Adenoviral vectors as genome editing tools : repairing defective DMD alleles

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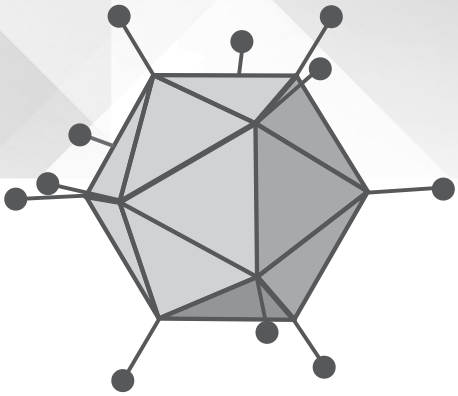

General introduction

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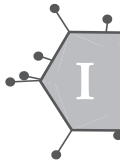
Achieving efficient, specific and accurate modifications of the genome of living cells represents the *holy grail* of genome editing. Fulfilling this goal can have an enormous impact in a broad range of applications in fundamental and applied research, including gene therapy.

Recently, significant advances in genome editing have been made possible by the development of sequence-specific designer nucleases, also known as programmable nucleases. Among them, the main platforms include zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and, more recently, RNA-guided nucleases (RGNs). When introduced in target cell nuclei, designer nucleases alone or together with donor DNA templates can trigger non-homologous end joining (NHEJ) or the homologous recombination (HR) pathways, respectively. The recruitment of these main DNA repair mechanisms is, ultimately, exploited for achieving targeted and permanent genetic modifications. For instance, gene knock-out, correction, replacement or tagging can be achieved depending on the specific goals and experimental settings. In this regard, **Chapter 1** provides a review of the recent progress in the field of genome editing and outlines the available options for introducing gene editing tools into target cells. Moreover, the specificity and fidelity attainable by deploying DNA-editing procedures in mammalian cells are also reviewed.

As discussed in **Chapter 1**, despite continuing advances, several hurdles limit the applicability of the current DNA editing tools in gene therapy. The work presented in the first part of this thesis contributes to tackling two of the major bottlenecks of genome editing technologies: (i) developing improved methods for delivering the sizable gene-editing tools, in particular RGN complexes, into target cells (**Chapter 2**), and (ii) increasing the specificity and fidelity of the gene-editing procedures (**Chapter 3**). The insights derived from these studies are further expanded in the second part of this thesis by testing adenoviral vectors (AdVs) encoding nucleases for repairing defective *DMD* alleles in muscle cell populations derived from patients with Duchenne muscular dystrophy (DMD) (**Chapter 4** and **Chapter 5**). Notably, loss-of-function mutations within the ~2.4 Mb dystrophin-encoding *DMD* gene constitute the molecular basis of DMD, which is one of the most frequent genetic neuromuscular disorders. Finally, the use of AdVs as *DMD*-editing tools is put in perspective by a review of the current status of viral vector-based strategies aiming at correcting faulty *DMD* reading frames (**Chapter 6**).

Throughout the work presented in this thesis, a key role is played by fiber-modified adenoviral vectors (AdVs). These engineered vectors, derived from members of the *Adenoviridae* family, contain a linear, protein-capped, double-stranded DNA genome packaged in an icosahedral non-enveloped capsid. By



capitalizing on the efficient cell entry mechanisms of their parental viruses, AdVs are frequently used for delivering foreign DNA into target cell nuclei. In the context of genome editing, the value of AdVs is further increased owing to their large cloning capacity and strict episomal nature for transient high-level expression of designer nucleases. Importantly, AdVs are amenable to cell tropism modifications and can transduce dividing and post-mitotic cells. When integrated with genome editing technologies, these features increase AdV potential for targeting and modifying the genome of a broad array of therapeutically relevant cell types, including those with myoregenerative capacity.

In this thesis, the utility of using AdVs as tools for genome editing involving the activation and recruitment of the HR and NHEJ repair pathways is investigated. Importantly, the insights derived from AdV-based gene editing have direct implications for the efficiency and accuracy with which genetic modifications of human cells can be carried out.