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Review Article

SMN2 Gene Based Treatments In Spinal Muscular Atrophy- A Review Of Recent Advancements

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ABSTRACT

Spinal muscle atrophy (SMA) is a neurodegenerative disease with autosomal recessive inheritance which is pathologically characterized by degeneration of α motor neurons from the anterior horn of the spinal cord resulting in muscle weakness, muscle atrophy involving mainly muscles of the trunk. SMA is commonly divided into types 1 through 4. In humans, SMN genes, (SMN1 and SMN2) are located on chromosome 5 in close proximity to each other. It has been assumed that the SMN2 locus is derived from a (evolutionarily) recent duplication event of a genomic region spanning 500 kb. The role of SMN2 gene as a phenotypic modifier of SMA has been studied in great details recently. Each patient affected with SMA retains at least oneSMN2 copy. From this perspective, it can be said that SMA arises due to the inability of SMN2 gene to fully compensate for the lack of functional SMN protein resulting from the mutation of SMN1. In this review article, we will focus on three of the treatment strategies using SMN2 gene which have been used as treatment strategies by various techniques. These strategies are: i) inducing the expression of SMN2, ii) modulating splicing of SMN2 derived transcript, and iii) stabilizing the full length SMN2 derived mRNA and/or protein.

Key-words: Self-emulsifying drug delivery system, oils, co-solvents, solidification, and surfactant.

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INTRODUCTION-OVERVIEW OF GENETICS AND PATHOLOGY OF SMA

Spinal muscle atrophy (SMA) is a neurodegenerative disease with autosomal recessive inheritance and is a leading cause of infant death globally secondary to a genetic etiology. This condition is pathologically characterized by degeneration of α motor neurons from the anterior horn of the spinal cord resulting in muscle weakness, muscle atrophy involving mainly muscles of the trunk. SMA is commonly divided into types 1 through 4. These types vary in age of onset and intensity of symptoms, but all are due to mutations in the SMN gene on chromosome 5. These types are therefore characterized in part, by a person's age at symptom onset and the highest motor milestone achieved (i.e., sitting, standing, walking).Approximately 60% of SMA patients have type I (1) disease, (2) which presents with severe generalized muscle weakness and hypotonia. The onset of illness is usually at birth or within the first few months of life and respiratory failure leading to death usually by 2 years of age. The Type II form accounts for ~27% of SMA (2) and its clinical presentation is variable, with some children presenting with severe respiratory insufficiency, whereas others having milder respiratory involvement who can retain their mobility with mechanical support. Individuals with type III SMA (3) have the mildest form of the disease. These patients experience benign symptoms like delayed motor milestones, mild muscle weakness and fatigue. The prevalence of SMA is 1 in 11,000 live births with a carrier frequency of 1 in 402. The etiology of SMA is the loss of functional SMN gene either due to the mutation or deletion resulting in lack of survival motor neuron (SMN).

In humans, SMN genes, (SMN1 and SMN2) are located on chromosome 5 in close proximity to each other. It has been assumed that the SMN2 locus is derived from a (evolutionarily) recent duplication event of a genomic region spanning 500 kb. In addition to SMN genes, this region contains additional genes and microsatellite markers. Both of these SMN genes (1 & 2) are comprised of nine exons and eight introns and encode an identical protein product. The molecular pathogenesis of SMA begins when a silent C–T transition in the exon 7 of SMN2 alters a critical exonic splice enhancer resulting in a strong reduction of exon 7 inclusion during splicing. Thus, 85% of the resulting mature mRNA lacks exon 7 (D7). The truncated protein is defective in SMN self-association and is degraded rapidly. More than 95% of all SMA patients have homozygous deletions of SMN1 gene resulting in absence of exons 7 and 8.

SMN1 gene produces full length functional SMN mRNA and protein which is required for normal function of all cell types. Although the copy gene SMN2 has only few nucleotides different than SMN1, the presence of. Its protein is vital for survival. The critical difference between SMN1 and SMN2 is a C to T transition at position 6 of exon7 in SMN2 which produces an alternative splice variant. SMN2 produces only ~10% of the full length functional SMN protein. Almost 90% of the mRNA synthesized by SMN2 gene lacks exon 7. This in turn gets translated into truncated, unstable non-functional protein called SMNΔ7. This protein gets ultimately degraded by the cells.

The lack of functional full-length SMN protein leads to the disease of SMA characterized by synaptopathy followed by the apoptotic death of motor neurons. All SMA patients have at least one copy of SMN2 gene, which produces functional SMN protein although in low levels. This ability to produce the functional SMN protein provides it the ability to act as a phenotypic or disease modifier of SMA. It has been observed that the SMN2 gene copy number and the disease severity of SMA follow an inverse relation (4). Type I patients of SMA usually have 1 or 2 copies of SMN2 gene compared to 2-3 copies in Type II and 3-4 copies of the SMN2 gene in Type III and IV categories. The disease severity decreases from Type 1 through Type 4 showing that more number of SMN2 gene copies protect from the manifestation of the disease. Infact, in individuals with more than 4 copies of the SMN2 gene, the disease does not manifest at all and such individuals are completely asymptomatic.

Presently, there is no effective treatment for SMA and all the strategies only act in a supportive manner. One of the recently developed treatment strategies for SMA has been to target SMN2 gene, which is capable of producing full length functional SMN protein as mentioned above. Thus this strategy can compensate in part for the absence of SMN1 gene. In this review article, we will elaborate these strategies. We will begin by elaborating the phenotypic modification properties of SMN2 gene. Further, we will focus on three of the treatment strategies using SMN2 gene which have been used as treatment strategies by various techniques. These strategies are: i) inducing the

expression of SMN2, ii) modulating splicing of SMN2 derived transcript, and iii) stabilizing the full length SMN2 derived mRNA and/or protein.

SMN2 GENE AS THE PHENOTYPIC MODIFIER OF SPINAL MUSCULAR ATROPHY

Before elaborating the SMN2 gene based treatment strategies in SMA, we will briefly address the role of this gene as a disease-modifier. This role of SMN2 gene as a phenotypic modifier of SMA has been studied in great details recently. Each patient affected with SMA retains at least oneSMN2 copy. From this perspective, it can be said that SMA arises due to the inability of SMN2 gene to fully compensate for the lack of functional SMN protein resulting from the mutation of SMN1. This is especially evident from the fact that even a small amount of full-length transcript generated by SMN2 accounts for a milder type II or III phenotype when the copy number of the SMN2 gene is increased.

The notion that SMN2 gene function and the SMA disease severity are associated, originated from the results of several studies which showed that there is an inverse relationship between the number of copies of SMN2 and clinical severity. This inverse relationship has been especially observed in several SMA mouse model researches. A general finding in these models has been that the mice lacking endogenous mouse SMN gene but expressing two copies of the human SMN2 gene develop severe SMA and die within one week of age. However, mice that express multiple copies of SMN2 do not develop the disease (5, 6, 7).Human researches have confirmed that most cases of SMA harbor homozygous deletions of the SMN1 gene but at the same time retain at least one copy of SMN2(8,9,10, 11,12,13,14,15). A second line of evidence shows that introduction of two copies of the human SMN2 transgene supports viability although these animals do develop motor function defects and an average life span of 4–6 days. Similarly, SMN−/− SMN2 mice have SMA-like phenotypes whose ***severity inversely correlates with the SMN2 copy number*** when the SMN2 transgene is introduced into them (5,16). Also, severe-SMA mice harboring only two SMN2 copies, or with an extra SMNΔ7 cDNA transgene (SMA Δ7 mouse model), develop early and rapidly progressive pathology, dying within 1–2 weeks postnatal (5,17,7,18). In contrast, the SMA phenotype mice harboring four SMN2 copies survive normally and do not develop paralysis, (5). In fact, Monian et al (7) found that 'high-copy' numberSMN2 transgenic mice were phenotypically 'normal'.

In addition to these animal researches, several human studies have also shown that the SMN2 copy number is related to severity of the disease (9, 19,20,21,22).In the normal population, the copy number varies from zero to three copies, with approximately 10%–15% of normal individuals having no SMN2. In the context of SMA patients, the milder types II or III have more copies of SMN2 than type I patients. Whereas the majority of patients with the severe type I form have only one or two copies of SMN2, most of the patients belonging to the type IIhave three SMN2 copies; and most patients with type III have three or four SMN2 copies. In fact, Prior et al (23) observed that three unaffected family members of SMA patients with confirmed SMN1 deletions had five copies of SMN2.

In addition to the copy number, some other modifying factors for theSMN2 gene influence the phenotypic variability of SMA. There are very rare reports of families in which markedly different degrees of disease severity occur in affected siblings with the same SMN2 copy number. This difference in disease severity could be because of the differences in splicing factors. Presence of some of these splicing factors allow more full-length expression from the SMN2 gene thus accounting for some of the variabilityobserved between discordant siblings with same genotypes(24).

THERAPEUTIC IMPLICATIONS OF SMN2 GENE FOR SMA

The SMN2 gene is of special therapeutic interest in SMA for two reasons. *Firstly*, SMN2 gene is universally present. Thus all SMA patients retain one or more copies of SMN2. *Secondly*, SMN2 has the potential to encode fully the functional SMN protein. Thus this copy gene is an invaluable therapeutic target. While early reports suggested that over expression of SMNΔ7 protein functioned in a dominant-negative manner, the generation of the SMNΔ7 mouse subsequently demonstrated that SMNΔ7 was neither toxic nor dominant-negative (17).These animals are homozygous null for murine SMN, possess two human SMN2 genes and contain an additional transgene that over expresses the cDNA for the SMNΔ7 iso-form (17).The addition of the SMNΔ7 cDNA extends survival to

approximately 14 days, as compared with approximately 5 days for the $SMN^{-/-}$; SMN2 SMA mice. Diverse treatment strategies aimed at improving the function of SMN2 have been envisioned. These strategies include, but are not limited to, 1) RNA based modulation of SMN2promotion of exon 7 inclusion in smn2 transcripts 2) SMN2 gene activation: histone deacetylase inhibitors and other drugs 3) SMN protein stabilization related strategies.

1) RNA-BASED MODULATION OF SMN2

RNA-based strategies to modulate SMN2 gene have been almost completely focused on the inclusion of exon 7 in subsequent transcription process. Modulating SMN2 pre-mRNA splicing is a direct approach to restore proper expression of the normal SMN protein. Much of stress in this RNA based modulation has been laid on reprogramming of the splicing of a faulty. SMN2 pre-mRNA splicing is a direct approach to restore proper expression of the normal SMN protein. The techniques described in this section will focus primarily on these direct and indirect methods of manipulating this pre-mRNA splicing.

Antisense oligonucleotide strategies: A non-pharmacological strategy which gained popularity recently for enhancing exon 7 inclusion is the use of synthetic antisense oligonucleotides (ASO). Antisense oligo-nucleotides (ASOs) are relatively short stretches of nucleic acid that recognize a target sequence with a high degree of specificity. While ASOs have often been used to knock-down expression of target mRNAs through siRNA pathways, they have been used for splice site-switching ASOs as a means to alter SMN2 exon 7 pre-mRNA splicing within the context of SMA. These ASOs bind to SMN2-derived transcripts and promote exon 7 inclusion during the splicing phase. An important precaution in this strategy has to be that the sequence targeted by the ASOs must be carefully selected to avoid masking any regulatory region that is crucial for exon 7 splicing.

The role of ASOs in SMA was initially limited to redirection of splice decisions by blocking the 3 splice site (ss) of exon 8 (25) and to inhibit the function of a negative splicing regulator (E1) within intron 6 (26). In a recent study, the ASO targeting the 3′ ss of exon 8 was incorporated into U7 snRNA by delivering it into SMA type-I patient fibroblasts (3813cells) using adeno-associated virus (AAV-5) (27) by the technique used by Madocsai et al (28). This resulted in an increased exon 7 inclusion and SMN protein levels.

This antisense strategy has been extended further by developing alternative chemistries and through the incorporation of an untethered binding platform for positively acting splicing factors to the exon 7 region of SMN2. These techniques involve combining the antisense region with either a covalently bound synthetic peptide (s16) or with a non-complementary ESE sequence acting as a binding platform for SR proteins (bifunctional RNAs) (29, 30).

Cartegni and Krainer (31) have used small chimeric molecules consisting of an antisense moiety that recognizes and hybridizes exon 7 sequence covalently linked to a peptide; these molecules mimic the function of SR proteins (a family of highly conserved splicing factors). At nearly the same time, Skordis et al (30) used oligo-ribonucleotides that are complementary to exon 7 and contain exonic splicing enhancer motifs to provide trans-acting enhancers. Both groups have demonstrated that such strategies can work in vitro to increase exon 7 inclusion. One of the main challenges of this approach in SMA patients will be in achieving efficient delivery of these constructs to motor neurons.

Through detailed molecular studies, an intertwined series of enhancers and silencers have been identified. In particular, the identification of intronic splicing silencers (ISSs) has greatly impacted the design of exon 7-stimulating ASOs and bifunctional RNAs. In addition to the use of mini gene systems, ASO arrays or tiling has proven to be a powerful and unbiased means to analyze ASOs in disease-specific cellular contexts (32, 33, 34, 35). ISS-N1 ASOs systemically injected into unaffected SMN2-transgenic, heterozygous SMA mice gave rise to 90% inclusion of exon 7 in the liver and kidney, whereas the effect was modest in thigh muscles and not evident in the spinal cord (32). Furthermore, a recent report indicated that multiple ICV injections of ISS-N1 ASO increased SMN protein levels in the brain and spinal cord of the SMAD7 mouse model. Remarkably, uptake of uncoupled 2′-O-methyl ASO was significantly greater as compared with ASO incorporated into previously described carriers. The weight and righting reflex, monitored until post-natal day 12, were also improved relative to a control group. As further confirmation of this regulatory sequence as a bona fide target, recently, an 8-mer ASO that binds to five

nucleotides of ISS-N1 was reported to increase SMN and SMN-associated proteins in 3813 cells (34). However, there are serious questions which need to be addressed such as if short ASO can function specifically in vivo or if the high number of cognate sites within the genome could result in unwanted off-targets effects.

ISS-N1 has also been targeted by ASOs composed of several different backbone, chemistries and of varying lengths (36, 37,38, 34, 39). Recently a backbone technology has been developed with a2′-O-2-methyoxyethyl-modified back-bone in their ASO-10–27 (ISIS SMNRx). This molecule has also been utilized with considerable efficacy. For example, in an asymptomatic SMN2-transgenic model, ASO-10–27 fully reversed the SMN2 splicing pattern when administered via an icv. injection (40). Subsequent work in an SMA mouse model resulted in a significant extension in survival from approximately 14 days to approximately 26 days following a single icv. Injection (41). Hua et al (42) found a surprising result when systemic administration of the same ASO resulted in nearly a 25-fold increase in survival in a mouse model. As opposed to the bifunctional RNAs that were designed to directly stimulate SMN2exon 7 inclusion by recruiting positively acting splicing factors, this alternative class of RNAs recruited negatively acting factors to the 5′ end of exon 8 as a means of shifting the equilibrium towards full-length expression. Intravenous injection into the temporal vein of SMA mice with these synthetic2′-O-methyl bifunctional RNAs enhanced SMN protein expression in the liver and kidney, whereas intracerebroventricular (ICV) injections increased SMN levels in brain tissue from treated SMA mice (43).

Other Nucleic acid-based therapeutics

The novel molecular entities including the nucleic acid-based therapeutics, have elicited a considerable amount of excitement based upon the encouraging results of studies in SMA animal models. While early works demonstrated that exon 7 splicing could be modulated by the over expression of transacting factors or the genetic removal of splicing inhibitors surrounding exon 7, the potential for this modulation for being used as therapeutic strategies have only begun recently. Especially these results laid the foundation for a whole new dimension of research using ASOs. As a result of these researches, several nucleic acid-based targets and strategies developed focusing on redirection of SMN2splicing.bifunctional RNAs; peptide–nucleic acid ESSENCE compounds; and trans-splicing RNAs (44, 45, 46, 47, 48, 30, 49). The bifunctional RNAs and trans-splicing RNAs deserve a special mention here.

The bifunctional RNAs: The bifunctional RNAs are the types of RNAs comprising of two domains: an antisense domain and a separate region that serves as a recruiting and binding substrate for splicing factors, such as hTra2-β1 or SF2/ASF. Ideally, a bifunctional RNA would have two modes of action: (i) the inhibition of a splicing silencer mediated by the antisense region, and (ii) the recruitment of SR proteins. Bifunctional RNAs have shown enhanced activation of SMN2 exon 7 splicing mediated by the recruiting platform over and above the activity conferred by the antisense domain. However, the extension of survival using these molecules was not very impressive. This could be attributable to chemistry or stability differences of these molecules. In an experiment, bifunctional RNAs were expressed from AAV-2 vectors, leading to increased SMN protein levels in cell-based models (29,43). The detection of the inhibitory element E1 within intron 6 led to the synthesis of a bifunctional RNA that played a dual role by simultaneously blocking E1 and recruiting SR proteins (36). The antisense moiety consisted of two non-sequential sequences designed to inhibit E1 by hybridizing to the flanking regions of E1. E1-bifunctional RNAs contained the exonic splicing enhancer for either ASF/SF2 or hTra2-b.

Furthermore, delivery of 2′-O-methyl bifunctional RNAs into the CNS of SMAD7 mice resulted in SMN levels comparable with that of carrier heterozygous mice in the brain and spinal cord. More importantly, E1-hTra2-b extended the lifespan and weight in a more severe mouse model of SMA (36). Plasmid-derived and 2′-O-methyl RNAs also increased SMN protein levels in 3813 cells. Utilizing small RNA molecules for reprogramming the splicing of a faulty pre-mRNA is an expanding area of research for a wide range of genetic diseases including SMA (50, 51).

The trans-splicing RNAs: Trans-splicing RNAs, which redirect SMN2 splicing from the endogenous pre-mRNA molecule to a vector-derived RNA in trans via a site-specific antisense domain, have shown promise in cell culture and modest activity in a severe mouse model of SMA. In large part, the high-throughput screening vectors that have been utilized captured either SMN2 promoter activity or SMN2 exon 7 splicing. Recently, a new reporter was developed that incorporated the SMN2 promoter, the SMN cDNA-spanning exons 1–6 and the genomic cassette comprised exons 6–8. In this system, multiple SMN-inducing mechanisms could be screened simultaneously, such as promoter activation, exon 7 splicing, or RNA stability (52).

2. SMN2 GENE ACTIVATION BY HISTONE DEACETYLASE INHIBITORS AND OTHER DRUGS

Recently there has been a significant interest in further stimulating the basal levels of SMN2 transcription for enhancing a global increase in transcription consequently boosting full-length and SMNΔ7 mRNA. For this purpose up regulation the SMN2 promoter has been seen as a viable means of elevating SMN protein Therefore several pharmacological strategies have focused on this upregulation of SMN2 promoter. However before moving on to the pharmacological targets specifically, we will elaborate the regulation of SMN2 gene expression which operates at multiple layers. This regulation thus tightly controls its gene expression. The SMN2 gene is regulated by a promoter that is nearly identical in sequence and activity to the SMN1 promoter (53,54). An approximately 150-bp region upstream of the translation initiation site contains the sequences necessary for minimal promoter activity, (55) but regulatory sequences relevant to *SMN* gene expression may extend to as far as 4.6 kb upstream of the transcription initiation site. An important component of this regulation is the SMN promoter. The *SMN* promoter contains binding sites for and binds the cAMP-response element binding protein, the Sp family of proteins, and the interferon regulatory factor, all of which can modulate the promoter activity. In addition, it has been demonstrated that the *SMN* promoter is associated with the histone deacetylase (HDAC) 1 and 2 proteins that may modulate the histone acetylation state at the *SMN* promoter and play arole in determining *SMN2* gene expression (56). This mechanism acts for regulation of gene expression by the mechanisms of the compaction and relaxation of DNA which is in part accomplished by histone acetylases (HATs). These HATs acetylate lysine residues found on histones, a primary building block for chromatin. Acetylation relaxes the DNA allowing for transcription while the activity of histone deacetylases (HDACs) promotes DNA chromatin compression and gene repression.

During the first high-throughput screen for SMA-specific compounds, two parallel screens were run in an attempt to capture compounds with two distinct modes-of-action: SMN2 promoter activation and SMN2 exon 7 splicing. The studies employing SMN2 promoter assay have identified a quinazoline structure that exhibited cell-based activity and led to a medicinal chemistry program that produced several compounds with drug-like properties (57,58). Among these layers of SMN2 gene expression, some targets have been specifically chosen for pharmacological manipulation. It is however important to realize that these targets are in no way exclusive as newer targets are being identified with each genetic research in this field. For the sake of simplicity, these pharmacological strategies can be divided into three categories:

A) **HDAC inhibitors:** One class of drugs that has been investigated for the ability to activate the SMN2 gene is the HDAC inhibitors group. These drugs utilize the fact that controlling the acetylation state of histones is an important epigenetic mechanism regulating gene expression in general. When the NH2-terminus of core histones is acetylated in a region of chromatin, this region acquires a more relaxed chromatin structure that is more transcriptionally active due to increased accessibility of DNA to the transcriptional machinery. The level of histone acetylation is determined by the balance of activities of histoneacetyl transferases, which acetylate histones, and HDACs, which deacetylate histones. During a small molecule screen of drugs that might increase SMN expression, Chang and colleagues (59) demonstrated that sodium butyrate, one of the earliest discovered HDAC inhibitors, increased full-length SMN2 transcript levels and protein levels in lymphoblastoid cell lines derived from type I SMA patients. In addition, when sodium butyrate was administered to the pregnant mothers of SMA transgenic mice, it improved survival in their offspring. Subsequent studies have shown that the *SMN2* promoter can be activated and full-length SMN mRNA and protein levels increased in SMA patient-derived cells by several other HDAC inhibitors, including Phenylbutyrate, Valproic acid, Suberoyl anilide and Hydroxamic acid. (60,61,56,62,57). Among them,

Phenylbutyrate and Valproic acid have been used in clinical practice for many years for other indications with well-established pharmacokinetic and safety profiles and good CNS penetration. Based on ideas driven by the safety as well as efficacy of these drugs, they have been used in early clinical trials in SMA patients, although efficacy in SMA animal models has not yet been proven. A pilot trial of Phenylbutyrate in 10 patients with SMA in Italy showed that the drug was well tolerated (63). There are currently ongoing or planned clinical trials of phenylbutyrate and valproic acid. There has also been interest in newer-generation HDAC inhibitorsAmong these drugs, sodium butyrate prodrugs have been shown to prolong survival in SMA mice (64). Interestingly, this survival benefit occurs without a demonstrable increase in SMN protein expression, indicating that this effect may be due to other neuroprotective effects, such as activation of anti apoptotic genes, as has been seen in a study of HDAC inhibitors in mice with ALS (65).

***b) Another drug that has been proposed to work by activating SMN2 gene expression is hydroxyurea*.** In the past this compound has been shown activate the fetal globin gene and is used to treat patients with sickle cell disease and thalassemias. Hydroxyurea was shown to increase the amount of full-length SMN transcript and protein and gems in patient-derived lymphoblastoid cell lines (66) and is currently being studied in clinical trials of SMA patients in several countries. Recently, a cell-based high-throughput screen of 550,000 compounds was performed to identify drugs that activate a 3.4-kb fragment of *SMN2* promoter present in a motor neuron–like cell line, NSC34 cells (57). Seventeen distinct compounds belonging to nine different scaffolds were identified. None appeared to be acting as HDAC inhibitors. Two of these scaffolds, an indole compound and the quinazoline compounds, were confirmed to increase full-length SMN transcript and protein in patient derived fibroblast cells lines. The quinazoline scaffold is more a more attractive option in this context as it showed the highest potency, minimal cellular toxicity, and was amenable to chemical modification. The lead compound using this scaffold was 2,4-diaminoquinazoline, was shown by Singh et al(67) to function through a distinct mechanism. They found that this molecule worked by inhibition of DcpS (67), which is an enzyme involved in 5′ cap-mediated degradation of mRNAs. Subsequently they tested the oral administration of this lead compound which is yet to receive a nam (RG3039). This led to a dose-dependent increase of SMN in SMA mice and extended survival by ~20–30% (68).

c) Some other drugs experimented act by uncertain mechanisms. Before addressing other drugs, we will at first highlight the drugs: Trichostatin A (TSA) (68), LBH589 (69), and suberoylanilide hydroxamic acid (SAHA) (70, 18). All of them have been shown to increase SMN protein levels from the human SMN2 gene and in some instances from the murine SMN gene. TSA and SAHA were able to provide significant extensions in survival as well as lessening overall disease severity in SMA mice (71, 72, 18). LBH589 elevated SMN protein by stimulating exon 7 inclusion and total SMN2 promoter expression, as well as by increasing hTra2-β1 levels (69). TSA plus a rigorous regimen of supportive and dietary care was further capable of extending survival by ~170% (72).

One of the first compounds to be examined in SMA models was *Aclarubicin* that demonstrated activity in cell-based assays (73). After a screen in patient fibroblasts, Andreassi et al. demonstrated that the chemotherapeutic drug aclarubicin stimulated exon 7 inclusion and consequently increased SMN protein levels. Salbutamol has also been shown to increase the relative ratio of full-length: Δ7 SMN transcript in cell-based models (74,75). As salbutamol is an approved compound, clinical trials were initiated based upon the positive SMN induction, demonstrating a high degree of tolerability yet showing only modest improvement in motor function (74,76).

It seems that that there could exist several pharmacological mechanisms for promotion of exon7 inclusion in SMN2 transcripts. As mentioned before, the SMN1 gene produces a full-length transcript, whereas the SMN2 gene predominantly produces an alternatively spliced transcript (exon 7 deleted) encoding an unstable protein (SMND7) that does not oligomerize efficiently (77, 78). The inclusion of exon 7 in SMN1 transcripts and exclusion of this exon in SMN2 transcripts is caused by a single nucleotide difference at þ6 in SMN1 exon 7. Although the C-to-T change in SMN2 exon 7 does not change an amino acid, it results in either the disruption of an exonic splicing enhancer (ESE) or creation of an exon silencer element (ESS), which finally results in the majority of transcripts lacking exon (20,77,79). Thus a silent cytosine-to-thymine (C–T) transition within exon 7 induces the alternative splicing event common to the majority of SMN2-derived transcripts. Therefore, a pharmacological strategy that

could be successful in increasing SMN protein levels would be to promote exon 7 inclusion in SMN2- derived transcripts. In a cell-free screen designed to identify compounds that increased SMN2 exon 7 splicing, a tetracycline derivative, PTK-SMA1, was identified that increased in vitro SMN2 splicing as well as exon 7 inclusion in mild SMA mouse model following intraperitoneal or iv. administration (80). The beta-lactam antibiotic Ceftriaxone has also been evaluated in SMA mice and was shown to modestly, but significantly extend survival and decrease the severity of disease in these mice (81). RNA transcripts were not examined in this report although total SMN protein levels were increased slightly. Ceftriaxone also exhibits a general in-vitro and in-vivo neuroprotective activity as it upregulates glutamate transporter expression, and in the G93A SOD1 amyotrophic lateral sclerosis mouse model it extends survival modestly and delays onset of disease (82). There are likely to be many exonic and intronic splice enhancer and silencer motifs that play roles in SMN transcript splicing.

A series of novel compounds has been identified that appear to function through different mechanistic pathways including increasing SMN2 exon 7 inclusion. SMN exon 7 inclusion was increased not just in the reporter system but from the endogenous SMN2 gene within SMA patient fibroblasts (83). Further confirmation of these new compounds is required to demonstrate in vivo activity and in disease relevant tissues. Because the ultimate goal of testing these compounds is to see their effects in neurons, their ability to reach inside neurons and their intracellular stability, will be key to the further development of these types of strategies in SMA as well as other neuro-degenerative disorders of the CNS.

3. SMN PROTEIN STABILIZATION RELATED STRATEGIES

SMN protein stabilization strategies for SMA treatment are aimed to increase the translation of SMN2-derived protein or to stabilize the protein that arises from the SMN2 gene. In this section, we will address these strategies which will also include pharmacological strategies. In a cell-based high-throughput screen of approximately 47,000 compounds, Indoprofen was shown to increase SMN2-derived protein, but not SMN1-derived protein (84). They hypothesized that this drug would be acting at the translational level rather than the transcriptional level perhaps by increasing the efficiency of translation of SMN2-derived transcripts. In a similar kind of work, Wolstencroft et al. (85) found that aminoglycosides increased SMN protein levels and gem counts in patient-derived fibroblasts. Although the exact mechanism by which aminoglycosides produce this outcome is yet to be understood, these molecules are known to alter translation by promoting read-through of stop codons thus enhancing the translation of the peptide. The authors put up the speculation that these drugs would be enabling read-through of the initial stop codon in exon 8 of SMN2 transcripts. This could have resulted in a SMN protein with a slightly elongated C-terminus, which promotes its stability. Unfortunately, both Indoprofen and Aminoglycosides have poor central nervous penetration.

In addition to the protein synthesis enhancing strategies, inhibition of SMN degradation has also been tried. Recently it has also been demonstrated that the SMN protein is degraded by the ubiquitin–proteasome system and that drugs that inhibit this pathway increase SMN protein levels in patient derived fibroblasts (74). Further understanding of how the SMN protein is degraded may lead to other SMA therapeutic.

To apply these protein translation/stabilization approaches to SMA patients, new compounds will need to be identified that retain this activity and penetrate the blood– brain barrier. targets.

CONCLUSION

In present review, we elaborated the role of SMN2 gene as a phenotypic modifier in Spino muscular atrophy patients. Additionally, we highlighted the ways in which this gene can be used in the treatment of SMA patients. There are several ways in which this goal may be achieved. Further elaboration and therapeutic testing is required for converting them into treatment strategies.

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