Gene Therapy (1997) 4, 273–274

Editorial

Suicide genes and bystander killing: local and distant effects

The mechanism underlying suicide gene therapy is not fully resolved but seems to be complex. Results from two recent studies[1,2] now demonstrate that the level of complexity may have further increased.

Suicide gene therapy is one of several approaches to treat cancer.[3,4] A suicide gene encodes a protein that is toxic to the cell. Some suicide genes encode products that are directly toxic to the cell, such as diphtheria toxin or *Pseudomonas* exotoxin, both of which inhibit protein synthesis. Other suicide genes give rise to enzymes that selectively convert nontoxic prodrugs to highly toxic metabolites. These genes have also been used in transgenic animals allowing researchers to target an inducible toxic phenotype.[5]

The current interest in suicide gene therapy is based on the 'bystander effect'. This phenomenon is caused by toxicity exerted on neighboring cells, which themselves are not genetically modified. Several investigators have demonstrated that it is sufficient to reach only a fraction of tumor cells to detect a pronounced bystander effect (reviewed in Refs 6 and 7). One of the major obstacles in current gene therapy is the inefficient transfer of the therapeutic gene. Cancer protocols are only likely to work if it is sufficient to genetically modify a fraction of the tumor cells.

Several different genes with these properties have been described[6,7] but the mechanism for toxicity is not fully understood and varies among the different products. The bacterial enzyme cytosine deaminase converts 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU), a drug currently used in cancer therapy. Normal mammalian cells do not contain this enzyme and are relatively resistant to 5-FC. The transfer of the cytosine deaminase gene into mammalian cells renders them selectively sensitive to 5-FC both *in vitro* and *in vivo*. A bystander effect has been obtained with this suicide gene as released 5-FU can diffuse to nearby tumor cells and pass through the cell membrane. The *E. coli* xanthine-guaninephosphoribosyl transferase gene (GPT) has also been used as a suicide gene with bystander capability. The prodrug 6-thioguanine is toxic when it is converted by GPT to a monophosphate metabolite and fed into the precursor pathways for RNA and DNA synthesis. The *E. coli DeoD* gene (purine nucleoside phosphorylase, PNP), converts a deoxyadenosine analog to 6-methylpurine, which is toxic to the cell with resultant bystander killing. *DeoD* has also been reported to effect nonproliferating cells.[8]

The tumor suppressor gene, *p53*, has recently been suggested to exert a bystander effect in a clinical trial in patients with lung cancer.[9] The same authors have previously shown both *in vitro* and *in vivo* experimental animal data indicative of a bystander effect. The underlying mechanism is not known but may be via soluble factors inducing apoptosis. During the initial phase of the original tumor development the preneoplastic cells are presumably resistant to the apoptotic effect, as the tumor, in fact, does develop. We would like to suggest that the fully neoplastic cells may have acquired sensitivity to *p53*-induced apoptosis while undergoing additional genetic changes.

The suicide gene most commonly employed, both in an experimental and a clinical setting,[6,7] is the thymidine kinase gene of the herpes simplex virus (HSV*tk*). HSV*tk* converts nucleoside analogs, such as ganciclovir, to monophosphate forms by phosphorylation. These are subsequently modified to toxic triphosphates by endogenous cellular enzymes and incorporated into nascent DNA causing chain termination and cell death. The HSV*tk* enzyme is almost three orders of magnitude more efficient at monophosphorylating GCV than endogenous thymidine kinase. Since phosphorylated GCV cannot pass through the plasma membrane, it is accumulated within the cell. The accumulation of phosphorylated GCV enhances the anticancer effects in HSV*tk* transduced tumors. More than a dozen clinical trials using HSV*tk* are underway[10] and some preliminary results have been reported.[6,7]

Several mechanisms underlying the bystander effect using HSV*tk* have been reported. They have been ascribed to direct influences of the suicide gene products as well as indirect effects by boosting the antitumor immune response. Toxic nucleoside analogs may either be transferred through cellular channels called gap junctions, or alternatively, through phagocytosis of apoptotic vesicles. Furthermore, some investigators have also reported that the bystander effect is impaired, or even absent, when experiments are conducted in immunocompromised animals, suggesting that an intact immune system is an important component for obtaining a bystander effect (reviewed in Ref. 6). These findings are corroborated by histological evidence of T lymphocyte and macrophage infiltrations in the tumors.

In a plasmacytoma model we could recently show that a bystander effect can be seen *in vivo* when human tumor cells are transferred to severe combined immunodeficient (SCID) mice.[1] However, an unexpected finding was that nontransduced tumors in a different location also showed evidence of regression, although they were not eradicated. These tumors had increased frequency of

apoptotic figures and decreased mitotic frequency. We called this phenomenon a 'distant' bystander effect, the traditional bystander phenomenon thus being referred to as a 'local' effect. In a recent publication, Wilson *et al*[2] report HSV*tk* gene therapy for head and neck squamous cell carcinoma in a nude mouse model. Interestingly, these authors also find that uninfected tumors regress, and intriguingly, they also refer to this phenomenon as a distant bystander effect. In both reports a direct effect of ganciclovir in the absence of HSV*tk* was ruled out as an explanation for this phenomenon. Another common finding was that the distant bystander effect was delayed as compared with the local bystander effect. PCR analysis of regressed nontransduced plasmacytoma specimens did not reveal any evidence of HSV*tk* genes indicative of homing of genetically modified cells from other locations (unpublished).

Thus, as these two independent reports, analyzing tumor cells of different cellular origins, describe a similar phenomenon it seems likely that this may be a general principle. Nude mice suffer from a transcription factor deficiency impairing thymus development rendering them deficient in T lymphocytes. Mice with the SCID mutation encode a deficient DNA-dependent protein kinase, which is involved in immunoreceptor gene recombination, causing a complete absence of functional T as well as B lymphocytes. This clearly indicates that lymphocytes are not involved in the distant bystander effect. What, then, might be the effector mechanism? Inflammatory cells and their products might be involved and the natural killer cell is another candidate. In preliminary studies, we did not find a correlation between the tumor necrosis factor α serum level and the distant bystander effect. Experiments are ongoing to address this issue, but at present we may simply conclude that the bystander effect seems to involve a novel component that could offer new ways of therapeutic intervention and at the same time increases the complexity of suicide gene therapy.

M Siraç Dilber[1,4] and CI Edvard Smith[2,3,4]
Departments of [1]Medicine and [2]Clinical Immunology, [3]Gene Therapy Research Centre, Clinical Research Centre, Huddinge Hospital, Karolinska Institute S-14186, and [4]Center for Biotechnology, Department of Biosciences, Karolinska Institute, NOVUM, S-14157, Huddinge, Sweden

References

1 Dilber MS *et al*. Suicide gene therapy for plasma cell tumors. *Blood* 1996; **88**: 2192–2200.
2 Wilson KM *et al*. HSV*tk* gene therapy in head and neck squamous cell carcinoma. *Arch Otolaryngol Head Neck Surg* 1996; **122**: 746–749.
3 Sikora K. Genetic approaches to cancer gene therapy. *Gene Therapy* 1994; **1**: 149–151.
4 Culver KW, Blaese RM. Gene therapy for cancer. *Trends Genet* 1994; **10**: 174–178.
5 Borrelli E *et al*. Targeting of an inducible toxic phenotype in animal cells. *Proc Natl Acad Sci USA* 1996; **85**: 7572–7576.
6 Freeman SM *et al*. *In situ* use of suicide genes for cancer therapy. *Semin Oncol* 1996; **23**: 31–45.
7 Caruso M *et al*. Gene therapy against cancer and HIV infection using the gene encoding herpes simplex virus thymidine kinase. *Mol Med Today* 1996; **2**: 212–217.
8 Sorscher EJ *et al*. Tumor cell bystander killing in colic carcinoma utilizing the *Escherichia coli* DeoD gene to generate toxic purines. *Gene Therapy* 1994; **1**: 223–238.
9 Roth JA *et al*. Retrovirus-mediated wild-type *p53* gene transfer to tumors of patients with lung cancer. *Nature Med* 1996; **2**: 985–991.
10 Human gene marker/therapy clinical protocols. *Hum Gene Ther* 1996; **7**: 1621–1647.