CCL2 at the crossroad of cancer metastasis

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Primary tumors can affect organ functions, either mechanically when they grow to a considerable size or via production of hormones. However, mortality of cancer patients is in most cases due to formation of secondary growths. Consequently, various drugs are currently employed in clinical trials to impair specific steps of cancer metastasis such as mesenchymal or amoeboid cell migration, intravasation and/or colonization. From the clinical point of view, targeting late metastatic processes such as extravasation or colonization might be required for cancer patients that bear already dormant micrometastases in their capillaries which have left behind earlier metastatic steps. Development of such drugs needs characterization of molecular targets implicated in distinct steps of cancer metastasis.

Extravasation of tumor cells resembles diapedesis of leukocytes which penetrate blood vessel walls at sites of inflammation. Similar to leukocytes, tumor cells attach to endothelial cells through adhesion molecules such as selectins. More ingenious, instead of inventing extravasation a new, tumor cells can attract neutrophils via chemotactic factors and use them as carriage horses to leave the capillaries. A related role has been attributed to platelets that are exploited by tumor cells for attachment to blood vessel endothelia. Attachment is mediated by a fibrinogen coat that can shield tumor cells from shear stress or lytic NK cell attack. Although platelets do not guide tumor cells through the blood vessel wall, they secrete factors such as TGFβ1 that promote epithelial to mesenchymal transition and extravasation. Additional support for extravasation is provided by vascular permeability factors such as vascular endothelial growth factor (VEGF). The founding member of the VEGF family, VEGF-A, was initially also termed “vascular permeability factor” (VPF) based on its ability to enhance the leakiness of blood vessels to plasma and plasma proteins. VEGF is produced by stromal cells but many cancer cells (including cells of hematologic malignancies) have acquired the ability to contribute to VEGF production. Thus, VEGF does not only promote angiogenesis and vascular permeability in primary tumors but also extravasation of disseminated micrometastases. Alternative factors that modulate endothelial barrier integrity are extracellular matrix proteins such as TGFβ-induced (TGFBI) or the protease thrombin that act on endothelial cells and result in cytoskeletal rearrangements (Fig. 1).

In a recent issue of Cancer Cell, Wolf and coworkers have reported about a novel mechanism in tumor cell metastasis that involves the chemokine CCL2. This mechanism goes beyond the well-characterized pro-metastatic functions of CCL2/CCR2-signaling in primary tumors and provides already disseminated cancer cells with an enhanced potential for extravasation and colonization. Chemokines are mediators of heterotypic interactions between tumor cells and their microenvironment. These interactions are essential for acquisition of invasive and metastatic behaviors. Chemokines bind to extracellular matrix compounds such as heparin and proteoglycans. They activate signaling in target cells via 7-span transmembrane chemokine receptors and trimeric G proteins. The activity of chemokines in metastasis is pleiotropic: they are implicated in...
many metastatic steps such as local invasiveness, intravasation, anoikis, extravasation, chemotaxis and colonization. The chemokine CCL2 (also termed monocytic chemotactic protein 1 or MCP-1) is implicated in immunosuppression and tumor angiogenesis. Moreover, CCL2 represents a major chemotactic factor for attraction of myeloid-derived suppressor cells (MDSCs), circulating monocytes and other immune cells to the tumor stroma. Consequently, CCL2 receptors CCR2 and CCR4 are expressed on immune cells (monocytes, macrophages, dendritic cells, NK cells and T cells). CCR2 is also expressed on epithelial and endothelial cells whereas CCR4 is not. Apart from the well characterized pro-metastatic activities in tumors (e.g., activation in endothelial cells promotes tumor neovascularization via matrix metalloproteinase MMP-14 activity), Wolf and coworkers discovered a distinct function of CCL2/CCR2-signaling in distant endothelia of capillaries that is triggered by CCL2-producing tumor cells. They used lung colonization by MC38 colorectal cancer cells in mice as model for identification of molecular events triggered by CCL2/CCR2 during tumor cell extravasation. The C57BL/6-syngeneic MC38 colonization model is not suitable for investigation of initial events of cancer metastasis such as local invasiveness or intravasation. However, it offers the advantage to appropriately manipulate gene expression in transplanted cancer cells, a task that is more difficult to achieve in primary tumor models. CCR2 functions were selectively ablated, either in metastatic target tissues (by using CCR2-deficient recipient mice) or in tumor cells (by shRNA-mediated knock-down of CCR2). Moreover, reciprocal bone marrow reconstitution experiments were performed to evaluate the implication of monocytic CCL2/CCR2-signaling in tumor cell extravasation. These experiments demonstrated that tumor cell-derived CCL2 promotes extravasation by a dual mechanism: (1) chemotraction of CCR2+ Ly6C+ monocytes (which might be used by tumor cells as carriers through the vessel wall) and (2) enhancement of local vascular permeability. Of note, the permeabilizing effect of CCL2 on the endothelial compartment in this metastasis model turned out to be more important for extravasation than chemotraction of leukocytes. CCL2 exerted this permeabilizing effect in a transient and non-systemic manner. Vascular endothelia became impermeable again after tumor cells have found their way through the vessel walls and established secondary growths in metastatic niches. The relevant CCL2/CCR2 downstream signaling pathways for extravasation were identified in vitro using tumor cell transmigration in the presence and absence of specific inhibitors. These experiments, together with additional experiments performed in mice, revealed the JAK2/STAT5 and p38MAPK pathways downstream of CCL2/CCR2 as crucial for induction of vascular permeability. p38MAPK is known to promote...
extravasation in a non-tumor cell-autonomous manner as reported by Matsuo and coworkers. They used heterozygous p38α+/- mice as recipients for tumor cell transplantation studies and observed impaired lung colonization of B16 melanoma cells when compared with p38α+/- recipients. At the molecular level, p38α was shown to regulate expression of P-selectin and E-selectin in endothelial cells thereby promoting endothelial adhesion of tumor cells. In contrast, the role of JAK-STAT activation in endothelial cells promotes metastatic processes leading to cell transformation, neurite outgrowth or STAT-mediated transactivation of platelet-derived growth factor α. JAK2/STAT5 enhances adhesiveness of cancer cells in cooperation with Ras/ERK signaling which might contribute to metastasis. In contrast, STAT1 inhibits Ras/PI3K pathways, promoting metastatic activities at distant sites. These studies might provide novel biomarkers for metastasis and increase the number of potential molecular targets for anti-metastatic therapies.

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