The Biology Behind

Use of Dendritic Cells to Immunize against Cancers Overexpressing p53

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Immunizing patients against their tumors has obvious appeal. A successful vaccine would generate systemic immunity, contribute to the elimination of primary tumors, and, most importantly, eliminate metastatic foci, all with minimal side effects. Given the significant progress that has been made with regard to our understanding of the induction and regulation of immune responses, tumor vaccines have evolved from first-generation whole-tumor preparations to the use of defined antigens and highly active antigen-presenting cell populations. For a review of vaccine strategies with particular reference to tumors see reference 1. Studies by Nikitina et al. (2) in this issue of Clinical Cancer Research use DCs transfected to express wild-type p53 to identify the presence of putative p53-responsive cytotoxic T-cell precursors in control and cancer patients. Furthermore, they also demonstrate the ability to activate and expand these cells after in vitro antigen stimulation. In this commentary, we will discuss the significance of the current study with regard to potential treatment of human malignancies as well as point out possible clinical limitations of immunizing against human p53.

The p53 tumor suppressor gene was originally identified more than 20 years ago as a cellular protein that interacted with large T tumor antigen in SV40-transformed cells (3, 4). Early studies also indicated that steady-state p53 protein levels are elevated in both SV40-transformed cells as well as in 40–50% of spontaneous human tumors not transformed with SV40. In addition, anti-p53 antibodies are detected in the serum of 10–20% of cancer patients (5). Therefore, before the realization that p53 is a tumor suppressor protein and “guardian of the genome,” it was commonly referred to as the “cellular tumor antigen” p53.

Although many cancer patients have a humoral immune response to p53, it is unlikely that such antibodies provide any therapeutic benefit. What is unclear is whether cancer patients can mount a CTL response to p53 to control the growth of tumors. In this issue of Clinical Cancer Research, Nikitina et al. (2) use DCs engineered to overexpress human p53 to stimulate T lymphocytes from cancer patients and demonstrate a CTL response that is specific for cancer cells expressing mutant p53. Therefore, it is possible, at least in vitro, to target p53 in cancer cells as a tumor antigen.

First, some p53 biology to explain why p53 in tumor cells would be considered a potential target for immunotherapy. p53 is a transcription factor that regulates the expression of a diverse array of genes involved in DNA repair, growth arrest, senescence, and apoptosis. The ability of wild-type p53 to diminish tumor growth results in selection pressure for loss of p53 function during tumorigenesis. Although nonsense p53 mutations and mutations affecting p53 RNA splicing occur in some tumors, the most common form of mutational events targeting p53 are missense mutations occurring in the central core DNA binding region of the protein (amino acids 102–292; Ref. 6). These missense mutants are incapable of transcriptionally activating p53 target genes such as mdm2. Mdm2 is known to participate in a feedback loop (7) that targets p53 for proteasome-mediated degradation (8, 9). Thus, one consequence of p53 missense mutations in cancer cells is increased expression of p53 (10).

Although p53 missense mutations are prevalent in human cancer, the frequency of a given mutation at a specific codon is low. For example, the most commonly mutated codon (Arg273) only accounts for approximately 7% of the p53 gene mutations reported. Almost every codon within the core domain was reported to be mutated at least once in a large database of p53 gene mutations (11). Therefore, at first glance, the large number of different p53 missense mutations would appear to limit the usefulness of immunization strategies targeting specific p53 mutations in cancer cells. However, studies first reported in 1996 (12), it was demonstrated that immunization of mice with wild-type p53 protected the mice from subsequent challenge with tumor cells expressing mutant p53. The scientific basis for these studies using wild-type p53 (rather than mutant p53) as an immunogen relied on the growing body of knowledge pertaining to the processing and presentation of endogenous antigens such as p53 via the class I MHC pathway. With regard to p53, elegant studies by Theobald et al. (13) demonstrated that the p53-derived peptides presented by class I MHC A2.1 were not solely confined to the core DNA binding domain but distributed throughout the molecule. Therefore, epitopes derived from wild-type p53 are potentially immunogenic, once tolerance to self-antigens is overcome.

The concept of immunizing to normal cellular proteins overexpressed in tumor cells is not new. For example, a number of studies demonstrated the ability to immunize melanoma patients to pigment-associated antigens (14) and prostate cancer patients to prostate-specific antigen (15). Central to the effectiveness of such an approach is the ability to deliver an immunogenic stimulus able to “break tolerance” to the wild-type proteins. This is dependent on optimal antigen presentation that requires not only the ability to process antigens but also the regulated expression of a number of costimulatory molecules and the elicitation of a favorable cytokine cascade. T cells recognize antigenic specificities in the context of MHC antigens. Thus, what is recognized is the antigen alone but the combined antigenic-peptide MHC-antigen complex. Presentation predominantly follows two pathways. Exogenous antigens

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2 The abbreviation used is: DC, dendritic cell.
are taken up by phagocytosis or pinocytosis and cleaved in vesicles with the resultant peptides binding to preformed MHC Class II molecules, and these are subsequently conveyed to the cell surface. Endogenously synthesized proteins, including virally encoded proteins, are cleaved in the cytosol, the resultant peptides transported to the endoplasmic reticulum where they are complexed with Class I molecules, and the complex exported to the cell surface. (Antigen presentation is reviewed in Ref. 16.) Whereas cells from a number of lineages have been shown to have antigen presentation capability, DCs have emerged as by far the most promising candidates. First described by the Steinman group, DCs phagocytize and process exogenous antigens; this results in presentation of antigenic peptides to CD4 T class of MHC class II antigens (17). DC also efficiently present exogenous proteins via the MHC class I pathway resulting in CTL generation. Critical to the antigen-presentation function of DCs, is the fact that they express high levels of both MHC class I and II antigens; costimulatory molecules such as B7.1 (CD80), B7.2 (CD86), and ICAM 1 (CD54), and in addition, produce immune stimulatory cytokines that dictate the nature of the resultant response.

Numerous studies examined strategies for DC “tumor-antigen loading.” These have focused on both Class I and Class II targeted approaches (Fig. 1). DCs, fed with tumor cell lysates, protein tumor antigens, and tumor cell apoptotic material, predominantly stimulate class II-dependent CD4 helper responses and may stimulate CTL responses by class I mechanism (cross-priming; Ref. 18). Two strategies have been used to express tumor antigens in the context of class I for CTL generation. First, binding motifs for a number of HLA Class I molecules have been identified by mod-
eling techniques that allow peptides to be generated from the defined tumor antigens and pulsed onto DCs (14). Although this has been shown to result in T-cell stimulation, the limited duration of expression and the requirement for expression of certain class I haplotypes have led to strategies by which the whole antigens can be processed and presented on a variety of haplotypes. Second, tumor antigen synthesis has been induced by transfecting DCs with tumor-derived DNA or RNA (18), fusing the DCs with tumor (19), or transfecting DCs with recombinant viruses encoding the desired antigen. All of these approaches have been shown to be effective in vitro, and a number having moved to clinical trials.

In the present study by Nikitina et al. (2), the entire wild-type p53 molecule was encoded into a nonreplicating adenovirus and was used to transfect DCs (Fig. 2). The p53 protein is produced in the proteasome and processed for class I presentation by the endoplasmic reticulum as well as by possible class II presentation. As noted above, using the viral transfection strategy and full-length p53 allows for prolonged antigen presentation and the potential presentation of a variety of antigenic determinants on varied class I haplotypes. Using this strategy, the authors demonstrate efficient antigen presentation based on the generation of CTLs to p53 determinants in vitro; these data support the use of this strategy for in vivo immunization.

The studies described by Nikitina et al. (2), provide the scientific basis for further experimentation including possible clinical trials. Several issues pertinent to p53 will need to be addressed to determine the clinical usefulness of this therapeutic approach. First, not all p53 missense mutations result in an accumulation of p53 protein necessary for immune targeting. This is probably because small differences exist between p53 response elements so that the residual transcriptional activity of some tumor-derived p53 mutants can still activate the mdm2 gene. Second, mouse studies indicate that one frequent mechanism for immune escape is through the loss of mutant p53 expression (12). Although mutant p53 may have a gain-of-function effect under some circumstances (20), it is also clear that a loss of wild-type p53 expression that is attributable to null mutations or gene deletion also contributes to tumorigenesis. Unlike mutant ras, tumor cells are unlikely to be dependent on mutant p53 for continued proliferation. Third, high levels of wild-type p53 are often present in normal adult cells in the spleen, thymus, testis, and lactating breast (21–23). This may not bode well if the only mechanism for overcoming tolerance is through overexpression of p53 in DCs. Fourth, if tolerance can be overcome it is unclear whether autoimmune disease will develop over time. This may be an issue if p53 vaccination will be used in the adjuvant setting in which survival may be several years. Therefore, although the current study is exciting it is premature to revert back to calling p53 a cellular tumor antigen.

REFERENCES

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