The Metastatic Capacity of Melanoma

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Metastasization is a multistep process in which cancer cells detach from the primary tumor (or other metastases) and spread to locoregional or distant lymph nodes, or to non-contiguous secondary sites. Here, if the tissue microenvironment allows them to survive, they generate a new tumor.

metastasis extravascular migratory metastasis vascular mimicry

1. Introduction

For many years the growth of solid tumors has been associated with their vascularization. The new vessels are needed to deliver oxygen and nutrients within the tumor mass. At the same time, these poorly stabilized vessels act as “Trojan horses” and open a way out for cancer cells. More recently, tumors have been identified whose growth appears to be independent of endothelial cell activity. Here we describe the ability of cancer cells to differentiate and reorganize themself in channels similar to blood vessels containing blood flow, overcoming the need for the angiogenic process for tumor vascularization. Together with the new vessels arising both from angiogenic and vasculogenic processes, these vessel-like structures can be exploited by tumor cells as a guide for migration and metastatic dissemination.

2. Non-Angiogenic Intratumoral Vascularization

Tumor vascularization resulting from the growth of vessels from pre-existing ones or the recruitment of circulant endothelial cell precursors (EPC) is fundamental for tumor growth [1]. Furthermore, the angiogenic process is an essential component of the metastatic pathway. The vascular bed within the tumor provides the principal route by which malignant cells exit the primary tumor site and enter into circulation. For many tumors, vascular and lymphatic density can provide a prognostic indicator of metastatic potential, where highly vascular primary tumors have a higher incidence of metastasis than poorly vascular tumors [2]. The vascular structure acts as a physical and mechanical impediment to the passage of cancer cells into the bloodstream, affecting their fate and the new destination tissue. Over the years, the process of tumor vascularization has been the target of numerous studies that led to the characterization of the sprouting phenomena of new vessels from pre-existing ones (the angiogenic process), and to the identification of possible longitudinal partition that divided the vessel into, two identified as intussusceptive microvascular growth. These phenomena, well characterized in blood vessels [3], have been recently described into the lymphatic torrent as well [4][5]. In contrast with the angiogenic process, intussusception in blood and lymphatic vessels facilitates tissue vascularization without modifying vascular permeability. In addition, the identification of EPC suggests that vasculogenesis could be an alternative process in tumor vascularization.

Indeed, EPCs, attracted into the tumor mass by the growth factors, leave the bloodstream to migrate into the tissues, finding a suitable microenvironment. Here they differentiate into mature endothelial cells and reorganize themselves into vascular structures. These new structures will reconnect to the pre-existing vessels of the tissue itself, allowing active circulation [6]. As already mentioned, the identification of these processes has for years supported the idea of tumor dependence on vessels, as suggested by Folkman in 1971. In the late 1990s, several studies suggested that brain and lung cancer growth proceeds independently from the neovascularization of the tissue and that the tissue vascular network in fact persists with the structure and distribution of pre-existing vessels. In these tumors, cancer cells can hijack existing blood vessels for tumor growth, survival, and metastasis [7][8][9], or exploit their high plasticity to reorganize themself in functional channels. These processes are termed vessel co-option and vascular mimicry (VM), respectively.

Vessel co-opting tumors differ from angiogenic ones in their ability to preserve the vascular scaffold of the surrounding normal tissue instead of inducing a destructive wound healing-like reaction along with angiogenesis, fibrosis and inflammation [10][11]. Usually, vascular co-option occurs in solid tumors, such as in the brain, breast, kidney, and lung [9], with a well-organized vascular bed. In this process, pericytes play an important role in supporting and stabilizing ECs [12]. Even if the molecular mechanisms driving vessel co-option are poorly understood, pericytes physically interacting with cancer cells support cancer invasion [10][13]. The identification of vascular channels lacking ECs was introduced by Maniotis et al., who reported the plasticity of aggressive cancer cells forming de novo vascular networks in highly aggressive uveal melanomas [14]. VM has been described in a plethora of tumors, including carcinomas of the breast [15], ovary [16], bladder [17], lung [18] and prostate [19], as well as sarcomas [20], glioblastomas [21], astrocytomas [22] and melanomas [23]. The functional channels (that are not vessels) are composed of tumor cells with stem cell features, and present characteristic patterns in addition to the erythrocytes inside them. The presence of VM is associated with a high tumor grade, short survival, invasion, and metastasis. Although the molecular mechanisms of VM are not entirely clear, the hypoxia-inducible factor 2α (HIF2α)/vascular endothelial (VE)-cadherin axes are a key pathway [2]. Microarray analyses highlighted in melanoma cell formation channel the downregulation of several melanoma-specific genes, such as melanoma-cell adhesion molecule (MCAM), melan-A (MLANA), and microphthalmia-associated transcription factor (MITF), suggesting a regression in the differentiation state of the tumors. On the contrary, EC-related genes including the tyrosine kinase receptor 1 (TIE1), epithelial-cell kinase (EPHA2), VE-cadherin (CDH5), neuropilin 1 (NPR1) and hypoxia-inducible factor 1α (HIF1α) are up-regulated [24].

3. The Angiotropic Process: Walking on the Abluminal Side of Vessels

Recently, pathologists described, in melanoma samples, the presence of single cancer cells or cell aggregates near vessels on their outer side. Based on these histological observations, Lugassy and Barnhill have hypothesized, for the first time, the phenomenon of angiotropism. Cancer cells, acquiring the typical forms and positions of pericytes, can migrate extravascularly, returning to a neural crest cell migratory phenotype [25]. According to this concept, melanoma cells closely associated with the endothelium in a pericytic location, which are

generally detected at the advancing front of the tumor and without evidence of intravasation, are defined as angiotropic melanoma cells [26][27]. Histological analysis has revealed that both micro and large vessels can be affected by the angiotropic phenomena, while data about neovessels or stabilized vessels are not conclusive yet. In this complex, the ECs show no signs of physiological damage or intravasation. Thus, in parallel to the classical metastatic dissemination, the migration of cancer cells outside the vessels has been referred to as extravascular migratory metastasis (EVMM) (**Table 1**).

Table 1. Short description of the different routes of cancer dissemination.

Metastatic Process	Features
1. Hematogenous	Metastatic cancer cells detaching from primary tumor approach capillaries or angiogenic blood vessels, degrade the basal lamina, invade the endothelium, and intravasate into the flow as single cells or small groups. Finally, they colonize receptive distant organs.
2. Lymphatic	Metastatic cancer cells detaching from primary tumor degrade the basal lamina of lymphatic vessels and intravasate. Metastatic cancer cells enter into the lymphatic system by active movement, pass up the lymphatic flow, and colonize the lymph nodes and other organs.
3. Transcoelomic	Metastatic cancer cells detached or exfoliated from the tumor remain as individual or groups of cells in the cavities. The spread of cancer cells into body cavities occurs via penetrating the surface of the peritoneal, pleural, pericardial, or subarachnoid spaces. In the cavities, metastatic cancer cells proliferate in suspension, generate ascites, and/or adhere to other tissues.
4. Extra Vascular Migratory Metastasis (EVMM)	In EVMM, metastatic cancer cells detaching from the primary tumor approach capillaries or angiogenic blood vessels. Once in the vessel, cancer cells migrate along the abluminal side without intravasating, degrading the basal lamina or altering the structure of vessels.
5. Vascular mimicry (VM)	Metastatic cancer cells, under hypoxic pressure, can form vascular channels interconnected with the tumor vasculature. These leaky structures give nutrients and oxygen to the tumor and support the spread of metastatic cells.

In this view, EVMM can be considered as an important alternative to tumor dissemination. EVMM and pericyte mimicry, mainly described in melanoma [26], have been reported in various tumors including cutaneous squamous cell carcinoma, prostatic adenocarcinoma, carcinosarcomas of the ovaries and endometrium, glioma, liposarcoma and glioblastoma [28][29][30]. During EVMM, the perivascular localization of melanoma cells has been associated with the acquisition of stem-like plasticity and the expression of specific pericytic markers, including PDGFRβ, CD146, CD44, CD73, CD105, and CD144 [31]. All these observations resume the glioblastoma behavior where EVMM occurs, and cancer stem cells give rise to up to 80% of the pericytic compartment. Despite these similarities, further effort is required to understand whether all the pericytes in both glioblastoma and melanoma exert similar migratory and metastatic abilities as well as play a role in vessel stabilization. A recent study suggested that up to 37% of cases of melanoma exhibit EVMM [32]. Therefore, EVMM is probably a common

phenomenon underestimated until now, likely due to technical limitations. Moreover, it has been observed that patients with progressive disease of melanoma that were sentinel lymph node-negative had progressive disease both in sentinel-basin and at distant sites. Therefore, it has also been hypothesized that EVMM, rather than hematogenous spread, might be responsible for the observed progressive disease with single organ involvement. For this, the understanding of the mechanisms of action of EVMM is of outstanding importance. For instance, differential analysis between angiotropic melanomas and non-angiotropic melanomas highlighted 15 critical genes involved in the modulation of EVMM [33]. Among these, KIF14 (kinesin family member 14), ECT2 (epithelial cell transforming sequence 2 oncogenes), and HMMR (hyaluronan-mediated motility receptor) seem to be implicated in cytokinesis. In addition, co-culture experiments demonstrated that the interaction of angiotropic melanoma cells with the abluminal vascular surface promotes the differential expression of genes related to cell migration (CCL2, ICAM1 and IL6), cancer progression (CCL2, ICAM1, SELE, TRAF1, IL6, SERPINB2 and CXCL6), EMT (CCL2 and IL6), stemness (CCL2, PDGFB, EVX1 and CFDP1), and pericytic recruitment (PDGFB) [34].

IIn conclusion, the paradigm for which tumor growth and dissemination are angiogenesis-dependent has been overcome by the identification of adaptive behavior of cancer cells described as vascular co-optation and vascular mimicry. These alternative strategies increase the supply of nutrients into the tumor mass and increase its invasive and migratory capacities both through the classical intra- and alternative extra-vascular routes. These alternative tumor behaviors assume greater importance if we consider that drugs with anti-angiogenic action directed against endothelial cells or their ligands are currently used in cancer therapy. More significant efforts should be devoted to elucidating these processes as they could be potential and exciting therapeutic targets.

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