Gene Therapy

Targeted Adenoviral Vectors

Many viral and nonviral vectors have been studied for their capacity to effect in vivo gene transfer. Adenoviruses are particularly attractive because they permit transient high-level gene expression upon in vivo delivery. The pharmacological parameters that influence therapeutic transgene expression from adenoviral vectors consist of the dynamics of adenoviral infection, namely the steps of the viral life cycle.

Virus binding to the cell surface via specific cell surface receptors must take place before any downstream event can happen. Binding may be considered the most important step in adenoviral gene transfer, and this article is focused on that step. The predominant high-affinity cell surface receptor for adenovirus, which has recently been identified, is called the Coxsackie-adenovirus receptor (CAR). Its expression has been shown to be essential for efficient gene transfer to a variety of cell types in vitro.

Adenoviral vectors may come into contact with both target cells and other cell types upon direct in vivo injection. To achieve efficient gene delivery, the vector must preferentially bind to the target cells. Such specific binding is predicated upon high-level expression of CAR only on the target cells, with other cell types ideally expressing low levels of the receptor.

Because receptor expression cannot be manipulated in patients, the profile of CAR expression in a particular anatomical compartment in vivo will ultimately dictate the distribution of adenoviral vector binding and hence of gene transfer. Delineation of the specific cell types that express CAR in vivo will be valuable in predicting the potential efficacy of gene transfer by adenoviral vectors and in predicting the potential toxicity of ectopic gene transfer to non-target cells that express CAR at high levels.

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Adenoviral gene transfer is currently being tried as adjunctive therapy following surgery for glioblastoma multiforme. The vector is injected directly into the cavity previously occupied by the surgically excised tumor. In the interest of preserving as much normal brain as possible, neurosurgeons do not remove wide margins along with the tumor mass. Tumor cells invariably remain and are the targets for adjunctive therapy.

Naturally, these cells are surrounded by normal brain components, including neurons, astrocytes, ependyma, and vascular structures. Adenoviral vectors have been shown to effectively transduce all of these cell types when directly injected into the brains of both tumor-bearing and normal mice.

Furthermore, adenoviral victors also efficiently transduce normal human astrocytes and neurons in culture, explaining why adenovirus is a promising vector for gene therapy of a number of nonmalignant neurological diseases such as Parkinson's disease and amyotrophic lateral sclerosis.

The profile of Coxsackie-adenovirus receptor expression in the human brain has yet to be determined, but these results imply that the cells constituting the normal brain express sufficient levels of CAR to permit efficient adenoviral gene transfer.

With primary tropism for CAR-expressing cells, current adenoviral vectors may bind and infect target and non-target cells alike. The number of non-target cells surrounding the tumor cells within the surgical margin should far outnumber the remaining tumor cells.

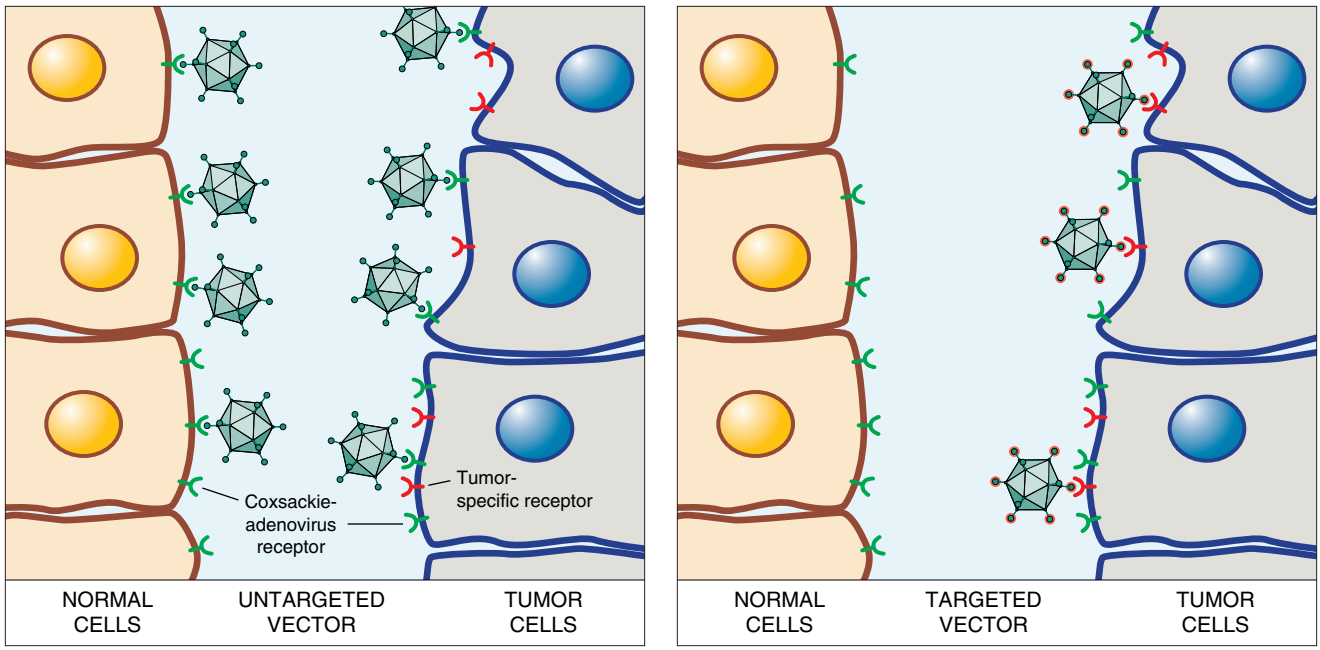
Consider a scenario in which the surgical debulking procedure is suboptimal and leaves as potential targets 10% tumor cells and 90% normal brain cells. Assuming that the affinity with which the adenoviral vector binds each of the different cell types is equal, it follows that the injected vector will predominantly bind and transfer genes to non-target cells, theoretically at a ratio of 1:9.

The effective dose of the vector in such a circumstance would be reduced by one log compared to the actual dose injected, thus requiring 10 times more virus to achieve a given level of gene transfer. Because adenoviral vectors induce a brisk immune response, the magnitude of which is directly proportional to the amount of input virus, increasing the dose will enhance the vector-induced immune response.

An assumption in this scenario is that the tumor cells intended to be transduced will uniformly express high levels of CAR, but this has proved not to be the case. Some primary tumor cells have been shown to lack CAR expression in vitro, including gliomas, melanomas, and bladder carcinomas.

High doses of adenoviral vector are required to efficiently transduce such CAR-deficient tumor cells, which could further complicate the vector-induced immune response when used in vivo. In a particular anatomical setting, therefore, the profile of CAR expression on tumor and surrounding normal cells may be suboptimal, preventing efficient transduction of the target cells.

An adenoviral vector targeted to a cell surface receptor specifically



expressed on tumor cells could potentially overcome this problem. Again consider a situation in which the ratio of tumor cells to normal cells is 1:9 and assume that the affinity with which adenovirus binds to both types of cells is equal. If a vector infected tumor cells 10 times more selectively than normal cells, then both tumor cells and normal cells would theoretically have an equal chance of being infected. A vector with a 100-fold selectivity for tumor cells would theoretically be 10 times more likely to infect tumor cells than normal cells. The greater the increase in tumor selectivity, the more the effective dose of the virus is increased, improving the efficacy of gene transfer, reducing the immune response, and reducing the toxicity caused by ectopic gene transfer.

A similar argument may be made for ovarian carcinoma, or for that matter any tumor confined to a specific anatomical compartment. Gene therapy is currently considered for patients with recurrent ovarian carcinoma following surgical debulking and adjunctive chemotherapy. In this setting, the target carcinoma cells remain adherent to the peritoneal cavity, ideally in nodules no larger than 1 cm^2. With nodules of that size, mesothelial cells comprising the peritoneal wall far outnumber the tumor cells. Mesothelial cells express CAR, so a highly tumor-selective adenoviral vector would be required to efficiently transduce tumor cells and spare surrounding normal tissue.

It is clear that a targeted adenoviral vector is necessary to achieve both efficient tumor-selective gene transfer and reduced vector-related toxicity when used for therapy of tumors confined to a specific anatomical space. Current technology permits the generation of targeted adenoviral vectors by two methods. The first employs bispecific targeting conjugates with specificity for both the adenoviral capsid and the target receptor, and the second is genetic modification of the viral capsid protein to incorporate ligands with target receptor specificity. Such vectors can now be rationally designed, but the foregoing discussion begs a question: what tumor-specific receptor(s) should be considered?

Since the advent of monoclonal antibody technology first suggested the possibility of targeted immunotherapy, investigators have looked for cellular receptors with tumor-specific expression. A number of promising markers for monoclonal antibody-based immunotherapy have been identified for a variety of tumors. Undoubtedly, the continuing investigation of monoclonal antibody therapy, together with newly developed phage display technologies, will yield even more attractive candidate tumor-specific markers in the future.

Obviously, any of these receptors would also be useful in the rational design of targeted adenoviral vectors. Yet in the volatile microenvironment of a tumor, cell surface receptor expression is heterogeneous on both the cellular level and the tumor level. To overcome receptor heterogeneity on the tumor cells, different targeted adenoviral vectors, each designed to bind to a distinct tumor-specific receptor, could be combined into a vector "cocktail" for use clinically.

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