Primer

Gene therapy

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In its purest form, gene therapy is the delivery and expression of a correct copy of a gene into cells in which the endogenous gene is non-functional. It can lead to cure — or significant enhancement of the quality of life — of the patient. The concept is highly attractive as it provides a therapeutic link between laboratory-based studies on the genetic basis of disease and attempts to alleviate patient suffering.

In this context, a prototypic disease for gene therapy is cystic fibrosis (CF). Several years of genetic analysis of affected patients led to the cloning of the *CFTR* gene, which contributes to a chloride ion transport channel in epithelial cells, and which is mutated in cystic fibrosis patients. Expression of the cloned, correct version of the gene in cultured cells in which CFTR is mutant corrects the pathological phenotype, and replacement of *CFTR* in only a proportion of affected cells corrects the physiological defect across the entire epithelial cell sheet. To correct the defect, expression of *CFTR* has simply to be on rather than off, and over-expression in cells which are not the primary site of the disease pathology does not seem to be detrimental. All that remains, therefore, is to translate these experiments in cultured cells into the context of a living patient — of which more to come later.

Other monogenic diseases are also candidates for treatment by gene replacement therapies, including Duchenne Muscular Dystrophy (DMD) and the severe immunodeficiency associated with the lack of adenosine deaminase (ADA). But not all monogenic diseases are as simple, even in theory, as these examples. For instance, thalassemia is caused by mutations in the genes which encode haemoglobin, causing a deficiency of functional α and β globin proteins relative to one another. But unregulated over-expression of, for example, β globin may simply convert a β thalassemia (relative lack of functional β globin) into an α thalassemia (relative lack of functional α globin). In such cases, regulated expression of the therapeutic gene is required, either quantitatively and/or temporally, to achieve effective treatment.

If you can't correct it, kill it

Increasingly, a range of different approaches aimed at curing genetic and acquired diseases are now falling under the umbrella of gene therapy, which make the definition as epitomized by CF, DMD or even the haemoglobinopathies difficult to recognize. For example, therapeutic genes may not be delivered to the cell type that is itself affected: human cells, engineered to express and secrete high levels of Factor IX, can be reimplanted in the body to act as factories of secreted products to treat haemophilia. But the most dramatic variation on the gene therapy theme comes from attempts at treating cancer or acquired, pathogen-related diseases such as AIDS. In these cases, the classical concept of gene therapy — the use of genes to correct disease — has been expanded to include the use of genes with a license to kill.

Gene therapy against viral diseases, such as targeting HIV in AIDS patients, uses nucleic acid sequences (anti-sense) with specificity against viral transcripts, or control sequences, so that the virus can no longer complete its life cycle. Similarly, gene therapy for cancer represents a dramatic swing away from the use of genes in a purely 'restorative' capacity. Populations of malignant cells contain multiple mutations, including both dominant mutations in oncogenes and recessive mutations in tumour suppressor genes. Such a multifactorial genetic disease could, theoretically, be tackled by conventional gene therapy; functional copies of mutated tumour suppressors (such as p53), or anti-sense sequences against oncogenes, could be delivered to cause the tumour cells' uncontrolled proliferation to become more orderly, invoking terminal differentiation or, at best, cell death by apoptosis.

But the genetic complexity of human tumours suggests that trying to correct them genetically may be much less effective than simply trying to kill them. Various strategies have been developed to achieve this, including the delivery of cytotoxic genes or genes which activate the immune system to recognize and kill tumour cells spread throughout the body. The preponderance of immunotherapy protocols reflects two major considerations. First, cancer is usually fatal because it is a disease of metastatic cells distributed throughout the body. The unrestricted access of immune cells to all body areas makes them attractive for recruitment to a 'search and destroy' capacity. Second, without the response amplification which an appropriately activated immune system can provide, there are currently no vector systems that can deliver enough toxic genes specifically to sufficient numbers of tumour cells to be effective. This latter point highlights the key block to effective gene therapy for just about any disease — that of gene delivery.

Nice message – shame about the messenger

The bottleneck into which all gene therapy protocols eventually funnel is that of gene delivery. Whatever therapeutic gene is chosen, it must be delivered to the cellular site of activity. Where therapeutic strategies allow, genes can be delivered to cells removed from the patient, which are

then transplanted *in vivo* (this approach is used for cytokine-modified anti-cancer vaccines or cell factories for the production of secretable proteins). But *ex vivo* cell modification is expensive, laborious and rarely appropriate. Hence, the ultimate goal is to develop vectors for direct *in vivo* delivery. This has proved to be a considerable hurdle and continues to be the main obstacle hindering effective treatment of disease using genetic therapy.

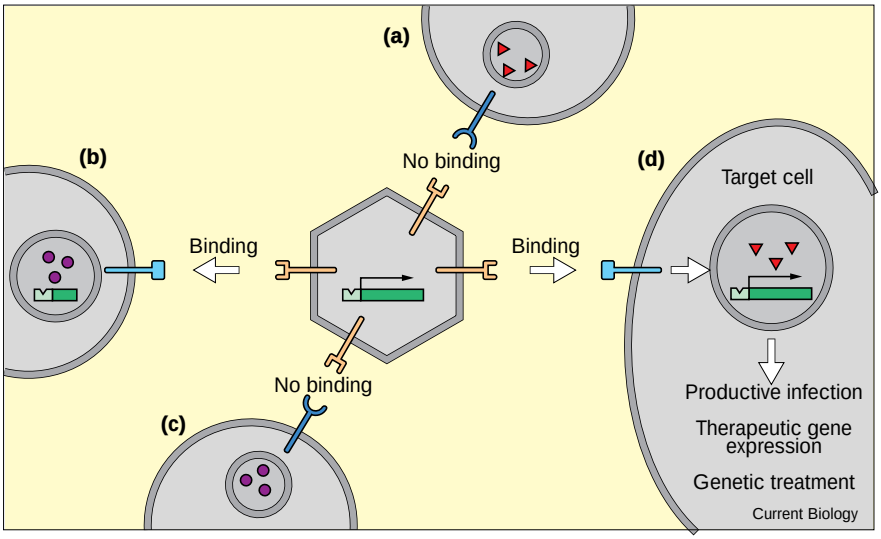
Vectors must be safe, efficient enough to transduce enough cells to exceed the therapeutic threshold and accurately targeted to reduce toxicity. The most commonly used vectors have been made from plasmid DNA — usually complexed in some form with liposomes to increase stability — retroviruses and adenoviruses, although others, such as adeno-associated, herpes or vaccinia viruses are increasingly coming into use. Each type of vector has its own advantages and drawbacks, which weigh differently, depending on the specific therapy required.

Thus, corrective gene therapy requires long-term, stable gene expression, ideally in the stem cell population of the diseased tissue. This requires a vector that integrates into the genome (plasmid, retro- or adeno-associated vectors), which itself poses a potential mutagenic risk. The non-integrating, rapidly diluted vectors, such as adenoviruses, would therefore be of limited value (unless the target cells never divide), although probably less mutagenic. But the adenoviral vectors come into their own in situations in which high levels of transient gene expression may suffice — for instance, in the delivery of immunostimulatory or cytotoxic genes to tumour cells, which should not live long enough for the lack of stable expression to be a problem. The titres of these adenoviral vectors (up to 10^{12} infectious units per ml) humble most other systems.

Vectors must also be accurately targeted to cell types *in vivo*. This can be achieved at various levels of sophistication. For example, topical administration (intratumoral injection for cancer, or airway lavage for CF) allows them to be concentrated at the site of action. Further targeting can be achieved by using natural tropisms of vectors, if they exist, although relatively few viruses infect specific cell types that coincide with the target tissues used by gene therapists.

Considerable effort is now dedicated to the molecular engineering of viral and plasmid vectors to restrict their tropisms to specific cell types. Where expression of a corrective gene is not toxic to non-involved cells, this restriction can be relatively loose (for example, *CFTR* delivered to the airway epithelial cells). By contrast, if a cytotoxic gene is being delivered to metastases in an organ of critical value to the patient, tumour cell targeting is essential. Molecular modification of vector sequences has already allowed recombinant viruses to be targeted to specific cells using, for example, transcriptional targeting with tissue-specific promoters and surface targeting by altering the binding properties of viral envelope proteins (Figure 1). Once again, different vector systems are differentially amenable to targeting strategies but there is now cause for genuine optimism that recombinant vectors can be effectively redirected to produce targeted infection or expression.

Figure 1

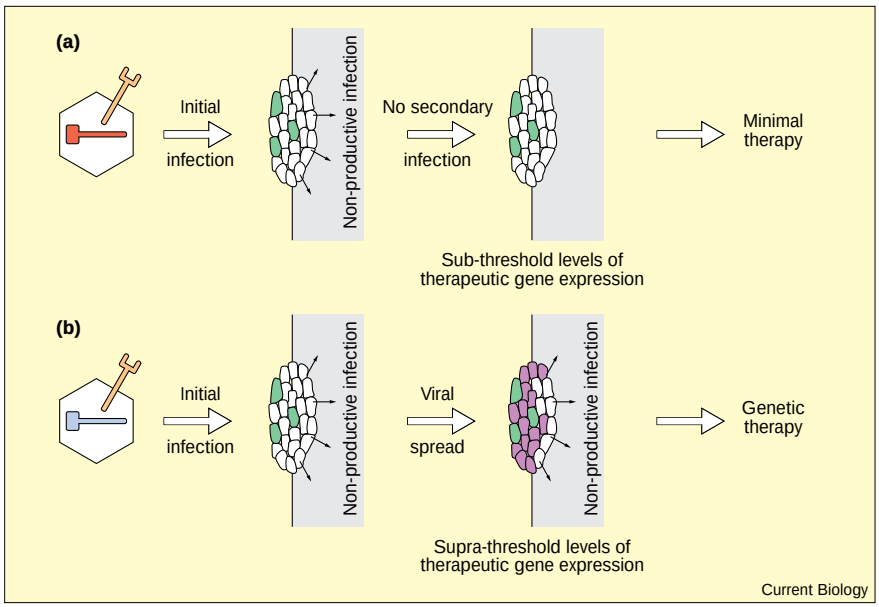


A vector targeted to at least two levels will productively infect only target cells that are compatible with both levels of targeting. The vector shown displays an engineered ligand (orange) that binds only to receptors known to be expressed on the target cell surface (light blue), and the therapeutic gene (green) is expressed from a promoter–enhancer combination that is activated by transcription factors (red) present in the target cell. But both cell-surface receptor and transcription factor are also probably expressed individually, but not in combination, in other non-target cells. When the vector is delivered to the target site, cells not expressing the receptor ((a) and (c)) will not be infected; cells expressing the receptor will be infected ((b) and (d)), but only cells expressing the receptor and the appropriate transcription factors (d) will also express the therapeutic gene (productive infection). The number of levels of targeting required to define absolute cell-type specificity for a vector will depend upon the target tissue type.

Innovation for the future

There is clearly a great discrepancy between what we want, and what we can have, using the vectors currently at our disposal. The attractive, but so far elusive, perfect vector would possess attributes from different vector systems which would

Figure 2



The advantages of using targeted replicating vectors for gene therapy. **(a)** With the recombinant vectors currently at our disposal, delivery of viral vector stocks to the target tissue will initiate a single round of infection (green). Titres are generally low relative to the numbers of target cells to be treated. Therefore, with time, the only expansion of the number of cells transduced with the therapeutic gene will come from division of cells infected at the first pass of the vector. The initial infection will be restricted to target cells because of the targeting features incorporated into the vector. In many physiological circumstances, vectors currently have titres too low to transduce enough target cells to obtain therapeutic benefits. **(b)** If a stock of targeted, but replicating, vector (blue) is delivered to the target tissue at the same initial titre as the recombinant vector in (a), the initial infection will transduce the same number of target cells. With time, however, the vector will spread through the target tissue to infect a large proportion of the cells (purple), such that the threshold level of infected cells required for therapeutic benefit will be exceeded. Because of the targeted nature of the vector, the spreading infection will cease at the boundaries of the target tissue.

themselves be tailored to the vector's clinical purpose. For example, gene therapy of CF requires both the high titre of adenoviruses and the long-term integrating capacity of retroviruses. Any vision for the future must now include the development of hybrid vectors that combine properties of several systems to produce designer vectors optimized for different clinical situations.

In addition, manipulations of vector structure or function often reduce the already poor efficiencies of the unmodified vector. With target-cell populations of many millions and vector systems with *in vitro* titres of, at most, 10^{10}–10^{11} infectious units per ml, the magnitude of the gene delivery task is already Herculean. But titres determined *in vitro* on ordered monolayers of readily exposed cells rarely match what will realistically be achieved in the patient. *In vivo*, target cells may be hidden beneath layers of other cells, embedded in a tight tissue or tumour architecture, or be below layers of mucus or other physiological barriers. It is hardly surprising, therefore, that virologists are wistfully turning to the concept of using viruses which have the ability to replicate selectively in target tissues. Here, a small vector inoculum would initiate a spreading infection which would be restricted at the boundaries of the target tissue by targeting features built into the vector (Figure 2).

Although the thought of replicating viruses often invokes visions of Frankenstein, there is both precedent and good scientific rationale behind investigation of such strategies. Indeed, it is indisputable that our ability to identify, clone and rebuild diseased genes into potentially therapeutically valuable pharmaceuticals has greatly outstripped our ability to package, deliver and express these genes in the appropriate cells of patients. The challenge for the immediate future is to manipulate targeting features into vector systems, while also modifying them to increase viral titres. In this way, useful numbers of cells will be safely, accurately and efficiently transduced. We should not be afraid to look at radical innovations, including the use of rigorously designed and tested replicating or hybrid vector systems, as these are now required to translate gene therapy beyond the sphere of virtual treatments and into the real clinic.

Key references

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