



# Adenoviral delivery of DNA plasmid for RNA silencing: pros and cons

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In the *Annals of Translational Medicine*, the paper titled “Oncolytic adenovirus-mediated dual knockdown of survivin and OCT4 improves therapeutic efficacy in esophageal cancer” (1) describes a gene therapy approach for treating esophageal cancer using an oncolytic adenovirus to deliver silencing short hairpin RNA (shRNA) targeting survivin and OCT4 genes. The study found that this approach led to a significant reduction in the expression of both genes and resulted in enhanced cancer cell death *in vitro* and tumor growth inhibition *in vivo* with direct intra-tumoral injection.

The authors used a dual-promoter adenovirus vector which allowed for the efficient delivery of two different shRNAs simultaneously to the cancer cells. The study provides a promising therapeutic strategy for esophageal cancer and highlights the potential of using oncolytic adenoviruses to deliver dual gene knockdown as a cancer treatment.

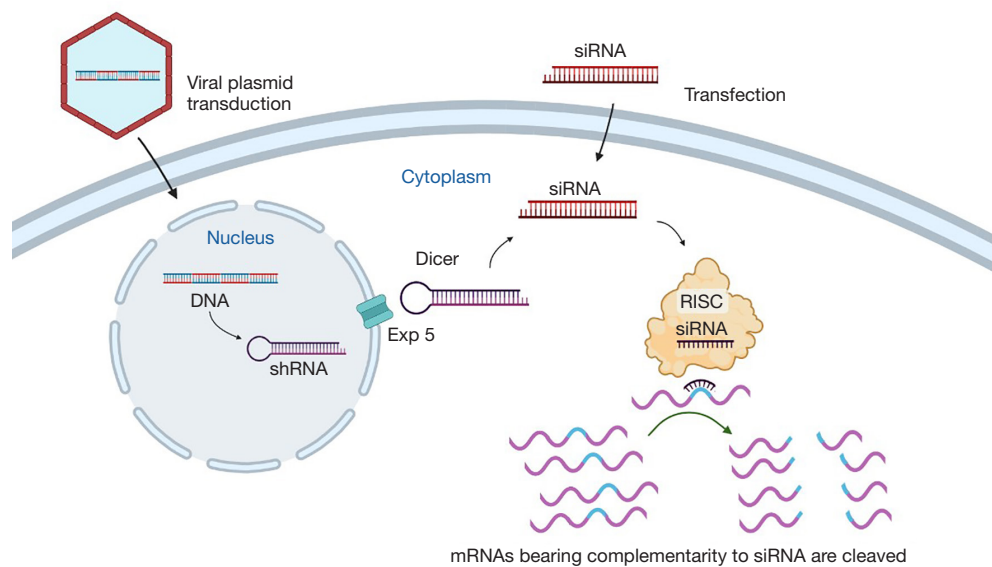
There are two main strategies for use in gene silencing, vector-based shRNAs and double-stranded small interfering RNAs (siRNAs). They have different mechanisms of action for protein knockdown. The pathway for shRNA is more complex. The adenovirus-DNA plasmids enter the nucleus. After that, shRNAs are produced and then exported to cytoplasm via exportin-5 after it is processed

by Drosha. Dicer removes the loop sequence of shRNA in the cytoplasm. Following this, the next steps are the same as for siRNAs. The siRNA associates with RNA-induced silencing complex (RISC) uses the siRNA as a template for recognizing complementary mRNA. It binds to the mRNA and cleaves it to silence its expression (*Figure 1*) (2,3).

The double-stranded siRNAs and vector-based shRNAs are two fundamental methods to RNA interfering. While both shRNAs and siRNAs can be used to inhibit protein expression, their methods of action differ. For shRNA, the pathway is more intricate. Plasmids with Adenovirus DNA reach the nucleus. After being expressed in the nucleus, shRNAs are processed by Drosha and transported to the cytoplasm by Exportin-5, where they interact with Dicer and the loop sequence is removed. They continue after this in a manner similar to that of siRNAs (introduced into cells as short duplexes and identified by Dicer). A multiprotein complex is known as the RISC. RISC recognizes complementary mRNA by using siRNA or miRNA as a template. Following the removal of one of the RNA strands by RISC, they target mRNAs with a complementary sequence, resulting in their degradation (*Figure 1*) (2,3).

Oncolytic adenoviruses are viruses that have been modified to selectively infect and kill cancer cells. They are designed to replicate within cancer cells, leading to their

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**Figure 1** The mechanisms of silencing for siRNA and shRNA. The figure is prepared by <https://www.biorender.com> under license. siRNA, small interfering RNA; shRNA, short hairpin RNA; RISC, RNA-induced silencing complex.

destruction. The safety of oncolytic adenoviruses is a major concern because of the potential for the virus to cause harm to normal cells. While the virus is generally safe and well-tolerated, there have been reports of serious side effects, including immune responses, and cytotoxicity, as well as cancer (4).

An alternative strategy to silence these genes is nanoparticle delivery. Nanoparticles are small particles (usually less than 100 nm in diameter) that can be engineered to deliver drugs or other therapeutic agents directly to cancer cells. They are generally considered safe because they are biocompatible, biodegradable, and can be eliminated from the body relatively quickly. However, there have been some concerns about the potential toxicity of certain types of nanoparticles, particularly if they accumulate in certain organs or tissues (5-7).

Another important consideration is the incorporation of the therapeutic agent into the patient's genome. While RNA delivered via nanoparticles does not integrate into the genome, DNA plasmids for shRNA as used in this paper can, and this can be a potential safety issue. If the virus integrates into the patient's DNA, it can function for long periods and could potentially cause mutations and lead to cancer or other diseases (8,9).

There are many challenges regarding using shRNA or siRNA. One disadvantage of siRNA delivery is that the siRNA concentration becomes diluted as cells divide. Also

due to high concentration of siRNA in cytoplasm, there is risk of off-target effects. There are many drawbacks besides the advantages of shRNA. Using shRNA may generate stable knockdown cell lines which is a danger. Stable shRNA production is time-consuming, since it may take months to prepare (10).

In addition, shRNA therapies are based on DNA plasmids. The shRNA is produced by DNA. But unlike RNA, DNA can be incorporated into the genome and function for a long time. RNA is quickly degraded, but not DNA. This raises safety concerns for the DNA approach (11-13).

ShRNA is often expressed abundantly. Cells cannot process it accurately. Overexpression can occur in loss of target-specificity. ShRNA treatment has the additional drawback of being unsuitable for certain cell types (10).

Overall, both nanoparticle and oncolytic adenovirus therapies have potential benefits and drawbacks, and safety is a critical consideration for both. Nanoparticles are generally considered safe and have a short duration of expression, while oncolytic adenoviruses have a longer duration of expression and also carry potential safety concerns, such as the risk of integration into the genome.

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