Exogenous and endogenous gene regulation for specific and efficient cancer gene therapy
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Chapter

Adenoviral gene therapy

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**Introduction**

Over the last decade gene therapy has evolved as a promising method for the treatment of many diseases. To deliver the genetic material into cells, a viral vector can be utilized. One of the most efficient vectors available at this moment is the adenovirus. Incorporation of specific transgenes into the genome of the adenovirus ensures that the genetic material will be delivered into the nucleus after viral infection. One multigenetic disease which receives much attention in the research area is cancer. Many attempts are made to tackle this still largely incurable disease with gene therapy. The adenovirus may serve as a vector to deliver toxic genes to the malignant cells, but, on the other hand, this virus can also be used as a tool by itself to kill these cells. In the latter approach, the virus may replicate specifically in tumor cells, causing cell lysis and release of new virus progeny.

**The adenovirus as gene therapy vector**

Our understanding about the biology of the adenovirus has increased enormously in the last decades, and today this virus is commonly used as a vector for gene transfer. This knowledge about the adenovirus has led to a fast progression in the development of new gene therapeutic agents. The adenovirus is known for its efficient infection of dividing and non-dividing cells, resulting in an efficient delivery of therapeutic genes to the site of action. Other advantages for the use of adenoviral vectors are the possibility to produce these viruses in very high titers and that they are relatively harmless.

To increase the safety measurements of adenoviral vectors in gene therapy, most adenoviral vectors used are unable to replicate. This elimination of replication is caused by the deletion of the E1A and E1B region of the adenoviral genome. This region is necessary for the adenovirus to replicate. The E3 region of the adenoviral genome may also be deleted to increase the space in the genome available for the insertion of transgenes up to 7.5 kb. The E3 region is not required for replication of the adenovirus, but includes genes that are able to interfere with the defence system of the host cell after infection.

Currently, the realization that clinical applications of adenoviral vectors encounter limitations becomes more and more clear. Although adenoviruses are among the most efficient vectors used in gene therapy, they are not always efficient enough. This is especially true for cancer gene therapy, where it is necessary to kill every tumor cell, otherwise the malignant tissue will reoccur. One intratumoral injected dose of adenoviral vectors will infect maximally 10% of the tumor cells, thus the therapeutic effect will be limited. Another major limitation of adenoviral vectors is the biodistribution of the adenovirus. When administrated intravenously, the majority of the virus ends up in the liver, where it can cause severe liver toxicity. Therefore a large proportion of research is conducted on the un-targeting of the liver.

There are two main routes to untarget the liver and to achieve the specific gene therapeutic effect in particular sites of the body. The first one is transdutional retargeting and the second one is transcriptional retargeting. Transductional retargeting changes the cell entry route of the virus, whereas transcriptional retargeting restricts the expression of genes to the target cells (Figure 1).
Retargeting of adenoviral vectors

The adenovirus contains a protein capsid with protruding fibers. These fiber proteins form a trimer and the C terminal region of these fibers is called the fiber knob. The fiber knob recognizes and binds to the host cell via the coxsackievirus–adenovirus receptor (CAR), which is present on many cell types. Upon binding to CAR, secondary interactions between the adenovirus and cell surface receptors cause the internalization of the adenovirus. By shielding or modifying the fiber of the adenovirus, the native tropism can be altered. One possibility is to construct bispecific antibodies that on one side bind to the adenoviral fiber knob and on the other side will bind to an antigen present on target cells. This way, the adenovirus will be retargeted to cells that express this specific antigen. A second possibility is to construct an adenobody, fusing an antibody directed against the fiber knob with a ligand for a receptor present on the cell type of interest. A third possibility to modify the tropism of the adenovirus is to genetically alter the fiber knob itself. It is possible to genetically insert sequences to be expressed within the fiber knob that are recognized by a certain receptor present on the cell type of interest without modifying the interactions within the trimer itself. However, it has to be noted that although interactions between the CAR receptor and the adenoviral fiber knob play an important role in cell uptake in vitro, these interactions might not play a crucial role in vivo.² Adenoviral particles containing a mutation within the fiber knob which abolished binding to the CAR receptor were systemically administrated in nonhuman primates. Surprisingly, the biodistribution of this ablated adenovirus was similar compared to the nonmodified adenovirus. This study showed that other receptors such as the heparin sulfate glycosaminoglycans (HSG) might play an important role in liver uptake. The construction of an adenovirus which is ablated for interactions between the virus and the HSG receptors can be a promising way to untarget the liver. Another approach to prevent uptake by the
liver is PEG-ylation of the adenovirus. Coating of the adenovirus by polyethylene glycol (PEG) will shield the adenovirus and prevent uptake by the liver. It is feasible to bind a homing device onto the PEG. This way the PEG-ylated adenovirus will be directed to the site of interest.

Besides transductional retargeting of the adenovirus to the tissue or cell type of interest, it is also possible to transcriptionally retarget the adenoviral vector. Transcriptional retargeting is the modification of the transgene expression profile to increase the specificity of gene transcription towards a certain tissue or cell type. Such increased specificity can be obtained by placing the gene of interest under control of a tumor- or tissue-specific promoter. This way, the gene of interest will only be transcribed in cells where the promoter is active.

**Oncolytic adenoviruses**

In the battle against cancer it is very important to kill every tumor cell present; otherwise, the remaining malignant cells will continue to grow and form new tumors. Although adenoviral vectors are one of the most efficient vectors used, one injected dose is thought to infect maximally 10% of the tumor cells. Thus it is necessary to increase the efficiency of adenoviral infection. An approach to increase this efficiency is to allow the virus to replicate, thereby increasing the amount of infectious particles. Replication of the adenovirus inside these cells will eventually lead to cell lysis, thereby releasing newly formed virions within the tumor mass. The subsequent viral generations will continue this cycle of infection, replication, and cell killing, thereby killing more and more tumor cells, resulting in the elimination of the tumor mass. Obviously, adenoviral replication should be confined to the tumor cells. Adenoviruses that can only replicate inside malignant cells are termed conditionally replicating adenoviruses (CRAds).

Two different approaches can be distinguished in constructing CRAds, generating either type I or type II CRAds. Type I CRAds contain a specific deletion in the adenoviral genome based on genes that are differentially expressed between tumor and normal cells. Due to this mutation normal cells are not permissive to adenoviral replication. In tumor cells this host cell defence mechanism is deregulated, thereby creating the perfect environment for the virus to replicate.

A well-known example of a type I CRAd is the adenovirus Onyx-015, also known as dl1520. This virus contains a deletion in the adenoviral E1B-region. As mentioned before, the E1A and E1B regions are important for the replication of the adenovirus. The adenoviral E1B 55-kDa gene codes for a protein that inhibits p53 function which is an essential step in viral replication. Onyx-015 is deleted for E1B 55 kDa, and thus unable to inhibit p53. Therefore in normal cells, where p53 is functionally present, Onyx-015 will not be able to replicate as p53 cannot be inhibited by this virus. However, in most tumor cells the function of p53 is already disturbed and the ability of the virus to inactivate p53 is not required anymore. This causes selective adenoviral replication in p53-deficient tumor cells. At this moment, phase I, II, and III clinical trials have been conducted with limited success.

Type II CRAds are adenoviruses where the expression of genes essential for adenoviral replication are under the control of a tumor-specific promoter. When this promoter is active, expression of this gene will result in replication of the virus. These promoters should mainly be active in tumor cells to ensure that replication predominantly occurs inside the malignant tissue. An example of a tissue-specific promoter is the prostate-
specific antigen (PSA) promoter which is highly active in PSA-producing prostate cells and shows limited activity in other tissues. Placing a gene essential for replication directly under the control of the PSA promoter directs adenoviral replication primarily to prostate cells that express PSA. Thus this adenovirus will replicate inside prostate (tumor) tissue while sparing the other tissues. At the moment, phase I and II trials are being conducted. Another example of a tumor-specific promoter is the telomerase promoter, which is active in more than 80% of all tumors. An adenovirus which has the expression of a replication essential gene under control of the telomerase promoter can therefore replicate in a broad range of tumor types. Other examples of tissue- or tumor-specific promoters are the epithelial glycoprotein-2 promoter, which is active in most epithelial-derived cancers making it a good candidate for the treatment of many tumor types, and the tyrosinase promoter, which is highly active in melanoma cells.

Although the theoretical idea behind type I and type II CRAds is very attractive, some drawbacks exist. In the case of an adenovirus where the expression of the replication essential gene E1 was restricted to melanoma cells using the tyrosinase promoter, replication was shown to be specific for melanoma cells at low infectious units. However, at a higher dosage replication was also demonstrated in nonmelanoma cells. This loss of specificity might be due to the presence of adenoviral promoter-like sequences upstream of the tyrosinase promoter. These sequences may interfere with the specific regulation of the tyrosinase promoter, causing activation of transcription in cells which lack actual tyrosinase promoter activity. Another possibility is the presence of E1-like proteins inside the cell, compensating for the lack of adenoviral E1 expression in nonmelanoma cells.

**Double-controlled conditionally replicating adenoviruses**

After the systemic administration of adenoviral vectors, most of the virus ends up in the liver. When adenoviral replication is not strictly restricted to certain cell types or tissues, severe liver damage might occur due to adenoviral replication and consequential lysis of the liver cells. Because of this importance to restrict adenoviral replication, solutions have to be found for the observed a-specific replication of CRAds. One solution is a double-controlled conditionally replicating adenovirus (dcCRAd). In a dcCRAd not one but two replication essential genes are controlled by two tumor-specific promoters. For example, both the adenoviral E1A and E1B genes have been placed under the control of two different tumor-specific promoters. Also, studies have already been conducted with both the E1A and the E4 gene under control of two different tumor-specific promoters. E1A, E1B, and E4 are all examples of adenoviral genes that are essential for replication of the adenovirus. The approach of a dcCRAd preferentially includes a more general promoter. For example, the telomerase promoter which has shown activity in more than 80% of all tumors and which is inactive in liver cells. The telomerase promoter might work in concert with a tissue-specific promoter such as the tyrosinase promoter to restrict the expression of two genes essential for adenoviral replication. Only in melanoma cells which contain both telomerase and tyrosinase activity will replication occur.

Another approach for designing a dcCRAd is to combine a type I and a type II CRAd. On one hand, these adenoviruses contain a deletion in the adenoviral genome to prevent replication in cells without a disturbed cell cycle. On the other hand, these viruses also contain a tumor-specific promoter to restrict the expression of adenoviral genes essential for replication to cells where this promoter is active.
Conclusion

Adenoviruses can be utilized as a vector for the delivery of genetic material into cells. They possess many valuable characteristics, making them good candidates as gene therapy vectors. However, a major obstacle for clinical applications of adenoviral gene therapy vectors is the biodistribution of the virus. When administrated systemically, over 90% of the administrated adenovirus ends up in the liver where it can cause liver toxicity. Two different approaches are being pursued to deal with this problem, referred to as either transductional or transcriptional retargeting.

Transductional retargeting is focused on preventing the liver uptake. Either by modifying or shielding the fiber knob, thereby inhibiting binding to the CAR receptor, or by shielding the complete adenovirus by means of PEGylation. The next step is the addition of a homing device for retargeting to the tissue or cell type of interest. Transcriptional retargeting does not prevent liver uptake, but gene expression will only occur in the target tissue or cell type. This can be accomplished by placing the transgene under the control of a tumor- or tissue specific promoter. When genes essential for adenoviral replication are placed under control of such a promoter selective replication will occur, thereby enhancing the efficiency of infection.

By combining these two fields of expertise it is possible to construct an adenovirus that, on one hand, circumvents the liver because it is transductional retargeted and homes to the tissue of interest. On the other hand, this adenovirus will selectively express the transgene in the targeted tissue. This means that specificity is increased and therefore it is possible to systemically administrate a higher dosage of the adenovirus, increasing the efficiency of adenoviral gene therapy. It is also feasible to transductionally retarget CRAds. In this case more infectious viral particles will reach the tumor environment, increasing the initial infectious dose and subsequent infection efficiency.

Although much progress is made in the transductional and transcriptional retargeting of adenoviral vectors, there are still hurdles to overcome. Success in the clinic using CRAds is not convincing. One problem might be that tumors not only consist of tumor cells but also of connective tissue impairing the spread of the adenovirus throughout the tumor tissue. However, the combination of adenoviral vectors and chemotherapy seems to result in synergistic antitumor effects. Compared to other nonviral and viral vectors, the adenovirus is among the most efficient vectors currently used and is relatively harmless. Progress is made very rapidly and the use of adenoviruses in the clinic is a promising method for the treatment of cancer.
References
