Targeting the Stroma by T Cells to Limit Tumor Growth

Bin Zhang

The Department of Medicine-Division of Hematology & Medical Oncology, Cancer Therapy & Research Center, University of Texas Health Science Center, San Antonio, Texas

Abstract

Solid tumors may modulate their environment and keep stromal cells in an immunosuppressive and tumor-promoting state. Recent findings indicate that targeting not only cancer cells but also nonmalignant stromal cells by T cells is required for the eradication of established tumor. Interestingly, a single adoptive transfer of effector T cells that recognize tumor antigen-loaded stromal cells, but not the cancer cells themselves, causes long-term inhibition of tumor growth associated with T cell-mediated killing of stromal CD11b+/Gr1+ myeloid cells. Therefore, targeting tumor stroma by T cells points to an important alternative approach to the effective control of tumor growth, particularly in the absence of direct targeting of cancer cells. [Cancer Res 2008;68(23):9570–3]

Stromal Destruction Is Required for the Eradication of Established Solid Tumors by T Cells

Solid tumors consist of malignant cancer cells that are embedded in stroma consisting of a variety of nonmalignant cells and extracellular matrix. It is often assumed that the major mechanism of tumor rejection is the direct recognition and killing of cancer cells by CD8+ CTLs. CTLs recognize MHC class I peptide complexes on target cells and lyse them with the help of perforin. Although killing of cancer cells is necessary for complete tumor rejection, it is usually not sufficient. For instance, tumor cells can escape immune recognition by loss of tumor antigen, called antigen loss variants (ALV). Moreover, tumor stroma might act as a barrier to antigen presentation and immune recognition by T cells (1–3).

We have recently showed that targeting not only cancer cells but also the tumor stroma is essential for eradicating ALVs in established solid tumors by adoptively transferred CD8+ effector T cells (4–6). When only the cancer cells (and not the stroma) were targeted, ALVs resistant to the T cells escaped and killed the host (4–6). Both the bone marrow–derived as well as the nonbone marrow–derived components of the stroma needed to be targeted for eradicating established tumors by CTLs. Tumor-specific T cells failed to eradicate the cancer and the tumor escaped destruction if the antigen could not be cross-presented because of inappropriate MHC class I, even if these T cells had extremely high affinity for the antigen on the cancer cells and killed all antigen-positive cancer cells (4–6). Similarly, tumor-specific T cells failed to eradicate the established tumor when cancer cells expressed insufficient levels of the tumor antigen to sensitize the stroma (6). Thus, when well-established solid tumors express low levels of tumor antigen, ALVs developed and the tumor escaped immune destruction, although each antigen-positive cancer cell in the tumor was eliminated by the T cells. Targeting cancer cells that express high levels of antigen, and simultaneously targeting the stroma, caused cancer elimination. Similar results were found in two different tumor models, each using analyzed different tumor rejection antigens (4, 5).

The mechanism for destruction of ALVs (indirectly in an antigen-independent way as “bystanders”) has not been fully determined (4–7). We speculate that endothelial cells might be the essential targets in the nonbone marrow–derived components of the stroma (7) because the effect of targeting exclusively the stromal cells that present antigen released from the cancer cells (without killing the cancer cells that release the tumor-specific antigen) closely mimics the effect of antiangiogenic agents (8).

It is interesting that well-established solid tumors have been treated using adoptively transferred T cells in combination with active immunization and interleukin 2, which, in contrast to the previous models, were not directed against tumor-specific antigens but were directed against a nonmutant differentiation antigen (9). Usually, the cancer recurred after a temporary yet potent antitumor effect by T cell therapy alone, and it is tempting to speculate that a failure of the T cells to target the stroma cells also prevented cure. Differentiation antigens and other self-antigens might bind poorly to the presenting MHC class I molecule (10) and therefore do not effectively sensitize stromal cells even if the antigen is expressed at high levels in the cancer cells.

A Model for T-Cell Targeting of Stromal Cells Cross-Presenting Tumor Antigen in the Absence of Direct Cancer Cell Recognition

Targeting both cancer cells and stromal cells are required for eradication of established tumors by T cells (4–6). However, cancer cells may not always be targeted, for example, when they have lost/down-regulated MHC class I molecules or antigens (ALV). By contrast, stroma cells are genetically much more stable, and targeting only tumor-derived stromal cells by T cells may contribute to effective control of tumor growth. To test this possibility, a model targeting the tumor stroma alone by T cells was generated (Fig. 1; ref. 11). In this model, antigenic cancer cells expressed MHC class I molecules not matched to the host. Antigen-specific T cells, therefore, were unable to recognize and kill them due to the intrinsic MHC restriction. Indeed, we found a long-term arrest or stabilization of progressive growth of established tumors by adoptive transfer of T cells in the absence of direct recognition of cancer cells (11). No tumors that escaped from this inhibition, or cancer cells reisolated from biopsy of these tumors, retained susceptibility to T cells. Clearly, targeting tumor stroma alone caused long-term suppression of tumor growth, but viable cancer cells may persist in the small residual tumors. This suspicion was
confirmed by injecting T cell–treated mice with anti-CD8 antibody. Cancer cells grew out progressively after antibody treatment. Thus, targeting tumor stroma by T cells caused remarkable regression of large tumors, which resulted in the small residual tumors that remained “dormant.” Because CTL failed to recognize and kill the cancer cells directly, CTL presumably achieved this effect indirectly through targeting tumor stromal cells. Moreover, this tumor growth inhibition relying on stromal destruction is long-lasting.

Histology of arrested tumors showed that most of the tumor mass was necrotic. This necrotic mass was surrounded by a rim of viable and mitotic cancer cells. Cancer cells were surrounded by lymphocytes directly adjacent to preexisting vasculature in the outer area. A similar rim structure of surrounding necrotic tumor masses was observed at different times after T cell transfer, indicating a state of dynamic equilibrium between host and cancer. Interestingly, continuous turnover of T cells and cancer cells was also observed during equilibrium. We assume that persistent killing of tumor stroma by T cells is associated with the equilibrium. Using specific high-affinity TCR tetramers to detect peptide-MHC complexes on cell surfaces by flow cytometry (6), we found that the tetramers specifically bound to freshly reisolated tumor-derived CD11b⁺ stromal cells, indicating these cells picked up tumor antigen from surrounding antigenic cancer cells and cross-presented antigen on the cell surface. Antigen-loaded (tetramer-positive) CD11b⁺ stromal cells from antigen-positive tumors can be lysed by T cells, and lysis was antigen specific because CD11b⁺ stromal cells from antigen-negative tumors were not lysed. Consistently, antigenic tumors contained more nonviable cells during equilibrium after T cells transfer than antigen-negative tumors. These nonviable cells in the tumor were largely CD11b⁺. These findings form a basis for our concept that tumor-derived stromal cells are recognized and killed directly by specific CTL. Therefore, during equilibrium, stromal cells in antigenic tumors are targeted continuously by CTL.

T cell–mediated destruction of stromal cells may be the reason for allowing the transferred T cells to remain effective despite the large tumor burden. The histologic appearance of arrested tumors—lack of resorption, persistence of T cells, and lack of neovascularization—may be a consequence of T cells eliminating antigen crosspresenting CD11b⁺ myeloid stromal cells. This very heterogeneous CD11b⁺ cell population is known to contain immature myeloid cells, macrophages, and bone marrow–derived endothelial precursors and other proangiogenic cells such as...
pericytes. Interestingly, these tumor-associated CD11b⁺ myeloid cells have been recently termed myeloid-derived suppressor cells including Gr1⁺ CD11b⁺ immature cells and CD11b⁺ F4/80⁺ macrophages, which hinder antigen processing and mediate resistance to antiangiogenic therapies (12). Indeed, our results showed that T cell–targeted myeloid cells suppressed antigen-specific proliferation, and directly promoted tumor growth (11). On the other hand, continuously adding Gr1⁺ immature myeloid cells that express irrelevant MHC-I molecules abrogates T cell–mediated inhibition of tumor growth.¹ Thus, we propose that T cell–mediated killing of myeloid-derived suppressor cell significantly contributes to the inhibition of tumor growth.

One might argue that T cell–mediated destruction of cells cross-presenting antigen violates an important basic safety mechanism. However, it is most unlikely for several reasons. For example, the destruction of cross-presenting tissue is completely antigen dose-dependent. This point is further supported by recent findings showing cross-presentation of intracellular peptides by transfer through gap junctions is limited to a few neighboring cells (13, 14). Although spread of apoptotic and necrotic material from killed cancer cells likely extends beyond neighboring cells, tumor stroma is distinguished from other stromal cells of normal organs and tissues by its proximity to cancer cells. There is no evidence to indicate the destruction of normal stroma away from the tumor. Tumor stroma contains activated fibroblasts (15, 16), recently formed immature and leaky capillaries, and many types of inflammatory cells comparable with those in a nonhealing wound (17). Antigen uptake and presentation is therefore likely to be different in such an active stroma. Thus, stroma may more readily acquire and cross-present highly expressed antigen to T cells.

Implications

Tumor stroma serves no beneficial purpose to the host but is necessary for tumor growth and therefore represents an ideal target. Targeting tumor stroma directly is quite profound and emphasizes the importance of the stromal elements in eradication of established cancers. Of course, killing of stromal elements may require overcoming some of the same immunosuppressive barriers that limit immunologic killing of tumor cells. However, the threshold for stromal cell killing may be lower than that for tumor cell killing, and initiation of stromal cell destruction by T cells may trigger a sequence of events that leads to better tumor-antigen cross-presentation and ultimately better direct tumor cell recognition and lysis. Similarly, pharmacologic inhibition of stromal elements may also potentiate immune-mediated elimination of tumor cells. Antiangiogenic compounds, such as antivascular endothelial growth factor monoclonal antibody, could theoretically stress tumor cells because of diminished perfusion and thus render T cell–mediated tumor cell killing more effective (18). Inhibition of other stromal support cells, including fibroblasts, may have a similar effect. PT-100 (talabostat) is an inhibitor of fibroblast-activating protein produced by tumor cells that has shown early evidence of clinical efficacy in cancer patients. Combining these or similar agents with vaccines should be explored to determine the potential for therapeutic synergy.

We previously reported that depletion of Gr1⁺ CD11b⁺ cells in vivo using anti-Gr1 antibody enhanced CD8⁺ T cell–mediated immune responses and resulted in the rejection of inocula of progressor tumor cells (19). This is consistent with the results of subsequent studies showing that Gr1⁺ CD11b⁺ bone marrow–derived cells give rise to myeloid-derived suppressor cells that inhibit CD8⁺ T cells are proangiogenic and stimulate cancer cell growth. However, using anti-Gr1 antibody in a therapeutic setting seems untenable due to wide expression of Gr1. Others have used antigens directly expressed by stromal cells (vascular endothelial growth factor receptor by endothelial cells (20), fibroblast activation protein by fibroblasts (21), or legumain by macrophages (22)) as targets for therapy. Therefore, it is possible to develop strategies for targeting stroma with T cells specific for antigenic epitopes presented by MHC I of stromal cells but not expressed by cancer cells themselves. Our approach of stromal targeting described here is unique in that it is effective against large established solid tumors, requires a single transfer of T cells and is tumor-specific because the peptide-MHC class I molecule we target on the stromal cells depends on the release of tumor-specific antigen from the cancer cells. These antigens are acquired locally by stromal cells in the tumor microenvironment preventing any significant bystander killing of normal tissues outside of the cancer.

No currently available stromal targeting strategies eliminate all cancer cells, leading to one major limitation of targeting stroma for cancer therapy. Indeed, tumors shrink yet often recur by antiangiogenic therapy such as anti–vascular endothelial growth factor. Interestingly, our approach succeeds in obtaining long-term equilibrium of well-established cancers after a single T-cell transfer. For aggressively growing rapidly lethal cancers that cannot be excised, long-term arrest is an acceptable goal. Tumor eradication is obviously preferable to tumor arrest. However, direct killing of cancer cells by CTL is not always feasible, for example, when they have lost MHC class I molecules or antigens. Thus, this mechanism of growth arrest should extend to cancers that have lost or downregulated the appropriate presenting MHC molecule, have defective antigen processing, or develop resistance to T cell–mediated lysis or apoptosis.

Our findings still need to be confirmed in physiologically more relevant settings. We will select mutated tumor-specific antigen rather than self-antigen as target. Procedures obtaining the tumor antigen–specific T cells ex vivo will be established to treat the hosts bearing autotchonous or transplanted tumors. We are also developing the way for enhancing the loading of the critical stromal cells with antigen because some tumors, though antigenic, may not express high enough levels of the relevant antigen for cross-presentation by stromal cells. Different strategies (e.g., tumor-specific antibody as probe) have been designed to deliver the antigen that will localize to the tumor microenvironment. The final goal is to introduce sufficient antigen for efficient stromal targeting by antigen-specific T cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 6/24/2008; revised 8/18/2008; accepted 9/3/2008.

I thank Dr. Hans Schreiber for his full support of the project and Dr. Tyler J. Curiel for his critical reading of the manuscript.

¹ B. Zhang, H. Schreiber, unpublished data.
References

Targeting the Stroma by T Cells to Limit Tumor Growth

Bin Zhang


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/68/23/9570

Cited articles
This article cites 21 articles, 9 of which you can access for free at:
http://cancerres.aacrjournals.org/content/68/23/9570.full#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/68/23/9570.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.