Gene transfer: Bax to the future for cancer therapy

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The Bax gene as a competitor in the marathon towards licensed cancer gene therapy

Intrapertoneal spread of gastrointestinal malignancies is a significant clinical problem and contributes to an incidence of distant relapse as high as 30% in gastric cancer. Local dissemination of tumour cells into the peritoneal cavity determines the outcome in advanced gastric cancer and diffuse-type carcinoma, and patients with negative peritoneal washings have a more favourable prognosis. Extensive lymph node dissection has been shown (by quantitative reverse transcription-polymerase chain reaction for carcinomaembryonic antigen and cytokeratin 20 combined with extensive intraoperative peritoneal lavage) to open lymphatic channels and spread viable cancer cells into the peritoneal cavity. Hence patient subpopulations that might benefit from intraperitoneal therapy regimens may be identified.

Malignant disease localised within the abdominal cavity has been a target for the staged development of clinical gene therapy approaches because of the smaller doses of experimental agent and increased safety margins over systemic administration. There has been extensive—and safe—experience of p53 gene therapy which culminated in a randomised phase III trial in which women with p53 null or p53 mutant ovarian cancer were randomised to chemotherapy alone or chemotherapy plus intraperitoneal Ad p53 following optimum debulking primary surgery. However, the first interim analysis indicated that not only did Ad p53 fail to improve effectiveness but it was also associated with increased toxicity. As a result, the study has been abandoned (reported in Zeitmer and Marth). Two broad possibilities exist to explain why the trials were relatively unsuccessful. Firstly, there is the perennial problem of suboptimal gene transfer. Secondly, there is the possibility that p53 is the "wrong" transgene. Although p53 mutations are found in many malignancies and defective p53 function may be causally linked to chemotherapeutic resistance, many aspects of p53 biology remain obscure, especially factors involved in the decision that determines whether cells undergo apoptosis or cell cycle arrest in response to p53 activation. There is some evidence that low level p53 expression, in the range likely after adenoviral gene transfer, causes cell cycle arrest rather than cell death. Also, the proapoptotic function of p53 depends on transactivation of genes such as Bax, Apaf-1, Fas, and PTK whose own expression or activity may be abnormal in tumour cells. Mutant p53 can act as a dominant negative in p53 tetramers, which could abrogate the effect of exogenous wild-type protein encoded by the transgene. Finally, polymorphisms of the p53 gene (especially codon 72) can determine the responsiveness of tumours to chemotherapeutic and radiotherapy by influencing inhibition of p73. Hence while p53 gene replacement was the early leader, it is likely to be overtaken by more robust competitors in the marathon towards a successful and licensed cancer gene therapy.

The work reported by Tsunemitsu and colleagues in this issue of Gut focuses on the potential of the Bax gene as a strong inducer of apoptosis, targeting gastric cancer growing as solid tissue deposits and as intraperitoneal disease [see page 554]. They show that a replication defective adenoviral vector expressing human Bax as a transgene could induce death even of p53 resistant gastric cancer. (Interestingly, treatment with a "control" virus expressing the reporter gene lac-Z was also observed to extend survival, a phenomenon that has been previously reported in the treatment of pancreatic cancer). However, it was evident that penetration of tumour deposits was only superficial after intraperitoneal instillation and, even though survival of treated animals was extended, cure was not achieved. Thus it appears that while Bax may be a more effective transgene than p53 for cancer gene therapy, its application might be restricted to lavage at the time of surgical dissection rather than the treatment of established bulk disease.

What further developments are on the horizon for the gene therapy of gastrointestinal cancers? We believe that advances will come from both improved gene delivery technologies and more powerful transgene combinations.

Replicating biological agents are the most promising means to improve the delivery kinetics in solid tumours, and both viruses and bacteria are being exploited for this purpose. The adenovirus E1B 55 kDa protein suppresses p53 function in infected cells21 and E1B 55K deleted adenoviral vectors replicate within and cause cytolysis of tumours with defective p53 function. In the past two years, six separate phase I/II trials of such a virus (variably known as dl520, Onyx-015, and CI-1042) have been published, in a range of tumour types, including colorectal,13, 14 ovarian,15 and pancreatic carcinomas,16, 17 and in patients with liver metastases from gastrointestinal malignancies. In combination with chemotherapy, some responses were seen; with 5-fluorouracil, eight patients with colorectal liver metastases demonstrated either partial or minor responses, at least five of whom had previously been refractory to 5-fluorouracil.15 In primary pancreatic carcinoma, two patients had partial responses in combination with gemcitabine.17 A second generation of selectively replicating adenoviral vectors specifically targeting retinoblastoma (Rb) function are approaching clinical trial. DNA tumour viruses such as adenovirus can infect and replicate in quiescent cells because viral proteins induce S phase entry. The G1-S phase cell cycle checkpoint is regulated by pRb and its related family members (p107, p130), and efficient progression from G1 to S phase requires binding and inactivation of the pRb family of proteins by the adenovirus E1A early viral protein. This interaction requires amino acids 121–127 of the E1A protein conserved sequence, in addition to amino acids 121–128. Partial responses in combination with chemotherapy have been described recently: dl922/947 is deleted in amino acids 122–129 while Δ24 is deleted in amino acids 121–128. Both have been assessed in vitro and in vivo models of cancer, and dl922/947 is capable of replicating with much greater efficiency within a panel of tumour cell lines (including gastrointestinal) than dl520, with minimal S phase induction in quiescent non-immortalised cells.20 Transcriptional targeting of viral replication is also possible through expression of cyclooxygenase 2 (COX-2) in gastrointestinal and pancreatic cancers has stimulated the construction and preclinical validation of selectively replicating adenoviruses incorporating the COX-2 promoter to drive expression of...
the viral early gene complex. Improved delivery kinetics for adenoviral agents can be achieved by incorporating ico- dexin solution in the carrier vehicle to prolong retention and distribution in the peritoneal cavity. More selective delivery of the viral agent can be achieved with bispecific single chain antibodies targeted toward epithelial cell adhesion molecule that is expressed on gastric tumours but not on normal gastric epithelium, allowing at least a 10-fold relative protection of normal tissues. Fibroblast growth factor receptors might also be exploited for targeting gastrointestinal tumours as it is perfectly feasible to incorporate receptor binding peptides into the coat of adenoviral particles. Although less highly developed in clinical applications, oncolytic herpes viruses also look potentially useful for treating peritoneal malignancies. An intriguing possibility is that bacteria engineered to invade epithelial cells could be used to deliver therapeutic genes and proteins, and this might have particular application to gastrointestinal tumours.

A variety of other transgenes with improved therapeutic potential are in development, including in combination with either chemotherapy or prodrug activating systems. Fhit expression is reduced in most cancers, and Fhit replacement by FHIT expression viruses in oesophageal and pancreatic cancers induces apoptosis in cancer cells. FHIT viral gene delivery prevents or retards development of carcinogen induced fore- stomach tumours and reverses development of established tumours in mice by 60–70% through an apoptotic pathway. Among the proapoptotic members of the family to which Bax belongs, its relative Bim is possibly the most powerful inducer of cell death, and combination gene therapy in which Bim was delivered in an adenoviral vector together with the herpes simplex virus thymidine kinase gene markedly enhanced its effectiveness. This complementary approach—combining viral and cellular elements—is further exemplified by a fusion protein containing a short peptide from the proapoptotic family member Bak fused to the herpes simplex protein VP22, which was shown to be very effective in entering cells and triggering apoptosis. It has also been shown that delivery of genes responsible for the downstream effector machinery of programmed cell death—Smac/DIABLO or caspase genes, for instance—can enhance sensitivity to chemotherapeutic agents that are other- wise ineffective.

The work reported by Tsunemitsu and colleagues in this issue supports the idea that apoptotic triggers will be useful in building cancer gene therapies of the future but it is clear that much effort is required for improved agent delivery and combinations with other therapeutic modalities to optimise their therapeutic effect.

**References**

11. Rigg AS, Lemoine NR. Adenoviral delivery of TIMP1 or TIMP2 can modify the invasive behavior of pancreatic cancer and can have a significant antitumor effect in vivo. *Cancer Gene Ther* 2001; 8: 869–78.