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REVIEW ARTICLE

Molecular Factors Involved in Spinal Muscular Atrophy Pathways as Possible Disease-modifying Candidates

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Abstract: Spinal Muscular Atrophy (SMA) is a neuromuscular disorder caused by mutations in the SMN1 gene. Being a monogenic disease, it is characterized by high clinical heterogeneity. Variations in penetrance and severity of symptoms, as well as clinical discrepancies between affected family members can result from modifier genes influence on disease manifestation. SMN2 gene copy number is known to be the main phenotype modifier and there is growing evidence of additional factors contributing to SMA severity. Potential modifiers of spinal muscular atrophy can be found among the wide variety of different factors, such as multiple proteins interacting with SMN or promoting motor neuron survival, epigenetic modifications, transcriptional or splicing factors influencing SMN2 expression. Study of these factors enables to reveal mechanisms underlying SMA pathology and can have pronounced clinical application.

Keywords: Spinal muscular atrophy, SMN, Genetic modifiers, DNA methylation, Actin cytoskeleton dynamics, Apoptosis.

1. INTRODUCTION

Spinal muscular atrophy is an autosomal recessive disorder characterized by degeneration of motor neurons of the spinal cord leading to progressive muscular weakness. It is the most common genetic cause of infant mortality with the incidence of 1 in 6,000-10,000 live births [1]. The carrier frequency for the disease was estimated at 1:40-1:60 with some variations among different ethnic groups [2, 3]. SMA is classified into four main clinical types based on the age of symptoms onset and attainment of motor milestones (types I-IV) [4, 5]. Also very severe SMA type 0 that develops prenatally and is defined by respiratory distress at birth is distinguished [5]. Type I SMA (acute form, Werdnig-Hoffmann disease) is characterized by generalized muscle weakness with onset in the first 6 months of life, no achievement of sitting without support and death within the first 2 years of life often caused by the respiratory failure [6]. Patients with type II spinal muscular atrophy, or intermediate SMA (often referred to as Dubowitz disease), with onset after the age of 6 months, can sit unsupported, but are never able to walk, and they survive beyond 2 years. Type III SMA (Kugelberg-Welander disease) is a milder form that develops after the age of 18 months; patients are able to walk unaided. This type is subdivided into IIIa, with disease manifestation before the age of 3, and IIIb, with onset beyond 3 years [7]. Lifespan is not significantly reduced compared with the normal population. Type IV SMA (mild adult form) has also been described. Patients usually present first symptoms in the second or third decade of life and have a normal life expectancy [5].

SMA is caused by mutations in the *SMN1* gene (OMIM 600354) located in the telomeric region of chromosome 5q13 [8]. About 95-98% of patients show homozygous deletions of the *SMN1* gene, where the rest exhibit small intragenic mutations [2, 9]. Approximately 2% of patients harbor *de novo* mutations, caused by high instability of this region of chromosome 5 [10]. The *SMN1* gene has a centromeric copy - the *SMN2* gene (OMIM 601627) - the result of duplication and inversion of the chromosomal segment of around 500 kb. *SMN1* and *SMN2* are almost identical except for five single nucleotide exchanges [8]. Only one difference is functionally important: a translationally silent transition at +6 in exon 7 (c.840C>T) that weakens the exonic splice site. This substitution causes exclusion of exon 7 from most of the *SMN2* transcripts, resulting in the production of a truncated SMN protein that is rapidly degraded [11, 12]. Only a small amount of *SMN2* transcripts are correctly spliced and produce full-length SMN protein.

SMN is a ubiquitously expressed protein located in both the cytoplasm and the nucleus, where it is localized in struc-

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tures called "gems" [13]. SMN complex participates in the assembly of small nuclear ribonuclear proteins (snRNPs), involved in the splicing of pre-mRNA [14]. SMN also fulfils unique functions in motor neurons, such as transport of mRNAs, in particular β-actin's mRNA, to the growth cones of axons [15-17].

The mechanism by which deficiency of housekeeping SMN protein leads to selective lower motor neuron degradation remains poorly understood, but there are two main hypotheses to explain SMA pathogenesis [13, 18]. One possibility is that motor neurons have a uniquely high demand for efficient messenger RNA splicing, the second hypothesis suggests that SMA is caused by disruption of specific for motor neurons functions of SMN [13].

The reason for considerable differences in symptoms severity of SMA patients is not quite understood as well; *SMN2* gene copy number variation seems to partially explain such differences. However there are factors, regulating the expression of the *SMN2* gene, such as epigenetic or transcriptional factors, that promote *SMN2* copies produce not equal amount of transcripts and may also be examined as potential disease modifiers [19, 20]. SMN interactome, especially proteins acting downstream from SMN are also of particular interest as possible modulators of disease pathogenesis in SMA [21]. Besides these proteins, regulating survival or functionality of the motor neurons independently of SMN, for example those involved in apoptotic pathway or cytoskeleton dynamics seem to be attractive targets to study in the context of potential SMA severity modifiers [22, 23]. Study of above-listed factors is actually important not only for better understanding the nature of SMA phenotype discrepancies, but for conclusions that we might make from such studies and use them for derivation of new targets for treating SMA.

First candidate phenotypic modifiers for SMA besides *SMN2* were *NAIP*, *p44* and *H4F5* genes, located in close proximity to *SMN* locus [9]. It was observed that about 50% of SMA type I patients had combined deletions of *SMN1*, *NAIP*, and *p44* exon 10 telomeric copy [24]. *NAIP* and *H4F5* gene (so called *SERF1*) were found to be more frequently deleted in severe SMA type I patients (68%-90% of deletions) than in milder forms II and III (20%-50% of deletions) [24-26]. Inverse correlation was observed between *H4F5* gene copy number and SMA type [27]. This gene encodes a protein homological to snRNP-interacting proteins like SMN, that made *H4F5* a candidate gene to influence SMA severity [28, 29]. Still there is no proof of *NAIP*, *p44* and *H4F5* genes involvement in the modification of SMA patients' phenotypes. Deletions of these genes in SMA type I individuals possibly reflect large-scale deletions encompassing *SMN1* gene and some copies of *SMN2* gene, that leads to severe phenotype due to lack of *SMN2* gene product [30-32].

Following studies have revealed other genes which can act as modifiers to the disease process, that were reviewed by Wirth and colleagues [33]. In this paper we aimed to summarize the update and expanded information about different factors potentially modifying SMA severity.

2. SMN2 GENE COPY NUMBER AS MAIN DISEASE MODIFIER

The ability of the *SMN2* gene to produce some amount of full-length SMN protein makes it the principal phenotype modifier in SMA patients. Homozygous absence of *SMN2* is found in 5% of healthy population and has no phenotypic effect [34]. However, patients with spinal muscular atrophy, who have both copies of the *SMN1* gene deleted or disrupted, harbor at least one copy of the *SMN2* gene. Complete absence of *SMN* genes is prenatally lethal [35]. The number of *SMN2* copies usually varies between 1 and 4 and rarely gains up to 8 copies [7, 36]. The increase of *SMN2* copy number is caused by duplication or gene conversion of *SMN1* into *SMN2* and was found to determine milder form of the disease [37]. Patients with SMA type 0 harbor 1 *SMN2* copy, about 80% of patients with type I SMA carry one or two *SMN2* copies, 82% of type II SMA patients have three *SMN2* copies, 96% of patients with type III SMA carry three or four *SMN2* copies and approximately 75% of type IV SMA patients harbor four *SMN2* copies [38-40]. Information concerning type IV patients is not as robust as for the other types, because adult form is relatively rare. Role of the *SMN2* gene as a main disease modifier can further be confirmed by several findings of asymptomatic individuals with the homozygous loss of the *SMN1* gene and increased *SMN2* copy number [41, 42].

Also inverse correlation has been observed between *SMN2* copy number and duration of survival. In the study of Feldkotter and colleagues for SMA type I patients, the mean survival of those who had one *SMN2* copy was about 7 months, while none survived beyond 11 months; two *SMN2* copies was associated with median age of survival of 8 months, most of the patients with two *SMN2* copies died before the age of 21 months; patients with three copies of the *SMN2* gene survived about 37.5 months [38]. Similar results were obtained in other studies [43, 44]. The Study carried out on a group of patients with infantile SMA (type I) showed differences in the age of disease onset between patients with 1 copy of the *SMN2* gene (SMA manifestation prenatally), 2 *SMN2* copies (onset at about 1.2 month of life) and 3 *SMN2* copies (onset at about 3.5 month of life) [43]. Examination of a cohort of adult SMA patients demonstrated that patients with three *SMN2* copies had significantly earlier age of onset of the disease compared to those who had 4 copies (4.4 ± 5.34 and 7.5 ± 6.0 years, respectively) [45].

SMN2 gene copy number was also shown to be associated with Hammersmith Functional Motor Scale (HFMS), Gross Motor Function Measure and SMA Functional Rating Scale (SMAFRS) scores determining the motor skills of the patients [45, 46]. Influence of *SMN2* copy number on denervation was observed in SMA patients: those who had less than 3 copies of the *SMN2* gene performed significantly lower Motor Unit Number Estimation (MUNE), maximum Compound Motor Action Potential amplitude (CMAP) values and worse functional outcomes compared to patients with more than 2 *SMN2* copies [47].

Analyzing genotype-phenotype correlation in SMA individuals Petit and colleagues evaluated two additional clinical criteria: brainstem involvement (for SMA types I-III) and ambulation loss (for SMA type III patients). Among SMA

patients with all three types, an inverse correlation was observed between *SMN2* copy number and brainstem involvement (manifesting itself as at least one of the following symptoms: facial weakness, chewing or swallowing difficulty, dysphonic voice, tongue fasciculation with lingual atrophy). Brainstem function was normal in patients who carried five copies of the *SMN2* gene. However, *SMN2* copy number did not correlate with brainstem dysfunction in patients within each SMA type group [44]. SMA type III patients who were able to walk had significantly higher number of *SMN2* copies compared to those who had lost ambulation [44, 45]. Among patients over 21 years of age (mean age about 32 years) only 31% of those with 3 copies of *SMN2* could walk unaided while 70% of patients with 4 copies were still ambulatory [45].

There are also evidences of heart defects in those patients who carry a single copy of the *SMN2* gene, not observed in patients with increased *SMN2* copies, revealing possible relevance of SMN protein for normal cardiogenesis [40, 48, 49].

Correlation between number of *SMN2* copies and disease severity has also been observed in transgenic SMA mice carrying various copy number of the human *SMN2* gene. Normally mice possess only one *Smn* gene - orthologue of the human *SMN1*, so to create SMA model the *SMN2* transgene was introduced in the mice genome on a Smn-knockout background [50]. Low number of *SMN2* copies (one or two) is associated with severe phenotype in mice, similar to that observed in SMA type I patients, reduced number of motor neurons and death within 6-8 days of life. Whereas increasing *SMN2* copy number up to eight completely rescues SMA phenotype [50, 51].

Besides being an essential prognostic criterion for the clinical course of SMA, quantification of the *SMN2* gene copy number may also be important in predicting the effect of pharmacological treatment in SMA patients. Up-regulation of the full-length *SMN2* mRNA represents a major goal of the pharmacotherapy for SMA. Several studies demonstrate that greater number of *SMN2* copies increases the efficiency of treatment with valproic acid and other therapeutic agents [52-55].

In spite of multiple evidences of *SMN2* copy number influence on SMA phenotype, there are some reports of families with marked differences in disease severity in siblings with the same number of *SMN2* copies [42, 56, 57]. Sometimes, less than five *SMN2* copies are found in asymptomatic carriers of a homozygous deletion of the *SMN1* gene and in some cases, six *SMN2* copies cannot rescue from SMA symptoms [7, 58, 59]. Some SMA type I patients carry three *SMN2* copies and SMA type III patients harbor two copies of the *SMN2* gene [59]. Such discrepancies can be determined by variability in expression between *SMN2* copies, caused by *SMN2* gene mutations or influence of splicing factors.

Also factors, interacting with SMN protein, regulating its stability, and those involved in different pathways influencing motor neuron viability are interesting targets for study as potential modifiers of SMA severity. By now, *SMN2* gene copy number is the only one approved modifier of SMA phenotype in patients. Meanwhile, there are multiple evidences of influence of additional factors on neuromuscular defects, neurite outgrowth and survival in SMA animal and cell models. Today it is far too early to speak about the reliability of these findings in context of modification of SMA severity, because these data have not been validated in patients. But these findings are worth paying attention because this field of research opens a perspective to disclose additional modifiers of SMA and potential targets for disease therapy. Factors and pathway connected with SMA pathology and interesting for further study as possible disease modifiers are outlined below. The summary of these factors is given in Fig. (**1**).

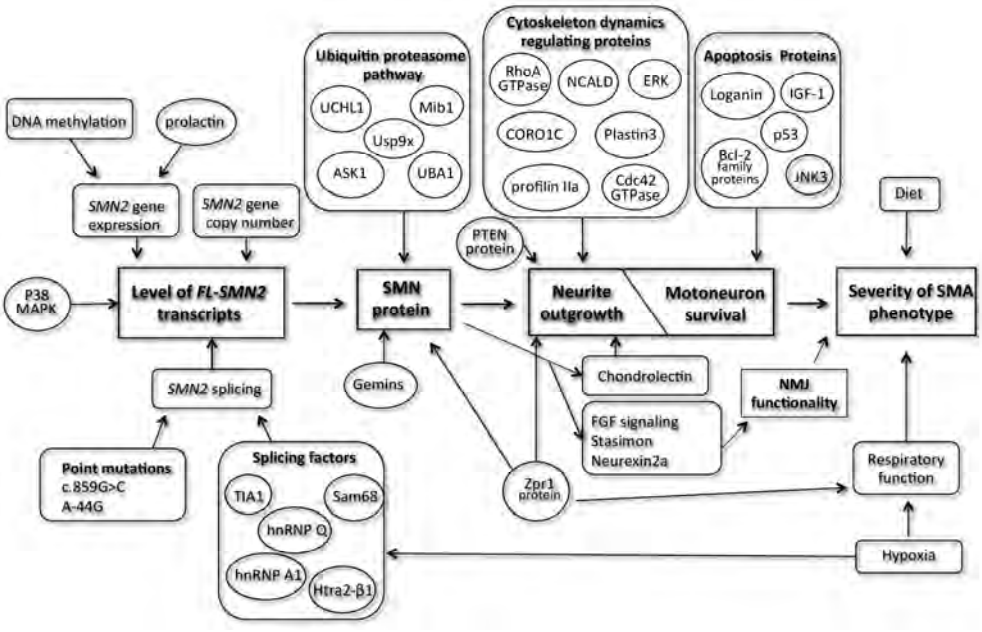

Fig. (1). Pathways and factors possibly involved in SMA pathogenesis. All comments and abbreviations are given in the text.

3. FACTORS AFFECTING SMN2 GENE SPLICING

Upregulation of exon 7 inclusion in *SMN2* mRNA can promote an increase in the amount of functional SMN protein, therefore factors influencing *SMN2* splicing are considered to be possible SMA modifiers. Indeed, chemical classes of small molecules modulating *SMN2* exon 7 inclusion were shown to ameliorate phenotype and prolong survival of SMA model mice as a result of restoration of *SMN2* splicing and SMN protein level [60].

Region of *SMN2* gene exon 7 and neighboring introns is rich with positive and negative *cis*-acting elements and trans-factors that bind regulation exon 7 inclusion in *SMN2* mRNA [61].

A transition (C-to-T) in exon 7 of *SMN2* gene disrupts Exonic Splicing Enhancer (ESE) - a binding site for splicing factor SF2/ASF, and creates Exonic Splicing Silencer (ESS) that is recognized by repressor protein hnRNP A1, resulting in exon 7 skipping [62, 63]. Lately, C-to-T transition in *SMN2* gene was also found to create a docking site for Sam68 protein, factor implicated in alternative splicing of several gene transcripts [64]. Overexpression of hnRNP A1 and Sam68 was shown to decrease the extent of *SMN2* exon 7 inclusion, and coexpression of Sam68 and hnRNP A1 together demonstrated cumulative effect, contributing to almost complete suppression of exon7 inclusion [65]. This action can be bypassed by depletion of Sam68 and hnRNP A1. Interestingly, interference with RNA-binding activity of Sam68 or with its binding to hnRNP A1 also restored *SMN2* exon 7 inclusion and SMN assembly in nuclear gems [65]. SMA mice lacking Sam68 demonstrated increased survival and motor activity with amelioration of NMJ defects [66]. These data show that SMA phenotype might be partially rescued by manipulating the level of splicing factors.

The opposite effect exerts SR-like splicing factor Htra2-β1, stimulating inclusion of *SMN* exon 7 through specific interaction with ESE in *SMN* exon 7 pre-mRNA [34]. Factors hnRNP G, SRp30c and TDP-43 were also shown to promote correction of *SMN* pre-mRNA splicing, but they elicited this effect *via* binding to Htra2-β1 rather than due to association with ESE [67-69]. Another splicing factor and potential SMA modifier is hnRNP Q, that exposed different expression level in severely and mildly affected patients [57]. Interestingly, different isoforms of this protein were shown to have opposite activity in the regulation of *SMN2* pre-mRNA splicing. HnRNP Q1 has beneficial effect on exon 7 inclusion, while minor hnRNP Q2 and Q3 isoforms antagonize this effect [70]. Additional evidence for the involvement of hnRNP Q as well as of highly related protein hnRNP R in SMA pathology is their interaction with wild-type SMN protein but not with the truncated form. Both hnRNP components of the spliceosome complex were found to co-localize with SMN in axons of motor neurons and presumably help SMN to perform neuron-specific functions such as mRNA trafficking [16, 71].

T-cell-restricted Intracellular Antigen 1 (TIA1) was shown to promote exon 7 inclusion by binding to U-rich motifs within intron 7 of *SMN2* and counteracting the inhibitory effect of polypyrimidine tract binding protein on *SMN2* exon 7 splicing. Depletion of TIA1 as well as hnRNP Q1 and Tra-2β noticeably increased exon 7 skipping, but injection of siRNA against TIA1 had the most pronounced negative impact on *SMN2* splicing [72].

Besides the above-listed elements, introns adjacent to *SMN2* exon 7 comprise other essential *cis*-elements, such as ISS-N1, A+100G, Element 1, that bind splicing repressors and represents a compelling targets for SMA treatment [61, 73, 74]. It is worth noting that the principle of splicing correction is the basis of major approaches to SMA therapy with antisense oligonucleotides (ASO). Importantly, the first approved treatment for SMA Spinraza is based on this principle, targeting ISS-N1 negative element [75]. Thus factors altering *SMN2* pre-mRNA splicing may be considered as SMA severity modifiers through changing the amount of functional SMN protein.

4. SMN MUTATIONS AND SEQUENCE VARIANCE MODULATING DISEASE SEVERITY

It was shown that the character of the mutation in *SMN1* gene can determine the severity of SMA phenotype. The majority of type I SMA patients carry real homozygous deletions of *SMN1*, while mildly affected SMA patients show absence of *SMN1* as a result of gene conversion of *SMN1* into *SMN2* [9]. The extension of the gene conversion may not be equivalent, that can alter the functionality of the alleles [76]. It has been proposed that chimeric genes as the result of fusion of exon 7 of the *SMN2* gene and exon 8 of the *SMN1* gene are associated with adult-onset SMA cases [77, 78]. Though exon 8 is untranslated, it may have regulatory function, influencing transcription or translation of *SMN* gene product [79].

Partial conversion of *SMN1* to *SMN2* confined only to C to T transition in position +6 of exon 7 with remaining sequence retaining as in *SMN1* gene was found to result in ~20% more full-length mRNA production compared to common *SMN2* gene copy [80]. A-44G substitution in *SMN2* gene was shown to exhibit marked positive effect on splicing due to preventing HuR protein docking that acts as a splicing repressor.

Differences in expression of *SMN2* gene copies may be caused by variance in *SMN2* sequence. Several studies describe the c.859G>C variant in exon 7 of the *SMN2* gene, associated with milder SMA phenotype [81, 82]. Single *SMN2* copy with c.859G>C transition produces 34% more full-length transcripts than each *SMN2* wild-type copy [83]. This effect is due to creation of an exonic splicing enhancer or disruption of an exon silencer element in *SMN2* exon 7.

In some cases, SMA is caused by *SMN1* point mutations, predominantly perturbing self-association of SMN protein or it's binding to other partners of SMN complex [9]. The positions of these mutations were shown to be related to different clinical phenotypes, so that mutations in exons encoding Tudor and YG box domains trigger the most severe symptoms [84, 85]. Clinical severity of SMA in patients with point mutations in *SMN1* gene was shown to be determined by the type and location of the mutation rather than *SMN2* copy number [86]. Importantly, it was shown that SMN alleles carrying missense mutations in different positions may complement each other resulting in the production of het-

eromeric SMN complex with greater function compared to homomers produced by either mutant allele on its own [87]. Thus, *SMN1* transgene carrying an A2G missense mutation as well as transgene with A111G substitution complements *SMN2* and modulates phenotypic severity in severe SMA mice [87, 88].

These data indicate that there are different intragenic factors such as point mutations and sequence variances in both *SMN1* and *SMN2* genes that influence SMN expression and disease severity, thus confirming the complexity of phenotype formation in SMA condition.

5. DNA METHYLATION AS POSSIBLE MODIFYING FACTOR OF SMA SEVERITY

DNA methylation is an important epigenetic modification regulating gene expression. In human, it occurs preferentially at cytosine residues within the CpG dinucleotides, which are concentrated in CpG Islands (CGIs) or are randomly distributed in the genome [89]. It is known that methylation of gene promoters leads to transcriptional inactivation, while gene body methylation facilitates transcription [90]. DNA methylation patterns can be changed by mechanisms of passive (replication-dependent) and active (*via* hydroxylation of methylated DNA) demethylation and subsequent remethylation. Global erasure and re-establishment of DNA methylation patterns occur in gametogenesis and preimplantation embryogenesis as a part of normal developmental program [91-93]. However, aberrantly altered DNA methylation patterns are associated with various pathologies, including neurodevelopmental and neurodegenerative disorders [94]. Methylation level of genes involved in pathogenesis of Alzheimer disease was similar in the nervous and peripheral blood cells, indicating that changes in methylation in lymphocytes may reflect those changes in target tissue and can possibly serve as a biomarker for neurodegenerative disease development [95].

For the first time, the involvement of DNA methylation in SMA pathogenesis was demonstrated by Hauke and colleagues in the study of severe and mild SMA patients with identical *SMN2* gene copy number [96]. *SMN2* gene expression was elevated by treatment of fibroblast cell cultures with DNA-demethylating agent. Four CpG islands with highly conserved methylation patterns were found within the area surrounding translational start site of *SMN2* gene. Significant differences in methylation level at the positions -296 and -290 in the CpG island 2 were observed between SMA type I and SMA type III patients' blood samples as well as fibroblast cell lines, so that the lower level of methylation correlated with milder SMA. The position -296 coincides with the first transcriptional start site of *SMN2* and methylation of this CpG may influence *SMN2* gene expression due to recruiting of methyl-CpG-binding protein 2 (MeCP2), a mediator of gene silencing [96].

In a recent study, methylation of four CGIs located ±3000 nucleotides from *SMN2* TSS was examined in SMA patients' blood samples. SMA type II patients demonstrated significantly higher methylation level of CGI 4 compared to SMA type III patients. In addition, differences were found in methylation level of CpG sites located in CGI 1 (nucleotides (nt) -871, -735) and CGI 4 (nt +999) between SMA type III and type I-II patients. Methylation of CpG site at the position -871 was shown to inversely correlate with *SMN2* full-length, Δ7 and total transcript levels. Inverse correlation was also observed between methylation of CpG unit encompassing nucleotides -290, -288, -285 and *SMN2* full-length transcripts' level, as well as between CpG site at the position +938 and FL/Δ7 ratio [97]. These data indicate the likelihood of methylation influence on *SMN2* transcription and splicing.

The first whole genome methylation analysis in DNA samples from peripheral blood cells of patients with severe and mild SMA types comparing to healthy individuals of the same age was carried out by our group to determine the association of DNA methylation status with the severity of the SMA phenotype [98]. We found significant differences in CpG methylation of about 40 genes between SMA patients with severe form and controls as well as between SMA patients with mild form and controls. Many of these genes can be involved in SMA pathogenesis. These genes encode proteins associated with the cytoskeleton system, processes of neuronal development and maintenance, apoptosis and transcriptional regulation. Further analysis of some of these genes in larger cohort study revealed significant differences in methylation level of CpG sites located in the regulatory regions of *SLC23A2* and *NCOR2* genes between SMA type I, II and III-IV patients' groups [99]. *SLC23A2* encodes SLC23A2 protein, a sodium/ascorbate co-transporter which provides high ascorbate concentration in the CNS. Ascorbate was shown to play an important role in neuronal maturation [100]. *NCOR2* encodes a subunit of complex connected with histone deacetylases (HDAC) [101]. Taking into account that HDAC are agents tested for SMA therapy, *NCOR2* might be involved in pathways regulating disease progression.

Recently significantly higher methylation level of *DYNC1H1* exon 37 has been found in mildly affected SMA patients samples compared to those with severe SMA form [102]. Exon 37 lies within CpG-rich region and apparently should be methylated for preventing spurious transcription initiations. Interestingly, mutations in *DYNC1H1* were shown to be associated with common neuromuscular diseases, such as Charcot-Marie-Tooth and dominant SMA, indicating the possibility of involvement of this gene into SMA pathogenesis [103-105]. Study of other genes previously revealed in genome-wide methylation analysis might help to identify more potential SMA modifiers.

These results support the likelihood of DNA methylation playing a role in SMA pathogenesis and disease development. In future, editing of DNA methylation might be considered as a possible therapeutic approach for SMA, though more studies are needed to verify this proposition and to check the extent of methylation impact on disease progression.

6. ACTIN-BINDING AND CYTOSKELETON DYNAMICS REGULATING PROTEINS AS PUTATIVE DISEASE MODIFYING FACTORS

SMA motor neurons are characterized by impaired neuritogenesis caused by perturbation in actin cytoskeletal pathway [106]. Several proteins were found to modulate actin polymerization thus likely playing a role in SMA pathogenesis.

Actin-binding protein plastin 3 was assumed as putative SMA modifying factor. Transcriptome-wide differential expression analysis revealed increased level of *PLS3* expression in unaffected individuals compared to their affected siblings with same *SMN1* deletion and *SMN2* gene copy number [22]. The level of F-actin also increased in these unaffected patients indicating the acting of plastin 3 through F-actin stabilization. Overexpression of plastin 3 in SMN-depleted neuronal-like PC12 and SMA animal models rescued neurite outgrowth [22]. Further studies demonstrated that PLS3-overexpressing mice displayed increase in end-plate and muscle fiber size and significant improvement of neurotransmission and motoric ability [107, 108]. A recent study enabled to reveal another F-actin binding protein CORO1C that interacts with PLS3 and demonstrates similar effect on SMA phenotype, ameliorating axonal defects caused by Smn deficit [108]. Endocytosis was shown to be the key mechanism, that being dramatically impared in SMA condition, is restored by overexpression of these proteins. In accordance with this position, knockdown of the negative regulator of endocytosis, NCALD improved axonal outgrowth and NMJ functionality in different SMA animal models [109].

Still there are disputable positions. Correlation was found between *PLS3* expression level and SMA type, as well as with the ability of patients to walk unaided, but this trend was observed only in females >3 years old [110]. In some studies, no correlation in *PLS3* expression was observed between examined tissues (when comparing between lymphoblasts, fresh blood cells and fibroblasts as well as between fibroblasts and iPSC-MN) and only slight increase in *PLS3* transcripts level was observed in lymphoblastoid cell lines of some asymptomatic SMA females compared to their affected siblings [111, 112]. Moreover, study of McGovern with colleagues on *PLS3* overexpressing SMA mice did not approve beneficial effect of high level of this protein on SMA phenotype [113].

Another actin-regulating protein that might influence SMA severity is profilin IIa, a neurospecific isoform of profilin. SMN protein was demonstrated to interact and colocalize with profilin IIa in gems and cytoplasm of cell body in motor neurons [114]. Profilin IIa was shown to negatively regulate actin dynamics while binding to SMN ameliorated this effect [115]. The depletion of SMN in PC12 cells, resulting in significant upregulation of profilin IIa mRNA and protein, also led to an increase in active RhoA and a decrease in active Cdc42 GTPases [106]. These proteins are known to play an important role in the neurite initiation and outgrowth through actin cytoskeleton dynamics regulation in neuronal growth cones [116]. The inhibition of Rho kinase (ROCK) - major downstream effector of RhoA GTPase led to partial rescue of neuronal outgrowth and differentiation of SMN-depleted PC12 cells [106]. Further studies demonstrated increased activation of RhoA-GTPase in the spinal cord of *Smn2B/-* mice at pre-phenotype and phenotype stages of the disease. Treating these mice with an inhibitor of ROCK contributed to an increase of lifespan and improvement in neuromuscular junction maturation [117]. Thus a model explaining SMA pathogenesis through SMN-profilin IIa interaction was proposed. According to this, the model reduced the amount of SMN in SMA patients, altering profilin II expression and functionality, which led to the upregulation of RhoA/ROCK, Cdc42 pathway with subsequent disruption in neuritogenesis processes [106].

Extracellular signal regulated kinase (ERK) pathway controlling actin cytoskeletal organization was also found to be dysregulated in SMA [118]. ERK was previously described to promote neurite outgrowth, thus antagonizing the ROCK-pathway activity in SMA [119]. However, ERK- as well as ROCK-inhibition both led to prolonged survival of SMA mice [117, 120]. Such effect of ERK pathway inhibition can be caused by subsequent upregulation of AKT/CREB pathway promoting enhanced SMN expression [120]. PTEN phosphatase, described below, was shown to demonstrate similar effect on AKT/CREB pathway [121].

Importance of cytoskeleton dynamics regulation in SMA pathogenesis is also supported by the observation that stathmin, a microtubule destabilizing protein, is upregulated in SMA and increased level of this protein correlates with disease severity [122]. Decrease in stathmin level improved motor functions and ameliorated neuromuscular junction defects in SMA-like mice, but did not extend their lifespan [122].

In whole genome methylation analysis, we observed changes in the methylation level of *CHML* and *ARHGAP22* genes that encode proteins regulating the activity of different Rab- and Rho-GTPases [98]. Using protein interaction databases, we showed that effectors of *CHML* and *ARHGAP22* gene products such as RhoB-GTPase and Rab6-GTPase may interact with profilin IIa and regulate the processes of actin polymerization and actin-myosin bundles formation.

Thus, there are multiple evidences that changes in actin dynamic and cytoskeleton regulation influencing axonal growth and branching are important events in SMA development. An approximate scheme of factors involved in this process is presented in Fig. (**2**). The data concerning factors engaged in these pathways that were shown to promote symptoms correction in SMA cell and mice models by overexpression or inhibition based on exposed effect may have pronounced therapeutic perspective. Yet the ambiguity of the relationships between these factors is obvious, so more detailed information about this issue is required. By now, encouraging data in SMA therapy area have been observed by different scientific groups while using combinational treatment of SMA mice with ASO and regulators of endocytosis. ASO aimed at correction of *SMN2* splicing supplemented by Plastin 3 overexpression demonstrated significantly greater effect on SMA mice survival, motor function and NMJ size compared to ASO administration solely [123, 124]. Likewise, NCALD reduction enhanced the positive impact of ASO in SMA mice, resulting in significant improvement of NMJ maturation and motor abilities [109]. These data indicate the relevance of investigation of SMA modifiers that might be a source of new targets for therapeutic strategies.

7. PTEN PHOSPHATASE IN REGULATION OF NEUROGENESIS

Recent studies have revealed the involvement of phosphatase and tensin homologue (PTEN) in the regulation of SMA severity. PTEN dephosphorylates PIP3, an inhibitor of

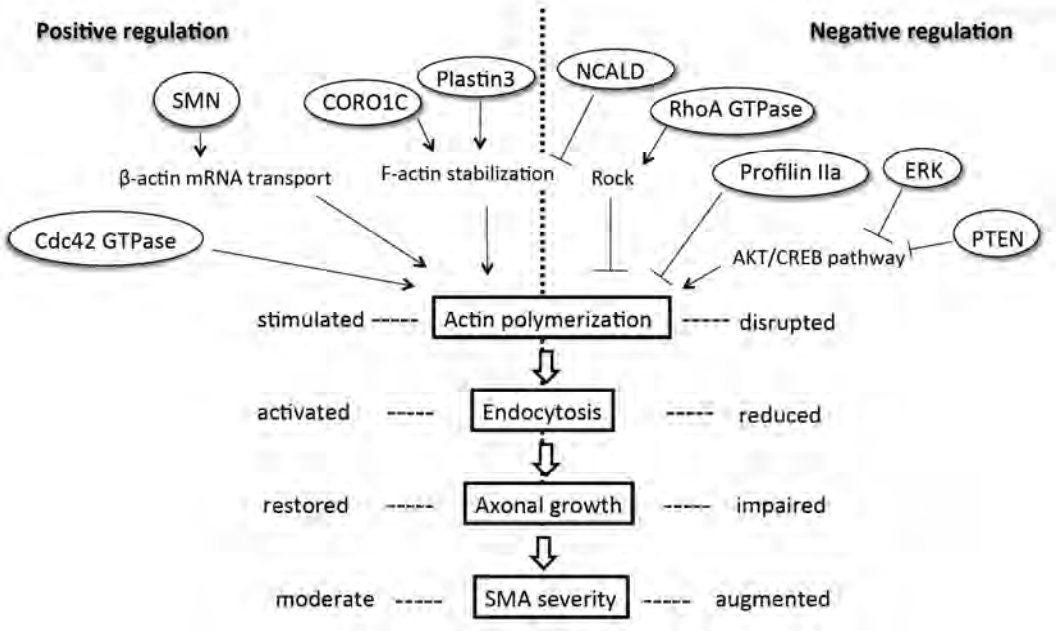

Fig. (2). Scheme of factors influencing actin polymerization as an important mechanism impaired in SMA condition. Arrow-headed lines indicate activation and bar-headed lines indicate inhibition of downstream pathways.

the Akt cascade - an essential pathway that, in particular, inhibits pro-apoptotic proteins and stimulates the expression of antioxidant enzymes thus displaying neural prosurvival activity [125]. Importantly, Akt signaling pathway was shown to stimulate SMN expression as well [120].

The effects of PTEN have been demonstrated to be cell-dependent. This protein is found in Central Nervous System (CNS) and peripheral nerves and plays a role in both developing and mature neural cells [126, 127]. PTEN expression is induced during neuronal differentiation and this molecule is implicated in moderating neurogenesis presumably in order to keep the CNS from cell-overgrowth and facilitate stable nerve-muscle interaction and synaptogenesis [128, 129]. Although this function is substantial for normal neural cells, it hinders axonal grows of neurons affected in degenerative conditions, or following injury to the nervous system [128, 130]. Inhibition of PTEN in injured cultured sensory neurons and in rat model with a complete nerve trunk transection injury resulted in an increase in the number and length of neurites [131]. In embryonic and adult neural stem cells, loss of PTEN increased proliferation and self-renewal capacity with no premature senescence or tumorigenic effect [132, 133].

PTEN downregulation was shown to prolong the survival of SMN-deficient motor neurons and increase axonal length and growth of cone size [134]. It is worth noting that it also stimulates restoration of β-actin protein level that is dramatically reduced in SMA motor neurons. Delivery of adeno-associated vector serotype 6 expressing (AAV6)-siPTEN into the hind limb muscles of mouse model of severe SMA led to amelioration of neuromuscular junction pathology and increased number of motor endplates [135]. Notably, this effect was not observed in healthy NMJs treated with AAV6-siPTEN, indicating that downregulation of PTEN was well tolerated by healthy motor neurons. Intravenous injection of this vector increased motor neuron survival and improved motor function of neonatal SMA mice resulting in significant gain of body weight compared to untreated littermates and about threefold extension of their lifespan [135].

These data show that PTEN inactivation can be a potential approach for moderating the severity of neurodegenerative diseases.

8. UBIQUITINATION OF SMN PROTEIN

The SMN protein is degraded through the ubiquitin (Ub)/proteasome pathway (Fig. **3**). This pathway includes the Ub-conjugating system and the 26S proteasome. The Ub-conjugating system carries out the ubiquitination of protein substrate through three-step mechanism, containing the Ub-activating enzyme E1, Ub-conjugating enzymes E2 and Ub-protein ligase E3. The inhibitors of Ub/proteasome system were shown to increase the level of the SMN protein in cytoplasm and nuclei of SMA patients' fibroblast cells [136]. Treatment of SMA model mice with proteasome inhibitor bortezomib blocked SMN degradation in the peripheral tissues and improved motor function [137]. SMN protein was found to be a substrate of Ubiquitin-specific Protease 9x (Usp9x) which is one of the largest deubiquitination enzymes. Usp9x deubiquitinates and stabilizes SMN protein and several other components of SMN complex - Gemins 2, 3 and 8. Knockdown of Usp9x resulted in the reduction of levels of SMN protein and SMN complex and decreased number of gems in nucleus [138].

Proteomic analysis revealed the differentially expressed ubiquitin carboxy-terminal hydrolase L1 (UCHL1) in type I SMA skin fibroblast cells comparedto the normal skin fibroblast cells [139]. Also, UCHL1 expression level correlated with SMA type. This protein was observed only in neurons and was found to be involved in the development of several

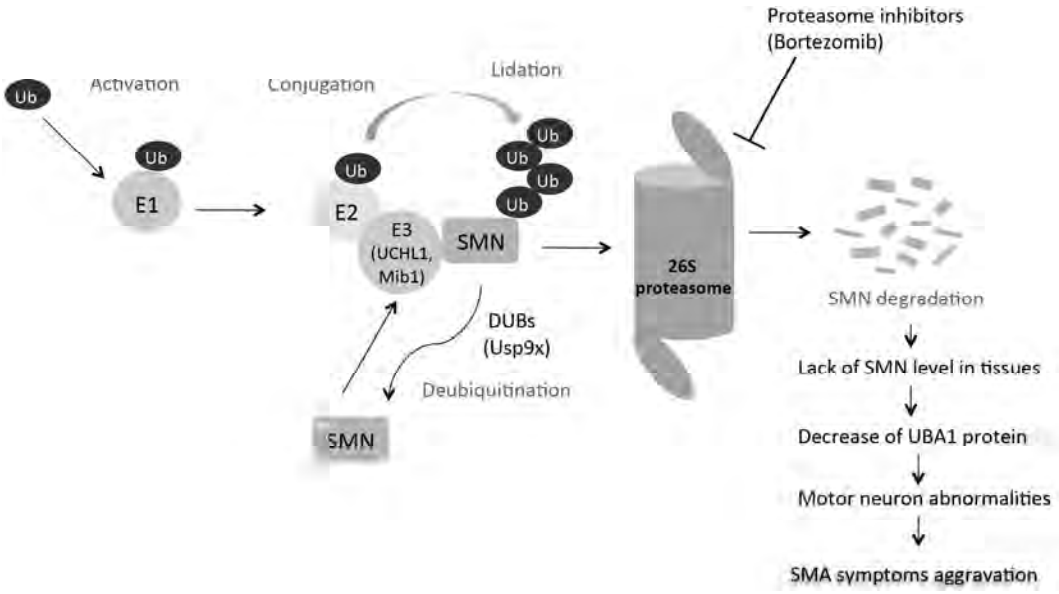

Fig. (3). Main factors playing a role in SMN protein degradation.

neurodegenerative diseases [140]. Previously considered a de-ubiquitinating enzyme, UCHL1 was also reported to function as an Ub ligase and a mono-Ub stabilizer. UCHL1 was shown to directly interact with SMN and over-expression of this enzyme decreased the level of SMN protein, while UCHL1 inhibition in SMA skin fibroblast cells augmented SMN level [139]. Similar effect on SMN protein level has demonstrated another E3 ubiquitin ligase mind bomb 1 (Mib1) previously shown to regulate neuronal morphogenesis [141, 142]. Decreasing level of Mib1 orthologue by RNA interference in *smn-1*-deficient *Caenorhabditis elegans* caused amelioration of the neuromuscular defects [141].

SMN was shown to be a target of a small ubiquitin-related modifier (SUMO1) and has a SUMO-interacting motif in the Tudor domain [143]. Disruption of this motif was demonstrated to impair the assembly of Cajal bodies and might have negative impact on snRNPs maturation and SMA phenotype.

It is interesting to note that whole genome methylation analysis revealed differences in the methylation level between SMA patients and healthy individuals in CpG site 05712748 located in region p34.2 of chromosome 1 (http://genome.ucsc.edu/GRCh37/hg19) [98]. *UBR2* gene, which encodes Ub-protein ligase E3, is situated approximately 3000 bp downstream. This position and its expression can be influenced by methylation of some regulatory elements upstream of the TSS [144].

The substrate specificity for E3 ubiquitin-protein ligases is dictated by variable F-BOX proteins which are the components of SCF (SKP1-CUL1-F-box protein) complex [145]. F-protein that provides the recognition of SMN protein and directs it to the Ub-conjugating system is still unknown. The identification of this F-protein may provide the additional target for SMA therapy based on SMN protein stabilization. We found differences in methylation level between SMA patients and healthy individuals in *FBXL17* and *FBXO3* genes which encode two members of F-box protein family [98]. It is proposed that these F-box proteins might participate in Ub/proteasome degradation of SMN or other proteins involved in SMA pathogenesis. Further studies are needed to determine whether these proteins interact with SMN and understand the way they recognize their substrates. The recognition of substrates to be ubiquitinated has multiple ways. Canonical way for the degradation is the presence of phosphodegron (the group of phosphorylated amino acids) in the target protein. But in some cases, the phosphorylated sites in protein may prevent its degradation, obstructing the access to degron motif for F-proteins [146]. The phosphorylation of SMN protein at threonine 25, threonine 122, and serine 290 was shown to contribute to its degradation inhibition [147]. These modifications might protect the degron domain in SMN protein against F-box protein connection and subsequent degradation.

An important role of ubiquitin-dependent pathways in SMA pathogenesis is supported by the fact that neuromuscular pathology can be ameliorated not only by regulating SMN protein stability, but also by manipulating the level of ubiquitination proteins acting downstream from SMN. Comparative proteomics revealed significantly decreased level of ubiquitin-like modifier activating enzyme 1 (UBA1) in SMA mice. It was shown that UBA1 level is influenced by SMN as a result of direct physical interactions between proteins *in vivo* [148]. UBA1 is responsible for regulating the conjugation of ubiquitin molecules to target proteins, such as β-catenin that was found to accumulate in SMA cells. Suppression of UBA1 recapitulated motor neuron abnormalities observed in SMA animal models, while inhibition of β-catenin signaling rescued motor axon defects [148]. Systemic delivery of UBA1 in SMA mice resulted in improved motor functions and neuromuscular pathology as well as increased survival [149].

These data are consistent with the fact that mutations in the *UBA1* gene cause a related disease X-linked infantile SMA and that ubiquitin proteasome system plays a fundamental role in the pathogenesis of other neurodegenerative

diseases [150-152]. Proteasome inhibitor bortezomib is now examined in mice as a prospective drug for treating SMA based on its beneficial effect on SMN level, motor performance and survival of SMA mice [153]. All these reports indicate a high probability of engagement of ubiquitin proteasome system in the modification of SMA symptoms severity and the possibility of modulation of disease phenotype by therapeutic agents targeted at different components of the ubiquitination pathway.

9. APOPTOSIS PROTEINS AS POSSIBLE MODIFYING FACTORS

Apoptotic pathway plays a crucial role in motor neuron loss and SMA development. In Drosophila S2 cell model, SMN deficiency was demonstrated to activate the caspase-dependent apoptotic pathway, which can be prevented by introducing caspase inhibitor [154]. The upregulation of Fas ligand-mediated apoptosis and increased caspase-8 and -3 activation was also present in induced pluripotent stem cell model of SMA, confirming the possible involvement of these factors in motor neuron degradation [155]. SMN protein was shown to possess anti-apoptotic activity, inhibiting caspase-3 activation, while mutant SMN proteins like SMNΔ7 or SMN-Y272C were found to lack this activity and according to some studies, are even proapoptotic, causing increased motor neuron loss and mice mortality [156]. Nevertheless, proapoptotic activity of SMNΔ7 was not verified in other studies and taking into account high instability of this protein form the extent of its influence on SMA phenotype is doubtful [157, 158].

Previously, Bcl-2 family proteins known to be key regulators of apoptosis were demonstrated to play a role in the pathogenesis of common motor neuron disease amyotrophic lateral sclerosis [159]. Bcl-2 family includes proapoptotic proteins (such as Bax, Bak, Bcl-xS) as well as antiapoptotic ones (such as Bcl-2, Bcl-xL) [160]. The expression study of Bcl-2 and Bcl-X proteins in motor neurons of SMA fetuses revealed a significant decrease in both protein levels, which may enhance neuronal apoptosis [161]. At the same time, the level of Bax protein was elevated in the spinal cord of SMA mice. To elucidate the role of Bax protein in SMA development model, mice were breed with Bax knockout mice. Littermate mice deficient for Bax gene had longer lifespan and less severe form of the disease [162].

The role of Bcl-2 in SMA pathogenesis is also supported by the fact that the Bcl-2 regulating protein WT-1 is expressed at lower levels in SMA mouse model [163]. Anderton and colleagues reported the co-regulation of SMN and Bcl-xL expression and their antiapoptotic effect in SH-SY5Y cells [164]. Over-expression of Bcl-xL ameliorated motor functions and prolonged the lifespan in SMA mice [165]. Similarly, high Bcl-xL level rescued mouse motor neurons from neurite degeneration and cell death *in vitro* [166].

SMN protein binds p53 - an important factor regulating neuronal apoptotic death. The decrease of SMN-p53 binding was observed in SMA-derived fibroblasts. Truncated SMN produced by *SMN2* gene in SMA patients does not manage to bind p53 properly. This leads to accumulation of p53 protein in the nucleolus rather than in Cajal bodies, typical for normal cells. The result is the high accessibility of p53, which can trigger activation of p53-dependent apoptosis of motor neurons [167].

Involvement in regulation of SMA severity was determined for members of MAP kinase cascades that are implicated in the generation of different cellular responses including apoptosis [168]. Apoptosis Signal-regulating Kinase 1 (ASK1), a ubquitiously expressed MAP Kinase Kinase Kinase (MAPKKK) that activates JNK and p38 MAP kinase pathways, was shown to stabilize SMN protein and upregulate neurite outgrowth [169]. Interestingly, this effect was not due to the kinase activity of ASK1, but was caused by protecting SMN from degradation by inhibiting its polyubiquitination. Considering the involvement of ASK1 in other neuronal diseases, such as Alzheimer's disease and Huntington's disease, ASK1 may play a role in SMA pathogenesis [170]. P38 MAPK pathway is known to play a role in modulation of apoptosis. It also functions as a regulator of gene expression. It affects mRNAs that comprise AU-rich element (ARE) in the 3'-UTR region through specific ARE-binding proteins [171]. SMN transcript that contains such ARE was demonstrated to be stabilized by the activation of p38 pathway. ARE-binding protein HuR stimulated by p38 interacts with SMN mRNA resulting in 2-3-fold increase in SMN transcript and protein levels in neuronal-cell lines [172]. Upregulation of p38 pathway in SMA mice prolonged lifespan and improved motor function of animals [173]. JNK signaling pathway was shown to be activated in SMA. JNK3 isoform is specifically expressed in neurons and is 10-fold more phosphorylated in cells lacking SMN. It was proposed that decrease in SMN level leads to cell stress resulted in the activation of JNK pathway and motor neuron apoptosis. JNK3 knockdown reduced degeneration of SMN-deficient cultured neurons and amelioration of symptoms in SMA mice [174].

Loganin was found to be a potential neuroprotective candidate that decreased the number of apoptotic cells and neurite damage in SMA cell model *via* stimulation of the expression of SMN, Gemin2, Akt and Bcl-2 [175]. IGF-1 was shown to play a key role in loganin neuroprotection. Previous studies demonstrated that IGF-1 overexpressed in muscle of SMA mice resulted in enlarged myofibers and prolonged survival [176]. Systemic administration of AAV1 vector encoding IGF-1 ameliorated motor neuron pathology and NMJ defects and improved motor function of SMA mice [177]. Strikingly, controversial results were obtained by Biondi with colleagues, demonstrating that reduction of IGF-1 suppressed the apoptotic processes, stimulated SMN protein expression in the spinal cords of SMA mice and in human myotubes [178]. Such contradictory responses on changing of IGF-1 level might be caused by the unexpected shift between ERK, JAK and AKT pathways, all regulated by IGF-1 and imbalanced in SMA condition [178]. Anyways, these aspects should be taken into account if designing further IGF-1-based treatment experiments on SMA models.

We have previously found the changes in the methylation profile between SMA patients and healthy individuals for several genes which are considered to be connected with the apoptosis process: *OPN3*, *CDK2AP1*, *CYTSB*, *PPP1R13L* and *WWTR1* [98]. *OPN3* is involved in the regulation of cell apoptosis through the Akt/Bcl2/Bax pathway [179].

CDK2AP1 and *CYTSB* genes might be implicated in the induction of apoptosis process [180, 181]. *PPP1R13L* is known as one of the most evolutionarily conserved inhibitors of p53 protein. *WWTR1* is a transcriptional coactivator shown to promote cell proliferation and inhibit apoptosis [182]. Further studies are needed to understand if some of these genes actually have any role in the context of SMA pathogenesis.

Taking into account the data on apoptosis proteins participating in regulation of *SMN* expression, neuronal viability, neurite outgrowth, SMN protein stabilization, these proteins seem to be interesting targets for further research of SMA modifiers. Manipulating the level of some of these factors in SMA mouse model caused changes in motor phenotype and duration of survival. Apoptotic pathway is known to play an important role in the pathogenesis of other neurodegenerative diseases and some apoptosis proteins are discussed as possible therapeutic targets for these diseases [159, 170].

10. SMN INTERACTOME AND SMN TARGET GENES AS POSSIBLE SMA MODULATORS

Being SMN as a ubiquitous protein, it is natural that decrease in SMN level has important consequences with respect to downstream molecular pathways and proteins. In order to achieve better understanding of the processes underlying SMA and identifying new potential targets for SMA therapy that can enhance SMN-focused therapy, SMN interactome has been examined. Large scale genetic and proteomic studies added by bioinformatic analyses enabled to reveal multiple genes and factors demonstrating conserved changes in SMA condition [21, 183]. As noticed by Sen and colleagues based on the results of their study, reduction of SMN seems to affect a wide range of programs regulating the development and maintenance of the whole neuromuscular system, such as ion channels functioning, synaptic vesicle recycling and intrinsic cellular functions control [183].

Among the important findings, one was identification of proteins, such as UBA1 (discussed above), GAPDH, GAP43, NCAM, LMNA, ANXA2, that demonstrated conserved changes in expression across several proteomic studies of SMA [21]. Lack of GAP43 protein essential for axonal pathfinding was observed in growth cones of SMA mice and restoration of GAP43 level rescued axonal outgrowth [184]. The cause of GAP43 deficiency was GAP43 mRNA mislocalization due to loss of SMN function. NCAM, a known regulator of neuronal growth, has been described in connection with different neurological disorders and seems to be an interesting target for further studies in relation to SMA phenotype modification [185]. Mutations in LMNA, a published interactor of SMN, were shown to be involved in the development of muscular dystrophies and mimic spinal muscular atrophy [186, 187].

Interesting data have been obtained concerning SMN target genes that displayed aberrant expression and were able to rescue SMA pathology when expressed normally. SMN was shown to control FGF receptor Htl and activation of FGF signaling demonstrated protective effect on neuromuscular junction defects in Drosophila model of SMA [188]. Impairment of SMN function in U snRNP assembly was found to affect splicing of critical neuronal genes that also influenced SMA phenotype. Among these genes are *stasimon* and *neurexin2a*, demonstrating altered expression and splicing in different animal models of SMA [189, 190]. Knock-down of *stasimon* and *neurexin2a* in animal models phenocopied motor axon and NMJ defects observed in SMA condition revealing these genes as plausible mediators of motoneuron pathology in SMA. Increase in Stasimon expression rescued motor neuron development and NMJ transmission in Drosophila and zebrafish smn mutants [189]. *Chondrolectin* was also found to be alternatively spliced in SMA mouse spinal cord and restoration of its expression demonstrated positive impact on neurite outgrowth in cell and zebrafish models of SMA [191].

Gemins, the known partners of SMN in chaperoning the assembly of snRNPs, have been considered as SMA modifying candidates and potential therapeutic targets for the disease [192]. Decreased level of Gemin2 in Smn heterozygous mice induced an enhanced motor neuron degeneration while specific knockdown of Gemin in motor units mimicked motor neuron pathology observed in Drosophila model of SMA [193, 194]. Interestingly, besides interacting with SMN protein in one complex, Gemin5 was shown to be an activator of SMN translation, thus influencing the level of SMN as well as its functionality [195].

Extensive obtained data in SMN interactome studies provide new information on molecular mechanisms perturbed in SMA but these data should be verified carefully by further experiments, highlighting “core” regulators of SMA pathogenesis and focusing on them.

11. ZPR1 PROTEIN AS MODULATOR OF SMA SEVERITY

ZPR1 is a zinc finger protein found both in the cytoplasm and nucleus and known to be involved in cell cycle regulation, pre-mRNA splicing, myelination and axonogenesis [196, 197]. In the nucleus ZPR1 co-localizes with SMN and their interaction is essential for SMN accumulation in subnuclear bodies [198]. Together with snurportin 1, importin β, SMN and Sm proteins, ZPR1 forms the cytoplasmic complex required for snRNPs transport in the nucleus and snRNPs biogenesis [199]. SMA patients were shown to have decreased level of ZPR1 protein [57]. The interaction between SMN and ZPR1 was also disrupted in SMA patient cells. Downregulation of ZPR1 caused snRNPs loss and inhibition of pre-mRNA splicing. Since ZPR1 possesses functions similar to that performed by SMN, reduced level of ZPR1 leads to the defects typical for SMN-deficient cells. *Zpr1* gene suppression in NSC-34 cells resulted in defective axons and growth cones [200]. ZPR1-deficient mice exhibited axonal degeneration, progressive motor neurons loss and phenotype that resembled those of SMA mice [197].

Increased caspase activation was found in ZPR1 deficient mouse embryos and their cells had apoptotic morphology [200]. Since apoptotic degeneration of motor neurons is characteristic for SMA patients and these patients have impaired expression of ZPR1, it has been proposed that ZPR1 deficiency might influence motor neuron death. This supposition was confirmed by observation of caspase activation and cell death in cultures of neurons transfected with *Zpr1* siRNA [200].

The study of influence of Zpr1 gene expression level on SMA mice phenotype showed that low level of ZPR1 protein contributed to the disease symptoms aggravation and the decline in animals' lifespan [201]. In detail, the decrease of ZPR1 protein causes hypermyelination and degeneration of axons in phrenic nerves reinforcing breath dysfunction in SMA. While the overexpression of ZPR1 leads to the increase of SMN and its nuclei accumulation. Thus the level of ZPR1 may modulate SMA severity.

12. PROLACTIN AS POSSIBLE MODIFYING FACTOR

Prolactin is a polypeptide hormone that activates JAK-STAT5 pathway and was shown to influence *SMN* gene expression [20]. STATs are proteins activated by JAK (Janus kinases) in response to cytokine stimulation and are involved in the regulation of different cellular responses including differentiation, cell growth and apoptosis. STAT5 plays a key role in CNS formation, particularly in the development of the spinal cord [202]. Constitutive activation of this protein was demonstrated to stimulate *SMN2* gene expression in SMA-like cells, resulting in increase in SMN protein level and number of gems, as well as amelioration of axonal defects in cultured SMA-like motor neurons [203]. Similar effect was observed in SMA mouse model after stimulation of STAT5 pathway by prolactin treatment [20]. Treated mice demonstrated improved motor function and prolonged survival. Other STAT5 activators such as histone deacetylase inhibitors have also been described, yet prolactin seems more safe and efficient [204]. Also the ability of prolactin to cross the blood brain barrier as well as the distribution of its receptors throughout the CNS make this hormone a perspective candidate for SMA therapy [20].

13. EXTRINSIC FACTORS INFLUENCING SMA PHENOTYPE

External factors have also been proposed to possibly contribute to SMA severity. In some studies it was shown that supplementary nutrition in combination with HDAC inhibitor as well as meal composition has positive impact on the duration of survival and motor functions of SMA mice, though these observations have not been fully replicated [205, 206].

Hypoxia was observed to negatively regulate SMA pathology. Respiratory distress is a feature of many severe SMA type I cases, leading to infant death, and ventilatory support was shown to increase survival [207, 208]. The study of hypoxia effect on SMA cell model demonstrated reduction in full-length *SMN2* transcripts and SMN protein level. This effect was found to be connected with upregulation of hnRNP A1 and Sam68 - factors promoting aberrant *SMN2* splicing [209]. Treating SMNΔ7 mice with hyperoxia resulted in weight gain and improved motor function. Interestingly, ZPR1 protein considered to be a SMA modifier is also likely implicated in the regulation of respiratory function. Reduced level of this protein observed in SMA mice resulted in impaired functionality of phrenic nerves innervating diaphragm [201]. Thus changing the level of factors mediating effect of hypoxia in SMA may influence disease severity.

CONCLUSION

High variability of clinical courses of SMA patients indicates that disease manifestation is apparently dependent on modifying factors. By now, *SMN2* gene copy number can contribute as supplementary criterion for SMA severity determination, but its prediction accuracy is not absolute [38, 49, 59, 210]. Detection of additional modifiers on the early stages of disease development can make the prognosis of clinical course more precise. Moreover, it can help in predicting the treatment efficacy, as it was shown to be dependent on several genes [54, 211]. Results obtained in studies of SMA modifiers provide new information about peculiarities of SMA development that lead to better understanding of cellular processes altered in SMA condition. It is gratifying to highlight recent studies demonstrating endocytosis as a major mechanism disrupted in SMA that found application in new treatment strategies [108, 109, 123]. Today, therapy approaches based on knowledge gained in study of different pathways and proteins involved in SMA pathogenesis are tested in SMA models. As vividly noticed and demonstrated by Kaifer and colleagues in their study high variation in SMA clinical spectrum may require a combinatorial treatment that includes SMN-independent approach together with increase of SMN level. Such therapy approach that besides stimulation of SMN production directed to compensate for a secondary defects in SMA seems more efficient than aimed at correction of SMN level solely [109, 123, 124]. This emerging revelation emphasizes the relevance of investigation of new SMA modifiers, that can be involved in different molecular pathways and contribute differently to the disease progression, but each of them might be important to study.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Ogino, S.; Wilson, R.B. Genetic testing and risk assessment for Spinal Muscular Atrophy (SMA). *Hum. Genet.*, **2002**, *111*(6), 477-500.

[2] Markowitz, J.A.; Singh, P.; Darras, B.T. Spinal Muscular Atrophy: A clinical and research update. *Pediatr. Neurol.*, **2012**, *46*(1), 1-12.

[3] Sugarman, E.A.; Nagan, N.; Zhu, H.; Akmaev, V.R.; Zhou, Z.; Rohlfs, E.M.; Flynn, K.; Hendrickson, B.C.; Scholl, T.; Sirko-Osadsa, D.A.; Allitto, B.A. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: Clinical laboratory analysis of > 72 400 specimens. *Eur. J. Hum. Genet.*, **2012**, *20*(1), 27-32.

[4] Zerres, K.; Wirth, B.; Rudnik-Schöneborn, S. Spinal Muscular Atrophy-clinical and genetic correlations. *Neuromuscul. Disord.*, **1997**, *7*(3), 202-207.

[5] Russman, B.S. Spinal Muscular Atrophy: Clinical classification and disease heterogeneity. *J. Child Neurol.*, **2007**, *22*(8), 946-951.

[6] Lunn, M.R.; Wang, C.H. Spinal muscular atrophy. *Lancet*, ***2008***, *371*(9630), 2120-2133. Available from: http://www.thelancet.com/

journals/lancet/article/PIIS0140-6736(08)60921-6/fulltext

[7] Wirth, B.; Brichta, L.; Schrank, B.; Lochmüller, H.; Blick, S.; Baasner, A.; Heller, R. Mildly affected patients with Spinal Muscular Atrophy are partially protected by an increased SMN2 copy number. *Hum. Genet*., **2006**, *119*(4), 422-428.

[8] Lefebvre, S.; Bürglen, L.; Reboullet, S.; Clermont, O.; Burlet, P.; Viollet, L.; Benichou, B.; Cruaud, C.; Millasseau, P.; Zeviani, M.; Le Paslier, D.; Frézal, J.; Cohen, D.; Weissenbach, J.; Munnich, A.; Melki, J. Identification and characterization of a Spinal Muscular Atrophy-determining gene. *Cell*, **1995**, *80*(1), 155-165.

[9] Wirth, B. An update of the mutation spectrum of the survival motor neuron gene (SMN1) in autosomal recessive spinal muscular atrophy (SMA). *Hum. Mutat*., **2000**, *15*(3), 228-237.

[10] Wirth, B.; Schmidt, T.; Hahnen, E.; Rudnik-Schöneborn, S.; Krawczak, M.; Müller-Myhsok, B.; Schönling, J.; Zerres, K. *De novo* rearrangements found in 2% of index patients with spinal muscular atrophy: Mutational mechanisms, parental origin, mutation rate, and implications for genetic counseling. *Am. J. Hum. Genet*., **1997**, *61*(5), 1102-1111.

[11] Lorson, C.L.; Hahnen, E.; Androphy, E.J.; Wirth, B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. *Proc. Natl. Acad. Sci. U.S.A*., **1999**, *96*(11), 6307-6311.

[12] Monani, U.R.; Lorson, C.L.; Parsons, D.W.; Prior, T.W.; Androphy, E.J.; Burghes, A.H.M.; McPherson, J.D. A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. *Hum. Mol. Genet*., **1999**, *8*(7), 1177-1183.

[13] Burghes, A.H.M.; Beattie, C.E. Spinal muscular atrophy: Why do low levels of survival motor neuron protein make motor neurons sick? *Nat. Rev. Neurosci*., **2009**, *10*(8), 597-609.

[14] Liu, Q.; Fischer, U.; Wang, F.; Dreyfuss, G. The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. *Cell*, **1997**, *90*(6), 1013-1021.

[15] Iwahashi, H.; Eguchi, Y.; Yasuhara, N.; Hanafusa, T.; Matsuzawa, Y.; Tsujimoto, Y. Synergistic anti-apoptotic activity between Bcl-2 and SMN implicated in spinal muscular atrophy. *Nature*, **1997**, *390*, 413-417. Available from: https://www.nature.com/articles/37144

[16] Rossoll, W.; Jablonka, S.; Andreassi, C.; Kröning, A.K.; Karle, K.; Monani, U.R.; Sendtner, M. SMN, the spinal muscular atrophy-determining gene product, modulates axon growth and localization of β-actin mRNA in growth cones of motoneurons. *J. Cell Biol*., **2003**, *163*(4), 801-812.

[17] Kariya, S.; Park, G.H.; Maeno-Hikichi, Y.; Leykekhman, O.; Lutz, C.; Arkovitz, M.S.; Landmesser, L.T.; Monani, U.R. Reduced SMN protein impairs maturation of the neuromuscular junctions in mouse models of spinal muscular atrophy. *Hum. Mol. Genet*., **2008**, *17*(16), 2552-2569.

[18] Sumner, C.J. Molecular mechanisms of spinal muscular atrophy. *J. Child Neurol*., **2007**, *22*(8), 979-989.

[19] Lunke, S.; El-Osta, A. The emerging role of epigenetic modifications and chromatin remodeling in spinal muscular atrophy. *J. Neurochem*., **2009**, *109*(6), 1557-1569.

[20] Farooq, F.; Molina, F.A.; Hadwen, J.; MacKenzie, D.; Witherspoon, L.; Osmond, M.; Holcik, M.; MacKenzie, A. Prolactin increases SMN expression and survival in a mouse model of severe spinal muscular atrophy *via* the STAT5 pathway. *J. Clin. Invest*., **2011**, *121*(8), 3042-3050.

[21] Fuller, H.R.; Gillingwater, T.H.; Wishart, T.M. Commonality amid diversity: Multi-study proteomic identification of conserved disease mechanisms in spinal muscular atrophy. *Neuromuscul. Disord*., **2016**, *26*(9), 560-569.

[22] Oprea, G.E.; Kröber, S.; McWhorter, M.L.; Rossoll, W.; Müller, S.; Krawczak, M.; Bassell, G.J.; Beattie, C.E.; Wirth, B. Plastin 3 is a protective modifier of autosomal recessive spinal muscular atrophy. *Science*, **2008**, *320*(5875), 524-527.

[23] Bowerman, M.; Anderson, C.L.; Beauvais, A.; Boyl, P.P.; Witke, W.; Kothary, R. SMN, Profilin IIa and Plastin 3: A link between the deregulation of actin dynamics and SMA pathogenesis. *Mol. Cell. Neurosci*., **2009**, *42*(1), 66-74.

[24] Amara, A.; Adala, L.; Ben Charfeddine, I.; Mamaï, O.; Mili, A.; Lazreg, T.B.; H'Mida, D.; Amri, F.; Salem, N.; Boughammura, L.; Saad, A.; Gribaa, M. Correlation of SMN2, NAIP, p44, H4F5 and occludin genes copy number with spinal muscular atrophy phenotype in Tunisian patients. *Eur. J. Paediatr. Neurol*., **2012**, *16*(2), 167-174.

[25] Velasco, E.; Valero, C.; Valero, A.; Moreno, F.; Hernández-Chico, C. Molecular analysis of the SMN and NAIP genes in Spanish Spinal Muscular Atrophy (SMA) families and correlation between number of copies of cBCD541 and SMA phenotype. *Hum. Mol. Genet*., **1996**, *5*(2), 257-263.

[26] Salahshourifar, I.; Shafeghati, Y.; Golkar, Z.; Najmabadi, H. Molecular analysis of the neuronal apoptosis inhibitory protein gene in families with spinal muscular atrophy. *Arch. Iran. Med*., **2007**, *10*(4), 509-513.

[27] Brkušanin, M.; Kosać, A.; Jovanović, V.; Pešović, J.; Brajušković, G.; Dimitrijević, N.; Todorović, S.; Romac, S.; Milić Rašić, V.; Savić-Pavićević, D. Joint effect of the SMN2 and SERF1A genes on childhood-onset types of spinal muscular atrophy in Serbian patients. *J. Hum. Genet*., **2015**, *60*(11), 723-728.

[28] Scharf, J.M.; Endrizzi, M.G.; Wetter, A.; Huang, S.; Thompson, T.G.; Zerres, K.; Dietrich, W.F.; Wirth, B.; Kunkel, L.M. Identification of a candidate modifying gene for spinal muscular atrophy by comparative genomics. *Nat. Genet*., **1998**, *20*(1), 83-86.

[29] Loan, J.J.M.; Connolly, S.D.; Haunschmidt, D.Z.; Bell, S.B.; Clarke, S.A.; Kelly, J.; Oswald, A.J.; Rae, V. Treatment options in motor neuron disease: Amyotrophic lateral sclerosis and spinal muscular atrophy. *J. Young Invest.*, **2012**, *24*(4), 33-54.

[30] Campbell, L.; Potter, A.; Ignatius, J.; Dubowitz, V.; Davies, K. Genomic variation and gene conversion in spinal muscular atrophy: Implications for disease process and clinical phenotype. *Am. J. Hum. Genet*., **1997**, *61*(1), 40-50.

[31] Ogino, S.; Wilson, R.B. Spinal muscular atrophy: Molecular genetics and diagnostics. *Expert Rev. Mol. Diagn*., **2004**, *4*(1), 15-29.

[32] Glotov, A.S.; Kiselev, A.V.; Ivaschenko T.E.; Baranov, V.S. Analysis of deletions in SMN1, SMN2, and NAIP genes in spinal muscular atrophy patients from the northwestern region of Russia. *Russ. J. Genet*., **2001**, *37*(8), 968-971.

[33] Wirth, B.; Garbes, L.; Riessland, M. How genetic modifiers influence the phenotype of spinal muscular atrophy and suggest future therapeutic approaches. *Curr. Opin. Genet. Dev*., **2013**, *23*(3), 330-338.

[34] Hofmann, Y.; Lorson, C.L.; Stamm, S.; Androphy, E.J.; Wirth, B. Htra2-Beta 1 stimulates an exonic splicing enhancer and can restore full-length SMN expression to survival motor neuron 2 (SMN2). *Proc. Natl. Acad. Sci. U.S.A*., **2000**, *97*(17), 9618-9623.

[35] Simic, G. Pathogenesis of proximal autosomal recessive spinal muscular atrophy. *Acta Neuropathol*., **2008**, *116*(3), 223-234.

[36] Vitali, T.; Sossi, V.; Tiziano, F.; Zappata, S.; Giuli, A.; Paravatou-Petsotas, M.; Neri, G.; Brahe, C. Detection of the survival motor neuron (SMN) genes by FISH: Further evidence for a role for SMN2 in the modulation of disease severity in SMA patients. *Hum. Mol. Genet*., **1999**, *8*(13), 2525-2532.

[37] Van der Steege, G.; Grootscholten, P.M.; Cobben, J.M.; Zappata, S.; Scheffer, H.; den Dunnen, J.T.; van Ommen, G.J.; Brahe, C.; Buys, C.H. Apparent gene conversions involving the SMN Gene in the region of the spinal muscular atrophy locus on chromosome 5. *Am. J. Hum. Genet*., **1996**, *59*(4), 834-838.

[38] Feldkötter, M.; Schwarzer, V.; Wirth, R.; Wienker, T.F.; Wirth, B. Quantitative analyses of SMN1 and SMN2 based on real-time lightCycler PCR: Fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. *Am. J. Hum. Genet*., **2002**, *70*(2), 358-368.

[39] Harada, Y.; Sutomo, R.; Sadewa, A.H.; Akutsu, T.; Takeshima, Y.; Wada, H.; Matsuo, M.; Nishio, H. Correlation between SMN2 copy number and clinical phenotype of spinal muscular atrophy: Three SMN2 copies fail to rescue some patients from the disease severity. *J. Neurol.*, **2002**, *249*(9), 1211-1219.

[40] Grotto, S.; Cuisset, J.; Guerrot, A.; Journel, H.; Morin, G.; Plessis, G.; Renolleau, S. Type 0 spinal muscular atrophy: Further delineation of prenatal and postnatal features in 16 patients. *J. Neuromusc. Dis.*, **2016**, *3*(4), 487-495.

[41] Jedrzejowska, M.; Borkowska, J.; Zimowski, J.; Kostera-Pruszczyk, A.; Milewski, M.; Jurek, M.; Sielska, D.; Kostyk, E.; Nyka, W.; Zaremba, J.; Hausmanowa-Petrusewicz, I. Unaffected patients with a homozygous absence of the SMN1 gene. *Eur. J. Hum. Genet*., **2008**, *16*(8), 930-934.

[42] Zheleznyakova, G.Y.; Kiselev, A.V.; Vakharlovsky, V.G.; Rask-Andersen, M.; Chavan, R.; Egorova, A.A.; Schiöth, H.B.; Baranov, V.S. Genetic and expression studies of SMN2 gene in Russian pa-

tients with spinal muscular atrophy type II and III. *BMC Med. Genet*., **2011**, *12*(1), 96. Available from: https://bmcmedgenet.biomedcentral.com/articles/10.1186/1471-2350-12-96

[43] Rudnik-Schöneborn, S.; Berg, C.; Zerres, K.; Betzler, C.; Grimm, T.; Eggermann, T.; Eggermann, K.; Wirth, R.; Wirth, B.; Heller, R. Genotype-phenotype studies in infantile spinal muscular atrophy (SMA) type I in Germany: Implications for clinical trials and genetic counselling. *Clin. Genet*., **2009**, *76*(2), 168-178.

[44] Petit, F.; Cuisset, J.M.; Rouaix-Emery, N.; Cancés, C.; Sablonnière, B.; Bieth, E.; Moerman, A.; Sukno, S.; Hardy, N.; Holder-Espinasse, M.; Manouvrier-Hanu, S.; Vallée, L. Insights into genotype-phenotype correlations in spinal muscular atrophy: A retrospective study of 103 patients. *Musc. Nerve*, **2011**, *43*(1), 26-30.

[45] Elsheikh, B.; Prior, T.; Zhang, X.; Miller, R.; Kolb, S.J.; Moore, D.A.N.; Bradley, W.; Barohn, R.; Bryan, W.; Gelinas, D.; Iannaccone, S.; Leshner, R.; Mendell, J.R.; Mendoza, M.; Russman, B.; Smith, S.; King, W.; Kissel, J.T. An analysis of disease severity based on SMN2 copy number in adults with spinal muscular atrophy. *Musc. Nerve*, **2009**, *40*(4), 652-656.

[46] Glanzman, A.M.; O'Hagen, J.; McDermott, M.; Martens, W.; Flickinger, J.; Riley, S.; Quigley, J.; Montes, J.; Dunaway, S.; Deng, L.; Chung, W.; Tawil, R.; Darras, B.; De Vivo, D.; Kaufmann, P.; Finkel, R. Validation of the expanded hammersmith functional motor scale in spinal muscular atrophy type II and III. *J. Child Neurol*., **2011**, *26*(12), 1499-1507.

[47] Swoboda, K.J.; Prior, T.W.; Scott, C.B.; McNaught, T.P.; Wride, M.C.; Reyna, S.P.; Bromberg, M.B. Natural history of denervation in SMA: Relation to age, SMN2 copy number, and function. *Ann. Neurol*., **2005**, *57*(5), 704-712.

[48] Rudnik-Schöneborn, S.; Heller, R.; Berg, C.; Betzler, C.; Grimm, T.; Eggermann, T.; Eggermann, K.; Wirth, R.; Wirth, B.; Zerres, K. Congenital heart disease is a feature of severe infantile spinal muscular atrophy. *J. Med. Genet*., **2008**, *45*(10), 635-638.

[49] Parra, J.; Alias, L.; Also-Rallo, E.; Martínez-Hernández, R.; Senosiain, R.; Medina, C.; Alejos, O.; Rams, N.; Amenedo, M.; Ormo, F.; Jesús Barceló, M.; Calaf, J.; Baiget, M.; Bernal, S.; Tizzano, E.F. Evaluation of fetal nuchal translucency in 98 pregnancies at risk for severe spinal muscular atrophy: Possible relevance of the SMN2 copy number. *J. Matern. Fetal. Neonatal Med*., **2012**, *25*(8), 1246-1249.

[50] Monani, U.R.; Sendtner, M.; Coovert, D.D.; Parsons, D.W.; Andreassi, C.; Le, T.T.; Jablonka, S.; Schrank, B.; Rossoll, W.; Prior, T.W.; Morris, G.E.; Burghes, A.H. The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in SMN(-/-) mice and results in a mouse with spinal muscular atrophy. *Hum. Mol. Genet*., **2000**, *9*(3), 333-339.

[51] Hsieh-Li, H.M.; Chang, J.G.; Jong, Y.J.; Wu, M.H.; Wang, N.M.; Tsai, C.H.; Li, H. A mouse model for spinal muscular atrophy. *Nat. Genet.*, **2000**, *24*(1), 66-70.

[52] Brichta, L.; Hofmann, Y.; Hahnen, E.; Siebzehnrubi, F.A.; Raschke, H.; Blumcke, I.; Eyupoglu, I.Y.; Wirth, B. Valproic acid increases the SMN2 protein level: A well-known drug as a potential therapy for spinal muscular atrophy. *Hum. Mol. Genet*., **2003**, *12*(19), 2481-2489.

[53] Weihl, C.C.; Connolly, A.M.; Pestronk, A. Valproate may improve strength and function in patients with type III/IV spinal muscle atrophy. *Neurology*, **2006**, *67*(3), 500-501.

[54] Tiziano, F.D.; Lomastro, R.; Pinto, A.M.; Messina, S.; D'Amico, A.; Fiori, S.; Angelozzi, C.; Pane, M.; Mercuri, E.; Bertini, E.; Neri, G.; Brahe, C. Salbutamol increases survival motor neuron (SMN) transcript levels in leucocytes of spinal muscular atrophy (SMA) patients: Relevance for clinical trial design. *J. Med. Genet*., **2010**, *47*(12), 856-858.

[55] Baranov, V.S.; Kiselev, A.V.; Vakharlovsky, V.G.; Zheleznjakova, G.J.; Komantzev, V.N.; Malisheva, O.V.; Glotov, A.S.; Ivashchenko, T.E.; Baranov, A.N. Molecular genetic basis of proximal spinal muscular atrophy and experience in its pharmaceutical treatment. *Russ. J. Genet.*, **2008**, *44*(10), 1148-1159.

[56] Prior, T.W.; Swoboda, K.J.; Scott, H.D.; Hejmanowski, A.Q. Homozygous SMN1 deletions in unaffected family members and modification of the phenotype by SMN2. *Am. J. Med. Genet*., **2004**, *130A*(3), 307-310.

[57] Helmken, C.; Hofmann, Y.; Schoenen, F.; Oprea, G.; Raschke, H.; Rudnik-Schöneborn, S.; Zerres, K.; Wirth, B. Evidence for a modifying pathway in sma discordant families: Reduced SMN level decreases the amount of its interacting partners and Htra2-beta1. *Hum. Genet*., **2003**, *114*(1), 11-21.

[58] McAndrew, P.E.; Parsons, D.W.; Simard, L.R.; Rochette, C.; Ray, P.N.; Mendell, J.R.; Prior, T.W.; Burghes, A.H. Identification of proximal spinal muscular atrophy carriers and patients by analysis of SMNT and SMNC gene copy number. *Am. J. Hum. Genet*., **1997**, *60*(6), 1411-1422.

[59] Cuscó, I.; Barceló, M.J.; Rojas-García, R.; Illa, I.; Gámez, J.; Cervera, C.; Pou, A.; Izquierdo, G.; Baiget, M.; Tizzano, E.F. SMN2 copy number predicts acute or chronic spinal muscular atrophy but does not account for intrafamilial variability in siblings. *J. Neurol*., **2006**, *253*(1), 21-25.

[60] Naryshkin, N.; Weetall, M.; Dakka, A.; Narasimhan, J.; Zhao, X.; Feng, Z.; Ling, K.; Karp, G.; Qi, H.; Woll, M.; Chen, G.; Zhang, N.; Gabbeta, V.; Vazirani, P.; Bhattacharyya, A.; Furia, B.; Risher, N.; Sheedy, J.; Kong, R.; Ma, J.; Turpoff, A.; Lee, C.; Zhang, X.; Moon, Y.C.; Trifillis, P.; Welch, E.; Colacino, J.; Babiak, J.; Almstead, N.; Peltz, S.; Eng, L.; Chen, K.; Mull, J.; Lynes, M.; Rubin, L.; Fontoura, P.; Santarelli, L.; Haehnke, D.; McCarthy, K.; Schmucki, R.; Ebeling, M.; Sivaramakrishnan, M.; Ko, C.; Paushkin, S.; Ratni, H.; Gerlach, I.; Ghosh, A.; Metzger, F. SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. *Science*, **2014**, *345*(6197), 688-693. Available from: http://science.sciencemag.org/content/345/6197/688

[61] Bebee, T.W.; Gladman, J.T.; Chandler, D.S. Splicing regulation of the survival motor neuron genes and implications for treatment of spinal muscular atrophy. *Front. Biosci.,* **2010**, *15*, 1191-1204. Available from: http://www.bioscience.org/2010/ v15/af/3670/list.htm

[62] Kashima, T.; Manley, J.L. A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. *Nat. Genet*., **2003**, *34*(4), 460-463.

[63] Cartegni, L.; Krainer, A.R. Disruption of an SF2/ASF-dependent exonic splicing enhancer in SMN2 causes spinal muscular atrophy in the absence of SMN1. *Nat. Genet*., **2002**, *30*(4), 377-384.

[64] Vogel, G.; Richard, S. Emerging roles for Sam68 in adipogenesis and neuronal development. *RNA Biol.*, **2012**, *9*(9), 1129-1133.

[65] Pedrotti, S.; Bielli, P.; Paronetto, M.P.; Ciccosanti, F.; Fimia, G.M.; Stamm, S.; Manley, J.L.; Sette, C. The splicing regulator Sam68 binds to a novel exonic splicing silencer and functions in SMN2 alternative splicing in spinal muscular atrophy. *EMBO J*., **2010**, *29*(7), 1235-1247.

[66] Pagliarini, V.; Pelosi, L.; Bustamante, M.B.; Nobili, A.; Berardinelli, M.G.; D'Amelio, M.; Musarò, A.; Sette, C. SAM68 is a physiological regulator of SMN2 splicing in spinal muscular atrophy. *J. Cell Biol*., **2015**, *211*(1), 77-90.

[67] Hofmann, Y.; Wirth, B. hnRNP-G promotes exon 7 inclusion of survival motor neuron (SMN) *via* direct interaction with Htra2-beta1. *Hum. Mol. Genet.*, **2002**, *11*(17), 2037-2049.

[68] Young, P.J.; DiDonato, C.J.; Hu, D.; Kothary, R.; Androphy, E.J.; Lorson, C.L. SRp30c-dependent stimulation of survival motor neuron (SMN) exon 7 inclusion is facilitated by a direct interaction with hTra2 beta 1. *Hum. Mol. Genet*., **2002**, *11*(5), 577-587.

[69] Bose, J.K.; Wang, I.F.; Hung, L.; Tarn, W.Y.; Shen, C.K.J. TDP-43 overexpression enhances exon 7 inclusion during the survival of motor neuron pre-mRNA splicing. *J. Biol. Chem*., **2008**, *283*(43), 28852-28859.

[70] Chen, H.-H.; Chang, J.-G.; Lu, R.-M.; And, T.-Y.P.; Tarn, W.-Y. The RNA binding protein hnRNP Q modulates the utilization of exon 7 in the survival motor neuron 2 (SMN2) gene. *Mol. Cell. Biol*., **2008**, *28*(22), 6929-6938.

[71] Rossoll, W.; Kröning, A.-K.; Ohndorf, U.-M.; Steegborn, C.; Jablonka, S.; Sendtner, M. Specific interaction of Smn, the spinal muscular atrophy determining gene product, with hnRNP-R and Gry-rbp/hnRNP-Q: A role for Smn in RNA processing in motor axons? *Hum. Mol. Genet.,* **2002**, *11*(1), 93-105.

[72] Singh, N.N.; Seo, J.; Ottesen, E.W.; Shishimorova, M.; Bhattacharya, D.; Singh, R.N. TIA1 prevents skipping of a critical exon associated with spinal muscular atrophy. *Mol. Cell. Biol*., **2011**, *31*(5), 935-954.

[73] Pao, P.W.; Wee, K.B.; Yee, W.C.; Dwipramono, Z.A. Dual masking of specific negative splicing regulatory elements resulted in maximal exon 7 inclusion of SMN2 gene. *Mol. Ther*., **2014**, *22*(4), 854-861.

[74] Singh, N.N.; Lee, B.M.; DiDonato, C.J.; Singh, R.N. Mechanistic principles of antisense targets for the treatment of spinal muscular atrophy. *Future Med. Chem.*, **2015**, *7*(13), 1793-1808.

[75] Chiriboga, C.A.; Swoboda, K.J.; Darras, B.T.; Iannaccone, S.T.; Montes, J.; De Vivo, D.C.; Norris, D.A.; Bennett, C.F.; Bishop, K.M. Results from a phase 1 study of Nusinersen (ISIS-SMNRx) in Children with spinal muscular atrophy. *Neurology*, **2016**, *86*(10), 890-897.

[76] Burghes, A.H.M. When is a deletion not a deletion? When it is converted. *Am. J. Hum. Genet*., **1997**, *61*(1), 9-15.

[77] Mazzei, R.; Gambardella, A.; Conforti, F.L.; Magariello, A.; Patitucci, A.; Gabriele, A.L.; Sprovieri, T.; Labate, A.; Valentino, P.; Bono, F.; Bonavita, S.; Zappia, M.; Muglia, M.; Quattrone, A. Gene conversion events in adult-onset spinal muscular atrophy. *Acta Neurol. Scand.*, **2004**, *109*(2), 151-154.

[78] DiDonato, C.J.; Ingraham, S.E.; Mendell, J.R.; Prior, T.W.; Lenard, S.; Moxley 3rd, R.T.; Florence, J.; Burghes, A.H. Deletion and conversion in spinal muscular atrophy patients: Is there a relationship to severity? *Ann. Neurol.*, **1997**, *41*(2), 230-237.

[79] Maamouri, W.; Hammer, M.B.; Bouhlel, Y.; Souilem, S.; Khmiri, N.; Nehdi, H.; Hentati, F.; Amouri, R. Spinal muscular atrophy due to double gene conversion event. *Int. J. Neurosci.*, **2011**, *121*(2), 107-111.

[80] Wu, X.; Wang, S.-H.; Sun, J.; Krainer, A.R.; Hua, Y.; Prior, T.W. A-44G transition in SMN2 intron 6 protects patients with spinal muscular atrophy. *Hum. Mol. Genet*., **2017**, *26*(14), 2768-2780.

[81] Prior, T.W.; Krainer, A.R.; Hua, Y.; Swoboda, K.J.; Snyder, P.C.; Bridgeman, S.J.; Burghes, A.H.M.; Kissel, J.T. A positive modifier of spinal muscular atrophy in the SMN2 gene. *Am. J. Hum. Genet*., **2009**, *85*(3), 408-413.

[82] Bernal, S.; Alías, L.; Barceló, M.J.; Also-Rallo, E.; Martínez-Hernández, R.; Gámez, J.; Guillén-Navarro, E.; Rosell, J.; Hernando, I.; Rodríguez-Alvarez, F.J.; Borrego, S.; Millán, J.M.; Hernández-Chico, C.; Baiget, M.; Fuentes-Prior, P.; Tizzano, E.F. The c.859G>C variant in the SMN2 gene is associated with types II and III SMA and originates from a common ancestor. *J. Med. Genet*., **2010**, *47*(9), 640-642.

[83] Vezain, M.; Saugier-Veber, P.; Goina, E.; Touraine, R.; Manel, V.; Toutain, A.; Fehrenbach, S.; Frébourg, T.; Pagani, F.; Tosi, M.; Martins, A. A rare SMN2 variant in a previously unrecognized composite splicing regulatory element induces exon 7 inclusion and reduces the clinical severity of spinal muscular atrophy. *Hum. Mutat*., **2010**, *31*(1), 1110-1125.

[84] Praveen, K.; Wen, Y.; Gray, K.M.; Noto, J.J.; Patlolla, A.R.; Van Duyne, G.D.; Matera, A.G. SMA-causing missense mutations in survival motor neuron (SMN) display a wide range of phenotypes when modeled in *Drosophila*. *PLoS Genet.*, **2014**, *10*(8), e1004489. http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1004489

[85] Takarada, T.; Rochmah, M.A.; Harahap, N.I.F.; Shinohara, M.; Saito, T.; Saito, K.; Lai, P.S.; Bouike, Y.; Takeshima, Y.; Awano, H.; Morioka, I.; Iijima, K.; Nishio, H.; Takeuchi, A. SMA mutations in SMN Tudor and C-terminal domains destabilize the protein. *Brain Dev.*, **2017**, *39*(7), 606-612.

[86] Yamamoto, T.; Sato, H.; Lai, P.S.; Nurputra, D.K.; Harahap, N.I.F.; Morikawa, S.; Nishimura, N.; Kurashige, T.; Ohshita, T.; Nakajima, H.; Yamada, H.; Nishida, Y.; Toda, S.; Takanashi, J.-I.; Takeuchi, A.; Tohyama, Y.; Kubo, Y.; Saito, K.; Takeshima, Y.; Matsuo, M.; Nishio, H. Intragenic mutations in SMN1 may contribute more significantly to clinical severity than SMN2 copy numbers in some spinal muscular atrophy (SMA) patients. *Brain Dev.,* **2014**, *36*(10), 914-920.

[87] Workman, E.; Saieva, L.; Carrel, T.L.; Crawford, T.O.; Liu, D.; Lutz, C.; Beattie, C.E.; Pellizzoni, L.; Burghes, A.H.M. A SMN missense mutation complements SMN2 restoring snRNPs and rescuing SMA mice. *Hum. Mol. Genet.*, **2009**, *18*(12), 2215-2229.

[88] Monani, U.R.; Pastore, M.T.; Gavrilina, T.O.; Jablonka, S.; Le, T.T.; Andreassi, C.; DiCocco, J.M.; Lorson, C.; Androphy, E.J.; Sendtner, M.; Podell, M.; Burghes, A.H.M. A transgene carrying an A2G missense mutation in the SMN gene modulates phenotypic severity in mice with severe (type I) spinal muscular atrophy. *J. Cell Biol.*, **2003**, *160*(1), 41-52.

[89] Illingworth, R.S.; Bird, A.P. CpG islands - 'A rough guide'. *FEBS Lett.*, **2009**, *583*(11), 1713-1720.

[90] Portela, A.; Esteller, M. Epigenetic modifications and human disease. *Nat. Biotechnol.*, **2010**, *28*(10), 1057-1068.

[91] Pendina, A.A.; Efimova, O.A.; Fedorova, I.D.; Leont'eva, O.A.; Shilnikova, E.M.; Lezhnina, J.G.; Kuznetzova, T.V.; Baranov, V.S. DNA methylation patterns of metaphase chromosomes in human preimplantation embryos. *Cytogenet. Genome Res.*, **2011**, *132*(1-2), 1-7.

[92] Efimova, O.A.; Pendina, A.A.; Tikhonov, A.V.; Fedorova, I.D.; Krapivin, M.I.; Chiryaeva, O.G.; Shilnikova, E.M.; Bogdanova, M.A.; Kogan, I.Y.; Kuznetzova, T.V.; Gzgzyan, A.M.; Ailamazyan, E.K.; Baranov, V.S. Chromosome hydroxymethylation patterns in human zygotes and cleavage-stage embryos. *Reproduction*, **2015**, *149*(3), 223-233.

[93] Efimova, O.A.; Pendina, A.A.; Tikhonov, A.V.; Parfenyev, S.E.; Mekina, I.D.; Komarova, E.M.; Mazilina, M.A.; Daev, E.V.; Chiryaeva, O.G.; Galembo, I.A.; Krapivin, M.I.; Glotov, O.S.; Stepanova, I.S.; Shlykova, S.A.; Kogan, I.Y.; Gzgzyan, A.M.; Kuznetzova, T.V.; Baranov, V.S. Genome-wide 5-hydroxymethylcytosine patterns in human spermatogenesis are associated with semen quality. *Oncotarget*, **2017**, *8*(51), 88294-88307.

[94] Ai, S.; Shen, L.; Guo, J.; Feng, X.; Tang, B. DNA methylation as a biomarker for neuropsychiatric diseases. *Int. J. Neurosci.*, **2012**, *122,* 165-176. Available from: https://www.tandfonline.com/doi/abs/10.3109/00207454.2011.637654

[95] Wang, S.; Oelze, B.; Schumacher, A. Age-specific epigenetic drift in late-onset Alzheimer's disease. *PLoS One*, **2008**, *3*(7), e2698. Available from: http://journals.plos.org/plosone/article?id= 10.1371/journal.pone.0002698

[96] Hauke, J.; Riessland, M.; Lunke, S.; Eyüpoglu, I.Y.; Blümcke, I.; El-osta, A.; Wirth, B.; Hahnen, E. Survival motor neuron gene 2 silencing by DNA methylation correlates with spinal muscular atrophy disease severity and can be bypassed by histone deacetylase inhibition. *Hum. Mol. Genet*., **2009**, *18*(2), 304-317.

[97] Cao, Y.-Y.; Qu, Y.-J.; He, S.-X.; Li, Y.; Bai, J.-L.; Jin, Y.-W.; Wang, H.; Song, F. Association between SMN2 methylation and disease severity in chinese children with spinal muscular atrophy. *J. Zhejiang Univ. Sci. B,* **2016**, *17*(1), 76-82.

[98] Zheleznyakova, G.Y.; Voisin, S.; Kiselev, A.V; Sällman Almén, M.; Xavier, M.J.; Maretina, M.A.; Tishchenko, L.I.; Fredriksson, R.; Baranov, V.S.; Schiöth, H.B. Genome-wide analysis shows association of epigenetic changes in regulators of Rab and Rho GTPases with spinal muscular atrophy severity. *Eur. J. Hum. Genet*., **2013**, *21*(9), 988-993.

[99] Zheleznyakova, G.Y.; Nilsson, E.K.; Kiselev, A.V.; Maretina, M.A.; Tishchenko, L.I.; Fredriksson, R.; Baranov, V.S.; Schiöth, H.B. Methylation levels of SLC23A2 and NCOR2 genes correlate with spinal muscular atrophy severity. *PLoS One*, **2015**, *10*(3), 1-14. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0121964

[100] Qiu, S.; Li, L.; Weeber, E.J.; May, J.M. Ascorbate Transport by primary cultured neurons and its role in neuronal function and protection against excitotoxicity. *J. Neurosci. Res.*, **2007**, *85*(5), 1046-1056.

[101] Codina, A.; Love, J.D.; Li, Y.; Lazar, M.A.; Neuhaus, D.; Schwabe, J.W.R. Structural insights into the interaction and activation of histone deacetylase 3 by nuclear receptor corepressors. *Proc. Natl. Acad. Sci. U.S.A.*, **2005**, *102*(17), 6009-6014.

[102] Maretina, M.A.; Zheleznyakova, G.Y.; Baranov, V.S.; Kiselev, A.V. DYNC1H1 gene methylation correlates with a severity of spinal muscular atrophy In: Abstracts of *European Human Genetics Conference* 2016; Barcelona, Spain, May 21-24, **2016**; pp. 307-308.

[103] Weedon, M.N.; Hastings, R.; Caswell, R.; Xie, W.; Paszkiewicz, K.; Antoniadi, T.; Williams, M.; King, C.; Greenhalgh, L.; Newbury-Ecob, R.; Ellard, S. Exome sequencing identifies a DYNC1H1 mutation in a large pedigree with dominant axonal Charcot-Marie-Tooth disease. *Am. J. Hum. Genet*., **2011**, *89*(2), 308-312.

[104] Harms, M.B.; Tuck, E.P.; Bell, S.; Ma, D.; Allred, P.; Miller, L.J. Mutations in the tail domain of DYNC1H1 cause dominant spinal muscular atrophy. *Neurology*, **2012**, *78*(22), 1714-1720.

[105] Tsurusaki, Y.; Saitoh, S.; Tomizawa, K.; Sudo, A.; Asahina, N.; Shiraishi, H.; Ito, J.I.; Tanaka, H.; Doi, H.; Saitsu, H.; Miyake, N.; Matsumoto, N. A DYNC1H1 mutation causes a dominant spinal muscular atrophy with lower extremity predominance. *Neurogenetics*, **2012**, *13*(4), 327-332.

[106] Bowerman, M.; Shafey, D.; Kothary, R. Smn depletion alters Profilin II expression and leads to upregulation of the RhoA/ROCK pathway and defects in neuronal integrity. *J. Mol. Neurosci.*, **2007**, *32*(2), 120-131.

[107] Ackermann, B.; Kröber, S.; Torres-benito, L.; Borgmann, A.; Pe-

ters, M.; Hosseini barkooie, S.M.; Tejero, R.; Jakubik, M.; Schreml, J.; Milbradt, J.; Wunderlich, T.F.; Riessland, M.; Tabares, L.; Wirth, B. Plastin 3 ameliorates spinal muscular atrophy *via* delayed axon pruning and improves neuromuscular junction functionality. *Hum. Mol. Genet.*, **2013**, *22*(7), 1328-1347.

[108] Hosseinibarkooie, S.; Peters, M.; Torres-benito, L.; Rastetter, R.H.; Hupperich, K.; Hoffmann, A.; Mendoza-ferreira, N.; Kaczmarek, A.; Janzen, E.; Milbradt, J.; Lamkemeyer, T.; Rigo, F.; Hammerschmidt, M.; Bennett, C.F.; Guschlbauer, C.; Bu, A.; Riessland, M.; Kye, M.J.; Clemen, C.S.; Wirth, B. The power of human protective modifiers: PLS3 and CORO1C unravel impaired endocytosis in spinal muscular atrophy and rescue SMA phenotype. *Am. J. Hum. Genet.*, **2016**, *99*(3), 647-665.

[109] Riessland, M.; Kaczmarek, A.; Schneider, S.; Swoboda, K.J.; Bradler, C.; Grysko, V.; Dimitriadi, M.; Hosseinibarkooie, S.; Torres-benito, L.; Peters, M.; Ho, I.; Garbes, L.; Upadhyay, A.; Biglari, N.; Kro, S.; Walter, M.; Gilissen, C.; Hoischen, A.; Nu, G.; Rigo, F.; Bennett, C.F.; Kye, M.J.; Hart, A.C.; Hammerschmidt, M.; Kloppenburg, P.; Wirth, B. Neurocalcin delta suppression protects against spinal muscular atrophy in humans and across species by restoring impaired endocytosis. *Am. J. Hum. Genet.*, **2017**, *100*(2), 297-315.

[110] Yanyan, C.; Yujin, Q.; Jinli, B.; Yuwei, J.; Hong, W.; Fang, S. Correlation of PLS3 expression with disease severity in children with spinal muscular atrophy. *J. Hum. Genet.*, **2014**, *59*(1), 24-27.

[111] Bernal, S.; Also-rallo, E.; Martínez-Hernández, A.L.; Alvarez-Rodríguez, F.J.; Millán, J.M.; Hernández-Chico, C.; Baiget, M.; Tizzano, E.F. Plastin 3 expression in discordant spinal muscular atrophy (SMA) siblings. *Neuromusc. Disord.*, **2011**, *21*(6), 413-419.

[112] Boza-Morán, M.G.; Martínez-Hernández, R.; Bernal, S.; Wanisch, K.; Also-Rallo, E.; Le Heron, A.; Alías, L.; Denis, C.; Girard, M.; Yee, J.-K.; Tizzano, E.F.; Yáñez-Muñoz, R.J. Decay in survival motor neuron and plastin 3 levels during differentiation of iPSC-derived human motor neurons. *Sci. Rep.*, **2015**, *5*, 11696. Available from: https://www.nature.com/articles/srep11696

[113] McGovern, V.L.; Massoni-Laporte, A.; Wang, X.; Le, T.T.; Le, H.T.; Beattie, C.E.; Rich, M.M.; Burghes, A.H.M. Plastin 3 Expression does not modify spinal muscular atrophy severity in the Δ7 SMA mouse. *PLoS One*, **2015**, *10*(7), e0132364. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0132364

[114] Giesemann, T.; Rathke-Hartlieb, S.; Rothkegel, M.; Bartsch, J.W.; Buchmeier, S.; Jockusch, B.M.; Jockusch, H. A role for polyproline motifs in the spinal muscular atrophy protein SMN Profilins bind to and colocalize with smn in nuclear gems. *J. Biol. Chem.*, **1999**, *274*(53), 37908-37914.

[115] Sharma, A.; Lambrechts, A.; Le, T.H.; Le, T.T.; Sewry, C.A.; Ampe, C.; Burghes, A.H.M.; Morris, G.E. A role for complexes of survival of motor neurons (SMN) protein with gemins and profilin in neurite-like cytoplasmic extensions of cultured nerve cells. *Exp. Cell Res.*, **2005**, *309*(1), 185-197.

[116] Negishi, M.; Katoh, H. Rho family GTPases as key regulators for neuronal network formation. *J. Biochem.*, **2002**, *132*(2), 157-166.

[117] Bowerman, M.; Beauvais, A.; Anderson, C.L.; Kothary, R. Rho-kinase inactivation prolongs survival of an intermediate SMA mouse model. *Hum. Mol. Genet.*, **2010**, *19*(8), 1468-1478.

[118] Hensel, N.; Stockbrügger, I.; Rademacher, S.; Broughton, N.; Brinkmann, H.; Grothe, C.; Claus, P. Bilateral crosstalk of Rho- and extracellular-signal-regulated-kinase (ERK) pathways is confined to an unidirectional mode in Spinal Muscular Atrophy (SMA). *Cell. Signal.*, **2014**, *26*(3), 540-548.

[119] Hausott, B.; Kurnaz, I.; Gajovic, S.; Klimaschewski, L. Signaling by neuronal tyrosine kinase receptors: Relevance for development and regeneration. *Anat. Rec.*, **2009**, *292*(12), 1976-1985.

[120] Branchu, J.; Biondi, O.; Chali, F.; Collin, T.; Leroy, F.; Mamchaoui, K.; Makoukji, J.; Pariset, C.; Lopes, P.; Massaad, C.; Chanoine, C.; Charbonnier, F. Shift from extracellular signal-regulated kinase to AKT/cAMP response element-binding protein pathway increases survival-motor-neuron expression in spinal-muscular-atrophy-like mice and patient cells. *J. Neurosci.*, **2013**, *33*(10), 4280-4294.

[121] Li, L.; Liu, F.; Ross, A.H. PTEN regulation of neural development and CNS stem cells. *J. Cell. Biochem.*, **2003**, *88*(1), 24-28.

[122] Wen, H.L.; Lin, Y.T.; Ting, C.H.; Lin-Chao, S.; Li, H.; Hsieh-Li, H.M. Stathmin, a microtubule-destabilizing protein, is dysregulated in spinal muscular atrophy. *Hum. Mol. Genet.*, **2010**, *19*(9), 1766-1778.

[123] Peters, M. *Combined therapy of SMN-ASO and Plastin 3 Overexpression Rescues Severe SMA in Mice*. Doctoral dissertation, University of Cologne: Cologne, Germany, **2016**.

[124] Kaifer, K.A.; Villalón, E.; Osman, E.Y.; Glascock, J.J.; Arnold, L.L.; Cornelison, D.D.W.; Lorson, C.L. Plastin-3 extends survival and reduces severity in mouse models of spinal muscular atrophy. *JCI Insight*, **2017**, *2*(5), e89970. Available from: https:// insight.jci.org/articles/view/89970

[125] Okouchi, M.; Ekshyyan, O.; Maracine, M.; Aw, T.Y. Neuronal apoptosis in neurodegeneration. *Antioxid. Redox Signal.*, **2007**, *9*(8), 1059-1096.

[126] Lachyankar, M.B.; Sultana, N.; Schonhoff, C.M.; Mitra, P.; Poluha, W.; Lambert, S.; Quesenberry, P.J.; Litofsky, N.S.; Recht, L.D.; Nabi, R.; Miller, S.J.; Ohta, S.; Neel, B.G.; Ross, A.H. A role for nuclear PTEN in neuronal differentiation. *J. Neurosci.*, **2000**, *20*(4), 1404-1413.

[127] Ross, A.H.; Lachyankar, M.B.; Recht, L.D. PTEN: A newly identified regulator of neuronal differentiation. *Neuroscientist*, **2001**, *7*(4), 278-281.

[128] Early, L. Axon regeneration: Using the PTEN deletion method. http://www.neuroblogspot.net/cit230/indv-design-docs/w2-indv-content-inventory.pdf (Accessed November 10, **2011**).

[129] Li, P.P.; Peng, H.B. Regulation of axonal growth and neuromuscular junction formation by neuronal phosphatase and tensin homologue signaling. *Mol. Biol. Cell*, **2012**, *23*(20), 4109-4117.

[130] Kreis, P.; Leondaritis, G.; Lieberam, I.; Eickholt, B.J. Subcellular targeting and dynamic regulation of PTEN: Implications for neuronal cells and neurological disorders. *Front. Mol. Neurosci.*, **2014**, *7*, 23. Available from: https://www.frontiersin.org/articles/10.3389/fnmol.2014.00023/full

[131] Christie, K.J.; Webber, C.A.; Martinez, J.A.; Singh, B.; Zochodne, D.W. PTEN inhibition to facilitate intrinsic regenerative outgrowth of adult peripheral axons. *J. Neurosci.*, **2010**, *30*(27), 9306-9315.

[132] Groszer, M.; Groszer, M.; Erickson, R.; Zack, J.A.; Kornblum, H.I.; Liu, X. Negative regulation of neural stem / progenitor cell proliferation by the PTEN tumor suppressor gene *in vivo*. *Science*, **2008**, *2186*(2001), 2186-2190. Available from: http://science.sciencemag.org/content/294/5549/2186/tab-figures-data

[133] Gregorian, C.; Nakashima, J.; Belle, J.L.; Ohab, J.; Kim, R.; Liu, A.; Smith, K.B.; Groszer, M.; Garcia, A.D.; Sofroniew, M.V.; Carmichael, S.T.; Kornblum, H.I.; Liu, X.; Wu, H. Deletion in adult neural stem/progenitor cells enhances constitutive neurogenesis. *Stem Cells*, **2009**, *29*(6), 1874-1886.

[134] Ning, K.; Drepper, C.; Valori, C.F.; Ahsan, M.; Wyles, M.; Higginbottom, A.; Herrmann, T.; Shaw, P.; Azzouz, M.; Sendtner, M. PTEN depletion rescues axonal growth defect and improves survival in SMN-deficient motor neurons. *Hum. Mol. Genet.*, **2010**, *19*(16), 3159-3168.

[135] Little, D.; Valori, C.F.; Mutsaers, C.A.; Bennett, E.J.; Wyles, M.; Sharrack, B.; Shaw, P.J.; Gillingwater, T.H.; Azzouz, M.; Ning, K. PTEN depletion decreases disease severity and modestly prolongs survival in a mouse model of spinal muscular atrophy. *Mol. Ther.*, **2015**, *23*(2), 270-277.

[136] Chang, H.C.; Hung, W.C.; Chuang, Y.J.; Jong, Y.J. Degradation of survival motor neuron (SMN) protein is mediated *via* the ubiquitin/proteasome pathway. *Neurochem. Int.*, **2004**, *45*(7), 1107-1112.

[137] Kwon, D.Y.; Motley, W.W.; Fischbeck, K.H.; Burnett, B.G. Increasing expression and decreasing degradation of SMN ameliorate the spinal muscular atrophy phenotype in mice. *Hum. Mol. Genet.*, **2011**, *20*(18), 3667-3677.

[138] Han, K.J.; Foster, D.G.; Zhang, N.Y.; Kanisha, K.; Dzieciatkowska, M.; Sclafani, R.A.; Hansen, K.C.; Peng, J.; Liu, C.W. Ubiquitin-specific protease 9x deubiquitinates and stabilizes the spinal muscular atrophy protein-survival motor neuron. *J. Biol. Chem.*, **2012**, *287*(52), 43741-43752.

[139] Hsu, S.H.; Lai, M.C.; Er, T.K.; Yang, S.N.; Hung, C.H.; Tsai, H.H.; Lin, Y.C.; Chang, J.G.; Lo, Y.C.; Jong, Y.J. Ubiquitin Carboxyl-Terminal Hydrolase L1 (UCHL1) regulates the level of SMN expression through ubiquitination in primary spinal muscular atrophy fibroblasts. *Clin. Chim. Acta*, **2010**, *411*(23-24), 1920-1928.

[140] Setsuie, R.; Wada, K. The functions of UCH-L1 and its relation to neurodegenerative diseases. *Neurochem. Int.*, **2007**, *51*(2-4 SPEC. ISS.), 105-111.

[141] Kwon, D.Y.; Dimitriadi, M.; Terzic, B.; Cable, C.; Hart, A.C.;

Chitnis, A.; Fischbeck, K.H.; Burnett, B.G. The E3 ubiquitin ligase mind bomb 1 ubiquitinates and promotes the degradation of survival of motor neuron protein. *Mol. Biol. Cell*, **2013**, *24*(12), 1863-1871.

[142] Choe, E.-A.; Liao, L.; Zhou, J.-Y.; Cheng, D.; Duong, D.M.; Jin, P.; Tsai, L.-H.; Peng, J. Neuronal morphogenesis is regulated by the interplay between cyclin-dependent kinase 5 and the ubiquitin ligase mind bomb 1. *J. Neurosci.*, **2007**, *27*(35), 9503-9512.

[143] Tapia, O.; Lafarga, V.; Bengoechea, R.; Palanca, A.; Lafarga, M.; Berciano, M.T. The SMN tudor SIM-like domain is key to SmD1 and coilin interactions and to Cajal body biogenesis. *J. Cell Sci.*, **2014**, *127*(5), 939-946.

[144] Lee, P.C.W.; Sowa, M.E.; Gygi, S.P.; Harper, J.W. Alternative ubiquitin activation/conjugation cascades interact with N-end rule ubiquitin ligases to control degradation of RGS proteins. *Mol. Cell*, **2011**, *43*(3), 392-405.

[145] Skowyra, D.; Craig, K.L.; Tyers, M.; Elledge, S.J.; Harper, J.W. F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell*, **1997**, *91*(2), 209-219.

[146] Skaar, J.R.; Pagan, J.K.; Pagano, M. Mechanisms and function of substrate recruitment by F-Box proteins. *Nat. Rev. Mol. Cell Biol.*, **2013**, *14*(6), 369-381.

[147] Burnett, B.G.; Muñoz, E.; Tandon, A.; Kwon, D.Y.; Sumner, C.J.; Fischbeck, K.H. Regulation of SMN protein stability. *Mol. Cell. Biol.*, **2009**, *29*(5), 1107-1115.

[148] Wishart, T.M.; Mutsaers, C.; Riessland, M.; Reimer, M.M.; Hunter, G.; Hannam, M.L.; Eaton, S.; Fuller, H.R.; Roche, S.L.; Somers, E.; Morse, R.; Young, P.J.; Lamont, D.J.; Hammerschmidt, M.; Joshi, A.; Hohenstein, P.; Morris, G.E.; Parson, S.H.; Skehel, P.A.; Becker, T.; Robinson, I.M.; Becker, C.; Wirth, B.; Gillingwater, T.H. Dysregulation of ubiquitin homeostasis and β-catenin signalling promote spinal muscular atrophy. *J. Clin. Invest.*, **2014**, *124*(4), 1821-1834.

[149] Powis, R.A.; Karyka, E.; Boyd, P.; Côme, J.; Jones, R.A.; Zheng, Y.; Szunyogova, E.; Groen, E.J.N.; Hunter, G.; Thomson, D.; Wishart, T.M.; Becker, C.G.; Parson, S.H.; Martinat, C.; Azzouz, M.; Gillingwater, T.H. Systemic restoration of UBA1 ameliorates disease in spinal muscular atrophy. *JCI Insight*, **2016**, *1*(11), e87908. Available from: https://insight.jci.org/articles/view/87908

[150] Ramser, J.; Ahearn, M.E.; Lenski, C.; Yariz, K.O.; Hellebrand, H.; von Rhein, M.; Clark, R.D.; Schmutzler, R.K.; Lichtner, P.; Hoffman, E.P.; Meindl, A.; Baumbach-Reardon, L. Rare missense and synonymous variants in UBE1 are associated with X-linked infantile spinal muscular atrophy. *Am. J. Hum. Genet.*, **2008**, *82*(1), 188-193.

[151] Korhonen, L.; Lindholm, D. The ubiquitin proteasome system in synaptic and axonal degeneration: A new twist to an old cycle. *J. Cell Biol.*, **2004**, *165*(1), 27-30.

[152] Halliwell, B. Proteasomal dysfunction: A common feature of neurodegenerative diseases? Implications for the environmental origins of neurodegeneration. *Antioxid. Redox Signal.*, **2007**, *8*(11-12), 2007-2019.

[153] Foran, E.; Kwon, D.Y.; Nofziger, J.H.; Arnold, E.S.; Hall, M.D.; Fischbeck, K.H.; Burnett, B.G. CNS uptake of bortezomib is enhanced by P-glycoprotein inhibition: Implications for spinal muscular atrophy. *Neurobiol. Dis.*, **2016**, *88*, 118-124. Available from: https://www.sciencedirect.com/science/article/pii/S0969996116300079

[154] Ilangovan, R.; Marshall, W.L.; Hua, Y.; Zhou, J. Inhibition of apoptosis by Z-VAD-Fmk in SMN-depleted S2 cells. *J. Biol. Chem.*, **2003**, *278*(33), 30993-30999.

[155] Sareen, D.; Ebert, A.D.; Heins, B.M.; McGivern, J.V.; Ornelas, L.; Svendsen, C.N. Inhibition of apoptosis blocks human motor neuron cell death in a stem cell model of spinal muscular atrophy. *PLoS One*, **2012**, *7*(6) e39113. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0039113

[156] Kerr, D.A.; Nery, J.P.; Traystman, R.J.; Chau, B.N.; Hardwick, J.M. Survival motor neuron protein modulates neuron-specific apoptosis. *Proc. Natl. Acad. Sci. U.S.A.*, **2000**, *97*(24), 13312-13317.

[157] Cisterni, C.; Kallenbach, S.; Jordier, F.; Bagnis, C.; Pettmann, B. Death of motorneurons induced by Trophic deprivation or by excitotoxicity is not prevented by overexpression of SMN. *Neurobiol. Dis.*, **2001**, *8*(2), 240-251.

[158] Vitte, J.; Fassier, C.; Tiziano, F.D.; Dalard, C.; Soave, S.; Roblot, N.; Brahe, C.; Saugier-Veber, P.; Bonnefont, J.P.; Melki, J. Refined characterization of the expression and stability of the SMN gene products. *Am. J. Pathol.*, **2007**, *171*(4), 1269-1280.

[159] Kostic, V.; Jackson-Lewis, V.; de Bilbao, F.; Dubois-Dauphin, M.; Przedborski, S. Bcl-2: Prolonging life in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Science*, **1997**, *277*(5325), 559-562. Available from: http://science.sciencemag.org/content/277/5325/559.full

[160] Burlacu, A. Regulation of apoptosis by Bcl-2 family proteins. *J. Cell. Mol. Med.*, **2003**, *7*(3), 249-257.

[161] Soler-Botija, C.; Ferrer, I.; Alvarez, J.L.; Baiget, M.; Tizzano, E.F. Downregulation of Bcl-2 proteins in type I spinal muscular atrophy motor neurons during fetal development. *J. Neuropathol. Exp. Neurol.*, **2003**, *62*(4), 420-426.

[162] Tsai, M.S.; Chiu, Y.T.; Wang, S.H.; Hsieh-Li, H.M.; Lian, W.C.; Li, H. Abolishing Bax-dependent apoptosis shows beneficial effects on spinal muscular atrophy model mice. *Mol. Ther.*, **2006**, *13*(6), 1149-1155.

[163] Anderson, K.; Potter, A.; Baban, D.; Davies, K.E. Protein expression changes in spinal muscular atrophy revealed with a novel antibody array technology. *Brain*, **2003**, *126*(9), 2052-2064.

[164] Anderton, R.S.; Price, L.L.; Turner, B.J.; Meloni, B.P.; Mitrpant, C.; Mastaglia, F.L.; Goh, C.; Wilton, S.D.; Boulos, S. Co-regulation of survival of motor neuron and Bcl-xL expression: Implications for neuroprotection in spinal muscular atrophy. *Neuroscience*, **2012**, *220*, 228-236. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0306452212006604

[165] Tsai, L.K.; Tsai, M.S.; Ting, C.H.; Wang, S.H.; Li, H. Restoring Bcl-xL levels benefits a mouse model of spinal muscular atrophy. *Neurobiol. Dis.*, **2008**, *31*(3), 361-367.

[166] Garcera, A.; Mincheva, S.; Gou-Fabregas, M.; Caraballo-Miralles, V.; Lladó, J.; Comella, J.X.; Soler, R.M. A new model to study spinal muscular atrophy: Neurite degeneration and cell death is counteracted by BCL-XL overexpression in motoneurons. *Neurobiol. Dis.*, **2011**, *42*(3), 415-426.

[167] Young, P.J.; Day, P.M.; Zhou, J.; Androphy, E.J.; Morris, G.E.; Lorson, C.L. A direct interaction between the survival motor neuron protein and p53 and its relationship to spinal muscular atrophy. *J. Biol. Chem.*, **2002**, *277*(4), 2852-2859.

[168] Zhang, W.; Liu, H.T. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Res.*, **2002**, *12*(1), 9-18.

[169] Kwon, J.E.; Kim, E.K.; Choi, E.J. Stabilization of the survival motor neuron protein by ASK1. *FEBS Lett.*, **2011**, *585*(9), 1287-1292.

[170] Song, J.; Park, K.A.; Lee, W.T.; Lee, J.E. Apoptosis signal regulating kinase 1 (ASK1): Potential as a therapeutic target for alzheimer's disease. *Int. J. Mol. Sci.,* **2014**, *15*(2), 2119-2129.

[171] Frevel, M.A.E.; Bakheet, T.; Silva, A.M.; Hissong, J.G.; Khabar, K.S.A.; Williams, B.R.G. p38 mitogen-activated protein kinase-dependent and -independent signaling of mRNA stability of AU-rich element-containing transcripts. *Mol. Cell. Biol.*, **2003**, *23*(2), 425-436.

[172] Farooq, F.; Balabanian, S.; Liu, X.; Holcik, M.; MacKenzie, A. p38 mitogen-activated protein kinase stabilizes SMN mRNA through RNA binding protein HuR. *Hum. Mol. Genet.*, **2009**, *18*(21), 4035-4045.

[173] Farooq, F.; Abadía-Molina, F.; MacKenzie, D.; Hadwen, J.; Shamim, F.; O'Reilly, S.; Holcik, M.; MacKenzie, A. Celecoxib increases SMN and survival in a severe spinal muscular atrophy mouse model *via* p38 pathway activation. *Hum. Mol. Genet.*, **2013**, *22*(17), 3415-3424.

[174] Genabai, N.K.; Ahmad, S.; Zhang, Z.; Jiang, X.; Gabaldon, C.A.; Gangwani, L. Genetic inhibition of JNK3 ameliorates spinal muscular atrophy. *Hum. Mol. Genet.*, **2015**, *24*(24), 6986-7004.

[175] Tseng, Y.; Chen, C.; Jong, Y.; Chang, F.; Lo, Y. Loganin possesses neuroprotective properties, restores SMN protein and activates protein synthesis positive regulator Akt/mTOR in experimental models of spinal muscular atrophy. *Pharmacol. Res.*, **2016**. *111*, 58-75. Available from: https://www.sciencedirect.com/science/article/abs/pii/S1043661815302188

[176] Bosch-Marcé, M.; Wee, C.D.; Martinez, T.L.; Lipkes, C.E.; Choe, D.W.; Kong, L.; Van Meerbeke, J.P.; Musarò, A.; Sumner, C.J. Increased IGF-1 in muscle modulates the phenotype of severe SMA mice. *Hum. Mol. Genet.*, **2011**, *20*(9), 1844-1853.

[177] Tsai, L.; Chen, C.; Ting, C.; Lin-chao, S.; Hwu, W.; Dodge, J.C.; Passini, M.A.; Cheng, S.H. Systemic administration of a recombi-

nant AAV1 vector encoding IGF-1 improves disease manifestations in SMA mice. *Mol. Ther*., **2014**, *22*(8), 1450-1459.

[178] Biondi, O.; Branchu, J.; Salah, A.B.; Houdebine, L.; Bertin, L.; Chali, F.; Desseille, C.; Weill, L.; Sanchez, X.G.; Lancelin, C.; Aïd, S.; Lopes, P.; Pariset, C.; Lécole, S.; Côté, J.; Holzenberger, M.; Chanoine, C.; Massaad, C.; Charbonnier, F. IGF-1R reduction triggers neuroprotective signaling pathways in spinal muscular atrophy mice. *J. Neurosci*., **2015**, *35*(34), 12063-12079.

[179] Jiao, J.; Hong, S.; Zhang, J.; Ma, L.; Sun, Y.; Zhang, D.; Shen, B.; Zhu, C. Opsin3 sensitizes hepatocellular carcinoma cells to 5-fluorouracil treatment by regulating the apoptotic pathway. *Cancer Lett*., **2012**, *320*(1), 96-103.

[180] Kohno, Y.; Patel, V.; Kim, Y.; Tsuji, T.; Chin, B.R.; Sun, M.; Bruce Donoff, R.; Kent, R.; Wong, D.; Todd, R. Apoptosis, proliferation and p12doc-1 profiles in normal, dysplastic and malignant squamous epithelium of the Syrian hamster cheek pouch model. *Oral Oncol*., **2002**, *38*(3), 274-280.

[181] D'agostino, L.; Giordano, A. NSP 5a3a: A potential novel cancer target in head and neck carcinoma. *Oncotarget*, **2010**, *1*(6), 423-435.

[182] Wang, L.; Chen, Z.; Wang, Y.; Chang, D.; Su, L.; Guo, Y.; Liu, C. WWTR1 promotes cell proliferation and inhibits apoptosis through cyclin A and CTGF regulation in non-small cell lung cancer. *Tumor Biol*., **2014**, *35*(1), 463-468.

[183] Sen, A.; Dimlich, D.N.; Guruharsha, K.G.; Kankel, M.W.; Hori, K.; Yokokura, T.; Brachat, S.; Richardson, D.; Loureiro, J.; Sivasankaran, R.; Curtis, D.; Davidow, L.S.; Rubin, L.L.; Hart, A.C.; Van Vactor, D.; Artavanis-Tsakonas, S. Genetic circuitry of survival motor neuron, the gene underlying spinal muscular atrophy. *Proc. Natl. Acad. Sci. U.S.A*., **2013**, *110*(26), E2371-2380.

[184] Fallini, C.; Donlin-asp, P.G.; Rouanet, J.P.; Bassell, G.J.; Rossoll, W. Deficiency of the survival of motor neuron protein impairs mRNA localization and local translation in the growth cone of motor neurons. *J. Neurosci.*, **2016**, *36*(13), 3811-3820.

[185] Gnanapavan, S.; Giovannoni, G. Neural cell adhesion molecules in brain plasticity and disease. *Mul. Scler. Rel. Disord.,* **2013**, *2*(1) 13-20.

[186] Rudnik-Schöneborn, S.; Botzenhart, E.; Eggermann, T.; Senderek, J.; Schoser, B.G.H.; Schröder, R.; Wehnert, M.; Wirth, B.; Zerres, K. Mutations of the LMNA gene can mimic autosomal dominant proximal spinal muscular atrophy. *Neurogenetics*, **2007**, *8*(2), 137-142.

[187] Iwahara, N.; Hisahara, S.; Hayashi, T.; Kawamata, J.; Shimohama, S. A novel lamin A/C gene mutation causing spinal muscular atrophy phenotype with cardiac involvement: Report of one case. *BMC Neurol*., **2015**, *15,* 13. Available from: https://bmcneurol.biomedcentral.com/articles/10.1186/s12883-015-0269-5

[188] Sen, A.; Yokokura, T.; Kankel, M.W.; Dimlich, D.N.; Manent, J.; Sanyal, S.; Artavanis-Tsakonas, S. Modeling spinal muscular atrophy in drosophila links Smn to FGF signaling. *J. Cell Biol*., **2011**, *192*(3), 481-495.

[189] Lotti, F.; Imlach, W.L.; Saieva, L.; Beck, E.S.; Hao, L.T.; Li, D.K.; Jiao, W.; Mentis, G.Z.; Beattie, C.E.; Mccabe, B.D.; Pellizzoni, L. An SMN-dependent U12 splicing event essential for motor circuit function. *Cell*, **2012**, *151*(2), 440-454.

[190] See, K.; Yadav, P.; Giegerich, M.; Cheong, P.S.; Graf, M.; Vyas, H.; Lee, S.G.P.; Mathavan, S.; Fischer, U.; Sendtner, M.; Winkler, C. SMN deficiency alters Nrxn2 expression and splicing in zebrafish and mouse models of spinal muscular atrophy. *Hum. Mol. Genet.,* **2014**, *23*(7), 1754-1770.

[191] Sleigh, J.N.; Barreiro-Iglesias, A.; Oliver, P.L.; Biba, A.; Becker, T.; Davies, K.E.; Becker, C.G.; Talbot, K. Chondrolectin affects cell survival and neuronal outgrowth in *in vitro* and *in vivo* models of spinal muscular atrophy. *Hum. Mol. Genet*., **2014**, *23*(4), 855-869.

[192] Borg, R.; Cauchi, R.J. Gemins: Potential therapeutic targets for spinal muscular atrophy? *Front. Neurosci*., **2014**, *8*, 325. Available from: https://www.frontiersin.org/articles/10.3389/fnins.2014. 00325/full

[193] Jablonka, S.; Holtmann, B.; Meister, G.; Bandilla, M.; Rossoll, W.; Fischer, U.; Sendtner, M. Gene targeting of Gemin2 in mice reveals a correlation between defects in the biogenesis of U snRNPs and motoneuron cell death. *Proc. Natl. Acad. Sci. U.S.A*., **2002**, *99*(15), 10126-10131.

[194] Borg, R.; Cauchi, R.J. The Gemin associates of survival motor neuron are required for motor function in *Drosophila*. *PLoS One*, **2013**, *8*(12), e83878. Available from: http://journals.plos.org/ plosone/article?id=10.1371/journal.pone.0083878

[195] Workman, E.; Kalda, C.; Patel, A.; Battle, D.J. Gemin5 binds to the survival motor neuron mRNA to regulate SMN expression. *J. Biol. Chem.*, **2015**, *290*(25), 15662-15669.

[196] Gangwani, L. Deficiency of the zinc finger protein ZPR1 causes defects in transcription and cell cycle progression. *J. Biol. Chem*., **2006**, *281*(52), 40330-40340.

[197] Doran, B.; Gherbesi, N.; Hendricks, G.; Flavell, R.A.; Davis, R.J.; Gangwani, L. Deficiency of the zinc finger protein ZPR1 causes neurodegeneration. *Proc. Natl. Acad. Sci. U.S.A.*, **2006**, *103*(19), 7471-7475.

[198] Gangwani, L.; Mikrut, M.; Theroux, S.; Sharma, M.; Davis, R.J. Spinal muscular atrophy disrupts the interaction of ZPR1 with the SMN protein. *Nat. Cell Biol*., **2001**, *3*(4), 376-383.

[199] Narayanan, U.; Ospina, J.K.; Frey, M.R.; Hebert, M.D.; Matera, A.G. SMN, the spinal muscular atrophy protein, forms a pre-import Snrnp complex with Snurportin1 and Importin β. *Hum. Mol. Genet*., **2002**, *11*(15), 1785-1795.

[200] Gangwani, L.; Flavell, R.A.; Davis, R.J. ZPR1 Is Essential for Survival and is required for localization of the survival motor neurons (SMN) protein to Cajal bodies. *Mol. Cell. Biol*., **2005**, *25*(7), 2744-2756.

[201] Ahmad, S.; Wang, Y.; Shaik, G.M.; Burghes, A.H.; Gangwani, L. The zinc finger protein ZPR1 is a potential modifier of spinal muscular atrophy. *Hum. Mol. Genet*., **2012**, *21*(12), 2745-2758.

[202] Markham, K.; Schuurmans, C.; Weiss, S. STAT5A/B activity is required in the developing forebrain and spinal cord. *Mol. Cell. Neurosci*., **2007**, *35*(2), 272-282.

[203] Ting, C.H.; Lin, C.W.; Wen, S.L.; Hsieh-Li, H.M.; Li, H. Stat5 constitutive activation rescues defects in spinal muscular atrophy. *Hum. Mol. Genet*., **2007**, *16*(5), 499-514.

[204] Lou, K.J. Prolactin for spinal muscular atrophy. *SciBX Sci. Exch*., **2011**, *4*(32), 1-2. Available from: https://www.nature.com/scibx/ journal/v4/n32/fig_tab/scibx.2011.896_F1.html

[205] Narver, H.L.; Kong, L.; Burnett, B.G.; Choe, D.W.; Bosch-Marcé, M.; Taye, A.A.; Eckhaus, M.A.; Sumner, C.J. Sustained improvement of spinal muscular atrophy mice treated with trichostatin a plus nutrition. *Ann. Neurol*., **2008**, *64*(4), 465-470.

[206] Butchbach, M.E.R.; Rose, F.F.; Rhoades, S.; Marston, J.; McCrone, J.T.; Sinnott, R.; Lorson, C.L. Effect of diet on the survival and phenotype of a mouse model for spinal muscular atrophy. *Biochem. Biophys. Res. Commun*., **2010**, *391*(1), 835-840.

[207] Birnkrant, D.J.; Pope, J.F.; Martin, J.E.; Repucci, A.H.; Eiben, R.M. Treatment of type I spinal muscular atrophy with noninvasive ventilation and gastrostomy feeding. *Pediatr. Neurol*., **1998**, *18*(5), 407-410.

[208] Chung, B.H.Y.; Wong, V.C.N.; Ip, P. Spinal muscular atrophy: Survival patterns and functional status. *Pediatrics*, **2004**, *114*(5), e548-553. Available from: http://pediatrics.aappublications.org/ content/114/5/e548

[209] Bebee, T.W.; Dominguez, C.E.; Samadzadeh-tarighat, S.; Akehurst, K.L.; Chandler, D.S. Hypoxia is a modifier of SMN2 splicing and disease severity in a severe SMA mouse model. *Hum. Mol. Genet*., **2012**, *21*(19), 4301-4313.

[210] Chen, W.J.; He, J.; Zhang, Q.J.; Lin, Q.-F.; Chen, Y.-F.; Lin, X.-Z.; Lin, M.-T.; Murong, S.-X.; Wang, N. Modification of phenotype by SMN2 copy numbers in two Chinese families with SMN1 deletion in two continuous generations. *Clin. Chim. Acta*, **2012**, *413*(23-24), 1855-1860.

[211] Garbes, L.; Heesen, L.; Hölker, I.; Bauer, T.; Schreml, J.; Zimmermann, K.; Thoenes, M.; Walter, M.; Dimos, J.; Peitz, M.; Brüstle, O.; Heller, R.; Wirth, B. VPA response in SMA is suppressed by the fatty acid translocase CD36. *Hum. Mol. Genet*., **2013**, *22*(2), 398-407.