

The dual adverse effects of TGF- β secretion on tumor progression

When a cancer escapes the growth-inhibitory effects of TGF- β secreted by cancer cells themselves or by cells in the local stroma, a further adverse outcome for the host is the associated TGF- β -induced suppression of anticancer T cell immunity. In addition to the previously described dampening of T cell activation and proliferation, TGF- β markedly and directly suppresses the transcription of genes encoding multiple key proteins of the “cytotoxic program” of CD8⁺ CTL, such as perforin and granzymes, cytotoxins that act through the granule exocytosis pathway. The findings described below suggest that TGF- β and its signaling pathways will be major targets for novel cancer therapeutics.

It has been appreciated for some time that the expression of TGF- β by some tumors or their surrounding tissue stroma can bring about dual undesired effects on tumor progression and metastasis. First, tumors that escape the growth-regulatory effects of TGF- β as a result of their inherent genetic instability have effectively uncoupled an important physiological “brake” on their own growth, resulting in greater local tumor invasiveness, an increased likelihood of metastasis, and the production of even more TGF- β (Figure 1). At some critical point, significant immune suppression may also occur as a result of the negative effects of TGF- β on T cell activation (Gorelik and Flavell, 2000) and a reduction of antigen presentation. For immunogenic tumors secreting TGF- β , rendering the tumor-reactive CD8⁺ T cells refractory to its inhibitory effects may be sufficient to induce tumor rejection (Gorelik and Flavell, 2001). Indeed, adoptively transferring such “disinhibited” CD8⁺ T cells to syngeneic tumor-bearing hosts also resulted in tumor regression. Therefore, blockade of these two major adverse effects of TGF- β might potentially provide an opportunity for therapeutic intervention for certain types of cancer.

In the current issue of *Cancer Cell*, Drs. Dori Thomas and Joan Massagué from the Sloan-Kettering Cancer Center in New York have defined some of the critical gene targets and begun to dissect the signaling pathways activated by TGF- β to adversely affect CTL effector mechanisms (Thomas and Massagué, 2005). Using EL4 thymoma cells that constitutively secrete TGF- β , these investigators showed that co-overexpression of a molecular “trap” comprising the extracellular domain of the TGF- β type II receptor by the tumor cells resulted in survival of the majority of mice inoculated with the tumor. Importantly, in this model, neither CD8⁺ T cell numbers nor their activation status appeared to be adversely affected by TGF- β . Gene microarray analysis of CD8⁺ T cells acti-

vated in vitro through crosslinking their T cell receptors and CD28 in the presence or absence of TGF- β revealed around 100 genes whose expression level was significantly (2- to 6-fold) influenced by TGF- β exposure. Among these were the genes encoding five key effector proteins of CTLs: perforin, granzymes A and B, Fas ligand, and interferon- γ , all of which were profoundly suppressed, resulting in a marked diminution of protein production. Perforin (a pore-forming protein) and granzymes A and B (serine proteases) are copackaged and released from CTLs by granule exocytosis following their conjugation of tumor cells. Granzymes

induce various apoptotic programs in target cells, some dependent upon, and others that do not require, caspase activation. But these activities are dependent on perforin, whose membranolytic properties are critical for granzymes to access and cleave their substrates (Trapani and Smyth, 2002). The Fas ligand can also induce tumor cell death by engaging its cognate death receptor (Fas) on the tumor cell, while the pleiotropic effects of interferon- γ include potentially enhancing MHC antigen expression on both the tumor and local antigen-presenting cells, rendering the tumor more visible to the immune system.

Thomas and Massagué went on to show that the genes encoding the major constituents of CTL effector mechanisms are targeted directly by TGF- β through the Smad signaling pathway. For instance, a short sequence within the granzyme B gene promoter proved to be the binding site of Smad partners ATF1 and CREB (which were induced by TGF- β despite cycloheximide treatment), as confirmed by chromatin immunoprecipitation (ChIP) analysis in primary T cells. Similar ChIP analysis showed inducible binding of ATF1 and Smad 2/3 (but not CREB) to the interferon- γ promoter in response to TGF- β . The inhibition of granzyme B expression by TGF- β was far less pronounced when the levels of ATF1 or CREB were specifically knocked down using siRNA oligonucleotides. IL-2 also largely restored granzyme B and interferon- γ expression in TGF- β -treated T cells, whereas IL-15 (which can also induce CD8⁺ T cell proliferation and differentiation) did not. Finally, the authors used an EL4 variant expressing the model antigen OVA to demonstrate that neutralization of TGF- β in vivo both increased the numbers of CD8⁺ CTL capable of detecting the OVA peptide SIINFEKL and augmented their expression of perforin, granzymes A and B, and interferon- γ . Surprisingly, Fas ligand expression was not restored, consistent with the recognized role for this molecule

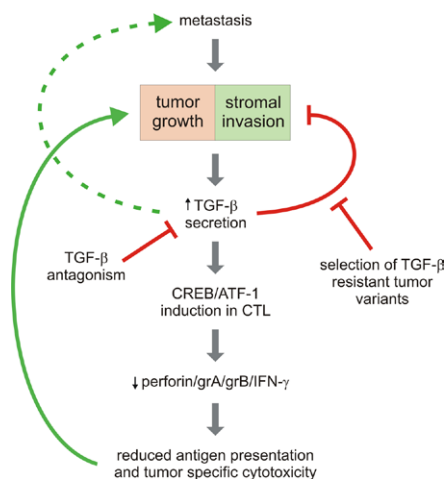


Figure 1. Dual adverse effects of TGF- β secretion by tumor cells

The selection of tumor variants resistant to the normal growth-regulatory activities of TGF- β results in unchecked growth, metastasis, and further TGF- β production at the tumor site. The cytokine also has marked immunosuppressive effects, among them the severe downregulation of CTL effector molecules perforin, granzymes A and B, interferon- γ , and Fas ligand. For immunogenic tumors, the abrogation of an effective CTL response further enhances tumor growth and spread. Strategies that inhibit TGF- β may therefore potentially play a valuable role in the therapy of some cancers. Green and red arrows indicate positive or negative effects, respectively.

in homeostasis of T cells, rather than a prominent role in tumor cell killing.

The findings of Thomas and Massagué are significant, as they both provide an additional rationale explaining the potent tumor-promoting properties of TGF- β and define a molecular framework to explain how certain T cell effector molecules known to protect against carcinogenesis (at least in mice) are inhibited by the same cytokine. At least two of the effector molecules identified by Thomas and Massagué as important targets of TGF- β , perforin and interferon- γ , have been shown independently to be critical for failed immune surveillance of primary lymphoid (Smyth et al., 2000; Street et al., 2001) and nonlymphoid (Shankaran et al., 2001) malignancies in mice, protecting against both spontaneous and carcinogen-induced neoplasia while also reducing the metastatic burden associated with several transplantable tumor cell lines. By contrast, mice and humans lacking a functional Fas ligand/Fas pathway develop B/T cell lymphoproliferation and antibody-induced autoimmunity, suggesting a crucial role for this death pathway in lymphoid homeostasis, activation-induced lymphocyte death, and deletion of autoreactive lymphocytes. Unlike perforin, a critical role for either granzyme A or granzyme B in tumor rejection remains controversial. Mice deficient in both granzymes have not been reported to be particularly tumor-prone as they age, but are exquisitely susceptible to the viral pathogen ectromelia. Interestingly, whereas primary B cell lymphomas that arise spontaneously in perforin-deficient mice are easily transplantable into syn-

genic perforin-deficient animals, these tumors are uniformly rejected by perforin-competent animals that lack both granzymes A and B (Smyth et al., 2003). In humans, the evidence for a causative role of perforin/granzyme dysfunction in neoplasia is far less well developed. Perforin-deficient children present in infancy with life-threatening hemophagocytosis in the bone marrow and liver and spleen enlargement due to infiltration of those organs with activated T cells and antigen-presenting cells, and generally do not survive to adulthood unless their cytotoxic capacity is restored by bone marrow transplantation (Stepp et al., 1999). While a recent report linked a perforin allele (Ala91Val) that has moderately reduced cytotoxic function (Voskoboinik et al., 2005) with childhood acute leukemia (Santoro et al., 2005), the functional significance of these findings requires further clarification, as several of the individuals identified possessed only a single copy of the defective allele. The tools for studying the molecular and cellular functions of perforin in humans are only beginning to become available (Voskoboinik et al., 2004), and the next several years may provide important insights into perforin and granzyme dysfunctions in a variety of diseases.

By identifying key molecules of the "cytotoxicity program" of CD8⁺ T cells as important targets of suppression by TGF- β , and beginning to unravel the signaling pathways responsible for this inhibition, the studies of Thomas and Massagué have provided a further impetus to efforts aimed at developing cancer therapeutics based on inhibiting TGF- β signaling.

Joseph A. Trapani^{1,*}

¹Cancer Immunology Program, Peter MacCallum Cancer Centre, St. Andrew's Place, East Melbourne, 3002, Australia

*E-mail: joe.trapani@petermac.org

Selected reading

Gorelik, L., and Flavell, R.A. (2000). *Immunity* 12, 171–181.

Gorelik, L., and Flavell, R.A. (2001). *Nat. Med.* 7, 1118–1122.

Santoro, A., Cannella, S., Trizzino, A., Lo Nigro, L., Corsello, G., and Arico, M. (2005). *Haematologica* 90, 697–698.

Shankaran, V., Ikeda, H., Bruce, A.T., White, J.M., Swanson, P.E., Old, L.J., and Schreiber, R.D. (2001). *Nature* 410, 1107–1111.

Smyth, M.J., Thia, K.Y., Street, S.E., MacGregor, D., Godfrey, D.I., and Trapani, J.A. (2000). *J. Exp. Med.* 192, 755–760.

Smyth, M.J., Street, S.E., and Trapani, J.A. (2003). *J. Immunol.* 171, 515–518.

Stepp, S.E., Dufourcq-Lagelouse, R., Le Deist, F., Bhawan, S., Certain, S., Mathew, P.A., Henter, J.I., Bennett, M., Fischer, A., de Saint Basile, G., and Kumar, V. (1999). *Science* 286, 1957–1959.

Street, S.E., Cretney, E., and Smyth, M.J. (2001). *Blood* 97, 192–197.

Thomas, D.A., and Massagué, J. (2005). *Cancer Cell*, this issue.

Trapani, J.A., and Smyth, M.J. (2002). *Nat. Rev. Immunol.* 2, 735–747.

Voskoboinik, I., Thia, M.C., De Bono, A., Browne, K., Cretney, E., Jackson, J.T., Darcy, P.K., Jane, S.M., Smyth, M.J., and Trapani, J.A. (2004). *J. Exp. Med.* 200, 811–816.

Voskoboinik, I., Thia, M.C., and Trapani, J.A. (2005). *Blood* 105, 4700–4706.

DOI: 10.1016/j.ccr.2005.10.018