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Nucleic Acids In Gene Delivery and Gene Regulation

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ABSTRACT

The concept of gene therapy, initially attributed to a technology that would allow the correction of inherited genetic disease, has evolved over the years. The realization of the immense technical hurdles to achieve genetic correction led to a broadening of the concept now including the transfer of genetic material whose expression will counteract, mitigate or revert a disease phenotype. This capacity to mitigate or revert the disease phenotype has also been achieved by gene expression regulation, at the level of the DNA or RNA, by the use of antisense or anti-gene technologies.

Therefore, at present, gene therapy encompasses not only the introduction and expression of therapeutic genes but also the regulation of gene expression itself which, when mediated by short oligonucleotides, gave rise to the concept of oligonucleotide-mediated gene therapy.

The evolution of gene therapy has been in straight connection with developments on gene delivery mechanisms and new nucleic-acid based chemistries, which have allowed progress in oligonucleotide based antisense / anti-gene methods.

Gene delivery mechanisms have relied mostly on viral-vehicles due to their known ability to carry and efficiently deliver their own genetic information to cells, a capacity perfected over the period of millions of years. However, production complexity and safety concerns have turned the attention to the possibility to use synthetically derived vehicles to achieve the same goal. These non-viral gene delivery methods, although regarded as safe, have not yet reached the efficiency of viral methods.

Increasing efficiency of non-viral vectors is a process involving mechanisms for protection and stabilization of the nucleic acid cargo (DNA, RNA, oligonucleotides) in extracellular biological fluids, as well as the intracellular release of the cargo. The cell itself presents several barriers for nucleic-acids cargo delivery such as the cellular membrane, endocytic vesicle release and the nuclear envelope.

In this thesis, in paper I, is presented a novel way to deal with nuclear membrane translocation when the nucleic-acid cargo needs to access the nuclear interior to exert its action. The developed method uses the cells own nuclear import machinery through the use of a synthetic nucleic-acid that acts itself as the nuclear localization signal. In paper II, we tackle the barriers formed by the cell membrane and endosomal vesicle release. In this paper a new type of cell-penetrating-peptide was developed for delivery of a splice-correction oligonucletide (single-stranded oligonucleotide). The CPP efficiently formed complexes with the oligonucleotide through non-covalent interactions, and these complexes were shown to have the capacity to efficiently be taken up by cells and be released from endocytic vesicles thus delivering the oligo to the intracellular environment.

We then turn to oligonucleotide-mediated gene regulation. In paper III we explore the use of oligonucleotides to correct an aberrant splicing of the *BTK* gene leading to loss of BTK protein production. The lack of BTK correlates with the absence of circulating B-cells thus causing the disease X-linked agammaglobulinemia. In paper IV we explore a new LNA (locked nucleic acid)-based oligonucleotide for double stranded DNA targeting and duplex invasion in order to develop a new tool for the anti-gene field.

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