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Mini review

Histone deacetylase 4 (HDAC4), an epigenetic target for spinal muscular atrophy

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Summary. Spinal muscular atrophy, a neurodegenerative recessive disease, is one of the leading genetic causes of death in early infancy and childhood worldwide, having an etiology in a mutation or deletion of the survival motor neuron 1 gene and deficient expression of the survival motor neuron protein. Being developed, spinal muscular atrophy is manifested in the denervation and consequent overexpression of histone deacetylase 4 in skeletal muscle, an epigenetic protein further having a role in the upregulation of two E3 ligases, atrogin-1 and MuRF1 via the myogenin-dependent pathway and leading to the structural and functional muscle protein breakdown through the ubiquitin-proteasome pathway. Being not medically treated, spinal muscular atrophy can progress toward the loss of movement and even death. Therefore, great efforts have been made so far to find an adequate therapy, with therapies already approved in Europe and the United States, yet of limited availability due to the high prices and severe side effects. In that sense, there are continuous efforts among the scientific community worldwide to develop novel, cost-efficient approaches in therapy. The development of selective histone deacetylase 4 inhibitors and their epigenetic modifying capabilities has been of high interest in an attempt to find potential candidates for the effective treatment of spinal muscular atrophy. Nevertheless, none of the histone deacetylase 4 inhibitors has been repurposed for treating spinal muscular atrophy.

Keywords: epigenetics, histone deacetylase 4, spinal muscular atrophy.

SPINAL MUSCULAR ATROPHY

Spinal muscular atrophy (SMA) is a rare, progressive neuromuscular disorder, and one of the leading genetic causes of death in early infancy and childhood (Lunke and El-Osta 2009; Kolb and Kissel 2015). Patients with SMA are born with the deficient expression of ubiquitously expressed survival motor neuron (SMN) protein, which plays a key role in the development of motor neurons, responsible for the transmission of signals from the brain and spinal cord, and enables the contraction of skeletal muscles, i.e., body movement.

Two nearly identical SMN genes expressed in human chromosome 5 at location 5q13 encode the SMN protein, a telomeric SMN1 and a centromeric SMN2 (Kolb and Kissel 2015). The most functional SMN protein is transcribed and translated from the SMN1 gene, while the biosynthesis from the SMN2 gene produces only a small amount of fully functional protein due to C to T substitution in an exonic splicing enhancer, whereby exon 7 is excluded from the transcription, resulting in a formation of truncated and unfunctional SMN, rapidly degraded in the cell (Monani et al. 1999). Approximately 10–15% of mRNA SMN2 gene transcripts contain exon 7 and can produce full-length SMN protein. Individuals affected by SMA are deficient in a functional SMN1 gene, due to the mutation or deletion occurring at exon 7 (Lunke and El-Osta 2009; Evans et al. 2011). However, almost all individu-

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als have at least one functional copy of the SMN2 gene and mostly have 2–4 of them, which can produce 10–20% of the usual level of the SMN protein, sufficient for the survival of some neurons. During the patient's life, the availability of the SMN protein reduces gradually in the anterior horn of the spinal cord and the brain, leading to the death of motor neuron cells. Consequently, skeletal muscles stop receiving the neural input from the central nervous system, normally obtained from these motor neurons, and exert less contractile activity, resulting in a decreased innervation (denervation).

A broad spectrum of SMA-diagnosed individuals is classified into clinical types based on the age of onset and disease progression (Kolb and Kissel 2015): Type 0 (usually fatal at birth); Type 1 (unable to sit independently; symptoms within the first few months of life); Type 2 (able to sit independently but not walk; symptoms between 7-18 months); Type 3 (independent walking; symptoms after 18 months) and Type 4 (independent walking and adult-onset). Moreover, the disease manifests in gradual but progressive muscle atrophy and loss of basic life functions, such as walking, digesting food, swallowing, and breathing. SMA does not affect the brain and cognitive functions (Day et al. 2022). Still, it is interesting to observe that SMA affects rather above-average intelligence and are very sociable newborns.

Not being medically treated, SMA leads to the progressive loss of movement and eventual death (Day et al. 2022). Three SMA drugs/therapies are currently approved by EMA (European Medicines Agency) and FDA (U.S. Food and Drug Administration): Spinraza™ (Nusinersen), developed by Biogen, Zolgensma™, developed by Novartis, and Evrysdi™ (risdiplam), developed by Roch. Hence, Spinraza™ represents an antisense oligonucleotide that modulates SMN2 splicing, via binding to a specific sequence in the SMN2 pre-mRNA and helps to increase the cellular concentration of stable SMN protein, therefore being an SMN-enhancing therapy (Kotulska et al. 2021). It is received by an intrathecal injection, directly into the cerebrospinal fluid through the lower back, four times within the first 2 months and every 4 months for the duration of the individual's life (maintenance doses). Despite being efficient, the medicine may cause serious unwanted effects (Erdos and Wild 2022).

Currently, the most efficient treatment for SMA, recommended for the administration to babies and young children under 2 years of age, is Zolgensma™ (onasemnogene abeparvovec-xioi). It is a medicament for so-called "gene therapy", i.e., adeno-associated virus serotype 9 vector, designed to address the genetic root cause of SMA, by supplying the damaged neurons identical copies of SMN1 genes by in vivo transfection. This way, Zolgensma™ stimulates the SMN1 gene transcription, and consequent translation of new SMN proteins, thus replacing the missing or non-working SMN1 genes and restoring their function (Kotulska et al. 2021). It's a one-time intravenous infusion therapy, yet readily expensive (one treatment costs approximately 2.125 million US dollars). To date, the epidemiological profile on the effects of treatment with Zolgensma™ is heavily understudied, which is confirmed in the paper of Thielen et al. (2022). Nevertheless, distinct treatments may cause numerous side effects (Erdos and Wild 2022).

The treatment with Evrysdi™ (risdiplam) is for children two months and older, a daily oral SMN-enhancing therapy designed to increase SMN protein levels in the central nervous system and throughout the body. The active pharmaceutical ingredient is a small molecule, pyridazine derivative risdiplam, which modifies the splicing of SMN2 mRNA to include exon 7 and helps to increase the amount of stable SMN protein made by this gene in vivo (Poirier et al. 2018). However, the drug exerts several side effects, such as pneumonia, upper respiratory tract infections, etc. (Erdos and Wild 2022).

Due to the serious side- and off-target effects of listed drugs (as well as the high prices and the unavailability of Zolgensma™), there are continuous efforts among the scientific community worldwide to develop novel, cost-efficient approaches for the treatment of SMA. Hence, a palliative-type therapy for SMA could be developed using targeted inhibition or degradation of histone deacetylase 4 (HDAC4), owed to the HDAC4's affinity to upregulate the high expression of two E3 ligases, Muscle Atrophy F-box protein (MAFbx, also known as Atrogin-1 and FBXO32) and Muscle Ring finger protein-1 (MuRF1, also known as TRIM63), crucial for structural and functional muscle protein breakdown during the SMA progression. To understand the HDAC4's significance in the SMA ongoing, a profound understanding of epigenetics and the enzyme's topology, as well as the topography is required.

EPIGENETIC REGULATION OF EUKARYOTIC GENE TRANSCRIPTION

Epigenetics refers to reversible heritable changes in gene expression without alterations in the underlying DNA sequence, i.e., modifications of chromatin able to switch genes "on" or "off" and control the production of proteins (Suppl et al. 2019). Epigenetic machinery comprises various processes included in the regulation of DNA-nucleosome assembly for subsequent gene expression. Gene expression is regulated by proteins, involved in modifications on histones classified as writers, erasers, and readers (Suppl et al. 2019). Writers (e.g., DNA methyltransferases, histone methyltransferases, and histone acetyltransferases) regulate the covalent modifications of amino acid residues within the histone tails. Eras-

ers (histone deacetylases, histone demethylases, etc.), on the other hand, conduct the opposite process, the removal of covalent modifications from the histone tails. Finally, readers (i.e., bromodomain, chromodomain, and Tudor proteins) are enzymatically inactive, yet able to recognize and bind to specific epigenetic modifications, preventing writers and erasers from accessing specific modifications (Drake and Søreide 2019).

The acetylation of histone tails, mediated by histone acetyltransferases as writers, neutralizes the positive charge of the surface lysine residues within several histones (H2AK5, H2AK9, H2BK5, H2BK12, H2BK20, H3K4, H3K9, H3K14, H3K18, H3K23, H3K27, H3K36, H4K5, H4K8, H4K12, H4K16, H4K20), making the chromatin structure less compact (Zhao et al. 2022), weakening the interactions between histones and DNA, and enabling the RNA polymerase to access DNA and perform gene expression (Park and Kim 2020). On the other hand, histone deacetylases (HDACs) as erasers, remove the acetyl moiety from the ε-amino group of lysine residues on the N-terminal extension of the histone protein, enforcing the tight condensation between the chromatin and DNA, and minimizing the chances for RNA polymerase-catalyzed gene expression (Mohseni et al. 2013). Published data show that silencing of HDAC by inhibitors VPA (targeting HDAC1, HDAC2, and HDAC3), PBA (targeting HDAC1 and HDAC2), M344 (targeting HDAC6), LBH589 (panHDAC inhibitor), SAHA (targeting HDAC1, HDAC2, HDAC3, HDAC8, and HDAC9), TSA (targeting HDAC5), romidepsin (targeting HDAC1 and HDAC2), resveratrol (targeting HDAC8), and curcumin (targeting HDAC8), act beneficially on the transcription machinery to the SMN2 promoter, by increasing the transcription activity at the SMN2 promoter and exon 7 inclusion (Evans et al. 2011; Mohseni et al. 2013). Consequently, treatment with the aforementioned inhibitors increases the transcription of mRNA bearing the genetic information for exon 7, resulting in an increased SMN expression. Unfortunately, most of these inhibitors exert severe side effects after treatment and this is a disadvantage for their use.

In addition to their essential role in gene transcription, histone acetyltransferases, and HDACs influence other cellular pathways as well, like differentiation, apoptosis, cell cycle control, signaling, and DNA repair through the modification of non-histone proteins (Lunke and El-Osta 2009).

An imbalance between histone acetyltransferases and HDAC activities can affect the improper expression of a specific gene, and result in genomic instability and epigenetic diseases (Park and Kim 2020). Therefore, the precise control of histone acetyltransferases and HDACs is required for the regulated expression of various genes associated with signal transduction, cell growth, and cell death.

HISTONE DEACETYLASES

Histone deacetylases, a subject of this paper, are a large family of proteases ubiquitously distributed within bacteria, fungi, plants, and animals (Silvestri et al. 2012). To date, at least 18 HDAC isoforms have been discovered in mammals, divided into four distinct classes (I, II, III, and IV) according to size, sequence homology, number of active sites, and localization in the cell. Classes I, II, and IV are zinc-dependent amidohydrolases, whereas class III members require NAD^+ as a cofactor for their catalytic activity (Park and Kim 2020). The members of class I, HDAC1, 2, 3, and 8, are predominantly expressed in the nucleus. The class II HDACs, namely HDAC4, 5, 6, 7, 9, and 10, are nucleocytoplasmic shuttling proteins, further divided, according to the domain organization, into class IIa (HDAC4, HDAC5, HDAC7, and HDAC9) and class IIb (HDAC6 and HDAC10). As for class I, Class IIa HDACs are extended with ~600 residues on the N-terminus, while the class IIb enzymes contain two catalytic domains (Bottomley et al. 2008). Class III is comprised of sirtuins (silent information regulators, SIRs) SIRT1-7, while class IV includes only HDAC11, as it is not highly homologous with yeast deacetylases. Besides histones, HDACs have shown the ability to deacetylate nonhistone proteins, predominantly transcription factors (Bottomley et al. 2008).

HDAC4: EXPRESSION, LOCALIZATION, SUBSTRATE SPECIFICITY, AND STRUCTURAL FEATURES

The highest expression of HDAC4 occurs in the brain, heart, and skeletal muscle. Different from other HDACs, it is localized both in the nucleus and cytoplasm and is an epigenetic regulator of diverse cellular functions (Wang et al. 2014). Its location in cells depends on an HDAC4 phosphorylation status, mediated by diverse kinases, namely, calcium/calmodulin-dependent protein kinase (CaMK), extracellular signal-regulated kinases 1 and 2, AMP-activated protein kinase (AMPK), and glycogen synthase kinase 3, as well as by proteolytic cleavage mediated by caspases (Huang et al. 2022). The non-phosphorylated form is the most abundant in the nucleus, whereas the phosphorylated form of the enzyme is located in the cytoplasm. Following phosphorylation, after binding to the chaperone protein 14-3-3, or HDAC4 shuttles from the nucleus to the cytoplasm (Fig. 1).

As a nuclear target, HDAC4 performs regulation of gene expression, cell growth, and proliferation. Still, its overexpression can lead to cancer development, like colon cancer, esophageal squamous cell carcinoma, gastric cancer, B-cell lymphoma, urothelial carcinoma, hepatocellular carcinoma, gliomas, etc. (Halkidou et al. 2004; Wilson et al. 2008; Ahmad et al. 2015; Amodio et al. 2016; Zeng et al.

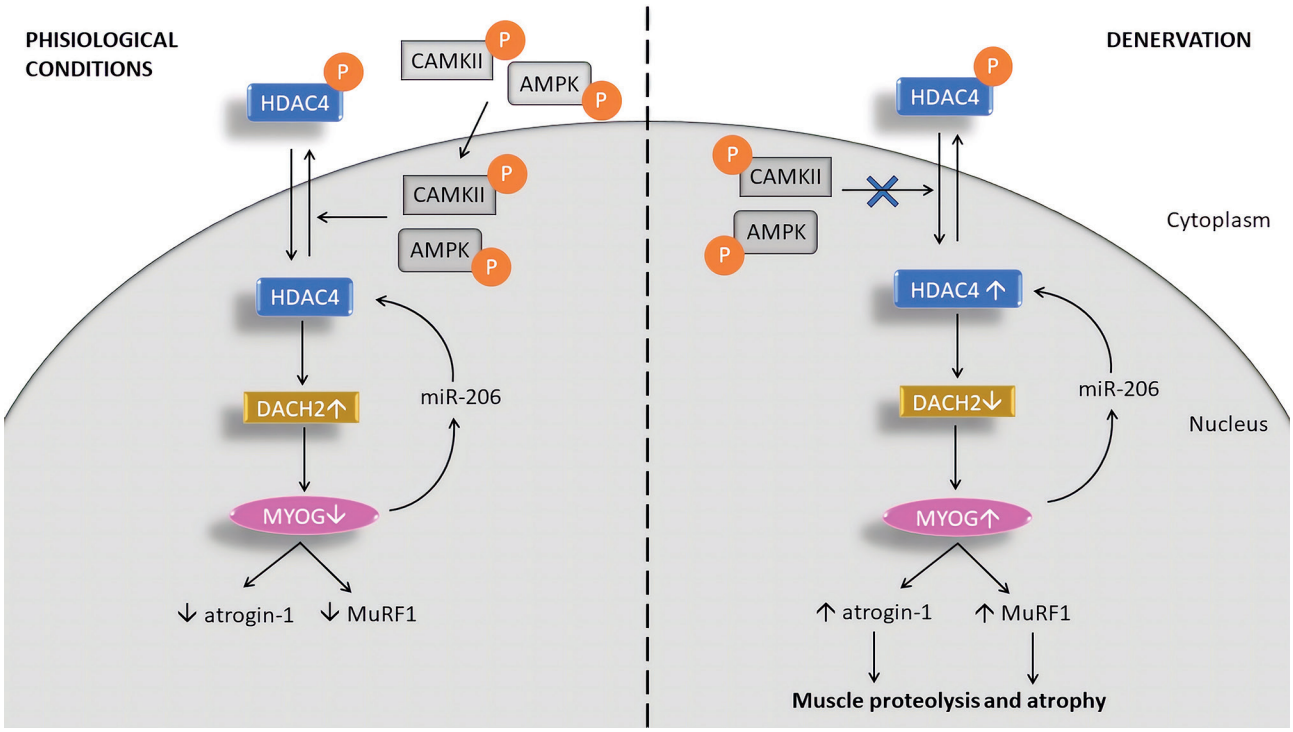

Fig 1. Upregulation of spinal muscular atrophy by HDAC4.

2016; Marampon et al. 2017; Milazzo et al. 2020). On the other hand, within the cytoplasm, HDAC4 is an important target for the treatment of cardiovascular, neurodegenerative, and muscle diseases (Wang et al. 2014; Ma et al. 2021; Huang et al. 2022).

The subcellular location of HDAC4 enables the deacetylation of the acetyl group from both histones and nonhistone proteins and consequently dictates the physiology and pathological role of the enzyme within cells and tissues. Still, the enzyme lacks substrate specificity, as it, differently from other HDACs, exerts very low enzymatic activity against acetylated lysine-containing histones likely due to the unique catalytic domain structural features (see below) (Bottomley et al. 2008). However, HDAC4 exerts the ability to deacetylase non-histone proteins: myosin heavy chain, peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC-1α), and heat shock cognate 71 kDa protein (Luo et al. 2019).

In general, HDAC4 shares conserved topology with other zinc-dependent HDAC isoforms, where the experimentally resolved crystal structures complexed with either hydroxamic acid inhibitors (PDB IDs: 2VQM (Bottomley et al. 2008), 2VQV (Bottomley et al. 2008), 4CBT (Bürli et al. 2013) and 6FYZ (Luckhurst et al. 2019)), trifluoromethylketone inhibitors 2VQJ (wild-type protein) (Bottomley et al. 2008), 2VQQ (mutated protein), and 2VQO (mutated protein) (Bottomley et al. 2008), and 4CBY (Bürli et al. 2013), and tetrasubstituted cyclopropane inhibitors (5A2S) (Luckhurst et al. 2016) reveal a hydrophobic channel leading to the enzyme active site that nests Asp196, Asp290, His198, and two water molecules coordinated to the catalytic Zn^{2+} ion (PDB ID: 2VQM, Fig. 2).

At the entrance of the catalytic site, there is another zinc-binding domain, specific only for the HDAC4 and other class IIa HDACs, which makes the active site more accessible compared to the other isoforms (Battomley et al. 2008; Park and Kim 2020). Within the active site of HDAC8 (PDB ID: 1W22) or HDAC6 (PDB ID: 5EDU), the acetamide carbonyl group of the acetylated lysine substrate replaces one water molecule and forms a hydrogen bond with the hydroxyl group of a tyrosine residue (Tyr306 in HDAC8, or Tyr782 in HDAC6), important for the stabilization of acetylated substrate via a tetrahedral intermediate (Fig. 2). However, in the binding pocket of HDAC4 (PDB ID: 2VQM) tyrosine (Tyr306/782), is replaced with histidine (His332) and rotated away from the binding site amino acids, leading to the change in the active site size, and the weaker stabilization of the acetylated substrate (PDB ID: 2VQM) in comparison to the other zinc-dependent deacetylases (HDAC8, PDB ID: 1W22 or HDAC6, PDB ID: 5EDU). Thus, reduced HDAC4 catalytic activity could be attributed to a Tyr306/782 to His332 active site mutation (Park and Kim 2020).

The unique characteristic of all class IIa HDAC members is an adapter domain in the N-terminus involved in

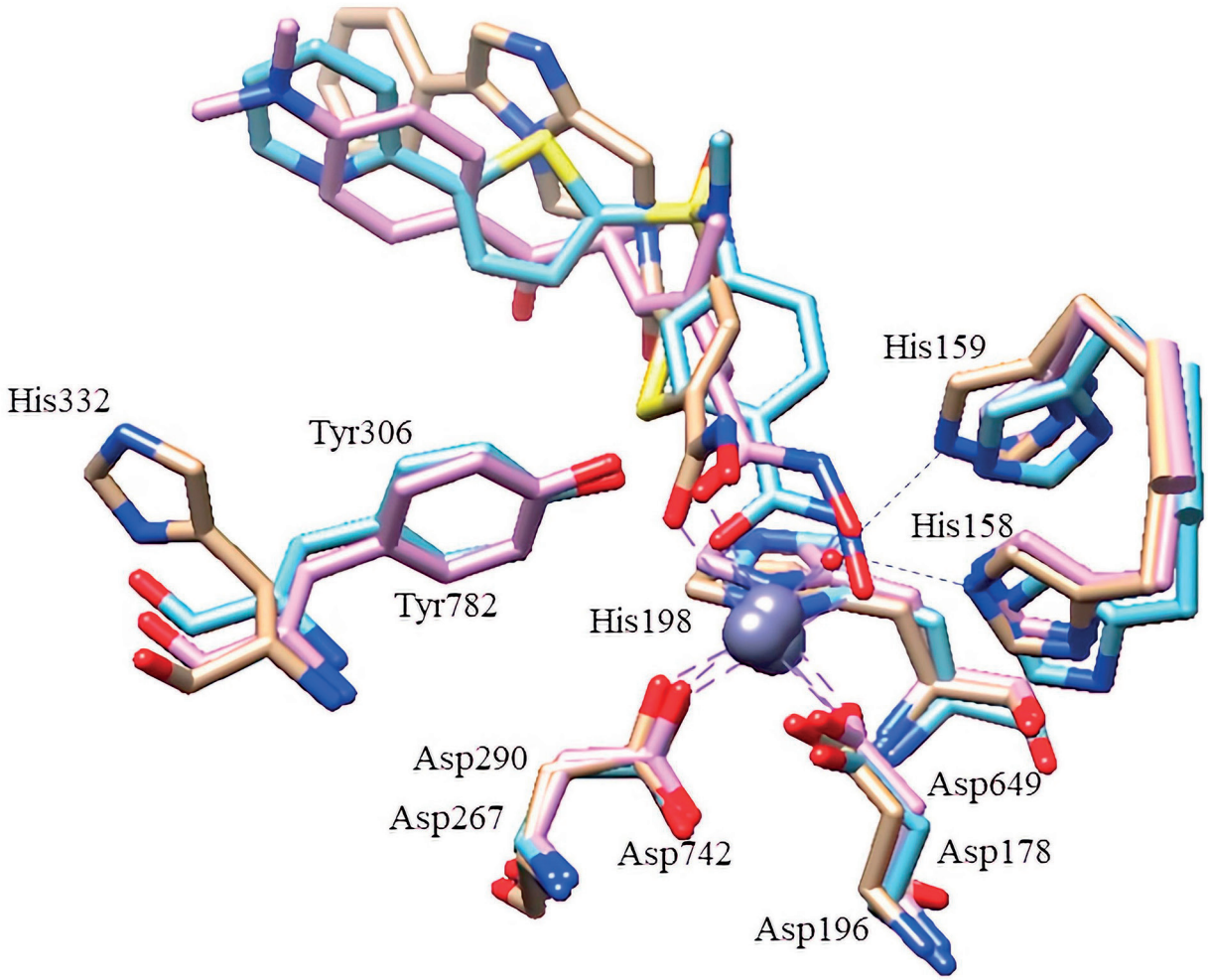

Fig. 2. Crystal structures comparison between the HDAC4 (beige chain, PDB ID: 2VQM, active site amino acids Asp196, His198, Asp290, His332, and His 158 and 159 involved in stabilization of water molecule), HDAC6 (violet chain, PDB ID: 5EDU, active site amino acids Asp649, His659, Asp742, Tyr782), and HDAC8 (blue chain, PDB ID: 1W22, active site amino acids Asp178, His180, Asp267, Tyr306).

binding to DNA via MEF2 transcription factor, which in addition enables enzyme phosphorylation, association with 14-3-3 proteins, and further shuttling between the cytoplasm and nucleus (Park and Kim 2020).

HDAC4 INVOLVEMENT IN SMA AND OTHER MUSCULAR DISORDERS (DENERVATING DISEASES)

As indicated above, SMA is characterized by motor neuron loss, skeletal weakness, and severe atrophy (Bricceno et al. 2012). During muscle atrophy, an increased expression of specific E3 ubiquitin ligases, atrogin-1 and MuRF1, occurs (Fig. 1), together with HDAC4 and myogenin as the upstream regulators (Bricceno et al. 2012). Thus, the atrogin-1 is involved in muscle growth and differentiation for degradation, whereas MuRF1 mediates the degradation of structural proteins, such as myosin heavy chain proteins, myosin light chains 1 and 2, myosin binding protein C, muscle creatine kinase, and other myofibrillar proteins. The high expression of distinct E3 ligases leads to structural and functional muscle protein degradation (breakdown) through the ubiquitin-proteasome pathway.

In more detail, an increment of HDAC4 following denervation suppresses the expression of transcription factor (TF) Dach2, a negative regulator of the myogenin promoter, and ends with increased myogenin expression (Fig. 1). Myogenin is a muscle-specific TF (regulatory protein) involved in myogenesis and is essential for muscle development, thus playing a dual role as both an inducer of neurogenic atrophy and a regulator of muscle development. HDAC4 can be positively regulated by myogenin, as well, via microRNA

involved in skeletal muscle development (miRNA-206) and can improve the ability of motor neurons in SMA patients and maintain the shape and number of the motor endplate in skeletal muscle (Ma et al. 2021).

In addition to the upregulation of atrogenes expression, HDAC4 is involved in the regulation of PGC-1α, a transcriptional cofactor important for energy metabolism and mitochondrial biogenesis (Luo et al. 2019). The regulation is based on the deacetylation of PGC-1α by HDAC4 and the decrease of PGC-1α level in myotubes.

This approach aiming to stop muscle denervation and degradation, the most severe symptoms of SMA by inhibiting HDAC4 has not been widely investigated, and it is completely different from the mechanism of action of previously published HDAC inhibitors, responsible for enhancing the SMN protein expression (Mohseni et al. 2013). Due to insufficient examination of HDAC4 as a target for SMA, the outcome can't be predicted.

AN OVERVIEW OF HDAC4 INHIBITORS

Denervation and other spinal muscular atrophy adverse effects can be mitigated by suppression of myogenin-dependent atrogene induction with HDAC4 selective inhibitors. Currently, besides the co-crystallized ones (Table 1), there are several classes of HDAC4 inhibitors, that still exert a low degree of isoform selectivity, and, to date, none of them have been tested against SMA (Table 2). For that reason, the development of selective HDAC4 inhibitors is one of the most pressing concerns to be addressed to minimize off-target toxicity (Park and Kim 2020). With selective HDAC4 inhibitors, the HDAC4-Dach2-myogenin pathway could be pharmacologically targeted to suppress atrogene induction and muscle protein breakdown.

CONCLUSION

HDAC4 appears to be an important enzyme in the regulation of spinal muscular atrophy and its inhibition could ameliorate the denervation and muscle decomposition within the proteasome pathway. Of currently available more than seventy HDAC4 inhibitors of which many of them (if not most of them) lack selectivity, none have been tested against SMA. Thus, the potential SMA treatment with HDAC4 inhibitors remains an unexplored field of medicinal chemistry, with great potential for further drug development.

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Table 1. An overview of compounds co-crystalized with HDAC4.

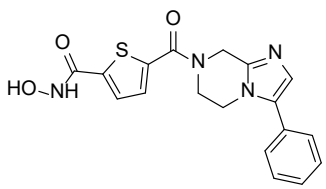
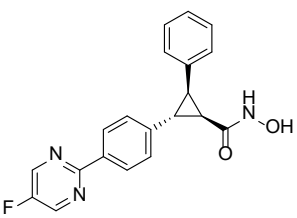
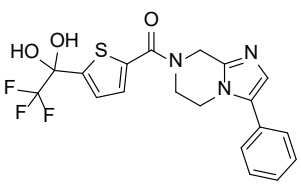
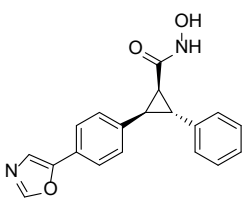
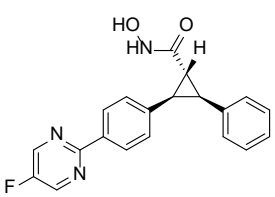
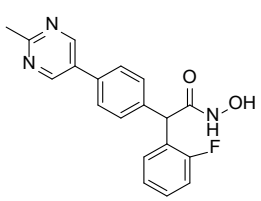
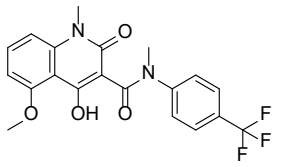
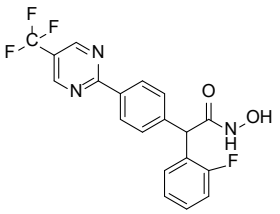
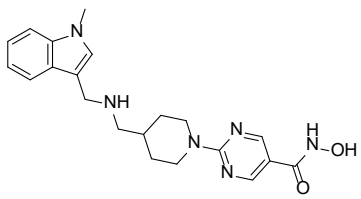
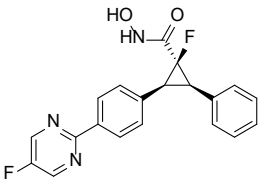
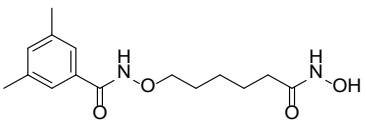
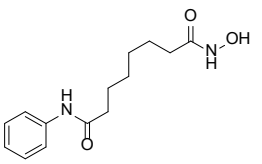
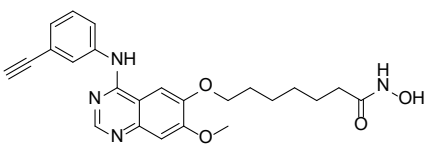
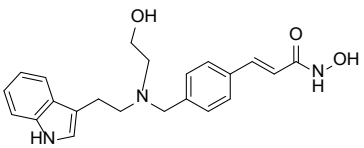
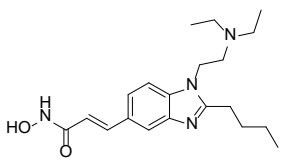
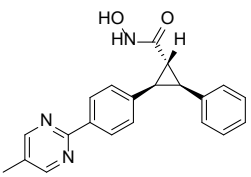
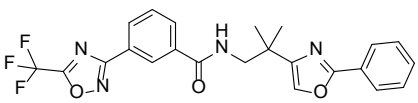
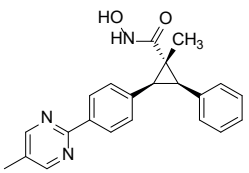
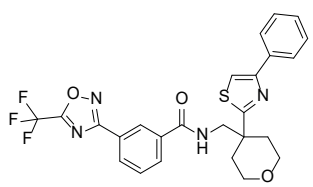
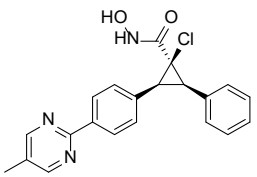
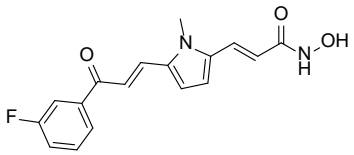
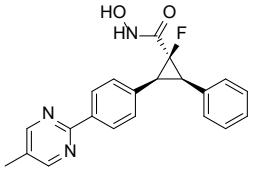
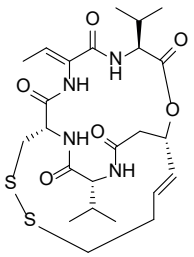
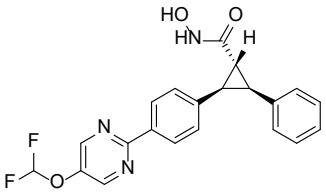
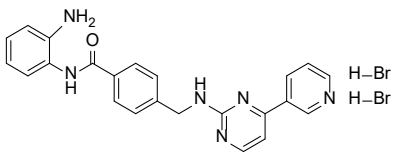
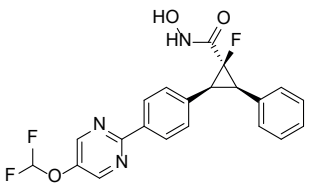
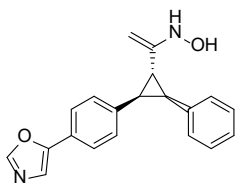
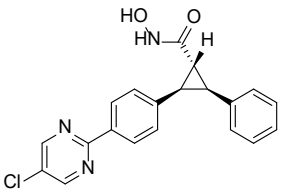
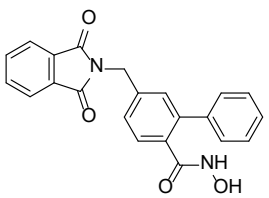
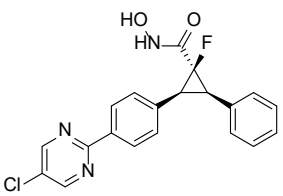
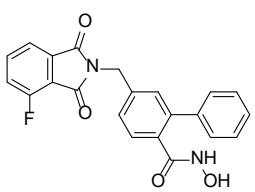
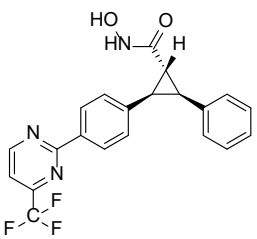
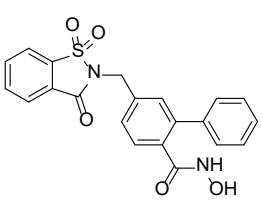
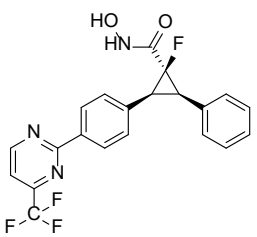
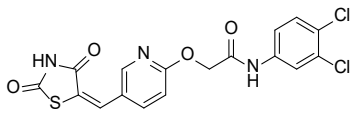
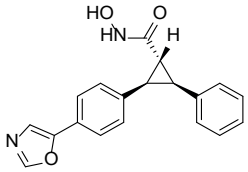
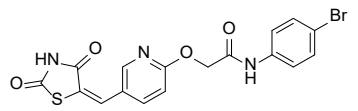
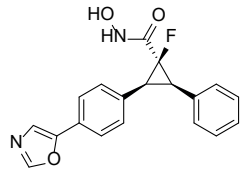
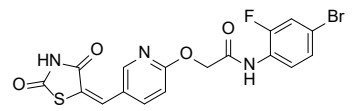
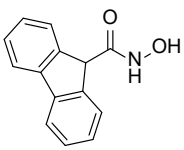
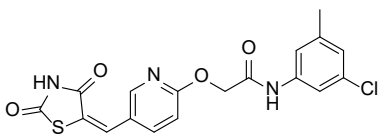
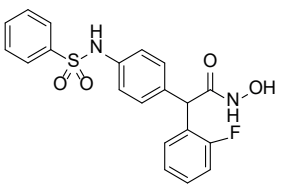
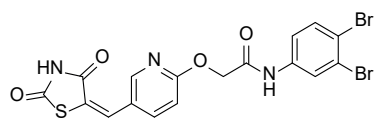
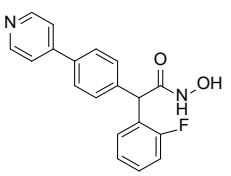
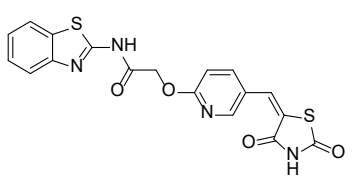
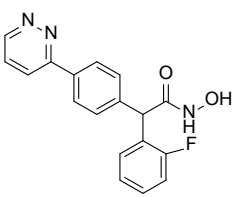
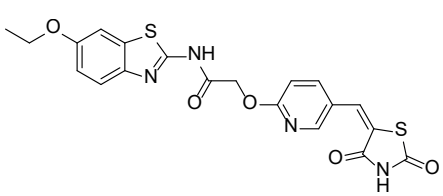
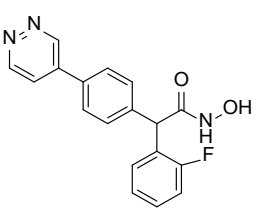
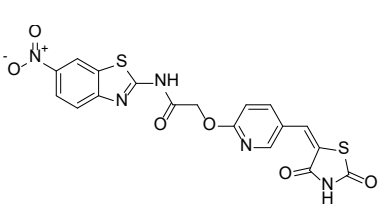
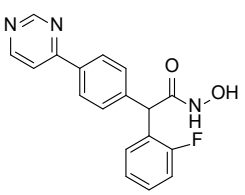
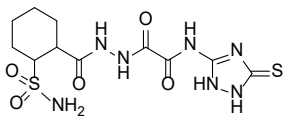
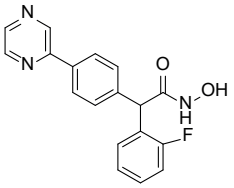
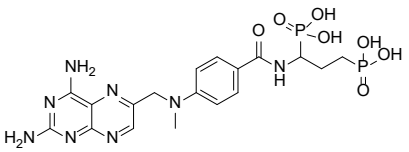
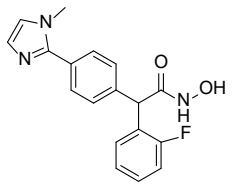
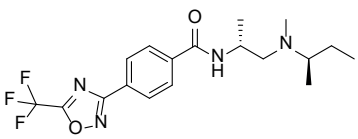
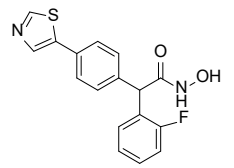
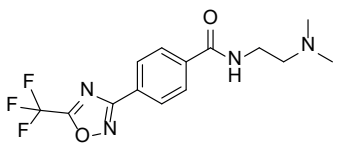
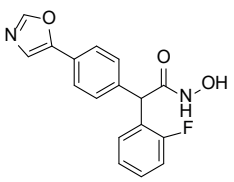
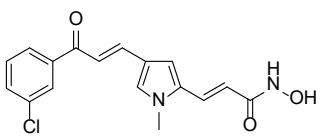
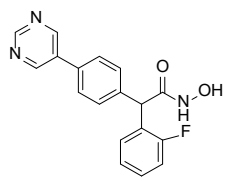
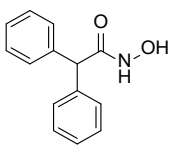
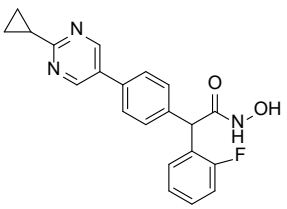
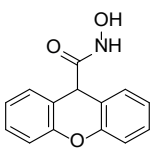
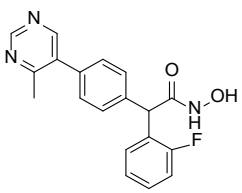
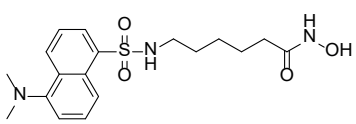
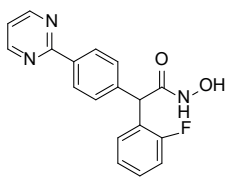
Structure	Activity (Ref.)	Structure	Activity (Ref.)
	IC_{50}: 978 nM (Bottomley et al. 2008)		IC_{50}: 50 - 60 nM (Bürli et al. 2013)
	IC_{50}: 367 nM (Bottomley et al. 2008)		IC_{50}: 20 nM (Bürli et al. 2013)
	IC_{50}: 10 nM K_d: 20 nM (Luckhurst et al. 2016)		IC_{50}: 39 nM K_d: 39 nM (Luckhurst et al. 2019)

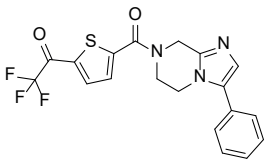
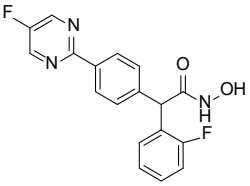
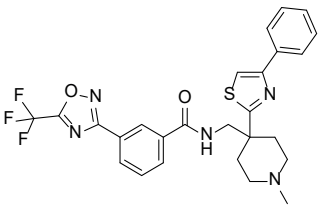
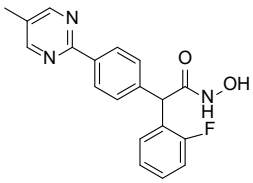
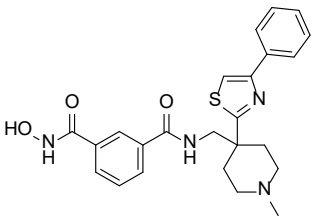
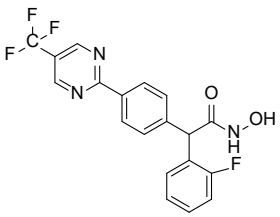
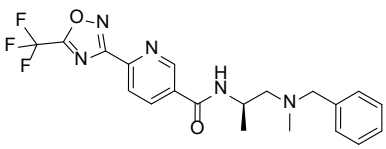
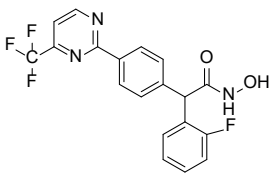
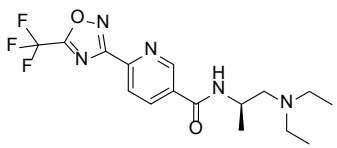
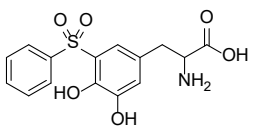
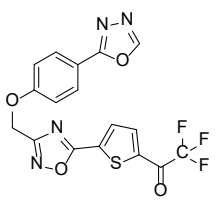
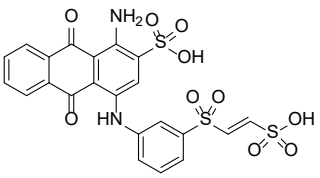
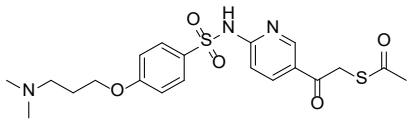
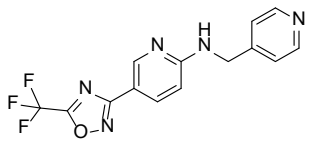
Table 2. Structures and activities of the literature available HDAC4 inhibitors.

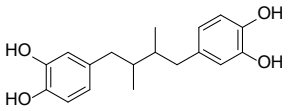
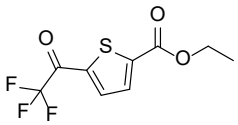
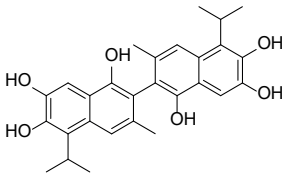
Structure	Activity (Ref.)	Structure	Activity (Ref.)
 Tasquinimod	K_d: 10-30 nM (Isaacs et al. 2013)		IC_{50}: 54 nM (Asfaha et al. 2019)
 Quisinostat	IC_{50}: 0.64 nM (Arts et al. 2009)		IC_{50}: 10 nM (Asfaha et al. 2019)
 LMK-235	IC_{50}: 11.9 nM (Huang et al. 2022)		IC_{50}: 41 μM (Macabuag et al. 2022)
 CUDC-101	IC_{50}: 13.2 nM (Liang et al. 2023)	 LAQ	IC_{50}: 0.50 μM (Tessier et al. 2009)
 Pracinostat (SB939)	IC_{50}: 56 nM (Liang et al. 2023)		IC_{50}: 0.18 μM (Luckhurst et al. 2016)
 TMP195	K_i: 59 nM (Huang et al. 2022)		IC_{50}: 50 nM (Luckhurst et al. 2016)
 TMP269	IC_{50}: 157 nM (Huang et al. 2022)		IC_{50}: 0.11 μM (Luckhurst et al. 2016)

Structure	Activity (Ref.)	Structure	Activity (Ref.)
 MC1568	IC_{50}: 220 nM (Kassis et al. 2016)		IC_{50}: 20 nM (Luckhurst et al. 2016)
 Romidepsin	IC_{50}: 25 nM (Liang et al. 2023)		IC_{50}: 150 nM (Luckhurst et al. 2016)
 Mocetinostat dihydrobromide	IC_{50}: 1 μM (Huang et al. 2022)		IC_{50}: 30 nM (Luckhurst et al. 2016)
 CHDI-00381817	IC_{50}: 20 nM (Huang et al. 2022)		IC_{50}: 140 nM (Luckhurst et al. 2016)
	IC_{50}: 28 nM (Mak et al. 2021)		IC_{50}: 40 nM (Luckhurst et al. 2016)
	IC_{50}: 13 nM (Mak et al. 2021)		IC_{50}: 80 nM (Luckhurst et al. 2016)
	IC_{50}: 49 nM (Mak et al. 2021)		IC_{50}: 40 nM (Luckhurst et al. 2016)

Structure	Activity (Ref.)	Structure	Activity (Ref.)
	IC_{50}: 6.7 μM (Tilekar et al. 2021)		IC_{50}: 30 nM (Luckhurst et al. 2016)
	IC_{50}: 15 μM (Tilekar et al. 2021)		IC_{50}: 10 nM (Luckhurst et al. 2016)
	IC_{50}: 15 μM (Tilekar et al. 2021)		IC_{50}: 0.33 μM (Luckhurst et al. 2019)
	IC_{50}: 8.8 μM (Tilekar et al. 2021)		IC_{50}: 22 nM (Luckhurst et al. 2019)
	IC_{50}: 4.2 μM (Tilekar et al. 2021)		IC_{50}: 103 nM (Luckhurst et al. 2019)
	IC_{50}: 0.75 μM (Tilekar et al. 2021)		IC_{50}: 33 nM (Luckhurst et al. 2019)
	IC_{50}: 4.9 μM (Tilekar et al. 2021)		IC_{50}: 31 nM (Luckhurst et al. 2019)
	IC_{50}: 2.3 μM (Tilekar et al. 2021)		IC_{50}: 22 nM (Luckhurst et al. 2019)

Structure	Activity (Ref.)	Structure	Activity (Ref.)
 A640973 Sulfone	IC_{50}: 17.4 μM (Hsu et al. 2017)		IC_{50}: 40 nM (Luckhurst et al. 2019)
 A363007 Phosphonic acid	IC_{50}: 4.1 μM (Hsu et al. 2017)		IC_{50}: 30 nM (Luckhurst et al. 2019)
 NVS-HD1	IC_{50}: 0.65 nM (Luo et al. 2019)		IC_{50}: 22 nM (Luckhurst et al. 2019)
 NVS-HD3	IC_{50}: 80 nM (Luo et al. 2019)		IC_{50}: 25 nM (Luckhurst et al. 2019)
 MC1575	IC_{50}: 5 μM (Giorgio et al. 2015)		IC_{50}: 57 nM (Luckhurst et al. 2019)
	IC_{50}: 750 nM (Giorgio et al. 2015)		IC_{50}: 55 nM (Luckhurst et al. 2019)
	IC_{50}: 250 nM (Giorgio et al. 2015)		IC_{50}: 49 nM (Luckhurst et al. 2019)
	IC_{50}: 330 nM (Giorgio et al. 2015)		IC_{50}: 25 nM (Luckhurst et al. 2019)

Structure	Activity (Ref.)	Structure	Activity (Ref.)
	IC_{50}: 320-370 nM (Asfaha et al. 2019)		IC_{50}: 25 nM (Luckhurst et al. 2019)
 TMP942	K_i: 75 nM (Asfaha et al. 2019)		IC_{50}: 17 nM (Luckhurst et al. 2019)
 TMP974	K_i: 23.8 μM (Asfaha et al. 2019)		IC_{50}: 54 nM (Luckhurst et al. 2019)
	IC_{50}: 18 nM (Asfaha et al. 2019)		IC_{50}: 119 nM (Luckhurst et al. 2019)
	IC_{50}: 28 nM (Asfaha et al. 2019)	 A226640 Sulfone	IC_{50}: 14.9 μM (Hsu et al. 2017)
	IC_{50}: 10 nM (Macabuag et al. 2022)	 A364365	IC_{50}: 2.5 μM (Hsu et al. 2017)
 KD 5170	IC_{50}: 26 nM (Payne et al. 2008)	 NVS-HD2	IC_{50}: 49 nM (Luo et al. 2019)

Structure	Activity (Ref.)	Structure	Activity (Ref.)
 A4291 Catechol	IC_{50}: 24.7 μM (Hsu et al. 2017)		IC_{50}: 320 nM (Giorgio et al. 2015)
 A159452 Catechol	IC_{50}: 4.4 μM (Hsu et al. 2017)		

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