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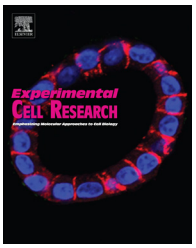

Review Article

Antisense-mediated exon skipping: Taking advantage of a trick from Mother Nature to treat rare genetic diseases

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ABSTRACT

Rare diseases can be caused by genetic mutations that disrupt normal pre-mRNA splicing. Antisense oligonucleotide treatment to the splicing thus has therapeutic potential for many rare diseases. In this review we will focus on the state of the art on exon skipping using antisense oligonucleotides as a potential therapy for rare genetic diseases, outlining how this versatile approach can be exploited to correct for different mutations.

Contents

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Introduction

Splicing is regulated by complex machinery that recognizes exons and removes introns from the pre-mRNA transcript [44]. Mutations interfering with proper splicing of the pre-mRNA underlie many genetic diseases. Antisense oligonucleotides (AON), small pieces of modified DNA or RNA that specifically hybridize to specific regions in the pre-mRNA are in development as potential treatment for many rare diseases. AON-mediated splicing manipulation is most advanced for two neuromuscular diseases, Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA) [10,40]. In this review we will provide an update for DMD and SMA, but will also focus on the current state of the art for AON-mediated splicing manipulation for other rare diseases to underline that AONs have broader therapeutic potential [17,40]. Due to space constraints we were unable to provide a comprehensive overview, but selected a few notable examples for the different ways exon skipping can be exploited.

Exon skipping to restore the open reading frame

Duchene muscular dystrophy

Duchenne muscular dystrophy (DMD) is a muscle wasting disease, caused by frame-shifting mutations in the *DMD*-gene that completely abolish the production of the dystrophin protein. The disease leads to severe disability and premature death [1]. The allelic disease Becker muscular dystrophy (BMD) is caused by in-frame mutations that allow production of internally deleted, partly functional dystrophins. Based on the fact that generally BMD patients have later disease onset, and slower disease progression [42], it was proposed that restoring the open reading frame for DMD patients might result in amelioration of DMD pathology.

Reading frame restoration can be achieved with AONs that can induce the skipping of an additional exon, to reframe the dystrophin transcript allowing the production of a BMD-like dystrophin. Proof of concept for this approach has been obtained in vitro in patient-derived cell cultures and in vivo in animal models and is currently in the clinical trial phase [1,4,25,26,15]. The focus is primarily on exon 51 skipping, since this would apply to the largest group of patients [2]. Two different AON chemistries are in clinical development: 2′-*O*-methyl phosphorothioate (2OMePS, drisapersen (GSK, Prosensa)) and phosphorodiamidate morpholino oligomers (PMO, eteplirsen (Sarepta)). For both chemistries, exon 51 skipping and dystrophin restoration was observed after intramuscular delivery of AONs in patients with amenable deletions [43,24]. Since muscle makes up about 35–40% of total body mass and is found all over the body [25] systemic delivery is a prerequisite.

In a dose escalation trial (0.5–20 mg/kg), intravenous delivery of eteplirsen for 12 weeks resulted in increased dystrophin levels in 7 of 19 treated patients [7]. In a follow up trail of 24 weeks the tested dosage was increased (30 and 50 mg/kg) and a placebo group was included ($n=4$ in all groups) [28]. Eteplirsen was well tolerated and no serious adverse events were reported. After 24 weeks, the percentage of dystrophin-positive fibers was modestly increased and the distance walked in 6 min (6MWD) was comparable between placebo and AON treated patients [28]. In an open-label extension study, placebo patients were dispersed over eteplirsen groups and all patients were treated for 24 more weeks. AON treatment significantly increased the percentage of dystrophin-positive fibers, also in the patients initially treated with placebo, albeit at a lower level. Patients have now been treated for up to 92 weeks and aside from 2 treated patients who lost ambulation early in the trial, the 6MWD appears to have stabilized after treatment. Sarepta hoped to obtain accelerated approval based on this trial, but the Food and Drug Administration (FDA, US) recently ruled that an additional, larger trial is required to confirm the results.

In a phase I/II dose-escalation trial (0.5–6 mg/kg) of 4 weeks, drisapersen was delivered subcutaneously and 10 out of 12 treated patients showed a dose-dependent increase in dystrophin [14]. Following this trial all 12 patients were enrolled in an open label extension study, where they were treated weekly with 6 mg/kg for 72 weeks, followed by 116 weeks of intermittent dosage. For 8 of 10 ambulant patients the 6MWD stabilized for the duration of the extension trial. Two phase II trials have been conducted as well in early disease stage patients, showing that patients treated with weekly 6 mg/kg doses for 24 or 48 weeks outperformed placebo-treated patients. Adverse events were reported, most notably injection site reactions and transient proteinuria for all patients and thrombocytopenia for some patients (for more details see (http://www.gsk-clinicalstudyregister.com/compounds/drisapersen#ps). Finally, a phase III trial (NCT01254019) was recently completed, involving 186 patients of varying disease stages, 125 of whom received 6 mg/kg drisapersen for 48 weeks and 61 receiving placebo. It has been reported that the primary endpoint (a significant difference in the 6MWD after 48 weeks of treatment) was not met (see link: http://www.gsk.com/media/press-releases/2013/gsk-and-prosensa-announce-primary-endpoint-not-met-in-phase-iii-.html). Secondary endpoints also did not show differences between drisapersen and placebo treated patients (e.g. 10-m walk/run test, 4-stair climb and North Star Ambulatory Assessment). Further analysis is currently ongoing and anticipated to be completed before the end of the year. Pending this, drisapersen dosing of patients in open label extension trials has been suspended. Meanwhile, trials assessing 2OMePS AONs to induce exon 44, 45 and 53 skipping are ongoing.

Antisense oligonucleotides to block gene expression

Myostatin knockdown

In contrast to the use in DMD, AONs can also be used to purposely reduce or knockdown the expression of a certain gene through skipping an out of frame exon. One such an approach is the use of AONs to knock down the expression of myostatin, a protein that inhibits muscle growth. Thus, through inhibition of the inhibitor, one might be able to increase muscle mass in Duchenne patients, thus increasing the amount of tissue that can generate dystrophin transcripts (i.e. targets for AON-mediated exon skipping). Indeed, animals without functional myostatin have increased muscle mass, but are otherwise healthy [23,21].

Skipping of exon 2 will disrupt the open reading frame, leading to loss of functional protein. In different cell lines both Kang et al.

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