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

Towards a double controlled conditionally replicative adenovirus for potent and melanoma-specific cell kill

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Abstract
To increase the efficiency of adenoviral cancer therapy, conditionally oncolytic adenoviruses are employed. However, these agents can cause toxicity due to replication in non-target tissues. To increase specificity, we designed trans-complementing adenoviruses: two non-replicating viruses were constructed which only allowed viral replication when both viruses infected the same target cell. Combining these two agents enhances the specificity and efficiency of adenoviral cancer therapy.
**Introduction**

The increase in incidence and mortality rates for melanoma are among the highest of all types of cancers worldwide, signifying the need for new therapies. A promising therapeutic approach is adenoviral cancer gene therapy. However, although adenoviral vectors are among the most efficient vectors currently available, they are not efficient enough to infect all tumour cells,\(^1\) which is a necessity for the treatment of cancer. To increase the efficiency, replicating adenoviruses can be utilized.\(^2\) Replication of the adenovirus results in cell lysis and spread of the viral progeny to neighbouring cells, continuing the cycle of infection, replication and cell lysis. These replication cycles profoundly enhances the efficiency of adenoviral agents. To restrict adenoviral replication to tumour cells, the expression of replication-essential genes can be controlled by a tumour specific promoter. These promoters are highly active in tumour tissue, but show limited activity in normal cells.\(^3\) A melanoma-specific promoter is the tyrosinase promoter, which was previously shown to retain its specificity for melanoma cells in an adenoviral context.\(^4\) We therefore used the tyrosinase promoter to control the expression of the adenoviral replication-essential E1 gene.\(^5\) The constructed conditionally replicating adenovirus did indeed efficiently replicate in melanoma cells, but some replication in non-melanoma cells was also observed. To increase the specificity towards replication in melanoma cells, we designed in this study a trans-complementing approach in which two non-replicating adenoviruses specifically replicate in melanoma cells, only if both viruses infect the same cell.

**Experimental methods**

To achieve specific adenoviral replication in melanoma cells, we constructed two adenoviruses (Figure 1A). Both viruses were based on an adenoviral backbone in which two replication-essential genes (E1 and pTP) were deleted.\(^6\) One adenoviral vector contained an expression cassette within this adenoviral backbone in which the tyrosinase promoter was used to control the expression of the replication-essential E1 gene. This adenovirus remained deleted for the replication-essential gene pTP (AdTyrE1ΔpTP) and therefore unable to replicate. In addition, we constructed a different virus containing the tyrosinase promoter controlling the expression of the replication-essential pTP gene, but deleted for the E1 gene (AdTyrpTPΔE1). Because adenoviral vectors need both the E1 and the pTP protein for replication, replication can only occur if both vectors are present in tyrosinase-promoter positive cells (Figure 1B). As a positive control for replication, wild-type adenovirus was used.

To test the functionality of the constructed viruses, 412 cells\(^6\) which complement for both the E1 gene as well as the pTP gene were infected with 10 plaque forming units (pfu)/cell AdTyrE1ΔpTP and/or AdTyrpTPΔE1 or cells were infected with 1 pfu/cell AdWT. In addition, the different viruses were tested for replication specificity in a tyrosinase-positive melanoma cell line and in a tyrosinase-negative glioma cell line. Cells were infected with 10 pfu/cell AdTyrE1ΔpTP and/or AdTyrpTPΔE1, or with 1 pfu/cell wild-type adenovirus. The remaining cells were fixated and stained with gentian violet.
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Results and discussion

We tested the functionality of the constructed vectors in 412 cells. As these cells complement for the both the replication-essential E1 and pTP gene, both viruses are expected to replicate, even if the tyrosinase promoter is not active. Indeed, after infection of either virus alone or in combination, efficient cell lysis was observed as detected by crystal violet staining (Figure 2).

Figure 1. Trans-complementing system.
A) Viruses have been constructed using an adenoviral backbone deleted for the E1 and the pre-terminal protein (pTP) gene. The virus AdTyr E1ΔpTP complements the E1 gene, while the adenovirus AdTyrpTPΔE1 complements the pTP gene. Both genes are under the control of the tyrosinase promoter. B) For tyrosinase positive (melanoma) cells, infection with either virus alone will not result in cell lysis through viral replication. Co-infection with both viruses simultaneously results in viral replication. In tyrosinase negative cells, infection with either virus alone or in combination will not result in viral replication and subsequent oncolysis, because the tyrosinase-promoter is not active. Wild-type adenovirus will replicate in both tyrosinase-positive and negative cells.

Results and discussion

We tested the functionality of the constructed vectors in 412 cells. As these cells complement for the both the replication-essential E1 and pTP gene, both viruses are expected to replicate, even if the tyrosinase promoter is not active. Indeed, after infection of either virus alone or in combination, efficient cell lysis was observed as detected by crystal violet staining (Figure 2).

Figure 2. The constructed adenoviral vectors can efficiently replicate in the E1 and pTP complementing cell line 412. AdTyrpTPΔE1 (A) alone or in combination with AdTyrE1ΔpTP (B) infected 412 cells at the indicated pfu/cell ratio. Remaining cells were fixated and stained with gentian violet.
Subsequently, the specificity of the constructed adenoviruses was examined by infection of tyrosinase-positive and negative cells. After infection with either AdTyrE1ΔpTP or AdTyrpTPΔE1, no cell lysis was observed in a tyrosinase-negative glioma cell line or in a tyrosinase-positive melanoma cell line (Figure 3). However when both vectors infected the same cells clear cell death was detected in the melanoma cell line, indicating that both vectors trans-complemented each other. As expected, in tyrosinase-negative cells no cell lysis was observed when both viruses infected the same cells (Figure 3). These results demonstrate that the trans-complementing system used in this study is an effective approach to restrict adenoviral replication to melanoma cells. This approach can be extended for many different types of cancer by using other tumour specific promoters. In this respect, the epithelial glycoprotein-2 promoter (EGP-2) has shown to be active in most carcinomas, including breast, colon, ovarian, and prostate cancer. Recently, we have demonstrated that the EGP-2 promoter retained its activity and specificity in an adenoviral context. Using this promoter to drive the expression of replication essential genes in a trans-complementing system can provide a novel therapy for broad range of tumour types.

**Figure 3.** Infection with two complementing viruses results in selective replication in tyrosinase positive cells only. Tyrosinase positive (melanoma) cells or tyrosinase-negative (glioma) cells were infected with AdTyrpTPΔE1 (A) alone or in combination with AdTyrE1ΔpTP (B), or with wild-type adenovirus at the indicated pfu/cell ratio. Remaining cells were fixated and stained with gentian violet.
In addition, the combination of two different tumour specific promoters might even further increase the specificity of replication. For example, it is feasible to combine the controlled expression of replication-essential genes using either the tyrosinase promoter or the EGP-2 promoter with a virus containing the telomerase promoter driving the expression of another replication-essential gene. The telomerase promoter is known to be active in over 85% of all tumours. Ultimately, two tumour specific promoters can be incorporated into one viral genome to drive the expression of the two replication-essential genes. Such constructed double-controlled conditionally replicating adenoviruses will greatly enhance the specificity of adenoviral replication towards the tumour tissue.

**Conclusion**

The trans-complementing approach used in this study demonstrated that replication could be restricted to melanoma cells. In addition to the treatment of melanoma, the above described examples illustrate that by using different promoters, the trans-complementing system can be utilized for the treatment of a wide variety of cancer types.
References


