The Role of Vascular Cell Adhesion Molecule-1 in Tumor Immune Evasion

T-C. Wu

Departments of Pathology, Oncology, Obstetrics and Gynecology, and Molecular Microbiology and Immunology,
The Johns Hopkins Medical Institutions, Baltimore, Maryland

Abstract

Tumor immune escape is a critical trait of cancer but the mechanisms involved have yet to fully emerge. One recent study has shown that tumor cells can escape T-cell immunity by overexpressing the endothelial cell adhesion molecule vascular cell adhesion molecule-1 (VCAM-1), which normally mediates leukocyte extravasation to sites of tissue inflammation. Renal cell carcinoma (RCC) was identified as one tumor type where VCAM-1 is commonly highly overexpressed. Together, our findings suggest that RCCs might exploit VCAM-1 overexpression for immune escape. [Cancer Res 2007;67(13):6003–6]

Background

Current therapies for cancer, such as surgery, chemotherapy, and radiation therapy, rarely result in long-term benefits in patients with metastatic disease. Cancer immunotherapy provides an alternative approach for the control of cancer. The ideal cancer therapy should have the potency to eradicate systemic tumors at multiple sites in the body, as well as the specificity to discriminate between malignant and normal cells. In both of these respects, immunotherapy is an attractive candidate.

Recently, several studies have focused on the mechanisms of tumor escape from immune surveillance and attack that may blunt the efficacious effects of active immunotherapy. These studies have identified many distinct loss-of-function and gain-of-function mechanisms of tumor immune evasion. For example, tumors can down-modulate multiple components of the MHC I antigen processing pathway to avoid recognition by tumor-specific CTL. In addition to antigen loss, down-modulation of proteasome subunits, transporter associated with antigen presentation, β2 microglobulin, and MHC I heavy chain can diminish presentation of MHC-peptide complexes on the tumor surface. In addition, transport of MHC-peptide complexes from endoplasmic reticulum (ER) through the Golgi to the cell membrane can be diminished (for review, see ref. 1).

Alternately, increased expression of a several molecules has been shown to mediate important mechanisms of tumor evasion. For example, expression of signal transducer and activator of transcription 3 (Stat3) by tumors has been identified as a central mediator of oncogenesis, angiogenesis, and immunosuppression. Recent studies have shown that Stat3 signaling in tumor cells promotes secretion of the immunosuppressive cytokines transforming growth factor-β (TGF-β) and interleukin (IL)-10, which inhibit the proliferation and function of tumor-specific cytotoxic T cells and are crucial in the accumulation of regulatory T cells in the tumor microenvironment (for review, see ref. 2).

Other important tumor immune evasion mechanisms are mediated by B7-H1 (3), indoleamine 2,3-dioxygenase (IDO) enzyme (4), and galectin-1 (5), as well as shedding of the natural killer cell receptors MIC-A and MIC-B (6), which affect the proliferation, survival, or function of effector T cells. Together, these studies have laid the foundation for understanding how tumors escape immune attack and interact with the immune system in general. However, it is clear that a better understanding of the molecular mechanisms that allow tumors to escape immune attack could have a major effect on small molecule and vaccine development strategies for cancer treatment.

Most of the studies involving tumor immune evasion mentioned above have followed an approach that involves the initial identification of a new immunoregulatory molecule that is thought to play a role in tumor evasion. The next step would be to show the dysregulation of this molecule in several cancers and finally provide evidence that the molecule plays a role in the impairment of the immune response against tumors. Several molecules have been identified in this fashion, including B7-H1, IDO, galectin-1, Stat3, TGF-β, and MIC-A/MIC-B.

Recently, we took a different approach to define immune escape molecules through the in vivo selection and characterization of an immune escape-resistant cancer cell line (7). We developed previously a human papillomavirus-16 (HPV-16) E7-expressing cancer cell line called TC-1/P0 as a mouse tumor model for testing the E7-specific cancer immunotherapy (8). In addition, we generated a HPV-16 E7-expressing vaccinia vaccine termed Sig/E7/lysosome-associated membrane protein-1 (LAMP-1), which encodes a fusion protein consisting of an ER signal sequence, HPV-16 E7, and the transmembrane and cytoplasmic domains of LAMP-1 to enhance antigen presentation and the development of cellular immunity (9). Vaccination with Sig/E7/LAMP-1 vaccinia led to a substantial increase in both E7-specific CD8+ and CD4+ T-cell immune responses compared with wild-type E7 vaccinia, preventing the growth of TC-1/P0 in 60% to 80% of immunized mice (8).

A tumor from one of the immunized mice exhibiting tumor growth was explanted and expanded in vitro. This escape variant cell line was designated P1 and injected into a new group of mice immunized with Sig/E7/LAMP-1 vaccinia. Again, a tumor from one of the immunized mice with tumor growth was explanted and expanded in vitro. This cell line was designated P2. These repeated injections with tumor cell lines allowed us to carry out in vivo immune selection and resulted in increasing resistance to prophylactic Sig/E7/LAMP-1 vaccinia immunization. After three rounds of in vivo immune selection, we obtained the P3 cell line, which was completely resistant to the vaccinia-induced
immune response. Both the P0 and P3 cell lines grew with similar growth kinetics. However, when these cell lines were injected into mice immunized with Sig/E7/LAMP-1 vaccinia, P3 was able to develop into a palpable mass in all of the mice within 7 days, whereas P0 only developed in two out of five mice after several weeks. Thus, we successfully generated an immune-resistant tumor model (P3), thereby developing a system that allows us to identify genes that may contribute to tumor escape from immune attack.

Using this system, we did a functional screening assay for the gene(s) responsible for this immune-resistant phenotype. Microarray analysis of P3 and P0 tumors revealed that vascular cell adhesion molecule-1 (VCAM-1) is one of the highly up-regulated genes expressed in the P3 immune-resistant variant compared with P0. VCAM-1, also known as CD106, is a molecule with a well-characterized role in the human immune system. It contains six or seven immunoglobulin domains and is expressed by many different cell types, including activated endothelial cells, bone marrow stromal cells, spleen stromal cells, thymic epithelial cells, peripheral lymph node (LN) and mesenteric LN high endothelial venules, and some dendritic cells in the spleen. Up-regulation of VCAM-1 in endothelial cells is induced by the cytokines IL-1β, IL-4, tumor necrosis factor-α, and IFN-γ. VCAM-1 is an endothelial ligand for very late antigen-1 (VLA-4; or α4β1 integrin) and α4β7 integrin. The interaction between VCAM-1 and VLA-4 or α4β7 integrin, expressed on leukocytes, is thought to be involved in the extravasation of leukocytes through the endothelium to sites of inflammation (for review, see refs. 10, 11).

Key Findings

The differential expression of VCAM-1 in immune-resistant P3 cells versus immune-sensitive P0 cells was confirmed using quantitative reverse transcription-PCR. Furthermore, we observed that retroviral expression of VCAM-1 in P0 tumors (P0-VCAM-1) significantly increased their resistance against vaccine-induced immune responses. In addition, knockdown of VCAM-1 in P3 tumors using small interfering RNA led to reduced tumor immune evasion, although the down-regulation of VCAM-1 did not completely abolish the tumor immune evasion phenotype of P3 tumors. Taken together, our data suggest that VCAM-1 is one of the important factors involved in tumor immune evasion in the P3 tumor.

Figure 1. Model for VCAM-1–mediated tumor immune evasion. A model is proposed for the role on VCAM-1 in tumor immune evasion. A, in the case of VCAM-1–expressing tumor cells, VCAM-1 on the tumor cell binds to α4β1 integrin, the receptor for VCAM-1 on the surface of the T cells. This interaction leads to migration of the T cells away from the tumor and decreased infiltration of T cells into the tumor, thus leading to tumor immune evasion. B, in the case of tumor cells not expressing VCAM-1, there is no interaction with α4β1 integrin on the T cells. The tumor cells present the tumor antigen through MHC class I molecule to the T-cell receptor on the CD8+ T cell, thus activating the T cells and leading to increased infiltration of the T cells into the tumor. This results in tumor killing (X).
We found that VCAM-1 expression by tumor cells led to decreased apoptosis of the tumor cells and a significant decrease in the number of tumor-infiltrating CD8+ T cells in the tumors expressing VCAM-1. In vitro Transwell studies showed that VCAM-1 can promote the migration of freshly isolated tumor-infiltrating CD8+ T cells and that this VCAM-1-mediated migration is diminished in the presence of an α4 integrin blocking antibody. Taken together, these findings suggest that tumor expression of VCAM-1 may modulate the motility of infiltrating CD8+ T cells.

We showed that mutation of α4β1 integrin binding sites on VCAM-1 completely abolished the immune resistance conferred by VCAM-1 expressed on P0-VCAM-1 tumors in vivo and restored the number of CD8+ T cells in mutated P0-VCAM-1 tumors similar to the number of CD8+ T cells in P0 tumors. Furthermore, using noninvasive bioluminescence imaging of luciferase-expressing E7-specific CD8+ T cells, we showed that significantly reduced numbers of E7-specific CD8+ T cells were present in P0-VCAM-1 tumors compared with P0 tumors. In addition, a significant number of E7-specific CD8+ T cells was observed within the P0-VCAM-1 tumors with mutation of α4β1 integrin binding sites, similar to the amount of E7-specific CD8+ T cells within the P0 tumors. Thus, our data indicate that VCAM-1 plays an important role in reducing the amount of antigen-specific CD8+ T cells in the tumors, through its interaction with its receptor, α4β1 integrin.

We then searched for human cancers with overexpression of VCAM-1 through analysis of the Oncomine Cancer Profiling Database and found that most human cancers do not up-regulate VCAM-1. However, human renal cell carcinoma (RCC) was found to be highly positive for VCAM-1 expression in comparison with other cancers or normal renal tissues. We characterized several human RCC cell lines and found that most RCCs stained positive for VCAM-1. Interestingly, we found that the only cell line that stained negative for VCAM-1 was derived from the only patient from a previous clinical trial (12) who responded to an irradiated granulocyte macrophage colony-stimulating factor–secreting RCC tumor vaccine. These findings suggest that at least a subset of human RCC express VCAM-1 and that the expression of VCAM-1 may serve as an indicator for the outcome of immunotherapy.

Implications

Our findings suggest that expression of VCAM-1 in tumors may promote T-cell migration away from tumors, resulting in decreased accumulation of T cells in the tumor microenvironment. This decreased accumulation of T cells around tumor cells may contribute to the ability of VCAM-1–expressing tumor cells to escape immune attack. Figure 1 depicts our model for VCAM-1–mediated immune escape. Our data showed that tumor expression of VCAM-1 represents a new mechanism of tumor immune evasion and has important implications for the development of immunotherapy against VCAM-1–expressing tumors, such as human RCCs.

One potential explanation for VCAM-1–mediated immune evasion is that VCAM-1 may promote T-cell migration through its interaction with α4 integrin on the T cells, thus minimizing the contact between T cells and the tumor cells. Several studies have confirmed that interaction of VCAM-1 with α4 integrin can promote the migration of CD8+ T cells. In addition, α4 integrin has also been shown to possess the unique property of opposing cellular spreading and focal adhesion formation while promoting cell migration (for review, see ref. 13). Specifically, it has been shown that binding of VCAM-1 to α4 integrin leads to the specific association of the cytoplasmic tail of α4 integrin to paxillin, a signaling adaptor protein. This association leads to the phosphorylation of paxillin, which can then phosphorylate and activate focal adhesion kinase (FAK; ref. 14). In turn, FAK can interact with Src kinases and regulate the disassembly of focal adhesions and integrin-dependent cell migration. In addition, binding of α4β1 integrin to VCAM-1 has also been shown to stimulate T-cell migration mediated by LFA-1 (α4β2 integrin; ref. 15). Thus, the expression of VCAM-1 on tumor cells may promote T-cell migration away from the tumor through these molecular mechanisms, resulting in tumor immune evasion. Although this mechanism for VCAM-1–mediated tumor immune evasion is highly plausible, we cannot exclude the possibility of other mechanisms contributing to the immune evasion mediated by VCAM-1.

This study represents the first report showing immune interference involving the dysregulation of a cell adhesion molecule by tumor cells. Because several cell adhesion molecules are known to be dysregulated in cancers, this study raises the possibility that dysregulation of other cell adhesion molecules may also play a role in tumor escape from immune attack (for review, see ref. 16). In addition, this study highlights the use of a novel approach of in vivo immune selection to study tumor immune evasion. Similar approaches may potentially be used to generate highly resistant variants from other murine cancer cell lines. Characterization of such resistant variants may aid in the identification of novel mechanisms of tumor immune evasion.

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References


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