Targeting the Tumor Vasculature to Improve the Efficacy of Oncolytic Virus Therapy

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Virus-mediated oncolysis is not a new concept. The idea goes back to the early 1900s, when it was noted that a flu-like disease coincided with a substantial drop in the number of tumor cells in a leukemic patient (1) and that rabies vaccination was followed by regression of cervical cancer (2). Additional anecdotal observations followed, but early attempts at viral therapy for cancer were unsuccessful (3,4). In principle, to be an effective weapon against cancer, an oncolytic virus must infect and kill cancer cells while sparing normal cells (5,6). Recent advances in biochemistry, molecular biology, and genetic engineering have brought oncolytic virus therapy back into focus. Some viruses that preferentially infect tumor cells that overexpress receptors for virus entry have been identified; other viruses have been genetically engineered to exploit specific characteristics of tumor cells, such as the expression of tumor antigens, tumor suppressor proteins, or altered signaling pathways (5,6). Although the in vitro results have been encouraging, preclinical animal models and early clinical trials have demonstrated little efficacy with oncolytic virus therapy and only limited spread of the virus infection beyond the primary site (6–10). Because safety reasons have dictated that oncolytic viruses be attenuated for clinical use, a concern has been raised that they may have largely lost their ability to effectively spread and kill the tumor tissue (6). Another concern is that innate host immunity may limit oncolysis and the spread of oncolytic viruses to the tumor tissue (5,6). There is evidence that the acute immune response that follows an initial oncolytic viral infection limits tumor oncolysis and viral replication, resulting in diminished antitumor efficacy (5). Indeed, depletion of complement activity or the use of the immunosuppressant drug cyclophosphamide was found to enhance virus oncolytic effects (11–13), and addition of interferon gamma or the activation of the cytokine interleukin 10 inhibited the replication of oncolytic virus in rat glioma cells (5,12).

In this issue of the Journal, Kurozumi et al. (14) sought to target the tumor vasculature to reduce tumor inflammation and improve the antitumor efficacy of oncolytic virus therapy. In their elegant studies, the authors treated established intracranial gliomas in rats with an oncolytic virus derived from type I herpes simplex virus. They observed that at 3 days after treatment, the tumor vessels had increased permeabililty and the tumor tissue was infiltrated with leukocytes. Gene expression profiling confirmed the presence of an inflammatory response: it showed increased expression of a number of mediators of inflammation, including interferon gamma. On the basis of previous results (11–13) that suggested that inflammation negatively impacts oncolytic virus therapy, the authors sought to reduce vascular permeability and inflammation by using a cyclic peptide of arginine-glycine-aspartic (cRGD), an antagonist of integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ that has been previously shown to inhibit angiogenesis and tumor growth (15,16). A single dose of cRGD peptide administered before oncolytic virus treatment resulted in reductions in tumor vessel permeability, leukocyte infiltration, and interferon gamma levels. Importantly, the titers of oncolytic virus were increased and the median survival of treated rats was statistically significantly extended. Thus, this study provides evidence that antiangiogenic therapy with cRGD peptides can improve the efficacy of oncolytic viral therapy with respect to targeting of tumor cells.

Recently, much attention has focused on the impact of the tumor microenvironment, particularly the tumor vasculature, on the growth and survival of tumor cells. We now know that certain microenvironments favor tumor growth and others suppress tumor growth and that tumor cells often alter their microenvironment to favor their progression (17). We have learned that stromal cells, inflammatory cells, and vascular endothelial cells within the tumor tissue can promote tumor growth and metastasis, and a number of molecules have been identified that mediate these functions. Cancer is not simply an autonomous cellular disease composed of transformed cells, and cancer treatment is beginning to target more than the tumor cells.

The tumor vasculature has recently emerged as a desirable target for the treatment of cancer in combination with chemotherapy, and several antiangiogenic agents are currently being developed. One group of agents incorporates the RGD cell adhesion motif that was originally discovered in the matrix protein, fibronectin, by Pierschbacher and Ruoslati (18). This peptide sequence was later identified in other matrix proteins, and it was found to mediate cell adhesion via specific members of a family of cell surface receptors named integrins (19–21). The $\alpha_v\beta_3$ integrin is expressed by vascular endothelial cells and is overexpressed in proliferating endothelium, particularly in tumor blood vessels (22). Synthetic RGD peptide antagonists of $\alpha_v\beta_3$ inhibit angiogenesis in a variety of tumor models (15,23). Currently, cRGD peptides are being tested in clinical cancer trials (24). The mechanism of action of all α_v integrin antagonists in blocking angiogenesis has been attributed to their ability to induce endothelial cell death in newly sprouting capillaries and to disrupt the enzymatic activity of metalloproteinases (16,22). Consistent with these earlier results, Kurozumi et al. (14) observed a reduction in the number of tumor blood vessels in

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DOI: 10.1093/jnci/djm234

Published by Oxford University Press 2007.

the cRGD-treated gliomas, which could explain the associated reduction of vascular permeability and immune cell infiltration they observed. Other inhibitors of tumor angiogenesis, particularly inhibitors of vascular endothelial growth factor (VEGF) function, markedly reduce vascular permeability (VEGF is also known as vascular permeability factor) and cause the tumor vasculature, which is characteristically abnormal, to transiently normalize both morphologically and functionally (25–28). Recently, cRGD peptide treatment was reported to reduce brain edema and inflammation after ischemic infarction in a rat model (29). However, blockade of integrin signaling by RGD peptides has also been linked to increased vascular permeability in some settings (30,31).

Another explanation for the improved antitumor efficacy of oncolytic therapy observed by Kurozumi et al. (14) is that cRGD peptides may possess anti-inflammatory activity. Although the processes of angiogenesis and inflammation are often linked, their relationship is complex (32–34). There is substantial evidence that many inflammatory responses are associated with increased angiogenesis and that inflammatory cells promote angiogenesis through different mechanisms (25). In addition, a recent study (35) has shown that angiopoietin-2, a well-known regulator of angiogenesis, can promote inflammatory responses, providing evidence that the same molecule can regulate both angiogenesis and inflammation. It is interesting that RGD peptides were found to statistically significantly inhibit leukocyte recruitment to synovial sites of chronic inflammation (36) and to reduce myeloid cell adhesion and transendothelial cell migration (37,38). Such activities have been attributed to the antiadhesive property of RGD peptides and appear to be distinct from their antiangiogenic activity.

The observations made by Kurozumi et al. (14) open a number of interesting possibilities about approaches to improve the efficacy of oncolytic virus therapy. Will other antiangiogenic treatments besides cRGD peptides be useful additives to oncolytic therapy? Will anti-inflammatory agents be useful? Will specific inhibitors of leukocyte migration be effective? As we learn more about the tumor microenvironment and its impact on tumor growth, we will be able to develop therapeutic strategies that combine specific tumor cell targeting as well as targeting the tumor microenvironment that facilitates tumor cell growth.

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