolysis.

Extravasation of cancer cells is a multistep process. The first step consists in the transient adhesion of cancer cells to the endothelium. It involves endothelial adhesion molecules such as E-selectin and P-selectin and their counter-receptors present on cancer cells. This step is associated with the rolling of the cancer cells on the endothelium. The second step consists in a firmer adhesion of cancer cells to ECs. It is mediated through chemoattractants and cell adhesion molecules on the endothelium and integrins on the cancer cells. The third step is characterized by the extravasation of cancer cells through endothelial cell–cell junctions.\textsuperscript{[20]}

Tumor cells once extravasated metastasize to specific organs. Organ tropism may be related to the following mechanisms:

Because the first step in extravasation is adhesion to the endothelium, tumor cells may have adhesion molecules whose ligands are expressed preferentially on the ECs of the target organ. Indeed, it has been shown that the ECs of the vascular beds of various tissues differ in their expression of ligands for adhesion molecules.

Chemokines also have a very important role in determining the target tissues for metastasis.\textsuperscript{[21]}

Thus, metastasis to a particular organ can be decided by:
• Mechanistic theory: determined by the pattern of blood flow
• “Seed and soil” theory: the provision of a fertile environment in which compatible tumor cells could grow.

**METASTATIC COLONIZATION**

To form metastases, extravasated carcinoma cells must survive in the foreign microenvironment that they encounter in the parenchyma of distant tissues. The microenvironment at the metastatic locus usually differs greatly from that present in the site of primary tumor formation. This dictates that disseminated cancer cells are, at least initially, poorly adapted to their newfound homes. These microenvironmental differences may include the types of stromal cells, ECM constituents, available growth factors and cytokines and even the microarchitecture of the tissue itself.

Some have proposed that carcinoma cells can address the problem of an incompatible microenvironment at the metastatic site through the establishment of a “premetastatic niche.”\textsuperscript{[22]} Primary tumors release systemic signals which are thought to occur before the arrival of carcinoma cells at the metastatic loci. Tumor cells deploy complex mechanisms to modify foreign microenvironments to initially survive at these ectopic locations.\textsuperscript{[23]} Accordingly, these predisposing changes convert distant microenvironments into more hospitable sites for future settling by disseminated tumor cells.

After the colonization and first proliferation of the secondary tumors, the neoangiogenesis takes place. Neoangiogenesis is the formation of new blood vessels in the direction of a neoplasia to provide it with nutrients and to remove metabolic waste products.\textsuperscript{[24]}

Neovascularization has a dual effect on tumor growth: perfusion supplies nutrients and oxygen, and newly formed ECs stimulate the growth of adjacent tumor cells by secreting polypeptide, growth factors such as IGFs and platelet-derived growth factor.

Tumor angiogenesis can occur in the following ways:
1. ECs sprout from host vessels in response to VEGF, basic FGF, angiopoietin and other proangiogenic stimuli
2. Bone marrow-derived circulating endothelial precursors migrate to the tumor in response to VEGF and differentiate into ECs while hematopoietic stem cells differentiate into leukocytes including tumor-associated macrophages that secrete angiogenic growth factors
and produce MMPs that remodel the ECM and release bound growth factors.

3. The pattern of vessel formation is haphazard: vessels are tortuous, dilated, leaky and branch in random ways. This leads to uneven blood flow within the tumor, with areas of acidosis and hypoxia (which stimulate release of angiogenic factors) and high intratumoral pressures that inhibit delivery of therapeutic agents.

Thus, with the accumulation of genetic and/or epigenetic alterations, carcinoma cells are capable of completing an intricate, multistep, cell-biological process that culminates in the formation of macroscopic, life-threatening growths at distant organ sites.

**METASTASIS OF ORAL CANCER**

Metastasis of oral squamous cell carcinomas is mostly to the regional lymph nodes of the neck but when a squamous cell carcinoma does metastasize through blood-borne routes, it will most often go to the lungs.

The mechanism of metastasis to the lungs is through the venous system. The route begins with the intravasation of cancer cells into a small vein. This cancer embolus may then drain into the pterygoid plexus or another local vein. It then drains into the larger veins such as the facial vein or retromandibular vein and on into the internal jugular vein. It then flows through the brachiocephalic vein, which forms at the junction of each internal jugular vein and the respective subclavian vein. The cancer embolus next passes through the superior vena cava into the right atrium of the heart. As the heart contracts, the cancer embolus is pumped past the tricuspid valve into the right ventricle. From there, further cardiac contractions pump it through the pulmonary valve into the pulmonary artery. The cancer embolus then passes further into the branches of the pulmonary artery system, which progressively narrows, until it physically wedges into a small arteriole or capillary. Many cancer emboli contain less than 100 cells. One or several of these cells then lyse the arteriole wall to extravasate into the lung and replicate into a metastatic focus. The lung remains the most common organ for distant metastasis as a result of this mechanism, where the lung’s pulmonary artery system serves as an effective filter.

The precondition for this purpose is further research in the field for a better understanding of these processes. This indication of an otherwise occult malignancy. It is estimated that 1% of all jaw tumors represent metastatic cancer.

In a study by Hirshberg et al. and Hirshberg et al., it was mentioned the most common site of origin of primary cancer in females was from breast, adrenal, colorectum and thyroid. For men, the primary site was the lung, followed by prostate, kidney, bone and adrenal glands.

The common route of metastasis from lower abdominal organs is through Batson venous plexus. These veins are valveless, offer an easy way for tumor emboli to spread with less resistance. Increase in intra-abdominal or intra-thoracic pressure causes a retrograde flow from the venous channels.

**BONE AS A UNIQUE ENVIRONMENT FOR METASTASIS**

In the jaw bones, metastasis is common in mandible (80%–85%) followed by maxilla, but both are involved in 5% cases.

An explanation for the mandibular predilection may be related to the larger amount of hematopoietic tissue having sinusoidal vascular spaces that provide easy access to tumor cells.

The bone matrix is a vast storehouse of growth factors such as IGF, TGF-β, bone morphogenetic protein, platelet-derived growth factor and VEGF. The release of these factors during bone remodeling may promote cell homing and appears to promote colonization and initial proliferation of tumor cells. Continuous and dynamic turnover of the bone matrix and bone marrow provides a fertile ground for tumor cells to utilize the vast available resources (cells, growth factors, cytokines and receptors) for their homing and subsequent proliferation. Both anatomic and molecular characteristics of bone make it a favorable site for metastasis.

**CONCLUSION**

The metastatic cascade represents a multistep process which includes local tumor cell invasion, entry into the vasculature followed by the exit of carcinoma cells from the circulation and colonization at the distal sites.

Metastasis is the leading cause of cancer mortality. New insights into the molecular processes of invasion and metastasis as well as the concept of cancer stem cells may pave the way for new, highly specific and successful drugs.
review demonstrates the necessity for investigation into the complexity of the regulatory networks which will allow the development of novel strategies to efficiently combat cancer cell dissemination.

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