

Spinal Muscular Atrophy and the Antiapoptotic Role of Survival of Motor Neuron (SMN) Protein

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Abstract Spinal muscular atrophy (SMA) is a devastating and often fatal neurodegenerative disease that affects spinal motor neurons and leads to progressive muscle wasting and paralysis. The *survival of motor neuron (SMN)* gene is mutated or deleted in most forms of SMA, which results in a critical reduction in SMN protein. Motor neurons appear particularly vulnerable to reduced SMN protein levels. Therefore, understanding the functional role of SMN in protecting motor neurons from degeneration is an essential prerequisite for the design of effective therapies for SMA. To this end, there is increasing evidence indicating a key regulatory antiapoptotic role for the SMN protein that is important in motor neuron survival. The aim of this review is to highlight key findings that support an antiapoptotic role for SMN in modulating cell survival and raise possibilities for new therapeutic approaches.

Keywords Survival of motor neuron (SMN) protein · Spinal muscular atrophy (SMA) · Antiapoptotic · Apoptosis · Prosurvival

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive infantile neurodegenerative disease that has an incidence of one in 6,000 live births. SMA is characterized by the progressive degeneration of motor neurons of the anterior horn of the spinal cord. Clinically typified by profound muscle

weakness, hypotonia, and trunk paralysis, SMA is classified into three main subtypes (I–III) based on age of onset and disease severity [1]. Type I SMA accounts for ≈50 % of SMA cases, manifests within 6 months of birth, and is usually fatal before the age of four. Patients with type II SMA develop muscle weakness before 18 months of age, often develop severe orthopedic and pulmonary complications [2], and may survive into adolescence [3]. Patients with type III SMA, typically display a later onset and suffer from a milder but still debilitating phenotype.

Approximately 95 % of SMA patients have a deletion or mutation in the *survival of motor neuron 1 (SMN1)* gene [4] that encodes the survival of motor neuron (SMN) protein. A second highly homologous copy of this gene, *SMN2*, differs by only a single translationally silent base change within exon 7. However, this base change causes aberrant splicing (exon 7 exclusion) in ≈90 % of *SMN2* transcripts and when translated, results in truncation, instability, and reduced activity of the SMN protein (Fig. 1). Full-length SMN protein is ubiquitously expressed and occurs in the cytoplasm and nucleus of most cells. Among other functions, SMN plays an important role in the biogenesis of spliceosomal small nuclear ribonuclear proteins (snRNPs) and pre-mRNA splicing. SMN also modulates apoptosis by directly blocking caspase activation and by affecting other key regulators of cell survival such as Bcl-2, p53, ZPR1, and Bcl-xL [5–9]. However, a clear understanding of SMA pathogenesis and the full functions of the SMN protein is still to be determined. In this review, we summarize the evidence that antiapoptotic and prosurvival pathways are affected by reduced SMN protein levels and explain the importance of targeting apoptosis as a potential therapeutic approach for SMA.

Genetics of Spinal Muscular Atrophy

In 1995, Lefebvre et al. [10] discovered that mutations in the *SMN1* gene caused SMA. The functional *SMN1* gene

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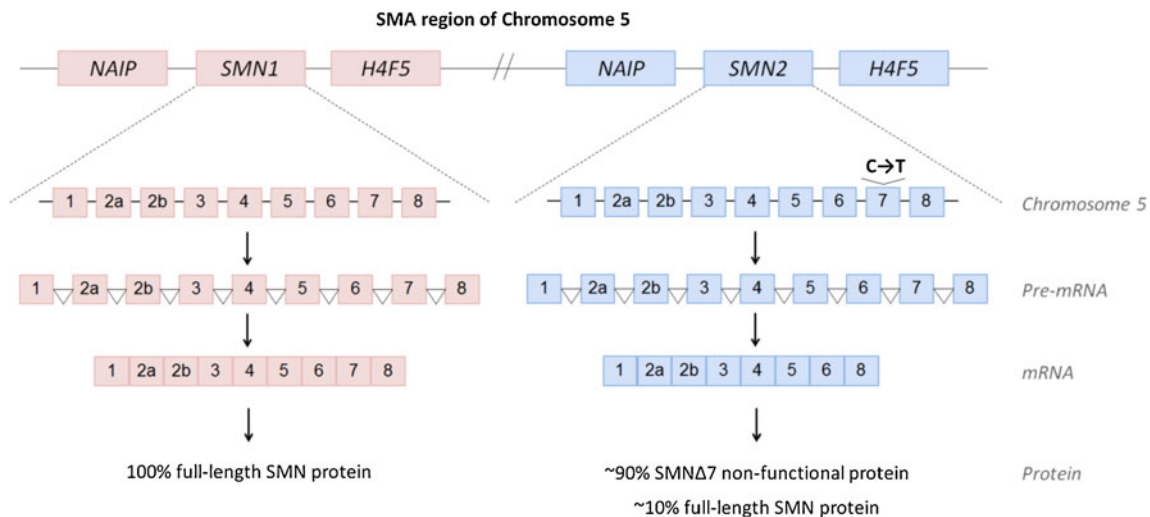


Fig. 1 Schematic diagram showing the *SMN1* and *SMN2* genes. Due to a 500-kb inverted duplication on chromosome 5, there exists two almost identical survival of motor neuron genes *SMN1* and *SMN2*. In unaffected individuals, the *SMN1* gene is normally spliced and produces a functional SMN protein. In contrast, the *SMN1* gene is mutated or deleted in 95 % of SMA patients. A single base change (C→T) affects

the splicing of the *SMN2* gene causing the majority of *SMN2* transcripts to lack exon 7. This translates to a mostly truncated SMN protein (SMN Δ 7) missing the amino acids encoded by exon 7. Therefore, SMA patients have much lower levels of full-length functional SMN protein and, consequently, show all the hallmark pathologies of SMA disease. [10]

encodes the SMN protein that is necessary for motor neuron development and survival. During human evolution, a second, almost identical, copy of the *SMN* gene, termed *SMN2*, arose following a 500-kb inverted duplication of the region containing *SMN1* on chromosome 5 [11]. Both the *SMN1* and *SMN2* genes contain identical promoter sequences and display enhanced neuronal expression [12]. Although the two genes differ by only five nucleotides, a critical translationally silent single C → T nucleotide base change in exon 7 of the *SMN2* gene causes exclusion of this exon in 90 % of *SMN2* RNA transcripts [13] (Fig. 1). Exclusion of exon 7 occurs due to disruption of a splice modulator site/sequence described as acting as an exon splicing enhancer (ESE) or an exon splicing silencer (ESS) [14, 15]. The C terminus of the SMN protein, which contains the region encoded by exon 7, is essential for survival, and when deleted, the mutant non-functional SMN protein (SMN Δ 7) fails to reduce cell death following injury [16]. The importance of exon 7 is further exemplified in conditional exon 7 knockout mice that demonstrate similar phenotypes to different SMA mouse models [17].

The Survival of Motor Neuron Protein and Spinal Muscular Atrophy Pathogenesis

The Survival of Motor Neuron Protein

The *SMN1* gene encodes a 38-kDa full-length and functional protein. In contrast, while encoding the same protein, alternative splicing of the *SMN2* transcript results in the

expression of relatively small amounts of full-length SMN protein (10–20 %; Fig. 1). The vast majority of protein encoded by the *SMN2* gene is missing the 16 carboxyl end amino acids encoded by exon 7, causing the protein to be less active and unstable [18, 19]. Ubiquitously expressed and developmentally regulated [20], full-length SMN protein is found in both the cytoplasm and nucleus. In the nucleus, SMN protein localizes to structures called Gemini of coiled bodies (gems) that coincide with Cajal bodies in most cell lines but are distinct from Cajal bodies in fetal tissues [21–23]. Cajal bodies and gems colocalize to some degree, and this relationship may be partly mediated by the interaction of SMN with the Cajal body protein coilin [24, 25]. The SMN protein is also present in axons and dendrites [26, 27] and independently localizes to the neuromuscular junction (NMJ) [28].

Spinal Muscular Atrophy Pathogenesis

While not all cell types are equally affected by reduced SMN levels, SMA is characterized by the degeneration of alpha motor neurons of the anterior horn of the spinal cord [29]. The increased vulnerability displayed by motor neurons may be due to a motor neuron-specific *SMN2* splicing inefficiency, whereby reduced SMN levels further exacerbates SMN exon 7 exclusion in motor neurons [30]. It is clear that reduced levels of oligomerization-competent intracellular SMN initiate the pathogenic cascade, but the sequence of events in SMA pathogenesis is debatable [31]. The most-studied function of the SMN protein is in forming a complex containing Gemin proteins (Gemin 2–8) and

small nuclear ribonucleoproteins (snRNP) assembly [21, 32–38]. The SMN complex specifically binds to and assembles Sm proteins (ribonucleoproteins) onto small nuclear RNAs (snRNAs), generating an active snRNP complex [25, 39–41]. As a component of the spliceosome [42], snRNPs play a role in pre-mRNA splicing that is essential for the expression of mature mRNA [43]. An important question is to what extent is SMN's role in RNA processing fundamental to SMA disease? Theoretically, the SMN protein impacts many mRNAs processed, suggesting defective splicing is playing an important role in the pathophysiology of SMA. Burghes and Beattie [44] suggest that disruption of snRNPs alters the splicing of genes critical to motor neuron function. Recently, Lotti et al. [45] identified an SMN-regulated U12 intron-containing gene, named *Stasimon*, responsible for normal synaptic transmission and motor neuron function. Restoration of *Stasimon* in a zebrafish SMA-like model rescued SMN-dependent motor neuron defects [45], suggesting dysregulation of *Stasimon* plays a key role in the motor neuron-specific pathology of SMA.

Another suggestion is that NMJ formation and axonal function is affected in SMA, leading to the degeneration and eventual cell death of the motor neurons [31]. This is supported by the axonal location and axonal transportation of the SMN protein [27, 46]. To this end, knockdown of SMN expression causes defects in motor neuron axonogenesis, branching, and NMJ formation independent of snRNP biogenesis [47–49]. In addition, anterograde transport of the truncated SMN Δ 7 protein does not occur and overexpression of SMN Δ 7 results in axonal defects [46].

While SMN appears important to motor neuron function, pathogenic models of SMA suggest a general requirement for the SMN protein in many other tissues. This review will delineate the antiapoptotic functions of the SMN protein and the contribution made by apoptosis to the pathogenesis of SMA. In addition, we will discuss the current and future therapeutic approaches aimed at achieving neuroprotection in SMA.

Apoptosis in Spinal Muscular Atrophy

Apoptosis, or programmed cell death, is a specific and deliberate cellular mechanism that results in nuclear fragmentation, chromatin condensation, and the formation of apoptotic bodies [50, 51]. This phenomenon is required during embryonic development and for the homeostatic maintenance of proliferative tissues [52, 53]. Apoptosis is essential for normal development in the Central Nervous System (CNS) and functions to rapidly remove redundant neurons and nerve cells that fail to establish the appropriate synaptic connections [54]. Apoptosis during development and later in adult life is strictly regulated by a balance of antiapoptotic and proapoptotic signals. Dysregulation of apoptosis due to a disturbance of this

balance can be associated with the development of cancer, autoimmune diseases, ischemia-related injuries, and neurodegenerative disorders [51].

There is increasing evidence that neurodegeneration in motor neuron disorders such as SMA and amyotrophic lateral sclerosis (ALS) may also involve apoptosis [55–57]. In genetically confirmed cases of SMA, examination of fetal tissue has revealed that during embryonic development, immature motor neurons undergo a more prolonged period of apoptotic programmed cell death, compared with motor neurons from control human fetal tissue [58, 59]. Furthermore, it is considered that the apoptotic loss of motor neurons in SMA patients after birth has been significantly underestimated due to the rapid elimination of these cells from spinal cord tissue [31]. Elevated levels of proapoptotic genes and apoptosis have also been observed in the CNS of SMA mouse models [60, 61]. Taken together, these human and animal studies provide strong evidence that the excessive loss of motor neurons by apoptosis plays an important role in the early stages of SMA.

The important function of SMN in regulating cell survival and apoptosis is supported in experimental studies showing that SMN levels are proportional to cell death rates following apoptotic stimuli [62]. For example, the SMN protein can protect various cell types against a range of apoptotic stimuli, including growth factor deprivation, PI 3-kinase inhibition, and camptothecin- and staurosporine-induced apoptosis [62–65]. In addition, SMN knockdown in neuronal-like NSC-34 cells results in reduced cell survival and elevated levels of apoptosis [62]. Therefore, irrespective of its other functions, it is clear that SMN can reduce apoptosis in normal cells and in cells induced to undergo apoptosis.

Proposed Antiapoptotic Mechanisms Used by Survival of Motor Neuron

Studies aimed at identifying SMN's specific antiapoptotic mechanisms are limited. Kerr et al. [66] first postulated that SMN may play a crucial role in modulating neuronal-specific apoptotic mechanisms. To date, the most well-defined antiapoptotic mechanism of action of SMN is through inhibition of active caspase-3 subunit formation. Caspase-3 is a key mediator of apoptotic cell death and is usually activated by the apoptosome complex consisting of cytochrome *c*, caspase-9, and Apaf-1. The activation of procaspase-3 (32 kDa) involves a two-stage process: calpain-mediated removal of the 3-kDa prodomain resulting in a 29-kDa subunit (p29), followed by spontaneous proteolytic cleavage of p29 into large and small subunits (17/12 kDa) by caspase-9 [67–69]. Caspase-3 is also activated by caspase-8 through the extrinsic pathway. This

pathway involves the activation of death receptors such as Fas and tumor necrosis factor (TNF), the recruitment of death receptor-associated molecules such as Fas-associated death domain containing protein (FADD), and the subsequent activation of the caspase-8 (reviewed in [51]). The ability of SMN to block calpain-mediated activation of procaspase-3 [65] and thereby reduce caspase-3 activation [62–65, 70] provides strong support for the view that SMN functions in an antiapoptotic caspase-3-dependent manner (Fig. 2). In support of this, human SMA motor neurons derived from induced pluripotent stem cells (iPSCs) exhibit increased levels of caspase subunits and caspase-3 cleavage [71].

What makes the involvement of caspase-3 and calpain potentially interesting is their ability to directly cleave full-length SMN protein following injury [66, 72, 73]. Caspases are predicted to cleave SMN at amino acid Asp-252, generating a \approx 29-kDa truncated protein that is present in cells

undergoing apoptosis in brain tissue [66]. Not surprisingly, mutating the SMN caspase cleavage site results in an SMN protein with enhanced antiapoptotic properties [66]. However, it is unclear whether caspase cleavage of SMN abolishes its intrinsic antiapoptotic properties or if the resulting SMN cleavage products have their own unique proapoptotic functions.

Cell Death and Prosurvival Proteins Affected in Spinal Muscular Atrophy

The Role of the Bcl-2 Protein

As a consequence of its multifunctional roles, the SMN protein impacts a variety of pathways and genes implicated in SMA pathogenesis. Of particular interest are the relationships and interactions between SMN and key proteins that are

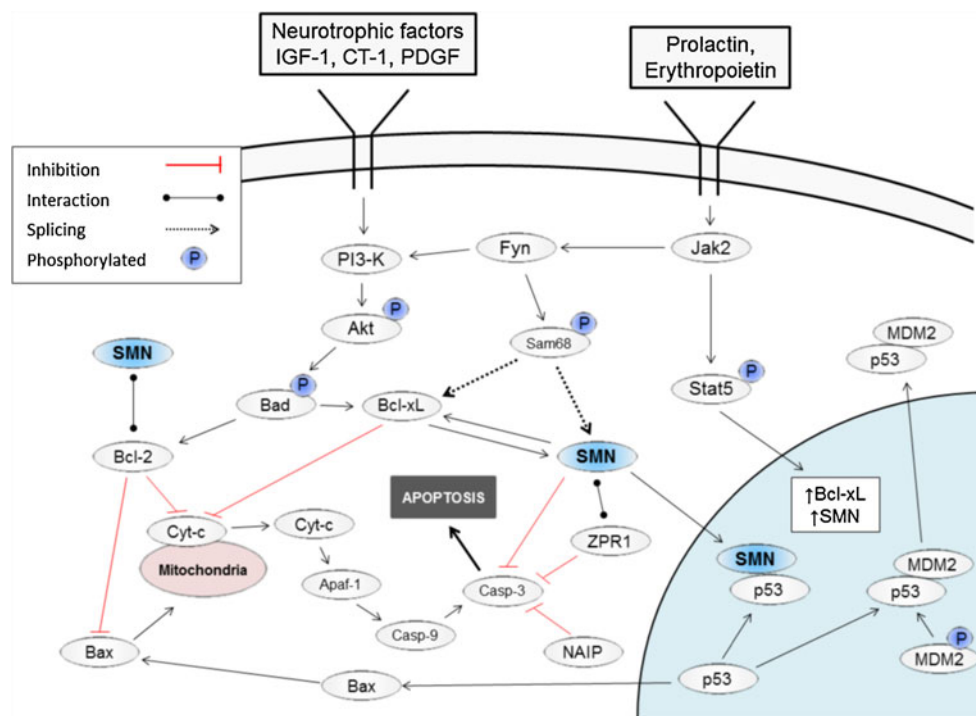


Fig. 2 Proposed cell signaling pathways linking SMN expression to the regulation of cell survival. Neurotrophic factors commonly signal through the PI 3-kinase pathway, phosphorylating Akt and preventing BAD sequestration of Bcl-2 and Bcl-xL. SMN interacts with Bcl-2 and regulates the expression of Bcl-xL, both of which prevent cytochrome *c* release from the mitochondria. The SMN protein also interacts with the tumor suppressor protein, p53, and when bound, colocalizes across the nuclear membrane. Under normal conditions, the p53 protein tightly regulates Bax activation, and the interaction between Bcl-2 and SMN synergistically prevents Bax-mediated apoptosis. In SMA patient cells, levels of the p53 modulator, MDM2, are reduced and unable to prevent p53 activation of Bax. Prolactin and erythropoietin signal via the Jak/Stat pathway. Prolactin increases SMN levels by stimulating Stat5, a transcription factor known to increase Bcl-xL

levels. Activation of this pathway by prolactin is also known to activate Fyn, a tyrosine kinase involved in the activation of the prosurvival PI 3-kinase pathway and the phosphorylation of the RNA binding protein, Sam68. Phosphorylated Sam68 splices Bcl-x and SMN transcripts, promoting the long Bcl-xL isoform and inclusion of exon 7 in SMN transcripts. End-stage apoptosis begins with the activation of caspase-9, via cytochrome *c*-mediated activation of Apaf-1 or by activation of cell surface death receptors. Activation of caspase-3 requires cleavage of the 3-kDa procaspase-3 prodomain, a step blocked by SMN. Expression of ZPR1 and NAIP are correlated with SMA severity and SMN protein levels, and both proteins can directly regulate caspase-3 activation. The overall inhibition of caspase-3, directly or indirectly by SMN, prevents cleavage of death substrates and apoptosis. [5, 6, 9, 65, 81, 92, 93, 101, 131, 159]

directly or indirectly involved in regulating cell survival (Table 1). For example, SMN is known to interact with Bcl-2, a well-characterized member of the Bcl-2 family that negatively modulates apoptosis by several mechanisms [74, 75]. The interaction of SMN with Bcl-2 provides a synergistic antiapoptotic action against Bax- and Fas-mediated apoptosis [5, 76]. Moreover, the truncated SMN Δ 7 protein is unable to produce a similar antiapoptotic effect, further supporting the importance of exon 7 to SMN protein functionality [5]. Finally, in SMA model mice, the transcription factor WT-1, a key regulator of Bcl-2 expression and apoptosis, is downregulated [77]. The WT-1 protein modulates apoptosis by upregulating Bcl-2 expression and by directly interacting with p53 to inhibit apoptosis [78, 79].

The altered expression of Bcl-2 in SMA is likely to have a significant impact on neuronal development. During murine embryogenesis, Bcl-2 expression peaks at embryonic day 11 and plays an essential role in regulating developmental apoptosis [53]. Interestingly, at 15 weeks of gestation, in the spinal cord tissue of SMA patients, expression of Bcl-2 is downregulated, a factor which is likely to lead to increased total motor neuron cell death [8, 58].

An Interaction with ZPR1

Another protein involved in negatively regulating caspase activation and known to interact and colocalize with the SMN protein is ZPR1 [7]. Like Bcl-2, expression of ZPR1 is reduced in SMA patients [80] and is also thought to play a role in SMA pathogenesis [81]. Similar to SMN, complete knockout of ZPR1 is embryonically lethal, supporting an

essential role for ZPR1 in cell survival [81]. Indeed, ZPR1 deficiency causes Cajal body defects, axonal abnormalities, defective embryonic growth, mislocalization of SMN, and increased apoptosis [7, 81, 82]. Interestingly, ZPR1 knock-down can induce caspase-3 activation and play a significant role in motor neuron degeneration in mice [82]. Taken together, these findings suggest that ZPR1 may play an important role in determining the extent of neuronal apoptosis in SMA and thereby contribute to disease pathogenesis.

The Role of p53

The SMN protein also interacts with the proapoptotic p53 protein [6]. The p53 protein is a sequence-specific transcription factor that targets both mitochondrial and death receptor-induced apoptotic pathways, such as Bax, NOXA, and PUMA, resulting in cytochrome *c* release and Apaf-1/caspase-9 activation [83]. Although p53 is not subject to SMN-dependent regulation [80], it is thought that high levels of SMN are responsible for p53 localization to nuclear bodies [6]. Young et al. [6] determined that the SMN/p53 interaction prevented p53-mediated apoptosis. Not surprisingly, the truncated SMN Δ 7 protein was unable to interact with p53 and prevent activation of p53 apoptotic pathways. The primary regulator of p53 is the MDM2 protein, which inhibits p53 transcriptional activity and targets p53 for proteasome degradation. MDM2 is a transcriptional target of p53 and, via an autoregulatory feedback loop, its expression is increased as p53 activity increases [84]. Interestingly, both MDM2 and its regulator, PSME3, are downregulated in SMA model mice [77], possibly secondary to reduced

Table 1 List of proteins affected in SMA that play a role in regulating cell survival

Protein	Function	Relationship	Reference
Bcl-2	Anti-apoptosis. Prevents Bax-mediated apoptosis, cytochrome <i>c</i> release and sequesters Apaf-1	Interacts, downregulated	[70, 71, 75]
Bcl-xL	Anti-apoptosis. Prevents cytochrome <i>c</i> release and can modulate SMN levels	Down/coregulated	[75–77]
Beta-synuclein	Protects against caspase activation in a TP53/p53-dependant manner [78]	Downregulated	[79]
Calmodulin	Mediates many processes such as apoptosis	Downregulated	[79]
DP-1	Forms a E2F-1/DP-1 complex that can mediate apoptosis	Downregulated	[72]
Hsp60	Necessary for cell survival following toxicity and stress	Downregulated	[79]
Htra2-beta	Binds to and blocks the action of inhibitor of apoptosis proteins (IAPs) [80]	Downregulated	[81]
Ku70	Suppresses mitochondrial translocation of Bax, inhibiting apoptosis [82]	Downregulated	[72]
MDM2	Inhibits TP53/p53-mediated apoptosis [83]	Downregulated	[72]
NAIP	Blocks apoptosis and the activation of caspase-3	Modifying gene	[84, 85]
P21	Cyclin-dependant kinase inhibitor. Involved in the cellular stress response	Upregulated	[86]
p53	Apoptosis. Induces Bax-mediated apoptosis	Interacts with	[72, 87]
p57/KIP2	Apoptosis. Inhibits cyclin-dependant kinases	Downregulated	[72]
p-JNK	Responsive to stress stimuli and play a role in the apoptosis pathway	Upregulated	[86]
WT-1	Transcription factor. Can modulate apoptosis by upregulating Bcl-2 [74]	Downregulated	[72]
ZPR1	Antiapoptosis. Prevents caspase-3 activation	Interacts, downregulated	[81, 88]

SMN and p53 levels. The importance of MDM2 as a regulator of p53 has been demonstrated in MDM2 knockout mice whereby p53-driven apoptosis causes embryonic lethality [85].

What Role Does the Bcl-xL Protein Play?

The Bcl-x gene encodes several alternatively spliced mRNA's, of which Bcl-xL is the predominant transcript [86, 87]. Unsequestered Bcl-xL targets the mitochondria, inhibiting cytochrome *c* release and Apaf-1-dependent caspase-9 activation, subsequently blocking apoptosis [88, 89]. Bcl-xL expression is high during embryogenesis [53] and is thought to play an essential role in CNS development, as Bcl-xL-deficient mice exhibit extensive apoptotic cortical and spinal neuronal loss [90]. In SMA, downregulation and irregular expression of Bcl-xL have been documented in human SMA fetal tissue [8] and SMA model mice [91]. However, since the identification of Bcl-2 as an interacting partner of SMN [5], Bcl-xL has been largely overlooked as playing a role in SMA pathogenesis. We recently reported that expression of Bcl-xL and SMN is coregulated, suggesting a common regulatory mechanism [9]. In addition, we reported that a regulator of both Bcl-x and SMN mRNA alternative splicing, the RNA-binding protein Sam-68, is reduced in SH-SY5Y cells when Bcl-xL or SMN is overexpressed [9]. Reduced levels of Sam-68 cause accumulation of both antiapoptotic Bcl-xL and full-length SMN2 transcripts [92, 93]. In SMA, Sam 68 regulation may be altered, resulting in abnormally high protein levels and potentially leading to the downregulation of Bcl-xL and increased exon 7 skipping in SMN2 transcripts.

The Role of the Neuronal Apoptosis Inhibitory Protein Gene

Lying immediately adjacent to the *SMN* gene, the *neuronal apoptosis inhibitory protein (NAIP)* gene is located within the 500-kb inverted duplication on chromosome 5. *NAIP* is the founding member of the human IAP protein family [94] that together inhibit many key caspases and procaspases. Interestingly, numerous studies have correlated *NAIP* deletions with SMA disease severity [95–100]. These studies show that up to 90 % of type I SMA patients have deletions in the *NAIP* gene. In contrast, deletions in *NAIP* are less frequent in types II and III SMA. Therefore, it is likely that *NAIP* gene expression is directly implicated as a modulator in SMA and absence of this gene exacerbates the SMA phenotype.

The NAIP protein directly inhibits caspase-3 and caspase-7 through the action of its baculovirus inhibitor of apoptosis protein repeat (BIR) [101]. Furthermore, NAIP suppresses apoptosis in neural tissues [102–104] and the loss of endogenous NAIP results in increased neuronal vulnerability [105]. In vivo, hippocampal pyramidal neurons from transgenic mice

lacking the *NAIP1* gene display an increased sensitivity to kainic acid-induced apoptosis [105]. However, these mice are morphologically normal and do not show any features or characteristics of an SMA phenotype. Attempts to determine the exact effects of NAIP deletions on SMA phenotype have, so far, not been successful, mainly due to the presence of six different *NAIP* genes [106], making in vivo modeling difficult.

Current Therapeutic Strategies Toward Treating Spinal Muscular Atrophy

Increasing Survival of Motor Neuron Protein Levels

Viral Delivery of Survival of Motor Neuron

The primary goal of current SMA therapeutics is threefold: firstly, to increase SMN protein levels; secondly, to promote the inclusion of exon 7 in SMN2 transcripts; and thirdly, to promote motor neuron survival. Considered to be the most promising of the current SMA therapeutic approaches, viral delivery of SMN presents an efficient method for systemic delivery of SMN protein. Demonstrating the feasibility of this approach for the first time, Azzouz et al. used a lentiviral vector to retrogradely increase SMN expression in motor neurons in an SMA mouse model [107]. Following this initial study, improvements in gene delivery efficiency have led to the rescue of a severe mouse model of SMA using an adeno-associated viral vector (AAV9), which can infect peripheral tissue as well as cross the blood–brain barrier [108]. However, it is still not clear what effects viral delivery will have on already diseased human motor neurons and if any immunological responses will occur.

Exon 7 Inclusion of Survival of Motor Neuron

An alternative approach to *SMN1* gene replacement is the use of antisense oligonucleotides to alter *SMN2* splicing. The antisense oligonucleotide approach targets pre-mRNA processing, reducing exon 7 exclusion in *SMN2* mRNA and increasing full-length SMN transcripts and protein levels. Antisense oligonucleotide technology has previously been used to alter gene expression in different forms of cancer (reviewed in [109]) and has been used successfully to restore dystrophin expression in Duchenne muscular dystrophy patients [110, 111]. Applying this methodology to treating SMA has proven successful both in vitro [112] and in vivo using SMA mouse models [113]. However, oligonucleotide delivery requires a direct route of administration to the CNS, such as intrathecal or intracerebroventricular injections, and will require additional optimization for an efficient delivery to peripheral organs.

Neuroprotection and Antiapoptotic Approaches

Survival of Motor Neuron-independent Therapies

While current strategies have focused solely on the importance of increasing SMN levels, the use of neuroprotective therapeutics as either a separate or complementary treatment for SMA also needs to be considered. For example, neurotrophic factors have demonstrated therapeutic benefits clinically and in animal models of neurodegenerative diseases, such as ALS, Alzheimer's disease, Parkinson's disease, and Huntington's disease (reviewed in [114]).

Cardiotrophin

Cardiotrophin-1 (CT-1) regulates apoptosis by blocking the proapoptotic functions of p53 and Bax, activating the Akt prosurvival signaling pathway and promoting Bcl-2 expression [115]. CT-1 delivery has proven to be neuroprotective in *in vitro* models of cerebral ischemia [116], while intramuscular delivery of an adenovirus-expressing CT-1 is able to delay motor impairment and muscle atrophy in an ALS mouse model [117]. With respect to SMA, Lesbordes et al. were the first to demonstrate the effective use of CT-1 treatment in an SMA mouse model [118]. In this study, overexpression of CT-1 in SMA mice resulted in increased survival, reduced NMJ disorganization, and diminished axonal degeneration [118]. Importantly, CT-1 is capable of reducing neuronal apoptosis by blocking caspase-3 and caspase-8 activation [119]. These findings suggest that there is potential for CT-1 to be used to reduce motor neuron apoptosis and prolong survival in SMA patients.

Insulinlike Growth Factor

Similarly, insulinlike growth factor (IGF-1) activates several prosurvival signaling pathways including PI 3-kinase/Akt and Erk1/2. In addition, IGF-1 enhances motor neuron axonal growth [120] and is thought to act in a retrograde manner to exert a neuroprotective effect on motor neurons. Expression of IGF-1 reduces muscle wastage in a mouse model of Duchenne muscular dystrophy (mdx) and causes significant improvements in life span and motor function in animals models of spinal bulbar muscular atrophy [121] and ALS [122].

In a severe mouse model of SMA, transgenic expression of IGF-1 in skeletal muscle tissue improved median survival and also resulted in improved overall motor function [123]. While significant improvements were observed in this study, they remain modest in comparison to recent studies using *SMN* gene replacement or correction of *SMN2* splicing using antisense oligonucleotide delivery [124–126]. In contrast, in a SMA type III mouse model, CNS-mediated delivery of IGF-1 did not improve overall survival or motor

function but did reduce motor neuron loss [127]. These results suggest the neurotrophic and antiapoptotic effects of IGF-1 therapy may be more beneficial in severe forms of the disease, where motor neuron loss is greater. Current therapeutic strategies are also using IGF-1 in combination with SMN-dependent approaches [128], demonstrating the importance of achieving neuroprotection and gene replacement for maximal therapeutic benefit in SMA.

Bcl-xL and Bcl-2

Both Bcl-2 and Bcl-xL expressions are reduced in SMA fetus spinal cords [8], potentially contributing to the irregular neuronal morphology and increased levels of apoptosis observed during SMA development [59, 129]. With this in mind, Tsai et al. [91] first postulated that Bcl-xL may compensate for a deficiency in SMN levels, showing that in transgenic SMA type III mice, increased Bcl-xL expression resulted in improved viability and reduced muscle atrophy. Recently, Bcl-xL has also been shown to phenotypically rescue mouse motor neurons exhibiting all the effects of reduced SMN levels [130]. Extending on these studies, we have demonstrated a relationship between Bcl-xL and SMN expressions, whereby coexpression of both proteins provided additive protection from apoptosis [9]. From these studies, we postulate that Bcl-xL is an important regulator of SMN expression and may be involved in mediating the antiapoptotic effects of SMN.

In addition to Bcl-xL, there also exists a relationship between the Bcl-2 and SMN proteins [5, 76]. Coexpression of Bcl-2 and SMN causes a synergistic reduction in Bax-mediated apoptosis [5]. Hence, it is not surprising that levels of proapoptotic Bax protein are elevated in the spinal cords of SMA mice and that inhibiting Bax in SMA mice results in improved motor neuron numbers and median survival [60]. Therefore, targeting apoptosis in SMA appears to be a viable treatment approach and the use of Bcl-xL and/or Bcl-2 in future SMA therapies should be considered.

Platelet-derived Growth Factor Prolactin and Erythropoietin

Recently, both platelet-derived growth factor (PDGF) and prolactin have been shown to elevate SMN protein levels through different pathways [131, 132]. PDGF was found to inhibit GSK-3, an important regulator of p53 activity, via activation of the Akt signaling pathway. In comparison, prolactin receptor activation stimulates the Janus kinase 2 pathway (JAK2), resulting in STAT5-mediated transcriptional activation of SMN and other antiapoptotic proteins [131, 133]. In this study, prolactin treatment of a severe mouse model of SMA resulted in elevated full-length SMN protein levels in the CNS, improved motor function,

and enhanced survival [131]. Signaling via the Jak2/Stat5 pathway, erythropoietin (EPO) is another growth factor that could potentially show therapeutic potential in SMA. EPO is able to cross the blood–brain barrier [134] and several studies have demonstrated its effectiveness to ameliorate or reduce neuronal injury in cell culture and/or animal models of stroke, epilepsy, spinal cord injury, and ALS [135–138]. Interestingly, both PDGF- and prolactin-stimulated pathways result in SMN upregulation and the regulation of key survival proteins. This suggests that molecular regulation of SMN can be tightly controlled, independent of a splicing function, via different cell signaling pathways.

Stem Cells to Treat and Model Spinal Muscular Atrophy

Embryonic Stem Cells

Human embryonic stem cells (hESCs) were first isolated in 1998 and have generated much interest in their use to develop in vitro disease models and for stem cell transplantation [139]. Pluripotent hESCs are derived from the “inner cell mass” of blastocyst stage embryos. While embryonic stem cells are relatively easy to obtain and differentiate, ethical challenges relating to their source need to be overcome if they are to be considered as a mainstream therapy. Recently, embryonic stem cell-derived neural stem cells were successfully transplanted into SMA model mice [140]. In this study, intrathecal transplantation of ESC-derived neural stem cells demonstrated appropriate migration patterns, differentiated into motor neurons, and increased overall survival [140]. In SMA, stem cell transplantation of donor-derived hESCs may replenish motor neuron numbers but will not target reduced SMN protein levels in peripheral tissues. Therefore, for this approach to be feasible, it will need to be used in conjunction with other SMA therapies.

Induced Pluripotent Stem Cells

Reprogramming of human fibroblasts presents another potential source of pluripotent stem cells and bypasses the ethical issues surrounding obtaining hESC's. While at present this method does not offer a therapeutic avenue, reprogramming of SMA fibroblasts into human SMA motor neurons has potentially opened the door to targeted drug screening and improved SMA therapeutics [141]. SMA-derived motor neurons show axonal deficits and increased levels of apoptosis but importantly respond to SMN-dependent therapies. In addition, SMA-derived motor neurons display increased caspase-3 activation and selectively blocking caspase-3 rescues these neurons from an apoptotic death [71]. Utilization of iPSCs to model SMA can potentially provide a reliable and accurate platform to identify and assess therapies aimed at increasing SMN levels and targeting apoptosis in SMA.

Clinical Trials

Completed and Potential Clinical Trials in Spinal Muscular Atrophy

Currently, there are no approved treatments to prolong the survival of SMA patients. Clinically, numerous compounds have been trialed to increase SMN transcript and protein levels, but to date, no therapeutic candidate has been adopted as a treatment for SMA. The first candidates identified for treating SMA were the histone deacetylase (HDAC) inhibitors, which were found to increase SMN2 expression [142]. Preclinical studies using HDAC inhibitors result in increased full-length SMN2 transcripts in vitro [143–145] and increased median survival in vivo [146]. However, the increased survival observed in HDAC-treated SMA mice could be due to an increased expression of several antiapoptotic genes as a result of the treatment [147]. Clinical trials of two HDAC inhibitors, phenylbutyrate and valproic acid, failed to show any significant clinical benefits in SMA patients [148–150]. Similarly, hydroxyurea enhances SMN2 expression in SMA patient cells [151] but has little or no clinical effects on SMA patients [152]. Currently, direct replacement of SMN using a scAAV9-expressing SMN appears the most promising therapeutic approach. Preclinical studies demonstrate dramatic improvements in systemic SMN expression and median survival in SMA mouse models [124, 125]. Furthermore, this approach results in sustained and systemic expression in nonhuman primates [153]. As a result of these studies and recent approval from the NIH Recombinant DNA Advisory Committee, phase I clinical trials using AAV9 to treat SMA are anticipated to commence in 2013.

Neuroprotective and antiapoptotic therapeutics could potentially target and reduce the early motor neuron loss that occurs in SMA patients. To date, clinical trials in SMA patients using the neuroprotective gabapentin have demonstrated negative or minimal results [154]. Neurotrophic factors have also been used in preclinical studies in numerous neurodegenerative diseases with success. However, in clinical trials of ALS, IGF-1, BDNF, and CNTF have failed to show any clinical benefit [155–158]. From these results, it appears that solely targeting apoptosis in SMA is not the answer. However, to combat the motor neuron loss due to elevated apoptosis occurring early in the disease, the addition of neurotrophic and/or antiapoptotic factors alongside SMN replacement may provide additional clinical benefits.

Conclusions

SMN is a multifunctional protein that interacts with and mediates the expression of numerous other proteins

involved in DNA repair, calcium handling, cell cycle, and apoptosis and which major function is the maintenance of motor neuron survival. Importantly, in SMA, the expression of critical modulators of cell viability is significantly affected (Table 1) that is likely to affect the disease phenotype. Expression of these genes in SMA may be affected through direct or indirect regulatory roles of the SMN protein and/or dysfunction of the snRNP complex, thereby causing altered splicing of these important genes [44]. The diverse range of proteins affected in SMA strongly supports a multifunctional role for the SMN protein. Further elucidation of SMN functions and downstream effects of SMN deficiency is likely to lead to a better understanding of the disease mechanisms that underlie impaired motor neuron survival in SMA.

It is important that SMN-independent approaches to promote neuronal survival should not be ruled out as they may complement therapies aimed at increasing SMN protein levels. In particular, neuroprotective and antiapoptotic proteins have shown promising results in promoting neuronal survival, rescuing axonal defects and increasing endogenous SMN protein levels. As summarized in this review, a variety of proteins involved in survival/apoptosis are affected in SMA mouse models and patient tissues. A more complete understanding of the signaling pathways involving SMN is required to substantiate the key cellular mechanisms involved in SMA pathogenesis. However, the role of apoptosis in SMA pathogenesis is well-established and targeting the early-stage apoptosis that occurs in SMA may be paramount to attempting to completely alleviate the disease.

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Conflict of interest None.

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