**CANCER AND THE METASTATIC SUBSTRATE**

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**Summary:** Seventy percent of cancer patients have detectable metastases when they receive a diagnosis, and ninety percent of cancer deaths result from metastases. These two facts highlight the urgency of research to study the mechanisms and processes that enable metastasis. We need to develop a greater understanding of the cellular and molecular mechanisms that cause metastasis, but we need to do more. We must also consider the micro- and macro-environmental factors that influence metastatic cancers. Studying this environmental context has led us to update the “*seed and soil*” hypothesis, which dates from the late 19th century. This theory describes cancerous *cells* as *seeds* and the *substrate* as the *soil* in target organs, and it may seem antiquated. Nonetheless, the tissue specificity that recent researchers have observed in metastatic colonization supports the validity of the *seed and soil* theory. We now know that metastatic potential of a tumor cell depends on multiple, reciprocal interactions between the primary tumor and distant sites. Those interactions determine tumor progression. Studies of metastasis have allowed us to develop treatments that focus on therapeutic effectiveness. These new treatments account for the frequent metastasis of some tumors to target organs such as bones, the lungs, brain, and liver. The purpose of this review is first to describe interactions at the cellular and molecular levels and in the target organ tumor environment that enable metastasis. A second aim is to describe the complex mechanisms that mediate these interactions.

***Key words:*** Cancer, metastasis, tumor micro-environment, tumor progression, tropism, exosomes, micrometastasis.

**Introduction**

The genetic paradigm of cancer posits that tumors result from multiple mutations in a single normal cell. These mutations alter the genotype of the cell and transform it into a malignant phenotype (1). The time required for this process to develop varies greatly, but it begins with the cloning of cells. Over months, years, or decades, this cloning results in the formation of a primary tumor (2). We now recognize that malignant mutation, development, and transformation can only occur in progenitor cells called *mother cells* or *stem cells* (3). Mutations in somatic cells thus do not produce cancer, as their short half-life illustrates. Cells differentiate, mature, carry out their functions, and complete their life cycles when they die in apoptosis. The aggressiveness and metastatic power of a tumor depends on the maturity level of the mother cell that produced the mutation. Tumors derived from mother cells in early maturity will have a more heterogeneous phenotype and will metastasize quickly. Tumors derived from a more mature mother cell will have a more homogeneous phenotype and are less metastatic (4). The biological heterogeneity of cellular populations that comprise malignant neoplasms varies widely. The notable properties of these cellular populations include their cellular surface, antigenicity, immunogenicity, proliferative index, and their sensitivity to antitumor agents. Also significant is phenotypic expression, which in combination with the aforementioned factors, allows tumors to invade other tissues.

The metastatic cascade begins in the primary tumor via local invasion characterized by several factors, including the mechanical pressure exercised by proliferating tumor tissue. The action of proteolytic enzymes reduces the molecular organization of barriers and lowers resistance to invasion. The capacity of metastatic cells to displace other cells is also a factor in the metastatic cascade (5). This dynamic invasion process produces a Darwinian evolutionary selection in which cells acquire changes to their genetic material. These changes confer an advantage, which over time becomes more common in the tumor through selection. Genetic instability thus characterizes these cells, and allows them to develop the capacity to invade and metastasize (6). Metastases develop and evolve as tumor cells spread and establish themselves in distant organs. Metastases determine the prognosis and life expectancy of patients, but they also dictate the clinical outcomes of most tumors (7,8). Current research that examines cellular and molecular processes is critical, but we must also study the cellular, tissue, and organ environments. All of these research areas are essential to understanding cancer and finding better and more effective treatments.

***I:* Metastatic dissemination**

*The spread of tumor cells:* Malignant tumors spread through the circulatory and lymphatic systems via the intravasation of tumor cells. Angiogenesis facilitates this process, and results from the development of microcirculation in neoformed vessels that form a fenestrated endothelium. These vessels are unstable intercellular unions, and form a discontinuous basal membrane that is sometimes absent (9). Tumor progression requires that invasion coincide with an increase in vascularization that provides nutrients and factors essential to the growth of tumor cells. Neovascularization occurs early in tumor progression and is detectable when diseases such as “*in situ”* carcinomas are incipient (10). Tumor cells stimulate endothelial cell proliferation and vessel formation through various factors. Among the best known of these factors are *vascular endothelial growth factor,* or VEGF, IL-8, and TNF-α. Endothelial cells also produce other growth factors, such as *fibroblast growth factor,* or FGF, which also promotes the growth of tumor cells (11).

Once tumor cells penetrate vessels, they can reach distant organs and proliferate. Metastasis is a highly inefficient process; even when millions of cells detach and migrate from a tumor, only a small fraction will survive and form a new tumor. This paradox is common in nature as all species use a large number of organs or specialized parts to survive. In cancer, great numbers of cells migrate from the primary tumor, ensuring that some have a chance to survive and form new tumors. Although inefficient, cell migration from primary tumors is so effective that metastases are the main cause of cancer deaths. Tumor cells that migrate may die from one of four fundamental causes. First, cells are usually linked with other cells or with the surrounding environment, while migrating cells are not. Consequently, migrating cells may die in a type of apoptosis that affects detached cells, *anoikis*. Tumor cells may also die because they are larger than blood cells, or they may undergo cytoskeletal changes that cause cell death. Finally, migrating tumor cells are also vulnerable to immunological mechanisms (12). The importance of epigenetic mechanisms in tumor progression is also becoming more evident, especially in the context of the cellular and extracellular environments (9). Angiogenesisisone of the distinct and crucial events in tumor progression and cancer malignancy (13).

*Adhesiveness between normal and tumor cells:* Research has examined specifically how tumor cells adhere to the microvascular endothelium of the target organ. Specific molecules on the surfaces of both types of cells are responsible for adhesion and determine the specific site of metastasis. Three studies identified specific characteristics of circulating tumor cells, endothelia, target organs, and the microenvironment of each organ. Data from these studies indicate that these characteristics play a role in determining the sites of secondary tumor foci (14, 15, 16). The cells capable of forming metastases are extravasated tumor cells in close contact with blood vessels. Soluble factors secreted by tumors induce the formation of tumor foci via endothelial activation. This process is mediated by FAK (which upregulates E-selectin) and promotes the adhesion of tumor cells to the endothelium (17). Moreover, endothelial activation by IL-1α, IL-1β, or TNF-α induces expression of E-selectin and P-selectin, as well as VCAM-1 and ICAM-1 on the surface of endothelial cells. The binding of these molecules to their ligands on tumor cells can promote contact and adhesion of tumor cells to endothelial cells (18). Malignant cells targeting the lungs express high levels of Angpt14 and VEGF-A factors, which hinder cell-endothelial cell unions and facilitate extravasation (19). EREG, COX2, MMP1, and MMP2 molecules also promote extravasation and metastasis (20). Extravasation can also result from the interaction of tumor cells and platelets, in which TGF-ß activates TGF / Smad and NF-kB in cancerous cells. This process induces the epithelial-mesenchymal transition in tumor cells, stimulating extravasation (21). Monocytes/macrophages recruited by tumor cells promote the establishment of metastatic breast cancer tumor cells in the lungs (22).

The F4 / 80 +, CD11b +, and Gr1 macrophages are associated with metastasis and secrete VEGF-A. This process promotes extravasation and the establishment and growth of tumor cells, possibly by increasing endothelial permeability (23). However, soluble factors secreted by primary tumors induce recruitment of bone marrow-derived cells, or BMDCs, which include immature myeloid cells, neutrophils, and monocytes. This process occurs in distant organs and results in the formation of a *premetastatic niche*, which ensures the survival and growth of tumor cells (24, 25, 26).

*Non-random metastatic distribution patterns:* Researchers have proposed two theories to explain the metastatic selectivity of tumor cells:

1. *The mechanistic theory:* The relative hemodynamic position of each organ in relation to the primary tumor site determines metastatic selectivity. This theory posits that tumor cells leave the primary tumor by following the circulatory and/or lymphatic drainage routes. Tumor cells then stop at the first organ they encounter, in a process that is non-specific. Consequently, the first organ along the drainage route will be the site of the greatest number of metastases. Anatomical and mechanical factors support this theory, and are significant in determining the metastatic patterns of multiple types of tumors. For example, gastrointestinal tumors start as a localized tumor in the digestive tract mucosa and grow. These tumors then invade the deepest layers of the digestive tract mucosa until reaching the serosa. When the tumor crosses the intestinal wall, it can then invade any organ within or outside the abdomen.
2. *The lymphatic dissemination theory:* Lymphatic dissemination occurs when tumor cells reach the network of lymphatic vessels that surround the colon, allowing lymph drainage to multiple lymph node regions. Dissemination via the lymphatic vessel network occurs in a sequence, affecting the closest lymph nodes first before spreading to more distant ones.