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CL-246

Review

Br. J. Surg. 1993, Vol. 80, May, 566–572

Gene transfer therapy in cancer

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Gene transfer techniques have now achieved clinical realization in the wake of recent advances in recombinant DNA technology, together with increased understanding of the molecular biology and immunology of cancer. These novel treatments, and their applications and limitations merit intensive study.

The current therapeutic techniques available for the treatment of patients with cancer are surgery, radiotherapy and chemotherapy. However, conventional chemotherapy has largely failed to have a significant impact on survival in the treatment of most solid tumours[1]. Surgery continues to be the most effective treatment for primary tumours and can be curative. A search has been conducted for effective and non-toxic adjuvant therapy for patients with minimal residual disease after operation.

Several observations suggest that a host immune response is often mounted in an attempt to reject the tumour. First, many solid tumours contain an immunological infiltrate, which in some is considered to be a good prognostic indicator[2–4]. Second, spontaneous regression of advanced disease sometimes occurs in immunogenic tumours such as melanoma and renal cell carcinoma[5]. Finally, immunosuppressed patients have an increased incidence of malignancy, although lymphoid tumours rather than carcinoma predominate in this susceptibility[6–8]. The therapeutic extrapolation of these findings has culminated, in the past decade, in the emergence of biological or immunological therapy for cancer[9–12]. Biological treatment represents a conceptual leap as the therapy does not itself have a direct tumoricidal effect but acts by stimulating host immune mechanisms to mediate tumour regression.

Several immunological prerequisites must be met before an effective immune response can be mounted against a tumour. There must be antigenic determinants that are expressed selectively, if not exclusively, on tumour as opposed to normal cells[13]. These tumour antigens must be presented to the immune system in the context of the major histocompatibility complex (MHC) antigens class I or II so that peripheral T cells can recognize them as foreign[14]. This has been the basis of many disappointing attempts at producing cancer vaccines in clinical studies[15]. In addition, the antigens must stimulate both helper T cells and cytotoxic T lymphocytes to ensure tumour destruction[16,17].

The cornerstone of biological therapy in the treatment of solid tumours has, until recently, involved the systemic administration of high-dose cytokines such as interleukin (IL) 2 and interferon (IFN)[18]. The rationale of this approach was initially either to generate direct killing of tumour cells (in the case of IFN) or provide activation of tumour-specific cytotoxic T lymphocytes by IL-2, thereby compensating for any defects in the helper T cell arm of the immune system that might be preventing tumour clearance *in vivo*. Although sporadically effective, these treatments are associated with severe side-effects and even fatalities[19]. The systemic levels of cytokines causing toxicity are well below the serum concentrations that are effective against tumours in animal models[12]. However, if high levels of these agents could be produced locally among tumour cells, they may effect tumour regression without the associated toxicity of systemic administration[20]. This possibility has formed part of the rationale for the initiation of clinical gene transfer studies in cancer. However, advances in understanding the molecular basis of the immunological response to cell-surface cancer antigens have also contributed to the emergence of therapeutic gene transfer for vaccination against some solid tumours[21]. Immunotherapy seeks to harness two unique characteristics of the immune system, namely antigenic specificity and response amplification, in selectively activating a strong immune response to induce tumour cell lysis.

Additionally, examination of tumour material has revealed alterations in several genes that are responsible for cell growth and proliferation. The molecular basis of cancer is now known to involve the activation of dominant oncogenes[22] and the inactivation of recessive tumour suppressor genes[23]. These genetic events can themselves be viewed as potential targets for cancer therapy.

In this paper, the current concepts of clinical gene transfer therapy in the treatment of solid tumours are reviewed and their potential or actual clinical applications discussed. The limitations, ethical considerations and future directions of this form of therapy are also addressed.

Therapeutic gene transfer

Gene transfer involves the insertion of novel genetic material into the genome of a target cell to produce a new cellular phenotype. Therapeutic gene transfer has evolved from the development of techniques for inserting and expressing genes in eukaryotic cells[24] and from advances in the understanding of the regulation of gene expression[25].

An essential prerequisite of any such technique is the efficient and stable delivery of the transferred DNA into the target cell. Retroviral expression vectors fulfil these requirements; they are retroviruses in which the viral genes have been replaced with therapeutic genes[26–28]. Viruses are naturally occurring genetic vectors and thus lend themselves to gene transfer. Retroviral vectors can efficiently carry foreign genes into the cell such that they become integrated and expressed in a predictable and, usually, very stable manner[27]. The precise site of integration of the vector in the host cell genome is, however, largely random. This is a source of concern for the use of retroviral vectors in gene transfer protocols, because of the small, but finite, risk of integration into a critical cellular gene. Retroviruses are also limited largely to the infection of replicating cells[29]. The retroviral vector is incapable of making any viral protein necessary for replication. Proteins required for initial infection of target cells are provided by retrovirus-packaging cells[28]. Introduction of the retroviral vector into the

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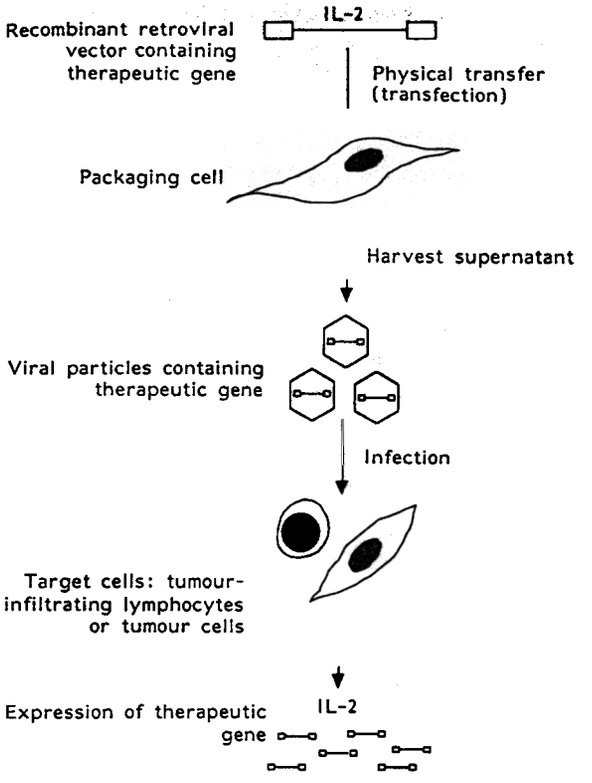

Figure 1 *Retrovirus-mediated gene transfer, exemplified for interleukin (IL) 2*

packaging cell results in the production of viral particles that can be used to infect the target cells but, importantly, no virus spread occurs after this initial infection[28,30]. This process is summarized in *Figure 1*.

The major disadvantage of retrovirus-mediated gene transfer is the potential for the production of replication-competent (helper) virus. This can occur by recombination between retroviral vector DNA sequences and viral coding sequences in the packaging cells[28]. However, this risk has been greatly reduced by careful design of the packaging cell lines[30], and in human trials thus far there is no evidence to indicate that patients have been exposed to replication-competent retroviruses[31,32].

Retrovirus-mediated delivery is currently the method of choice in clinical gene transfer studies[31]. However, other techniques are under investigation using as vectors other types of virus that can infect non-replicating cells such as central nervous system tissue[33]. There are also physical methods by which genes can be directly delivered to cells either *in vitro* or *in vivo*, including coating of DNA in liposomes or a precipitate of calcium phosphate, both of which increase the efficiency with which DNA traverses the cell membrane. The efficiency of such methods is, however, significantly lower than that which can be achieved using viral-based techniques.

Protocols of immunotherapy

A tumour deposit consists of different cellular components. From a therapeutic perspective, these can be broadly divided into malignant cells and immunological cells that are frequently present in tumour infiltrate (*Figure 2*). It has been assumed that the presence of such an infiltrate is indicative of an

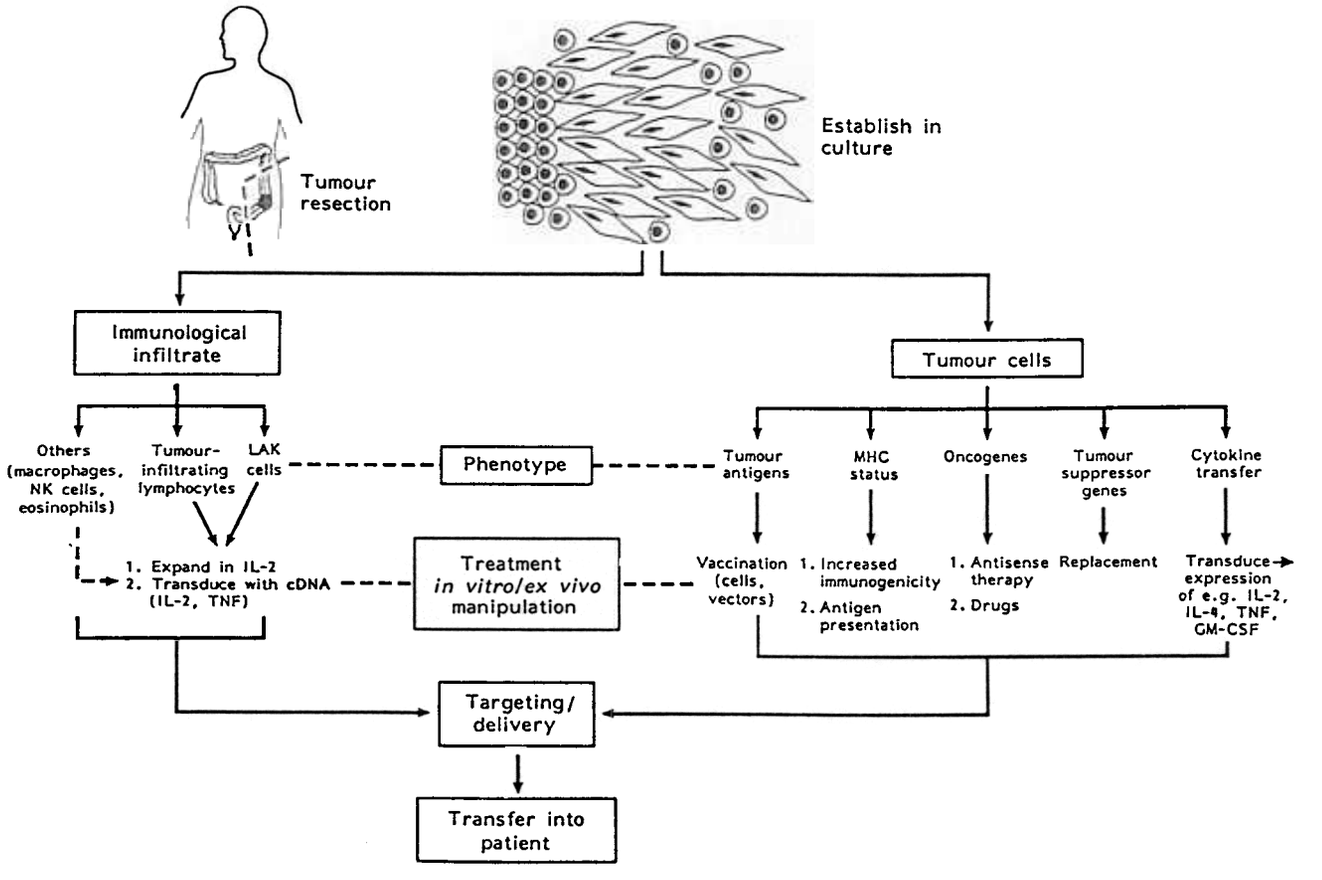

Figure 2 *Potential manipulations of immunological infiltrate or tumour cells, from tumour resection to transfer of altered cells into the patient. MHC, major histocompatibility complex; NK, natural killer; LAK, lymphokine-activated killer; IL, interleukin; TNF, tumour necrosis factor; GM-CSF, granulocyte–macrophage colony-stimulating factor*

antitumour response[2–4]. Moreover, the infiltrating cells presumably also have a natural tumour-localizing specificity, a property that can be exploited therapeutically[34,35].

The patient's ability to mount an effective response to the tumour can be enhanced by increasing the antitumour potency of the infiltrating cells and/or by manipulating the tumour cells to become more immunogenic. Advances in molecular technology have resulted in characterization of some of the genes of the putative tumour antigens themselves[21], of accessory molecules involved in their immunological presentation[36] and of immunomodulatory cytokines[37]. Additionally, novel genetic vehicles and vectors now exist with which to present these components to the immune system[28].

Tumour-infiltrating immune cells

The discovery of the T cell growth factor IL-2 led to the ability to expand T cells *in vitro* and *in vivo*. As a result, clinical trials of IL-2 for cancer therapy were rapidly established[38,39]. Systemic administration of IL-2 led in some cases to a dense lymphoid infiltration of the tumour and, in some instances, tumour regression was observed[40–42].

Such observations show that T cells play a pivotal role in the immune response to cancer. The next step[34,43] was to use recombinant IL-2 to expand populations of immune cells recovered from resected tumours followed by adoptive transfer of these populations back into the patient together with high doses of systemic IL-2. These cells consist of tumour-infiltrating lymphocytes and lymphokine-activated killer (LAK) cells. Subsequent studies revealed that tumour-infiltrating lymphocytes are 50–100 times more effective in destroying tumour than are LAK cells[35,44]. Such regimens have been associated with spectacular tumour regression in some patients with advanced metastatic disease from melanoma, but at the expense of severe side-effects[32,34,45].

The cloning of many cytokine genes has made the prospect of manipulating immune effector cells to express high levels of potentially therapeutic cytokines or growth regulators a clinical reality. These cells can be administered back to the patient, where they are expected to secrete these proteins in the tumour deposits in locally high, but systemically negligible, concentrations. Tumour-infiltrating lymphocytes target tumour tissue with high efficiency as this is the basis of their operational definition[32,44]. Furthermore, the specificity of such immune cells for their tumour targets makes tumour-infiltrating lymphocytes attractive candidates for treating clinically undetectable metastases[34]. Response rates of 40 per cent have been reported in patients with advanced melanoma treated with tumour-infiltrating lymphocytes but long-term survival rates remain disappointing[34].

In an initial study of the use of gene-modified T cells, tumour-infiltrating lymphocytes were recovered from melanomas in six patients and genetically marked by infection with a recombinant retroviral vector encoding a reporter gene[32]. (Reporter genes serve no therapeutic purpose and are inserted into a cell simply to mark it biologically, usually with a gene for drug resistance. This allows an investigator to follow the fate of the cell in the body.) Reinfusion of these marked tumour-infiltrating lymphocytes back into patients revealed that they could be detected in tumour deposits and the peripheral circulation for several weeks following transfer. The presence of the marker gene had no effect on the efficacy of the tumour-infiltrating lymphocytes, which induced a response in half of the patients, but importantly demonstrated their innate tumour specificity and also the lack of harmful side-effects associated with treatment by cells modified by recombinant retroviral vectors[32]. Use of adoptively transferred tumour-infiltrating lymphocytes as vehicles for gene therapy is under way, and such lymphocytes from patients with melanoma have been infected with a retroviral vector expressing tumour necrosis factor (TNF)[46,47]. It is too early to assess this trial, but initial results are awaited. Success in this study will promote the use of other recombinant cytokine genes (e.g. IL-2, IL-4) for transduction of tumour-infiltrating lymphocytes.

There is variation in both the density and composition of the tumour infiltrate between different tumours of the same histopathological type. Thus both helper T cells and cytotoxic T lymphocytes, as well as natural killer cells, LAK cells, eosinophils, macrophages and neutrophils, are present in varying proportions[48–50]. Some of these immune cells mediate non-MHC restricted lysis of tumour cells and may prove to be of as much value in immunotherapy as tumour-infiltrating lymphocytes, which have been the principal cell type studied so far. Indeed, the nature of the infiltrate may predict appropriate manipulation of the tumour cells required to recruit greater numbers of relevant immune cells.

Targeting of tumour cell

The alternative to manipulating the immune infiltrating cells is to study the malignant cells themselves and define tumour-specific characteristics that can be used to induce cell death directly or promote tumour cell rejection (cancer vaccination) (*Figure 2*).

Tumour antigens and vaccination. To date, candidate cancer vaccines have used autologous (MHC matched) or allogeneic (MHC mismatched) tumour cells, or tumour cell-derived preparations, in conjunction with an adjuvant (e.g. bacille Calmette–Guérin vaccine) to enhance antitumour immunity[51,52]. In the absence of purified tumour antigens of known efficacy, tumour cells have been the best way to ensure that any tumour-specific antigens are presented to the immune system. The heterogeneity of tumour phenotypes, even between different tumour deposits in the same patient, necessitates the presentation of as large a variety of antigens as possible to produce a vaccine of even moderate efficacy. The synergy produced by combinations of tumour-associated antigens on tumour cell preparations is crucial to generate strong cell-mediated rejection immunity against the tumour. Apart from isolated reports of encouraging responses[53], the use of whole tumour cells and preparations derived from them as vaccines has yielded disappointing results[51].

Ideally, a cancer vaccine should possess a broad antigenic complexity, as well as a high degree of specificity of antigenic stimulation. However, whole cell preparations contain a plethora of irrelevant antigens, which induce redundant immune responses that can hinder tumour-specific activity. Nevertheless, it is now possible to clone, re-express and assess potentially clinically relevant tumour antigens to determine their role in tumour cell rejection, leading to the isolation of elements that produce the greatest therapeutic benefit by inclusion into 'designed' vaccines[13,21]. So far, melanoma is the only human malignancy in which a tumour antigen has been defined at a molecular level that is recognized by autologous cytotoxic T lymphocytes from the patient. Immunological techniques are now able to define antigens that are recognized *in vitro* by cytotoxic T lymphocytes derived from a patient with a tumour. Using gene transfection techniques and a cytotoxic T lymphocyte-based selection assay, Van der Bruggen and colleagues[54] cloned a gene for a melanoma antigen called MZ2-E. The MZ2-E antigen is expressed on melanoma and several other histological types of tumour but not on a panel of normal tissues. As MZ2-E has been cloned using cytotoxic T lymphocytes from patients with melanoma, it is expected that this tumour-specific antigen will have relevance in *in vivo* antitumour immunity. Immunological presentation of MZ2-E was also found to be human leucocyte antigen (HLA) A restricted[54] (that is, only one type of MHC molecule will present the antigen to the immune system). Therefore, cancer vaccines must also take into consideration the MHC genotype status of the patient. It is hoped that similar approaches will be applied to the cloning of other tumour-specific antigens for other carcinomas. Tumour antigens need not necessarily be proteins. Indeed, some carbohydrate moieties expressed in glycoproteins or mucins on epithelial cancer cells are known to be

immunogenic in man, and a vaccine expressing a sialomucin antigen is undergoing trials in patients with colorectal cancer[55].

Mutated oncogene products and tumour suppressor genes are also theoretical candidates as rejection antigens in a range of tumours[56]. The immunogenicity of such mutated proteins has not yet been demonstrated in human cancers, but the predominance of oncogene activation might make these proteins immunogenic targets for vaccines.

Major histocompatibility complex antigens. Experimental evidence has demonstrated that a decrease in the expression of cell-surface MHC class I antigens increases the tumorigenic potential of various transformed cells. For instance, the tumorigenicity of several cancer cell lines in mice can be partially abrogated by transfection of a single MHC class I gene into the tumour cells[57,58]. Importantly, transfected class I-positive tumour cells were able to sensitize a syngeneic mouse host to reject an otherwise tumorigenic dose of class I-negative parental tumour. This is an absolute prerequisite if MHC replacement is to offer a realistic vaccine strategy, as delivery of an appropriate MHC gene to every tumour cell in the body is not possible. These results are consistent with the known role of MHC class I in the presentation of foreign or tumour-associated antigens on the tumour cell surface to cytotoxic T lymphocytes[59]. Therefore, decreased expression of MHC molecules, class I in particular, might allow transformed cells to escape immune detection and allow tumour progression[60].

Clinical observations also suggest that loss of MHC may enhance tumorigenicity. The HLA class I antigens are downregulated in about 10 per cent of primary melanoma lesions and in 30 per cent of metastatic nodules[61]. Moreover, there is a significant association between low levels of class I antigens and a poor outcome in melanoma[62]. Losses of HLA have also been reported in a variety of other solid tumours[63]. It is possible, therefore, that the delivery of MHC molecules to tumour cells, and the expression of the corresponding antigens, may increase their recognition by the immune system and augment their rejection. Indeed, this strategy has formed the therapeutic basis of the initiation of a clinical study using liposome-mediated transfer of an HLA-B27 molecule directly into the tumour deposits of patients with advanced melanoma[64].

Tumour cells engineered to express cytokines. Failure of the immune system to destroy malignant cells is the result of either poor presentation of any available tumour antigens, as discussed earlier, or insufficient immunological activation. Therefore, many groups have attempted to create a locally increased level of immune activation within the tumour by transfer of cytokine genes directy into tumour cells[20]. In animal models, an enhanced immune response to weakly immunogenic tumours has been generated by *ex vivo* transfer of cytokine genes into tumour cells followed by immunization. This has been successfully accomplished by transfer of genes for IL-2, IL-4, IFN, TNF and granulocyte colony-stimulating factor[65]. Local secretion of a cytokine critical for activation of cytotoxic T lymphocytes can compensate for defects in the helper T cell arm of the immune system to permit recognition of the poorly immunogenic tumour by class I restricted cytotoxic T lymphocytes or other effectors of tumour cell clearance such as eosinophils and macrophages[66]. In animal models, poorly immunogenic tumours engineered to secrete IL-2 or IL-4 at high local concentrations generated tumour-specific cytotoxic T lymphocytes, which conferred systemic immunity against non-transfected tumour cells at distant sites[66–69]. This induction of immunological memory in cytotoxic T lymphocytes is pivotal if non-modified tumour cells at remote sites are also to be destroyed.

Different cytokines induce different immune effector mechanisms. For instance, the tumour infiltrate in a murine renal cell tumour secreting IL-4 consisted predominantly of macrophages and eosinophils, whereas the secretion of IL-2 by either a murine colon tumour line or a murine melanoma stimulated an MHC class I restricted cytotoxic T lymphocyte response against the parental tumour[66–69].

Although cytokine gene transfer offers an attractive approach to cancer vaccination, its use must be based on a clear understanding of both the tumour type and the immune response that the patient is able to mount against it. This may vary even between patients with the same tumour and obviously limits clinical adoption of this strategy. Finally, the choice of therapy should also be guided by the growth requirements of the tumour, as the local overproduction of certain cytokines can lead to enhanced growth of some tumours through an autocrine mechanism[70]. Thus the delivery of an inappropriate cytokine to tumour cells may actually promote aggressive cell growth. Work is under way to rationalize the use of cytokine gene transduction of tumour cells to produce optimal stimulation of tumour-infiltrating effector cells.

Gene correction therapy. The transformation of a normal cell never occurs in a single step and cannot be attributed exclusively to the mutation of only one gene[71]. Rather, there must be a series of mutations in several different genes that usually fall into one of at least two classes: the proto-oncogenes and the tumour suppressor genes[71]. In addition, mutations frequently occur in other classes of gene, which lead to acquisition of the metastatic phenotype[72].

Oncogenes are altered cellular genes encoding proteins that participate in normal pathways of cellular proliferation[22]. Tumour suppressor genes negatively regulate cell proliferation and promote cell differentiation[23]. Mutations in cellular proto-oncogenes lead to a dominant gain of function of the mutant protein, whereas deletions in tumour suppressor genes produce loss of function. These two genetic changes constitute part of the molecular basis of cancer development. Oncogenes are genetically dominant in that mutation in one of the two alleles is sufficient to induce neoplastic transformation. Tumour suppressor genes, however, require both alleles to be deleted or inactivated and function in a recessive manner. Whether or not these findings will contribute to new approaches in therapy remains speculative[73]. For this to be realistic requires either the abrogation of the activity of oncogenes or the replacement of tumour suppressor genes.

Oncogenes have been considered as targets for treatment both by rational drug design and by antisense oligonucleotide therapy. More than 60 oncogenes have been discovered and their protein products act at many different sites in the cell[22]. The elucidation of the crystal structure of one such protein, the oncogenic form of the *ras* protein, offers the chance to design a drug that will be highly selective in its action against mutant *ras* proteins while sparing normal *ras* proteins in non-cancer cells[74–76].

Antisense therapy is based on tumour-cell targeting of oligonucleotides that are complementary to the messenger RNA species encoding the oncogene products[77]. These oligonucleotides can be made highly specific by base pairing with the oncogene messenger RNA in the cancer cell, preventing translation (expression) of the oncoprotein product (*Figure 3*). *In vitro* studies have confirmed that antisense oligonucleotides directed against oncogenes can inhibit the growth of tumour cell lines, but major technical obstacles limit their application *in vivo*[73].

The challenges presented by tumour suppressors are different and require the delivery of correct copies of the missing gene to tumour cells. In this respect, clinical trials are under way to deliver a functional gene to replace a defective gene in T cells for adenosine deaminase deficiency and are also being considered for other genetic diseases, such as cystic fibrosis, Duchenne muscular dystrophy and the thalassaemias[31,78]. In cancer cells, this approach may ultimately prove to be more successful than current attempts to neutralize the effects of aberrantly expressed oncogenes[79,80], but the appropriate techniques are still remote. Reintroduction of the normal tumour suppressor genes *Rb*, p53 and *DCC* into appropriate

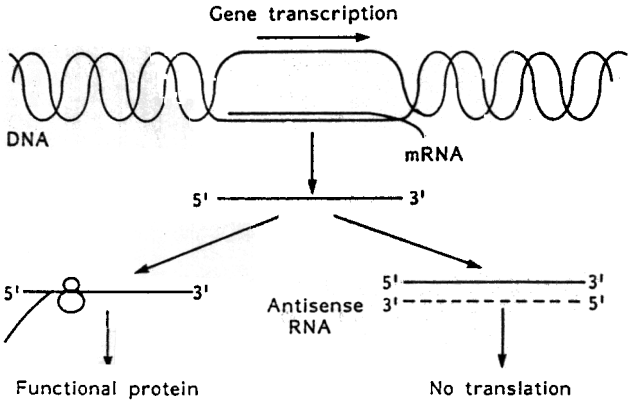

Figure 3 *Probable mechanism of action of antisense RNA. Binding to the messenger RNA prevents translation into functional protein*

tumour cell lines[79–82] has resulted in suppression of tumorigenicity and growth rates. However, replacement of these genes has not led to complete reversion to the untransformed phenotype nor has it been demonstrated to be a general phenomenon[23].

Despite encouraging experimental results of interventions directed against oncogenes and tumour suppressor genes, there remain major conceptual problems with their curative use. As carcinogenesis is a multistep process it is improbable that the correction of a single genetic defect will reverse the malignant phenotype. Indeed, by the time a patient presents to the clinic the dependence of tumour cells for their proliferation on any single genetic defect is very limited. Additionally, an absolute prerequisite for such therapy is the delivery of the therapeutic gene to all tumour cells in the body. Currently, no genetic delivery system can attain such efficiency. Thus, at present, it is difficult to conceive that correction of oncogene or tumour suppressor gene defects will achieve clinical realization in the short term other than as an adjunct to conventional therapy.

Delivery and targeting

The recurring shortfall of conventional cancer chemotherapy is the inability to deliver sufficient levels of a therapeutic agent specifically to all tumour cells while sparing their normal non-transformed counterparts. Similar problems exist for protocols of *in vivo* gene transfer to tumour cells, with one notable exception. In replacement of tumour suppressor genes or correction of oncogenes all malignant cells must be targeted. However, immunotherapy requires only that a significant fraction of either tumour cells or infiltrating cells is successfully transduced by gene transfer. Thereafter, the inherent specificity of the immune response to a given target antigen, together with its *in vivo* amplification, should result in efficient and accurate destruction of distant micrometastases.

The direct and specific delivery of therapeutic genes *in vivo* to tumour or infiltrating cells is an ideal that remains to be realized. This would obviate the need for the recovery, isolation and propagation of cells in culture while gene transfer is performed. Such protocols are time consuming and expensive but, more importantly, significant alterations in the phenotype of the recovered cells can occur during cellular adaptation to growth in culture.

Despite the efficiency and safety of retrovirus-mediated gene delivery, this method still lacks target-cell specificity. This property is conferred by the presence of cell membrane receptor proteins by which viruses gain entry into cells. Most viruses employ receptors that are expressed on a wide variety of cells, thus facilitating their propagation[83,84]. No known viruses infect exclusively tumour cells, although tumour cell-specific gene expression can be achieved with some viral vectors[85]. However, there are hopes that *in vivo* gene transfer will become a clinical reality as protocols already exist in which the gene is delivered directly into the tumour either by injection of DNA[64,86] or by administration of retrovirus stock[87].

In summary, the optimal protocol for gene transfer in the treatment of solid tumours currently involves the recovery of target cells (either tumour cells or the infiltrate) from the patient, their *ex vivo* expansion during which therapeutic gene transduction is achieved as efficiently and rapidly as possible by retroviral infection, followed by systemic administration of these altered cells back into the patient.

Patient selection

Preclinical studies in animal models have indicated that active immunization to pre-existing tumours is most effective when the tumour load is small and less effective against widespread cancer[67,88]. As a result, many investigators have proposed that, optimally, such therapies should be given early in the treatment of patients with cancer[65,89]. Clinical evidence for this seemingly rational proposal, although scant, does suggest that these treatments appear to be most effective in patients with early disease[90,91]. Most clinical studies of active specific immunization against cancer have been performed in patients with advanced tumours, so anything other than a dramatic response to therapy is likely to be concealed[32]. These protocols are ethically sound as such patients have failed to benefit from any other form of treatment, either alone or in combination. It is therefore appropriate that these, often young, patients with end-stage cancer should be offered the opportunity to participate in the initial clinical studies, provided that such novel therapy does not itself result in additional morbidity.

If the initial promise of these innovative strategies is to be translated into clinical reality there must be major changes in patient selection. Therapeutic gene transfer in patients with cancer ultimately needs to be brought into the context of adjuvant therapy[89,92], i.e. in the treatment of patients with minimal residual (microscopic) disease following radical resection of a primary tumour. Clearly, such a policy poses many ethical issues that must now be addressed. Not least of these is the problem of exposing relatively healthy patients to experimental therapies.

Evaluation of the results of these treatment protocols will require the definition of clear response endpoints, as initial tumour regression is not always associated with increased survival[89]. It is improbable that any of the therapeutic regimens discussed will be effective in isolation, given the complexity of host–tumour immune interactions. A combined approach will almost certainly be required, involving existing therapy and manipulation of both tumour cells and the immunological infiltrate[18,93,94].

Despite significant advances in the understanding of the molecular basis of cancer and the immunological response that a tumour elicits, widespread clinical application of this work is remote. However, it has become clear that gene transfer is, at the very least, a realistic treatment adjunct in some patients with cancer. Such therapies are currently in their infancy but the results of experimental studies have already been translated into clinical practice. Six gene therapy trials using retrovirus-mediated gene transfer in the treatment of patients with solid tumours have been established in the USA[31]. These trials have been approved by the Recombinant DNA Advisory Committee of the National Institutes of Health and the safety of such approaches is now widely accepted[31]. The benefits of treating selected patients with solid tumours with tumour-infiltrating lymphocytes are clear, but it remains to be seen whether using gene-modified tumour or infiltrating cells can improve on these results. Laboratory-based work has provided some clear strategies for the future in this field; the next decade will reveal whether they are to be of real value to patients with cancer.

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Paper accepted 30 October 1992