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Review

Peptidic tools applied to redirect alternative splicing events

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ABSTRACT

Peptides are versatile and attractive biomolecules that can be applied to modulate genetic mechanisms like alternative splicing. In this process, a single transcript yields different mature RNAs leading to the production of protein isoforms with diverse or even antagonistic functions. During splicing events, errors can be caused either by mutations present in the genome or by defects or imbalances in regulatory protein factors. In any case, defects in alternative splicing have been related to several genetic diseases including muscular dystrophy, Alzheimer's disease and cancer from almost every origin. One of the most effective approaches to redirect alternative splicing events has been to attach cell-penetrating peptides to oligonucleotides that can modulate a single splicing event and restore correct gene expression. Here, we summarize how natural existing and bioengineered peptides have been applied over the last few years to regulate alternative splicing and genetic expression. Under different genetic and cellular backgrounds, peptides have been shown to function as potent vehicles for splice correction, and their therapeutic benefits have reached clinical trials and patenting stages, emphasizing the use of regulatory peptides as an exciting therapeutic tool for the treatment of different genetic diseases.

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Abbreviations: ANP, atrial natriuretic peptide; ASBNP, alternatively spliced BNP; ASO, antisense oligonucleotides; BNP, B-type natriuretic peptide; CBD, calmodulin binding domain; CPP, cell penetrating peptides; DMD, Duchenne muscular dystrophy; ESE, exonic splicing enhancer; ESS, exonic splicing silencer; GFR, glomerular filtration rate; IAS, intronic activators of splicing; ISE, intronic splicing enhancer; ISS, intronic splicing silencer; LMN, lamin; NA, nucleic acids; NLS, nuclear localization signal; ON, oligonucleotides; Pen, penetratin; PID, primary immunodeficiency disease; PMO, phosphorodiamidate morpholino oligos; PNA, peptide nucleic acid; PPMO, peptide-PMO; PTD, protein transduction domains; siRNA, small interfering RNA; snRNA, small nuclear ribonucleic acid; snRNP, small nuclear ribonucloprotein particles; SCO, Splice-oligonucleotides; SR, serine–arginine rich domains; SSO, splice-switching oligonucleotides; TP, transportan.

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Introduction

Defects in splicing play a significant role in several diseases and genetic disorders [73,106]. Due to the importance of this mechanism on regulating gene expression, splicing is consequently an emerging therapeutic target. One of the first attempts to regulate splicing was the development of synthetic oligonucleotides (ON) designed to target specific genes in order to regulate alternative splicing events [1,18]. The main limitation of the use of many types of synthetic ON and their analogs as therapeutic antisense agents has been their poor cellular delivery. In order to achieve successful ON transfection, efficient delivery vectors are generally necessary. Many types of such vectors have been designed to aid ON delivery both for cell culture and *in vivo* [41,70]. Amongst such strategies, conjugation to cell penetrating peptides (CPP) has received attention recently [58,78]. At present, multiple drugs that rely on the antisense splicing redirection principle involving the use of CPPs as carriers are in advanced phases of clinical trials [80,108,110]. Characteristically, CPPs are short, cationic peptides that often have amphipathic properties and share structural features with antimicrobial peptides [47]. These CPPs, developed as vectors for cargo delivery into eukaryotic cells, have been extensively applied as delivery agents for splicing regulatory molecules [11,31]. According to their nature, some CPPs exhibit antimicrobial action toward bacteria, parasites, and fungi *in vitro* besides the effect on splicing regulation. Such antimicrobial activity is observed at higher concentrations than the one required for translocation across eukaryotic membranes [46]. In this review, we will discuss not only the evidence related to CPPs, but we will also present other efforts that target splicing regulation by the use of natural and synthetic molecules, inspired in nature-existing peptides.

Alternative splicing mechanism

Pre-mRNA splicing is a nuclear process that occurs in all eukaryotes whereby the intervening sequences in nascent transcripts, called introns, are removed while the flanking coding sequences are joined together to produce a mature mRNA molecule that is subsequently exported to the cytoplasm where it serves as a template used in protein translation. The splicing mechanism is catalysed by a large ribonucleoprotein complex called spliceosome [102]. The core components of the spliceosome include four ribonucleoprotein particles (snRNP): U1, U2, U4/U6 and U5. Each particle contains the correspondent RNA molecule (snRNA) and a set of specific and common proteins [111]. The spliceosome complex also contains multiple auxiliary factors that are essential for complex assembly and splicing catalysis [54]. These regulatory factors include the SR family of proteins, which are commonly known as splicing facilitators [64] and the heterogeneous group of hnRNP proteins [72]. The assembly of the splicing factors on the pre-mRNA conforms the active spliceosome that would be ready for catalysis [74]. RNA splicing depends on the proper recognition of exons, which is a challenge, given that the average size for a human exon is 100–300 nucleotides. Exon recognition is guided in part by conserved sequence elements at exon–intron boundaries, named 5′ and 3′ splice sites, but it also depends on sequence elements within exons and on intronic elements distinct from the splice sites, which have been divided into four functional categories: exonic splicing enhancers (ESEs), exonic splicing silencers (ESSs), intronic splicing enhancers (ISEs) also known as intronic activators of splicing (IASs) and intronic splicing silencers (ISSs). These elements and the factors that recognize them are employed for the definition of introns and exons (Fig. 1A). This combinatorial control regulates splicing decisions [40]. Alternative splicing expands the coding capacity of the human genome given the ability to generate diverse protein isoforms from a single gene. In many cases, the specific function of each isoform is not yet understood. There are several types of alternative splicing (Fig. 1B). Inclusion or skipping of a complete exon is the most common case of alternative splicing. In some cases, a whole intron is removed or retained to generate two different isoforms. A portion of an exon can also be present or absent in the final isoform. Additionally, different transcription start sites or polyadenylation signals could be used according to the spliced sequences. A combination of more than one alternative splicing event can occur in the same gene [53]. According to the information obtained from the Human Genome Project, approximately 75% of the human genes undergo some type of splicing [61]. Data from the Human Gene Mutation Database suggest that near 10% of the mutations annotated impinge on splice sites [103]. However, these percentage underestimates the number of mutations that affect splicing given that they do not consider the intronic and exonic regulatory elements described before. In addition, mutations associated with disruption of recognition sites for regulatory factors can result in global splicing defects, which can generate very different phenotypes. With all these considerations, it is reasonable to suggest that a large fraction of all human mutations affect splicing. Therapeutic tools have been developed to correct some of these anomalous splicing events. Some examples of the developments involving peptidic molecules are presented here.

Small molecules designed to specifically modulate splicing events.

The development of strategies to study the mechanisms that regulate gene expression has focused on modifying or modulating this process at the post-transcriptional level using nucleic acids (NA) or nucleic acids analogs such as short oligonucleotides (ONs), designed either to silence or to enhance gene expression. Nucleic acids analogs could be single stranded antisense oligonucleotides (ASOs) designed as a complementary molecule that targets a specific mRNA blocking gene expression both *in vitro* and *in vivo*, through the inhibition of translation or by enzymatic cleavage of the target mRNA. Moreover, there are double stranded RNAs such as short or small interfering RNAs (siRNAs) used to hydrolyze mRNA and to avoid the translation of a specific messenger into protein. These oligonucleotides could be modified to improve their properties as modulators of gene expression conffering an increased resistance to nucleases [29], the most common modifications are summarized in Fig. 2A [24]. Interestingly, some of these have been successfully tested on the splicing process. A modified NA analog is a non-RNase-H inducing 2′-*O*-methyl oligonucleotide (2′-OMe), useful to conserve the target RNA intact in splicing redirection [51]. PNA (peptide nucleic acid) molecules, which are neutral oligonucleotide analogs, are considered potential antisense drugs, and could be excellent candidates for gene therapy in AIDS patients [85] or in redirecting splicing [25]. Morpholino oligomers or phosphorodiamidate morpholino oligos (PMOs) are antisense nucleic acid analogs frequently used to modulate pre-mRNA splicing by originating a steric antisense blockage without activating RNase-H. PMOs have proven to be effective agents inside cells [110] and have been effective inducing exon-skipping in Duchenne muscular dystrophy (DMD) patients [20]. Some of the applications of ASOs are further discussed.

Anomalous splicing events corrrected using ASOs

It has been mentioned that pre-mRNA splicing is a tightly coordinated process that can be disrupted by ONs in a highly specific manner to suppress aberrant splicing, redirect exon recognition or intron removal and it can also bypass nonsense or frame-shifting mutations to alter isoform ratios [1]. ASOs can bind to target sequences in mRNA by standard nucleic acid base-paring. In other cases, AMOs can bind to a target site and blocking the interaction