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Cancer Gene Therapy

The power of negative thinking

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Promising new results in a well-established murine model of transplantable prostate cancer could lead to strategies that seek to exploit a fourth pathway for the gene therapy of cancer.

Cancer is fundamentally a genetic disorder associated with modification of genes that block apoptotic pathways, thereby causing abnormal necrotic cell death and expression of genes which enable unscheduled cell growth, particularly in nominal cancer stem cells, which have learned and inculcated these dark secrets.[1] Thus, much of the excitement about gene therapy for cancer at its beginning posited developing strategies to replace these altered genes (the first pathway) thereby returning a normal phenotype. Although attractive, this notion was limited by the bilateral Achilles heels of targeting (getting the right genes into the right cells in the right places) and the irksome immune response to the more effective viral delivery systems. Perhaps the most intriguing current strategy of this ilk is to deliver antisense constructs to tumors to limit expression of antiapoptotic genes such as Bcl-2, which has indeed met with some success in the clinic.[2]

A second pathway is the use of genetic constructs to confer novel properties on a cell, often to elicit an effective immune response to the tumor using cytokines or antigens, an approach that our group and others have championed for quite some years[3–10] with a modicum of success. For example, our studies in the late 1980s (the first gene therapy studies in the United States) focused on the adoptive transfer of genetically modified antitumor T cells first designed solely to mark them,[5] and subsequently to use them to deliver additional gene products such as TNFα. The importance of such attempts may have been primarily in elucidating the underlying immune mechanisms that are complex, highly evolved, remarkably redundant and synergistic. The application of GM-CSF as an immune adjuvant, for example, was first identified using such gene transfer strategies[11] and we can imagine future gene transfer studies using newly identified cytokines and costimulatory molecules to similarly help us define the biology of these factors. The third pathway of cancer gene therapy, and actually one of the first applied clinically, is to use nominally tumor-selective viruses that mediate oncolytic effects on the tumor.[12]

A fourth emergent pathway for cancer gene therapy focuses on strategies designed to modify the biology of the tumor-reactive inflammatory cells. Zhang *et al*.[13] new study, recently published in *Cancer Research,* is an excellent example of one such strategy to make T cells resistant to the altered and nominally disordered tumor microenvironment (see below). The authors adoptively transferred T cells which recognized the prostatic tumor, but that had been genetically modified to bind but not respond to TGFβ. The notion that TGFβ is important in cancer has a long history, with many tumors being able to both produce it and limit their own responsiveness to it by downregulating receptors. The immunologic consequences of local TGFβ elaboration by the tumor most likely involve both direct and indirect immunosuppression as well as the ability of TGFβ to promote tissue repair and wound healing. TGFβ limits NK and T-cell production of IFNγ, thereby checking the so-called TH1 response. Limiting this response, in turn, leads to inhibition of tumor growth and enhancment of the cytolytic response to the tumor. TGFβ is delivered not only by the tumor but likely also by the resplendent and renascent T-suppressor cell, found abundantly within various tumors.[14,15] This same group first tested the strategy of blocking TGFβ effects by engineering bone marrow cells to express a dominant-negative receptor in all emergent T cells. However, this strategy could be dangerous because it could lead to autoimmune T cells as well as anti-tumor T cells arising.[16] By contrast, the new strategy of introducing TGFβ into tumor-specific T cells is more likely to be clinically applicable.

There is strong evidence from murine models and inflammatory infiltrate, which indicates that early events in tumorigenesis are associated with dendritic cell (DC) and T-cell infiltrates. It is clear then that cancers in adults arise most often as the end stage of chronic inflammation.[17,18] Cancer in adults is thus an immunologic disorder, which generates multiple neoepitopes and common tumor antigens.

These findings fueled burgeoning academic and biotechnology industries that were designed to develop antigen and cytokine therapies, some as gene therapies, based on the very effective therapies in murine models. The average epithelial neoplasm has over 10 000 mutations as a consequence of emergent genomic instability, enabling a cornucopia of antigens for exploration. One example of such a cancer is prostate cancer, the fifth highest cause of cancer death in the USA. Prostate cancers[13,19] develop resistance to TGFβ during their development[20] from a variety of mechanisms including downregulation of the receptor itself or associated signaling pathways during their inflammatory genesis. This increased resistance protects them from the TGFβ elaborated in the tumor microenvironment. Not so for the susceptible T cell. Alternatives to genetically modifying the T cells to protect them from tumor-elaborated TGFβ are to apply reagents that modify the tumor TGFβ receptors themselves or to antagonize the local release by antisense constructs or antibodies.[21,22] Although intriguing, the global role of TGFβ and its ablation, being associated with lethal inflammation in murine models as well as its critical role in tissue remodeling and healing, limit enthusiasm for this strategy. Modification of the T cell confers specificity (through the cognate T-cell receptors specific for cancer antigen/MHC complexes) and limits the biologic effects of these cells nominally to the tumor itself. So it appears that TGFβ in the

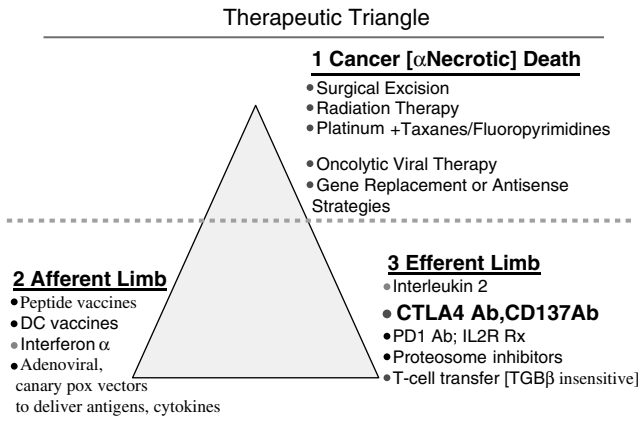

Figure 1 Cancer therapeutic triangle. The past 50 years of evolving cancer therapies have primarily focused on means of either locally extirpating cancer (1) by surgical excision or radiation therapy. Adjuvant, therapeutic or, increasingly, neoadjuvant strategies to elicit apoptotic (non-necrotic) cell death most frequently uses chemotherapeutic agents, most commonly platinum-based regimens, often with taxanes or fluoro-pyrimidines. An alternative strategy that uses direct application of oncolytic viruses has been dealt with extensively in this journal and others.[12,27–32] One of the major issues with this approach is that TGFβ[33] and HMGB1[34–37] often mediate the therapeutic response: paradoxically, these molecules can also suppress immune reactivity and promote reparative cell growth with associated angiogenesis and stromagenesis. Gene replacement or antisense strategies are similarly designed to enhance apoptotic death of tumors. Immune approaches that have been explored in earnest for 20 years typically try to elicit a novel immune response (2) by targeting the afferent limb using peptide or dendritic cell (DC) vaccines, systemic delivery of interferon α or use of adenoviral, canary pox or other viral vectors to deliver antigens or cytokines. The emergent and perhaps most interesting approaches predicate an already established immune response. These strategies are designed to promote survival and function of the (3) efferent limb of the immune response, which is deficient in the setting of the tumor microenvironment, perhaps as a consequence of HMGB1 and TGFβ local elaboration. Interleukin 2 administration likely overcomes some of the effects of suppressor T cells, which can be supplemented by antibodies to important immunological molecules regulating the immune response including antibodies to CTLA4, CD137, PD1 or the IL2R. Proteosome inhibitors appear to promote apoptotic death in the tumor targets and may limit elaboration of immunosuppressive factors. Making the adoptive transfer of T cells bullet-proof by making them TGB-β insensitive, as described, would represent one of the versions of enhancing effector pathways as a third emergent pathway in cancer gene therapy.

tumor microenvironment might indeed modify the ability of T cells to mediate their effector function.[23]

The tumor microenvironment is replete with the ancient cytokines TGFβ and HMGB1, which are important in wound repair and inflammation. Intriguingly, TGFβ is highly conserved, with only a single amino-acid difference between man and mouse. This molecule modifies inflammation in important ways.[24,25] Similarly, the nuclear transcriptional regulatory factor, high-mobility group B1 (HMGB1), appears to promote inflammation when released from necrotic cells[26] and to drive the initial events in eliciting immune reactivity. HMGB1 differs only in two amino acids when comparing mouse and man. Like TGFβ, HMGB1 is probably in an evolutionary box that it cannot get out of. So both these cytokines are critically important for tissue homeostasis and the response to injury or damage.

To sensibly coordinate and direct cancer therapies, we need to recognize the critical role of these cytokines in normal tissues and the response to injury, while (Figure 1) promoting tumor eradication (1), eliciting an immune response (2), and/or promoting immune effector function (3). Future strategies will need to remain aware of these three coordinate approaches when confronting the problem of cancer. Nascent gene therapies should similarly pay heed to these notions, applying all three approaches coordinately when possible. ■

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1 Hahn WC, Weinberg RA. Modelling the molecular circuitry of cancer. *Nat Rev Cancer* 2002; **2**: 331–341.
2 Cotter FE. Unraveling biologic therapy for Bcl-2-expressing malignancies. *Semin Oncol* 2004; **31**: 18–21; discussion 33.
3 Lotze MT. Trials and tribulations: the cost of developing gene therapies. *FASEB J* 1991; **5**: 3013–3014.
4 Culver K *et al*. Lymphocytes as cellular vehicles for gene therapy in mouse and man. *Proc Natl Acad Sci USA* 1991; **88**: 3155–3159.
5 Rosenberg SA *et al*. Gene transfer into humans – immunotherapy of patients with advanced melanoma, using tumor-infiltrating lymphocytes modified by retroviral gene transduction. *N Engl J Med* 1990; **323**: 570–578.
6 Zitvogel L *et al*. Construction and characterization of retroviral vectors expressing biologically active human interleukin-12. *Hum Gene Therapy* 1994; **5**: 1493–1506.
7 Tahara H, Lotze MT. Antitumor effects of interleukin-12 (IL-12): applications for the immunotherapy and gene therapy of cancer. *Gene Therapy* 1995; **2**: 96–106.
8 Tzeng E, Shears II LL, Lotze MT, Billiar TR. Gene therapy. *Curr Problems Surg* 1996; **33**: 961–1041.
9 Lotze MT, Kost TA. Viruses as gene delivery vectors: application to gene function, target validation, and assay development. *Cancer Gene Therapy* 2002; **9**: 692–699.
10 Tanaka F *et al*. Therapeutic and specific antitumor immunity induced by co-administration of immature dendritic cells and adenoviral vector expressing biologically active IL-18. *Gene Therapy* 2002; **9**: 1480–1486.
11 Dranoff G *et al*. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte–macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci USA* 1993; **90**: 3539–3543.
12 Zeh HJ, Bartlett DL. Development of a replication-selective, oncolytic poxvirus for the treatment of human cancers. *Cancer Gene Therapy* 2002; **9**: 1001–1012.
13 Zhang Q *et al*. Adoptive transfer of tumor-reactive transforming growth factor-beta-insensitive CD8+ T cells: eradication of autologous mouse prostate cancer. *Cancer Res* 2005; **65**: 1761–1769.
14 Laouar Y, Sutterwala FS, Gorelik L, Flavell RA. Transforming growth factor-beta controls T helper type 1 cell development through regulation of natural killer cell interferon-gamma. *Nat Immunol* 2005; **6**: 600–607.
15 Chen ML *et al*. Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals *in vivo*. *Proc Natl Acad Sci USA* 2005; **102**: 419–424.
16 Shah AH *et al*. Suppression of tumor metastasis by blockade of transforming

growth factor beta signaling in bone marrow cells through a retroviral-mediated gene therapy in mice. *Cancer Res* 2002; **62**: 7135–7138.
17 Lotze MT. Future directions for recombinant interleukin-2 in cancer: a chronic inflammatory disorder. *Cancer J Sci Am* 1997; **3**: S106–S108.
18 Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. *Nat Rev Immunol* 2004; **4**: 641–648.
19 Zhang F *et al*. Blockade of transforming growth factor-beta signaling suppresses progression of androgen-independent human prostate cancer in nude mice. *Clin Cancer Res* 2005; **11**: 4512–4520.
20 Zhang Q *et al*. Insensitivity to transforming growth factor-{beta} results from promoter methylation of cognate receptors in human prostate cancer cells (LNCaP). *Mol Endocrinol* 2005, (in press).
21 Lahn M, Kloeker S, Berry BS. TGF-beta inhibitors for the treatment of cancer. *Expert Opin Investig Drugs* 2005; **14**: 629–643.
22 Kontani K *et al*. Spontaneous elicitation of potent antitumor immunity and eradication of established tumors by administration of DNA encoding soluble transforming growth factor-beta II receptor without active antigen-sensitization. *Cancer Immunol Immunother* 2005: 1–9; Epub ahead of print 20 July 2005.
23 Ahmadzadeh M, Rosenberg SA. TGF-beta 1 attenuates the acquisition and expression of effector function by tumor antigen-specific human memory CD8 T cells. *J Immunol* 2005; **174**: 5215–5223.
24 Mule JJ *et al*. Transforming growth factor-beta inhibits the *in vitro* generation of lymphokine-activated killer cells and cytotoxic T cells. *Cancer Immunol Immunother* 1988; **26**: 95–100.
25 Moseman EA *et al*. Human plasmacytoid dendritic cells activated by CpG oligodeoxynucleotides induce the generation of CD4+CD25+ regulatory T cells. *J Immunol* 2004; **173**: 4433–4442.
26 Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002; **418**: 191–195.
27 Wakimoto H, Fulci G, Tyminski E, Chiocca EA. Altered expression of antiviral cytokine mRNAs associated with cyclophosphamide's enhancement of viral oncolysis. *Gene Therapy* 2004; **11**: 214–223.
28 Rivera AA *et al*. Combining high selectivity of replication with fiber chimerism for effective adenoviral oncolysis of CAR-negative melanoma cells. *Gene Therapy* 2004; **11**: 1694–1702.
29 Chen MJ *et al*. Enhanced efficacy of *Escherichia coli* nitroreductase/CB1954 prodrug activation gene therapy using an E1B-55K-deleted oncolytic adenovirus vector. *Gene Therapy* 2004; **11**: 1126–1136.
30 Huang TG *et al*. Telomerase-dependent oncolytic adenovirus for cancer treatment. *Gene Therapy* 2003; **10**: 1241–1247.
31 Heinzerling L *et al*. Oncolytic measles virus in cutaneous T-cell lymphomas mount anti-tumor immune responses *in vivo* and target interferon resistant tumor cells. *Blood* 2005, Epub ahead of print 14 June 2005.
32 Chan MM, Iglehart JD. Viral oncolysis: tumor-tailored therapy. *Curr Surg* 2005; **62**: 25–30.
33 Lin AH *et al*. Global analysis of Smad2/3-dependent TGF-{beta} signaling in living mice reveals prominent tissue-specific responses to injury. *J Immunol* 2005; **175**: 547–554.
34 Lotze MT, DeMarco RA. Dealing with death: HMGB1 as a novel target for cancer therapy. *Curr Opin Investig Drugs* 2003; **4**: 1405–1409.
35 Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol* 2005; **5**: 331–342.
36 Tsung A *et al*. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia–reperfusion. *J Exp Med* 2005; **201**: 1135–1143.
37 Zeh III HJ, Lotze MT. Addicted to death: invasive cancer and the immune response to unscheduled cell death. *J Immunother* 2005; **28**: 1–9.