Enzyme/Prodrug-Based Tumor Vaccination: All Politics (and Immunity) Are Local

John C. Morris

A major focus of gene therapy for cancer has been the effort to introduce into cancer cells a number of foreign genes that encode enzymes that will selectively convert nontoxic prodrugs into toxic compounds, producing high local concentrations that result in tumor cell killing—so-called "suicide" gene therapy. A number of enzyme/prodrug systems have been described [reviewed in (1)], including herpes simplex virus-thymidine kinase (HSV-tk)/ganciclovir (GCV) and Escherichia coli cytosine deaminase (CD)/5-fluorocytosine. A surprising early observation in many of these systems was that not every cell in a tumor need express the transgene to achieve meaningful cell killing and tumor regression (2,3). This phenomenon, the bystander effect, is defined as the ability of the genetically modified cells, in the presence of the prodrug, to cause cytotoxic effects in cells that lack the suicide gene. The result is that the fraction of cells killed is in excess of the fraction in the tumor that actually expresses the suicide gene (4). The bystander effect is a powerful enhancement of many suicide gene/prodrug systems that compensates for the inability of current vector systems to transduce all but a small fraction cells in a given tumor (5).

The degree and mechanism of the bystander effect differ with various cell lines and the enzyme/prodrug system studied. At the cellular level, several explanations have been advanced for the bystander effect; however, current evidence supports direct transfer of activated prodrug from the transgene-expressing cells to untransduced wild-type cells (6–8). In the HSV-tk/GCV system, in which the activated prodrug is highly ionized and is unable to diffuse across cell membranes, the bystander effect appears to be mediated by transfer of phosphorylated GCV through cellular gap junctions (6,8,9). Nonadherent cells and those with few gap junctions do not exhibit a significant HSV-tk/GCV bystander effect. 5-Fluorouracil (5-FU), the activated prodrug of the CD/5-fluorocytosine system, is a small nonpolar molecule that passively diffuses across the cell membrane down its concentration gradient, from CD+ cells to untransduced cells (7,10). The clinical importance of the bystander effect is illu-
treated by an early trial that involved transfer of the HSV-tk gene by stereotactic implantation of murine retroviral vector producer cells into patients with refractory brain tumors who were subsequently treated with GCV (11). Despite an estimated transduction efficiency of under 5%, responses were seen in five of 19 treated lesions.

Even with the ability to achieve local responses, any clinically useful gene therapy must address the fact that most cancer deaths are the result of uncontrolled metastatic disease. There is currently no gene delivery system that can approach the transduction efficiency and selectivity required for successful systemic treatment of metastatic cancer. These issues have prompted investigation of the potential vaccine effect of enzyme/prodrug therapy. Killing of cancer cells by coexpression of foreign bacterial or viral proteins may lead to enhancement of the immune response to normally weak tumor antigens (12,13). Several groups (14–16) have reported the development of resistance to challenge with wild-type cells from the same tumor cell line in animals that had been successfully treated with tumor cells transduced with either the HSV-tk or the CD gene for enzyme/prodrug therapy. Researchers (17,18) have also described regression of wild-type tumors implanted in a remote site within an animal after GCV treatment of HSV-tk-transduced tumors. This phenomenon has been termed the “bystander effect at a distance.” Histologic examination of the regressing tumors shows inflammatory infiltrates, predominantly composed of CD8+ T cells and macrophages (14,19). These findings have been confirmed in animals bearing CD-expressing tumors that have been treated with 5-fluorocytosine (15). Treatment of HSV-tk-expressing tumors with GCV has been shown to increase the expression of major histocompatibility complex class I molecules, the costimulatory surface molecule B7, which is the ligand for the CD28 receptor on T cells, and surface intercellular adhesion molecules (16,20) as well as to induce development of a tumor-specific cytotoxic T-lymphocyte response (20).

In this issue of the Journal, Pierrefite-Carle et al. (21) address the anticancer vaccine effect of the CD/5-fluorocytosine enzyme/prodrug system. CD deaminates the nontoxic antifungal 5-fluorocytosine to generate the antitumor agent 5-FU (3,23). Several groups (14–16) have reported the development of resistance to challenge with wild-type cells from the same tumor cell line in animals that had been successfully treated with tumor cells transduced with either the HSV-tk or the CD gene for enzyme/prodrug therapy. Researchers (17,18) have also described regression of wild-type tumors implanted in a remote site within an animal after GCV treatment of HSV-tk-transduced tumors. This phenomenon has been termed the “bystander effect at a distance.” Histologic examination of the regressing tumors shows inflammatory infiltrates, predominantly composed of CD8+ T cells and macrophages (14,19). These findings have been confirmed in animals bearing CD-expressing tumors that have been treated with 5-fluorocytosine (15). Treatment of HSV-tk-expressing tumors with GCV has been shown to increase the expression of major histocompatibility complex class I molecules, the costimulatory surface molecule B7, which is the ligand for the CD28 receptor on T cells, and surface intercellular adhesion molecules (16,20) as well as to induce development of a tumor-specific cytotoxic T-lymphocyte response (20).

In this issue of the Journal, Pierrefite-Carle et al. (21) address the anticancer vaccine effect of the CD/5-fluorocytosine enzyme/prodrug system. CD deaminates the nontoxic antifungal drug 5-fluorocytosine to generate the antitumor agent 5-FU (22). Cells that express CD are sensitized, by 500-fold to 2000-fold, to 5-fluorocytosine (3,23). Treatment with 5-fluorocytosine causes regression of CD-expressing tumors and has been shown to have a strong local bystander effect in many cell lines, with the presence of as little as 2% CD+ cells in a tumor resulting in cure of the animals (3,7,23,24). The CD/5-fluorocytosine system is currently undergoing clinical evaluation (25).

Pierrefite-Carle et al. (21) generated CD+ rat DHD/K12/PROb (PROb) colon cancer cells by plasmid transfection and selection. Not surprisingly, tumor cells that expressed CD (PRObCD+ cells) and that were injected into the liver of rats failed to form tumors after the rats were treated with 5-fluorocytosine beginning 24 hours after inoculation. Wild-type PROb cells readily formed measurable tumors despite 5-fluorocytosine treatment. None of seven animals given an injection of wild-type PROb cells in the opposite lobe of the liver of rats receiving 5-fluorocytosine treatment for previously implanted PRObCD+ tumors developed tumors, whereas all of the control animals developed measurable tumors. Rats that developed wild-type PROb liver tumors and that were inoculated with PRObCD+ cells in the opposite lobe of the liver and then treated with 5-fluorocytosine had an improved survival when compared with the survival among control rats (median survival, 154 days versus 91 days; P<.0001). These results are suggestive of a vaccine effect against wild-type PROb cells stimulated by the 5-fluorocytosine treatment and subsequent regression of the PRObCD+ tumor cells. Immunohistochemical studies of PRObCD+ tumors in animals treated with 5-fluorocytosine found the tumors to be extensively infiltrated with cells that stained for natural killer (NK) antigens. In contrast to the work of other investigators (14–16), few CD8+ cells were seen in the treated lesions, but rather these cells were restricted to the periphery of the tumor. Corticosteroid treatment significantly lowered the number of circulating T cells in the rats but had little effect on the response of the PRObCD+ and wild-type tumors to 5-fluorocytosine, suggesting that the vaccine effect was not directly mediated by CD8+ cells. This contrasts with the work of Consalvo et al. (15), which indicated that monoclonal antibody treatment directed against CD8+ cells reduced the effectiveness of the CD/5-fluorocytosine system. In the present study by Pierrefite-Carle et al. (21), when animals were treated with a monoclonal antibody (anti-asialo GM1) directed against NK cells, the vaccine effect was abrogated, which suggested that NK cells were the primary mediators of the distant bystander effect in this model. Although described previously with the HSV-tk/GCV system, to our knowledge, this report (21) is the first demonstration of a bystander effect at a distance in the CD/5-fluorocytosine enzyme/prodrug system.

The study by Pierrefite-Carle et al. (21) suffers from some limitations. The very small tumors treated in this preclinical model are unlikely to be treated at this stage in the clinic. The 5-fluorocytosine treatments were initiated 24 hours after inoculation, at a time the tumor burden was low. A more realistic approach would have been to see if larger, established tumors, both PRObCD+ and wild-type, could be effectively treated by use of this strategy. The CD/5-fluorocytosine system requires prolonged therapy. In these experiments, continuous 5-fluorocytosine treatment was administered to the animals for 30 days. Evidence supporting an important role for an NK cell antitumor immune responses in suicide gene therapy comes from the work of other investigators (17), who found similar distant bystander effect responses with the HSV-tk/GCV system in strains of mice with severe combined immunodeficiency, which lack T and B cells but which manifest NK cell activity (26). Other workers analyzing the immune response to CD/5-fluorocytosine suicide gene therapy have found the effects to be mediated by CD8+ T lymphocytes and granulocytes (15,27) as well as through the animals’ ability to generate a long-lasting immune memory to the tumor, which requires the activity of CD4+ lymphocytes (15). Pierrefite-Carle et al. found little evidence of a role for the activity of any T cells in their “vaccination” effect. Further work is required to reconcile these different findings.

While the work of Pierrefite-Carle et al. points out an important direction for systemic gene therapy for cancer, the clinical effectiveness of this strategy is uncertain. The CD/5-fluorocytosine system is already in clinical trials against colorectal (25) and other carcinomas (28). As of yet, there are no results available from these trials. The published results (11,29) of HSV-tk/GCV trials for patients with brain tumors and malignant mesothelioma have been disappointing. Perhaps the level of cellular transduction and/or gene expression in these trials was...
insufficient to result in adequate cell killing to elicit a vaccine response (30), or perhaps the tumor burdens seen clinically are large enough to render the patient immunologically unresponsive. Melcher et al. (31) have shown that tumor cell killing by suicide genes via apoptotic cell death-independent pathways generates a stronger immune response than cell killing proceeding through apoptotic mechanisms. While tumor vaccination by enzyme/prodrug therapy may hold great promise, for this promise to be realized, this strategy will likely need to be combined with other strategies that increase local tumor killing and antigen exposure. Manipulation of the enzyme/prodrug system, such as by genetic (32) or pharmacologic modifications (33) of gap junctions, by combinations of gene therapy and chemotherapy (34) or radiation therapy (35), or by the use of replication-competent vectors, which would improve tumor transduction through multiple rounds of viral replication and oncolysis (36,37), may help to enhance the potential vaccine effect of this approach.

REFERENCES


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