Intercellular spread of the transgene product to improve the efficiency of cancer gene therapy

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General Introduction
Cancer Gene Therapy

Cancer is still one of the major causes of death in the western world, despite the progress that has been made in the treatment of this disease. Because many patients are not cured, and because the current treatment options are invasive and have severe side effects, there is a need to develop new types of therapy for cancer. Gene therapy is a promising new type of treatment for many diseases, including cancer. In gene therapy, genetic material is introduced into cells. By introduction of this genetic material (DNA or RNA), a therapeutic effect may be achieved.

The therapeutic effect can be achieved in a number of ways. For monogenetic diseases, (re)introducing a gene that has been lost or damaged is usually the goal. For other diseases, like cancer, a number of different approaches can be applied. The possible strategies include introducing a regulatory gene such as protein involved in cell cycle arrest like p53, a dominant negative variant of a protein involved in cell proliferation, for example Ras of Raf, or an (artificial) transcription factor, oncolytic viruses, genetic vaccination and suicide gene therapy.

Suicide Gene Therapy

In suicide gene therapy, a gene is introduced into the cancer cells, which encodes for a protein that is able to kill the cell. For the purpose of suicide gene therapy, many transgenes have been investigated. Although apoptosis inducing proteins like p53 have been studied as suicide genes, the best anti-tumor effects were obtained when using enzymes that can activate a prodrug into a toxic drug. The most ubiquitously used gene is the thymidine kinase (TK) gene, derived form the Herpes Simplex virus. TK has been used in numerous pre-clinical and clinical studies. The enzyme produced from the TK gene is able to activate a prodrug (ganciclovir, GCV), routinely used to fight herpes infections. The activated drug kills cancer cells by being incorporated into the new strand during DNA replication and halting the process, causing the cell to go into apoptosis. Pre-clinical studies employing TK in animal models of cancer were very convincing. The enzyme was able to eradicate tumors in mice very efficiently because the effect was enhanced by the so-called ‘bystander effect’, in which the activated drug migrates to neighboring cells through gap junctions. This migration results in cell death of the neighboring cells, in addition to the transduced cells. Clinical studies however, have until now shown modest efficacy of TK gene therapy in cancer patients, due to limitations inherent to current gene therapy protocols.
Current Limitations of Cancer Gene Therapy

In many cases, the outcome of gene therapy is dependent on the number of cells that receive the new genetic material. This is even more important in the case of cancer gene therapy where, if not all tumor cells are killed, the disease will re-manifest itself at a later time. To treat cancer with gene therapy, it is therefore paramount to reach all cancer cells with the effect of the therapy. Only a small percentage of the tumor cells will receive and express the transgene, even when a gene therapy vector is injected directly into a tumor, and the affected cells will almost exclusively be located near the needle tract\textsuperscript{10}. When administered systemically, only a small fraction of the vector ends up at the target site\textsuperscript{11}. This problem is caused by shortcomings of the vectors used for gene delivery, in addition to many physiological barriers that are present in the human body and within the tumor mass. For example; Adenovirus type 5 (Ad5) is the most efficient gene therapy vector in current gene therapy protocols. If a vector based on Ad5 is injected intravenously, most of the virus will be taken up in the liver, and only a very small fraction will be able to reach the tumor site\textsuperscript{12}. An additional drawback of this vector is the harmful effect it may have on the healthy liver cells, and other healthy tissues, either by infection of healthy cells, or by causing an inflammatory reaction.

Possible Solutions to the Limitations of Cancer Gene Therapy

The above described problems of efficiency and selectivity of gene transfer need to be solved before any truly effective cancer gene therapy protocols can be designed. Many solutions have been designed for the problem of specificity of gene therapy. This thesis will mainly deal with the lack of efficiency. There are a number of approaches to increase the efficiency of gene transfer. This increased efficiency could be achieved by the design of more competent vectors, that are able to transfer genes to a larger number and more types of cells\textsuperscript{13,14}, or by using replicating viruses\textsuperscript{3} that multiply locally and thereby increase the effective concentration of vector at the target location. Viruses with an increased competency however, will still be limited by the physiological and immunological barriers, and replicating vectors will raise safety concerns.

Transmission to Increase the Efficiency of Cancer Gene Therapy

A different approach to increasing the efficiency of gene therapy is transmission. Transmission is the spread of the produced protein from the infected cell to the surrounding cells\textsuperscript{15,16}. The critical steps in transmission are production of the
therapeutic protein, secretion, uptake and endosomal escape. These steps are described in this thesis and illustrated in fig. 1.

In some cases, where the protein is able to have its therapeutic effect outside of the cell, secretion of the protein alone may be enough to affect the surrounding cells. However, many therapeutic proteins, such as TK, have to be present in either the cytoplasm or nucleus of the cell to be active. Therefore, not only does the protein need to be secreted from the producing cell, it also has to translocate into the target cell and end up inside the cell intact and functional.

Fig 1. Schematical overview of critical steps in the process of transmission.
1. Gene transfer by the gene therapy vector
2. Transcription and translation to produce the protein
3. Secretion of the protein (Chapter 3 of this thesis)
4. Possible direct internalization of the transgene product, bypassing conventional endocytosis, by fusion to protein transduction domain \(^{17,18}\) (Chapter 2 of this thesis).
5. Internalization of the protein (Chapter 4 & 5 of this thesis)
6. Escape from the lysosomal pathway
7. Effect of the protein (Chapter 6 of this Thesis)
Imaging of Gene Therapy

To determine the effectivity of gene therapy and evaluate new strategies, research into gene expression after the vector is administered to patients is required. Many reporter systems for gene therapy have been developed, but most have serious drawbacks. Methods are needed to quantify and localize gene expression in a non-invasive way and to allow for easy, repeated and patient friendly imaging. Especially the use of non-human reporter genes like Green Fluorescent Protein\textsuperscript{19}, luciferase\textsuperscript{20} and TK\textsuperscript{21} may cause immune reactions and undesired effects in patients. Non-linear tracer uptake, as in the case of TK, can also hamper the use of an imaging technique in human gene therapy\textsuperscript{22}. There is a need for improved systems using human reporter genes and state of the art quantifiable imaging techniques such as Positron Emission Tomography (PET).

Thesis Overview

The aim of this thesis was to improve the effectivity of cancer gene therapy by transmission. To this end we constructed a secreted and internalizing suicide enzyme. We have selected TK as a model suicide gene, because it has been studied extensively, and has already been used with limited success in clinical gene therapy protocols. In addition we investigated a novel reporter gene-tracer combination for non-invasive imaging of gene expression after gene therapy. Certain proteins have been identified that contain sequences, termed protein transduction domains (PTDs) that are internalized into cells, without the use of a specific transporter. The utility of these PTDs for gene therapy is described in chapter 2 of this thesis.

We constructed a secreted form of HSV TK and demonstrate in chapter 3 that it is possible to build a TK protein that is secreted after gene transfer by Ad5. We further show that secretion has a detrimental effect on the enzymatic activity of the protein.

In addition, we studied two PTDs for their usefulness in gene therapy. We investigated thymidine kinase (TK) fusion proteins including the HIV TAT and HSV VP22 PTDs, by comparing the intercellular trafficking and efficiency of oncolysis of these fusion proteins to the original protein. From the results of this research we conclude that these PTDs are not suitable candidates to establish transmission of TK. These results and conclusions are detailed in chapter 4 and 5.

Finally, in chapter 6 we show in an animal model that an improved system for imaging of gene therapy can be developed using state of the art imaging techniques and a human reporter gene. Better imaging systems for gene transfer will result in more insight in the factors that influence gene transfer and gene expression, and increase the controllability of clinical gene therapy protocols.
Chapter 1

Reference List


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