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Kidney regeneration in vivo

Gene based therapies for kidney regeneration

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ABSTRACT

In this review we provide an overview of the expanding molecular toolbox that is available for gene based therapies and how these therapies can be used for a large variety of kidney diseases. Gene based therapies range from restoring gene function in genetic kidney diseases to steering complex molecular pathways in chronic kidney disorders, and can provide a treatment or cure for diseases that otherwise may not be targeted. This approach involves the delivery of recombinant DNA sequences harboring therapeutic genes to improve cell function and thereby promote kidney regeneration. Depending on the therapy, the recombinant DNA will express a gene that directly plays a role in the function of the cell (gene addition), that regulates the expression of an endogenous gene (gene regulation), or that even changes the DNA sequence of endogenous genes (gene editing). Some interventions involve permanent changes in the genome whereas others are only temporary and leave no trace. Efficient and safe delivery are important steps for all gene based therapies and also depend on the mode of action of the therapeutic gene. Here we provide examples on how the different methods can be used to treat various diseases, which technologies are now emerging (such as gene repair through CRISPR/Cas9) and what the opportunities, perspectives, potential and the limitations of these therapies are for the treatment of kidney diseases.

1. Introduction

All the cells in our body fulfill a particular function, express numerous genes and respond in a different way to their environment. In a healthy situation, cells will adequately react to changes in oxygen, temperature, pH, metabolites, hormones, cytokines, pressure and more. However, in case of a genetic defect or in a chronic kidney disease, some of these pathways will be affected and can result in loss of cell function or cell death. When the genes or molecular pathways in these processes are known, gene based therapies can be used to target the defective pathway on a RNA, DNA or epigenetic level to restore cell function. With gene based therapies we refer to all therapies in which recombinant DNA is delivered to a cell to produce a therapeutic protein or RNA sequence. In this way, gene based therapies can be used to supplement a missing gene, inhibit a gene from being translated into a protein, change splicing of a specific gene, permanently repair or even delete a genetic sequence.

The hallmark of genetic therapies is that it requires knowledge of the mechanism underlying the disease. For genetic kidney diseases the most important step is identification of the affected gene, which is greatly facilitated by the availability of fast and cost effective whole genome sequencing techniques. The greater our knowledge on disease mechanisms, the more pathologies will become realistic targets for gene therapy. Currently, clinical trials are being conducted using gene based therapies in a wide variety of diseases which can be categorized in four main groups: monogenetic diseases, infectious diseases, cardiovascular diseases and cancer www.abedia.com/wiley and (Ginn et al., 2013). Anticancer therapies (Sterman et al., 2016) represent the biggest group and here gene therapy is used to either directly damage the cancer cells, empower the immune system to induce a specific immune response against the tumor, or to protect sensitive tissues from high doses of chemotherapy (Salem et al., 2015). Monogenetic diseases are the second biggest disease category targeted by gene therapy. Here, cDNA of the affected gene is transiently or stably introduced into cells to restore cell function and halt disease progression. A new tool that has recently become available and may prove very valuable for the treatment of both dominant and recessive genetic diseases is nuclease induced gene repair. To battle infectious diseases and to reduce chronic inflammation in cardiovascular diseases, recombinant therapeutic proteins are

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produced by liver or muscle cells and released in the bloodstream of patients.

In nephrology, therapies may be directed to a defect that directly affects the kidney cells, to target the production of toxic metabolites produced by other cells, or to ameliorate a defect in the immune system leading to chronic kidney inflammation. Some of these approaches have already shown to be effective in preclinical studies of kidney diseases. For other applications a proof-of-principle study in another disease will pave the way to new therapies that could also be applied to the kidneys. Here we will discuss the various different ways in which gene therapy can be used to target a disease and how delivery methods play an important role in the effectivity and specificity of a treatment.

2. The molecular toolbox of gene therapy

The proteins or RNA sequences required for gene therapy are delivered to the cell through recombinant DNA sequences that represent a functional gene-expressing unit, including a promotor and the gene that should be expressed (Fig. 1). When introduced into a cell and transported to the nucleus this DNA sequence is recognized as a gene and, depending on the promotor incorporated in the recombinant DNA, it is transcribed. The promotor sequence functions as an on/off switch and if the application requires the recombinant gene to be activated in all cells, a constitutively active promotor can be used. However, in some cases the recombinant DNA should only be transcribed in a subset of cells or only under a specific condition. This principle is also commonly used in animal models in which a transgene only becomes active in one particular tissue or cell type after a specific signal, such as tamoxifen induced Cre expression (Ly et al., 2011). Specific promotor sequences exist for the proximal tubules, cortical tubules, and podocytes, which makes it possible to express the recombinant gene only in a specific part of the kidney. The recombinant DNA can be delivered to the cell for temporary gene expression, or stably integrated in the genome of the cell. This depends on the therapy and the delivery method (see also Section 3). The routes of gene therapy delivery are normally intravenously, intramuscularly, intra-ocular or *ex-vivo*. For *ex-vivo* therapy blood cells or stem cells from the patient are manipulated outside of the body and transplanted back into the patients. In this way only a specific cell type is targeted and quality checks can be performed before cells are placed back. Here we provide an overview on how gene based therapies can be used to express a recombinant protein in the cell (Section 2.1), to permanently modify the genome of a cell (Section 2.2), or to regulate gene expression (Section 2.3) (Fig. 2).

2.1. Gene addition

Inducing the expression of a recombinant protein can be done to compensate for a genetic defect (Fig. 2E) or to trigger a pathway that will ameliorate disease development (Fig. 2F). Some therapies, like enzyme replacement therapies, insulin injections and immunoglobulin therapy, depend on regular intravenous or subcutaneous injection of recombinant proteins produced by pharmaceutical companies. These diseases are currently candidates for

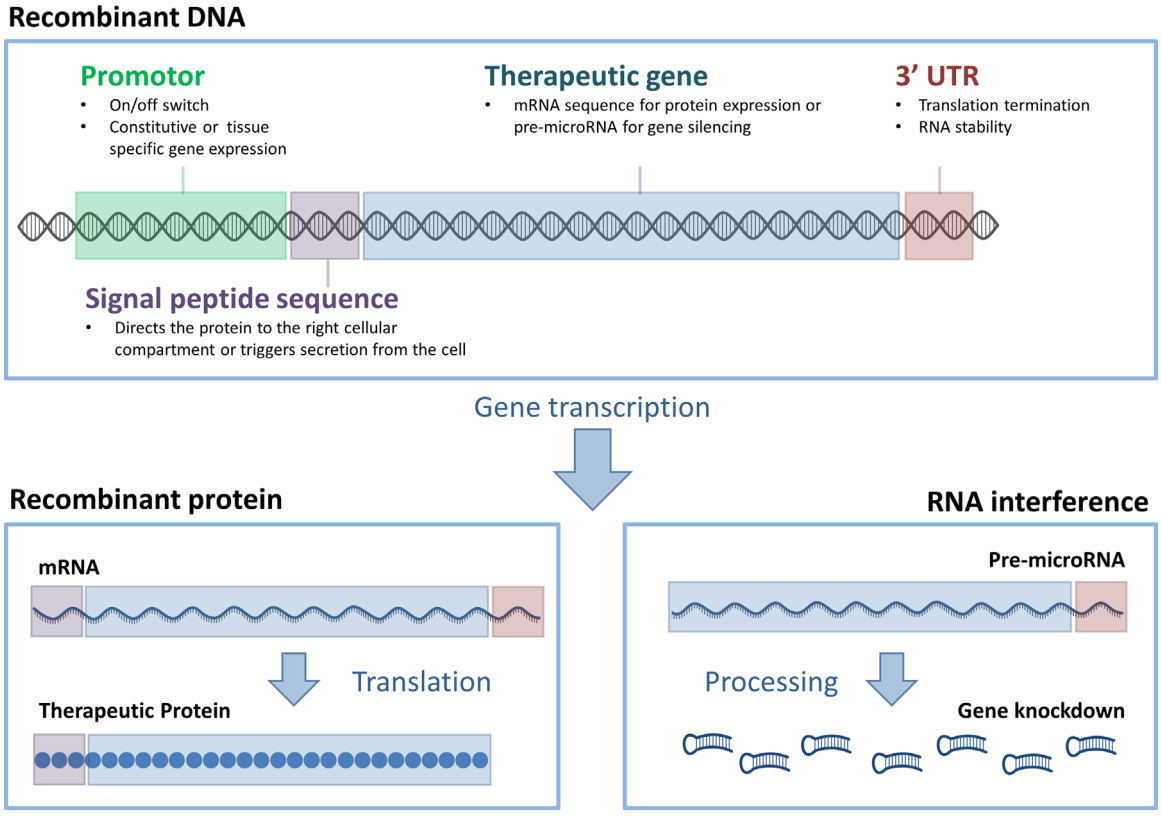

Fig. 1. Expression of recombinant DNA. Recombinant DNA resembles an endogenous gene and will be recognized as such by the cell resulting in the expression of a recombinant protein or the production of pre-microRNA. The promotor sequence will be bound by available transcription factors in the cell and the transcription machinery will produce the mRNA or pre-microRNA. After transcription termination the RNA sequences will be capped with a poly-A tail. The mRNA will be transported from the nucleus to the cytoplasm for translation. Depending on the presence of a signal peptide the protein will stay in the cytoplasm or be directed to the Golgi where the protein will be either transported to specific cell organelles or be secreted from the cell. The pre-microRNA will be processed in the nucleus into short hairpins which are transported to the cytoplasm where they will be substrate for the RNA interference machinery. The sequence of the RNA hairpins will be used as a template for the destruction of specific mRNA molecules (target genes) and in this way prevent their translation into protein (gene knockdown).