Molecular Pathways: VCAM-1 as a Potential Therapeutic Target in Metastasis

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Abstract

Interactions between disseminated tumor cells (DTC) and stromal cells in the microenvironment are critical for tumor colonization of distal organs. Recent studies have shown that vascular cell adhesion molecule-1 (VCAM-1) is aberrantly expressed in breast cancer cells and mediates prometastatic tumor-stromal interactions. Moreover, the usefulness of VCAM-1 to DTCs in 2 different organs—lung and bone—is based on distinct mechanisms. In the lungs, VCAM-1 on the surface of cancer cells binds to its counter-receptor, the α4β1 integrin (also known as very-late antigen, VLA-4), on metastasis-associated macrophages, triggering VCAM-1–mediated activation of the phosphoinositide 3-kinase growth and survival pathway in the cancer cells. In the bone marrow, cancer cell VCAM-1 attracts and tethers α4 integrin–expressing osteoclast progenitors to facilitate their maturation into multinucleated osteoclasts that mediate osteolytic metastasis. These findings highlight the importance of direct interactions between DTCs and stromal cells during tumor dissemination and draw attention to the possibility of targeting the α4 integrin/VCAM-1 interactions in metastatic breast cancer. Anti-α4 integrin inhibitors have been developed to treat various diseases driven by massive leukocyte infiltrates and have gained U.S. Food and Drug Administration approval or are undergoing clinical trials. Testing these drugs against tumor-stromal leukocyte interactions may provide a new strategy to suppress lung and bone relapse of breast cancer. Clin Cancer Res; 18(20); 5520–5. ©2012 AACR.

Background

Over the past several decades, early detection, surgical removal, and targeted therapy have improved treatment outcome for primary breast tumors. However, breast cancer still has a high mortality rate that is primarily due to metastatic disease. Lung, bone, brain, and liver are the most common sites of distant dissemination (1, 2). Metastasis from primary sites to distal organs is a complex process that involves a series of sequential steps: invasion and intravasation of tumor cells from the primary tumor sites to enter the circulation, extravasation of these circulating tumor cells (CTC) into distant tissues, and final colonization of the seeded organ (3, 4). Even though CTCs are present in large numbers in the bloodstream, only a small proportion of these cells succeed in forming distal metastasis (1, 5). As envisioned in the "seed and soil" hypothesis (6, 7), the organ tropism of metastasis depends on the complex interplay between tumor cells and the unique microenvironments of different distal organs. The underlying mechanisms of this interplay are just beginning to be elucidated.

VCAM-1 as an adhesion molecule in endothelial cells

VCAM-1 is an immunoglobulin (Ig)-like adhesion molecule with 7 extracellular Ig domains that are mainly expressed in endothelial cells (13, 14). While expressed at low level on resting endothelial cells, VCAM-1 is strongly induced by several inflammatory cytokines (15, 16). VCAM-1 binds with high affinity to the integrins α4β1 (also known as very-late antigen, VLA-4) and α4β7. α4β1 in particular is a well-studied counterreceptor expressed on the surface of several cell types of the hematopoietic lineage including lymphocytes, monocyte/macrophages, and eosinophils (17). VCAM-1 plays a critical role in the inflammatory response by recruiting leukocytes to acute and chronic inflammatory sites (15, 16). In addition to
mediating leukocyte adhesion on endothelial cells. VCAM-1 can activate signaling pathways to facilitate leukocyte passage from blood to tissue. The short intracellular tail of VCAM-1 interacts with Ezrin (also known as cytofilin or villin-2), a member of the ERM (Ezrin/Radixin/Moesin) protein family (18). ERMs are cytoplasmic adaptor proteins that link various transmembrane proteins to the actin cytoskeleton (18). In endothelial cells, VCAM-1 clustering, either by antibody cross-linking or integrin binding, triggers the activation of Rac1, which is a Rho-like GTPase (19). The activation of Rac1 results in the rearrangement of the cytoskeletal network, which is thought to remodel the tight junctions between vascular endothelial cells and consequently facilitate transendothelial migration. Circulating melanoma cells expressing α4 integrin have been shown to interact with VCAM-1 on endothelial cells to facilitate extravasation and metastasis in distal organs (20–22). Therefore, in addition to passive entrapment of tumor cells by size restriction (4), capillary beds may actively participate in the extravasation into disseminated sites of CTCs via the adhesion molecules on vascular endothelial cells.

**Role of VCAM-1 in lung metastasis of breast cancer**

Studies combining animal models and analyses of clinical samples identified a lung metastatic gene signature (LMS) as a set of 18 genes whose expression is associated with lung relapse in estrogen receptor–negative tumors and mediates lung metastasis in xenograft models (23). Understanding the molecular mechanisms of how the LMS genes mediate lung metastasis can provide novel treatment modalities for lung metastasis of breast cancer. VCAM-1 is among these 18 genes, and its expression in breast cancer cells enhances the ability to form metastases in the lungs (8, 23). The obvious question is why do cancer cells with a high propensity to form lung metastasis abnormally over-express a vascular endothelial adhesion molecule? Our work addressing this question indicates that the high expression of VCAM-1 in tumor cells does not influence tumor cell intravasation from the primary sites, the number of CTCs in the bloodstream, or the CTC extravasation into the lungs. Instead, VCAM-1 promotes lung colonization by providing juxtacrine survival signaling that protects tumor cells from death (Fig. 1).

Similar to its adhesive function in endothelial cells, VCAM-1 expressed on the surface of tumor cells tethers leukocytes that express α4 integrins. Analyses on leukocyte subpopulations in lung metastatic lesions indicate that tumor-associated monocytes and macrophages not only prominently express α4 integrins but also express higher levels of α4 integrins compared with T and B lymphocytes. Macrophages have been shown to play important roles in primary tumor growth and lung metastasis at different steps of the lung metastatic process, including promoting tumor angiogenesis, tumor invasion at the edge, tumor cell intravasation into the bloodstream, and lung colonization (24, 25). Most of these studies have been done on breast tumors and show that the cross-talk between tumor cells and tumor-associated macrophages (TAM) is mediated by various soluble factors secreted by tumor cells and TAMs. However, recent studies have shown that depleting lung macrophages or preventing the recruitment of macrophages into lung by blocking CCL2-CCR2 signaling in mouse models significantly decreases lung metastasis via unknown molecular mechanisms (26, 27). The recent work on VCAM-1 provides one such mechanism (8). Tumor cells entering the lung parenchyma are immediately surrounded by macrophages, probably as a result of an innate immune response. VCAM-1 itself does not appear to play an active role in recruiting macrophages to the vicinity of tumor cells. However, the close proximity of macrophage and tumor cells subsequently facilitates contact between the α4 integrins and VCAM-1. This direct contact initiates juxtacrine stimulation by clustering VCAM-1 on tumor cells to activate prosurvival AKT signaling. VCAM-1 engagement by α4 integrins recruits Ezrin to the VCAM-1 cytoplasmic tail, leading to phosphorylation of Ezrin on tyrosine. Once activated, Ezrin serves as an adaptor that binds both phosphoinositide 3-kinase (PI3K) and its downstream mediator, AKT, leading to activation of AKT-mediated cell survival signaling (8). A similar survival advantage from VCAM-1 was observed in TAM-enriched primary tumor sites when lung metastatic tumor cells were back-seeded into primary tumors (8). Therefore, α4 integrin-expressing TAMs create a favorable microenvironment for high VCAM-1–expressing tumor cells in lungs and also in areas of primary tumor infiltration. A direct interaction between lung macrophages and disseminated tumor cells via VCAM-1 evokes tumor cell prosurvival signaling and promotes lung metastasis.

**Role of VCAM-1 in bone metastasis of breast cancer**

Another set of studies identified a role of VCAM-1 in osteolytic bone metastasis by breast cancer cells that have emerged from latency in the bone marrow (9). Although high expression of VCAM-1 is observed in clinical tissue samples of lung metastasis and bone metastases compared with brain metastasis tissues, VCAM-1 did not directly support the survival of bone metastatic breast cancer cells in the marrow in mouse models (8). However, VCAM-1 was highly expressed in aggressive clones that emerged from an indolent bone metastatic breast cancer cell line (9). Hyperactive NF-kB signaling appears to drive VCAM-1 expression in these cells. Regardless, a high level of VCAM-1 expression mediates the aggressive osteolytic phenotype of these “postdormancy” breast cancer cells. VCAM-1 also mediated bone metastasis in other mouse and human breast cancer cell lines. Notably, systemic administration of blocking antibodies targeting either α4 integrin or VCAM-1 decreased bone metastasis in these mouse models (9).

VCAM-1 expression had no effect on the proliferation of breast cancer cells in the bone marrow (9). So how does VCAM-1 facilitate bone metastasis in these postdormancy cells that egressed from the indolent stage? Bone metastasis proceeds with the outgrowth of micrometastases in the bone marrow and areas adjacent to the bone matrix. As the lesion grows, it engages osteoclasts in the destruction of
bone matrix, causing the release of bound TGF-β and other growth factors that further stimulate the cancer cells to release more osteoclast activating factors. This is referred to as the “vicious cycle” of bone metastasis (28, 29). Thus, osteolytic metastasis is dependent on the differentiation of osteoclast precursors and their maturation into multinucleated osteoclasts. Bone metastatic cells have been shown to increase the activity of osteoclast progenitors through RANKL (receptor activator of NF-κB ligand; refs. 29, 30).

The study by Lu and colleagues showed that osteoclast progenitors in the bone marrow express α4β1 integrin. The tethering of these cells to the VCAM-1 rich surface of the cancer cells apparently stimulates osteoclast differentiation by facilitating cell fusion into multinucleated, mature osteoclasts (Fig. 1; ref. 9).

Clinical–Translational Advances

The prevention and treatment of metastatic breast cancer remain a challenge. The recent findings on the roles of VCAM-1 in lung and bone metastasis provide one potential target to control the disease. Drugs that disrupt the binding of α4 integrins to VCAM-1 have already been in development to treat diseases involving massive influx of leukocytes into inflammatory sites (31, 32). Natalizumab, a monoclonal antibody against α4 integrin, is approved by the U.S. Food and Drug Administration for the treatment of relapsing multiple sclerosis and inflammatory bowel disease. Furthermore, small molecule inhibitors against α4 integrin are in clinical trials. On the basis of the highlighted studies, these drugs have potential usefulness in treating metastatic breast cancer by interrupting α4 integrin-VCAM-1 interactions.

Natalizumab

Natalizumab (Tysabri; Elan Pharmaceuticals and Biogen Idec) is a humanized monoclonal antibody that binds to α4 integrin chains and prevents formation of the α4β complexes. Natalizumab is generated by fusing the
complementary determining regions of antibody AN100226m and human IgG4 framework to reduce immunogenicity and increase the half-life of the drug in patients (33, 34). Its efficacy in treating MS was preclinically showed using an animal model of autoimmune encephalomyelitis (35). Cerebral microvessels in these animals, as well as in patients with multiple sclerosis, display high VCAM-1 expression before perivascular leukocyte infiltration (36). AN100226m suppressed and reversed the disease progression in the encephalomyelitis animal model (37). Natalizumab can block α4 integrin binding to both MadCAM-1 and VCAM-1, the 2 adhesion molecules mediating leukocyte trafficking in gut-associated lymphoid organs and inflammatory sites (15, 38, 39). According to the public accessible website from NIH (ClinicalTrials.gov), Biogen Idec additionally conducted a clinical trial testing the utility of natalizumab as a potential therapy for multiple myeloma (40). In animal models of multiple melanoma, anti-α4 integrin treatment decreases the growth of multiple myeloma in bone marrow (41–43). This suppressive effect is due to the disruption of α4 integrin interactions between tumor and bone marrow stromal cells as well as tumor cells and extracellular matrix proteins (43–47).

Although no significant adverse events were observed in earlier clinical trials of natanizumab, a small proportion of patients developed progressive multifocal leukoencephalopathy (48). This is a rare but fatal neurologic condition that occurs in patients with a severe immune-deficient condition (49). Therefore, natalizumab is not approved in combination with immunosuppressive agents. Immunosuppression is a common adverse effect of many chemotherapeutic agents used to treat breast cancer, creating a significant challenge for the potential use of natalizumab to treat metastatic breast cancer.

**Small-molecule inhibitors against α4 integrin**

Small-molecule inhibitors against α4 integrin have been developed, largely by structure-based design. They are in clinical testing to determine their efficacy in treating autoimmune diseases, but this research is still in a much more preliminary stage than research related to antibody-based therapy. Importantly, these small molecules have several advantages over antibody-based therapy. Although the human IgG4 framework used to humanize natalizumab does not activate the complement cascade and has low affinity to Fc receptors, antibodies against natalizumab still emerged in 11% of multiple sclerosis patients and 7% of inflammatory bowel disease patients in the clinical trials (50, 51). This problem is largely averted with small molecules. Furthermore, long-term treatment is required for autoimmune diseases and potentially in breast cancer patients with metastasis. Natalizumab needs to be administered intravenously more than 1 hour every 4 weeks, whereas small-molecule inhibitors of α4 integrin make oral bioavailability possible (52). Despite these advantages over natalizumab, the application of small-molecule inhibitors against α4 integrin targets a biologically important interaction and, therefore, would need to be closely monitored for possible adverse reactions. Currently, an orally active α4 integrin antagonist, AJM300, reportedly is undergoing a clinical trial in patients with inflammatory bowel disease (53), and a non-oral small inhibitor, ELND002, is being investigated in a phase 1 clinical trial in patients with multiple sclerosis (54).

Disrupting α4 integrin–VCAM-1 interactions between cancer cells and stromal components of the tumor microenvironment offers a possibility to suppress the outgrowth of disseminated breast cancer cells. Additional studies are needed to better identify the efficacy of this approach, but the recent reports (6, 9) provide encouraging evidence in support of this possibility.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

Conception and design: Q. Chen, J. Massagué
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Q. Chen, J. Massagué
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Q. Chen, J. Massagué
Writing, review, and/or revision of the manuscript: Q. Chen, J. Massagué
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