

Research on the Progress of Spinal Motor Neuron Development and Motor Neuron Diseases

Lingling Li¹, Yulong Tang¹, Jinan Chen¹, Youjia Wu^{2,*}, Yuqin Zheng^{3,*}

¹ Department of Pediatrics, The Second People's Hospital of Nantong, Nantong, China

² Department of Child Health, Children's Hospital of Nanjing Medical University, Nanjing, China

³ Department of Pediatrics, Affiliated Hospital of Nantong University, Nantong, China

***Correspondence:** Youjia Wu, Department of Child Health, Children's Hospital of Nanjing Medical University, Nanjing 210008, China; Yuqin zheng, Department of Pediatrics, Affiliated Hospital of Nantong University, Nantong 226001, China.
E-Mails: 2231310084@stmail.ntu.edu.cn, francis_nt@163.com, and 403006970@qq.com

Abstract

Objective: This review aims to summarize the developmental dynamics of spinal motor neurons (SMNs), with a focus on key signaling pathways (including Sonic Hedgehog [Shh], fibroblast growth factor [FGF], and bone morphogenetic protein [BMP]) and transcription factors such as pancreas Homeobox protein 9 (Hb9), Insulin Gene Enhancer Protein 1 (Isl1), and LIM Homeobox Protein 3 (Lhx3). This review also examines the pathological associations with amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), and it evaluates emerging therapies.

Methods: A comprehensive literature search was conducted in PubMed, Web of Science, Embase, and Scopus from database inception to December 2025. The search strategy combined terms related to SMN development, signaling pathways, transcriptional regulation, amyotrophic lateral sclerosis, spinal muscular atrophy. Two authors independently performed screening and data extraction, after which the evidence was synthesized narratively.

Results: SMNs connect the central nervous system to peripheral muscles. Their development is governed by a complex molecular network in which Shh, FGF, and BMP signaling synergize with Hb9, Isl1, and Lhx3 to regulate origin, differentiation, migration, and synapse formation. Genetic factors and extracellular microenvironment abnormalities are linked to ALS and SMA. Gene therapy and stem cell transplantation show translational potential.

Discussion: This review provides a theoretical framework for understanding neural development and identifying therapeutic targets for motor neuron disorders. Integrating developmental signaling, pathological factors, and translational strategies may advance research and treatment of SMN-related diseases.

Keywords: spinal motor neurons; signaling pathways; transcriptional regulation; amyotrophic lateral sclerosis; spinal muscular atrophy

The development of nervous system relies on the precise coordination of multidimensional molecular events. Among these processes, the development of spinal

motor neurons (SMNs) is particularly critical. SMNs arise from ventral progenitor cells within the neural tube and ultimately form neuromuscular junctions

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(NMJs) enabling the transmission of motor commands through a series of tightly regulated steps, such as differentiation, migration, and axonal guidance^[1,2]. In recent years, breakthroughs in single-cell sequencing^[3] and gene editing technologies^[4] have revealed the intricate interplay between transcription factor networks and signaling pathways during SMN development^[5-7]. Moreover, research incorporating population-specific characteristics and environmental factors has paved the way for tailored therapeutic strategies for domestic patient populations. Concurrently, the integration of traditional Chinese medicine with modern biomedical approaches offers novel avenues for intervention^[8]. These advances not only deepen our understanding of neurodevelopmental mechanisms but also provide fresh insights into disorders linked to developmental abnormalities, such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). This review synthesizes the latest domestic and international research to systematically delineate the molecular and cellular mechanisms underlying SMN development and their pathological implications. Furthermore, it outlines future directions for translational research and proposes innovative strategic frameworks and therapeutic paradigms for the treatment of related neurological disorders.

Molecular regulatory mechanisms of SMN development

Origin and early induction

Embryonic Stem Cells (ESCs) have garnered significant research interest due to their capabilities for self-renewal and pluripotency, conferring the potential to differentiate into diverse cell types. Under appropriate inductive conditions, ESCs can further differentiate into Neural Stem Cells (NSCs), which not only possess self-renewal capacity but are also multipotent, enabling their development into various cell types of the nervous system, including SMN progenitors^[9]. During this process, early signaling pathways play an indispensable role. These pathways, mediated by specific signaling molecules such as Sonic Hedgehog (Shh)^[10] and fibroblast growth factor (FGF)^[11], critically induce the differentiation of NSCs into SMN progenitor cells.

Shh signaling pathway

Shh signaling pathway plays a pivotal role in the induction of SMNs. During critical stages of embryonic development, the Shh protein is secreted by the notochord and floor plate cells of the neural tube, establishing a concentration gradient in the surrounding tissue. The presence of this concentration gradient enables varying levels of Shh to activate distinct sets of transcription factors, thereby precisely directing the fate of NSCs and determining the specific neuronal subtypes they will become. Specifically, exposure to lower concentrations of Shh promotes the differentiation of NSCs into dorsal neuron populations, which are primarily involved in sensory information processing. In contrast, higher concentrations of Shh signal direct NSCs to adopt a SMN progenitor fate, which subsequently mature into motor neurons responsible for controlling muscle movement^[12].

FGF signaling pathway

FGF signaling pathway regulates the fate determination of SMN progenitors through multi-dimensional mechanisms. Studies demonstrate that FGF ligands (e.g., FGF8) significantly promote the survival and proliferation of NSCs by activating FGF receptors^[11]. Key downstream effector molecules include the Mitogen-Activated Protein Kinase-Extracellular Signal-Regulated Kinase (MAPK/ERK) pathway, which regulates cell cycle progression, and the Phosphoinositide 3-Kinase-Protein Kinase B pathway, which controls the expression of anti-apoptotic genes. Concurrently, the c-Jun N-terminal kinase pathway is involved in establishing axonal growth polarity^[13]. Notably, FGF and Shh signaling form a dynamic synergistic network in the ventral neural tube: the Shh gradient establishes the foundational ventral identity of progenitor cells, while FGF enhances Shh signaling efficacy by inhibiting the GLI family zinc finger 3 (Gli3) repressor. These pathways collaboratively drive the expression of motor neuron-specific transcription factors oligodendrocyte transcription factor 2 (Olig2) and NK6 homeobox 1 (Nkx6.1). This integrated signaling mechanism ensures the spatiotemporally precise specification of motor neuron progenitor cells^[14].

Bone Morphogenetic Protein signaling pathway

As a member of the TGF- β superfamily, bone morphogenetic protein (BMP) plays a pivotal role in establishing the dorsoventral patterning of the neural tube. BMP4/7 ligands bind to type II (BMPRII) and type I (ALK2/3/6) receptors, activating the Smad1/5/8 phosphorylation cascade and thereby driving the differentiation of NSCs toward dorsal interneurons, such as sensory neurons^[15]. Its function exhibits significant regional specificity: in the dorsal neural tube, BMP signaling upregulates *Msx1* and *Msx2* transcription factors to suppress the expression of motor neuron markers (e.g., *Olig2*); whereas in the ventral neural tube, BMP forms a dynamic antagonistic network with Shh and FGF signaling—Shh counteracts the dorsalizing effect of BMP by inhibiting the Gli3 repressor, while FGF8 downregulates Smad activity via the MAPK pathway. These interactions collectively delineate the generative boundary of motor neuron progenitor cells^[16]. Furthermore, BMP signaling maintains the proliferation of neural precursor cells by inducing Id protein expression and modulates the size of the progenitor cell pool by activating anti-apoptotic genes (e.g., Bcl-xL) of the Bcl-2 family^[17].

Bidirectional regulatory role of the notch signaling pathway

The Notch signaling pathway plays a critical role in the proliferation and differentiation of NSCs. By modulating the balance between self-renewal and differentiation in NSCs, activation of the Notch pathway can suppress neuronal differentiation and influence the formation of SMN progenitor cells^[18]. In spinal cord injury models, therapeutic strategies such as pharmacological intervention, electroacupuncture, cell transplantation, or genetic modification to inhibit Notch signaling activation have been shown to alleviate pathological progression, promote tissue repair, and facilitate the recovery of motor function^[19–21].

Differentiation and transcriptional regulation of SMNs

Core regulatory role of transcription factors

The differentiation of SMNs is precisely driven by a conserved cascade of transcription factors. Among

these, Jessell^[22] posited that spinal neuronal specification is governed by a combinatorial transcriptional code instructed by graded inductive signals, thereby providing a conceptual foundation for understanding Wnt-dependent patterning. Maurice and Angers^[23] subsequently delineated the mechanistic basis of Wnt/ β -catenin pathway activation and signal transduction, encompassing ligand–receptor engagement, β -catenin stabilization, nuclear translocation, and transcriptional regulation. Wnt/ β -catenin signaling, activated by Wnt ligands, acts as a key regulator of cell proliferation, differentiation, and fate determination. Hb9 (encoded by the *MNX1* gene), as a key determinant of motor neuron identity, directly activates genes associated with cholinergic function (such as ChAT and VACHT) while suppressing the expression of interneuron markers (e.g., *Pax*)^[24,25]. *Isl1* and *Lhx3* form a LIM-homeodomain complex that synergistically activates the motor neuron-specific program by binding to the enhancer region of the *MNX1* gene^[26]. Single-cell transcriptome analyses reveal that *Isl1*/*Lhx3* double-positive cells are fated to differentiate into SMNs, whereas *Lhx3* single-positive cells differentiate into V2 interneurons, demonstrating the decisive role of this combinatorial code in determining cell fate^[27]. Furthermore, Homeobox (*Hox*) gene clusters (e.g., *Hoxc6*, *Hoxc9*) regulate the segment-specific differentiation of SMNs through chromatin spatial reorganization, unveiling the multi-layered regulatory mechanisms orchestrated by transcription factors^[28].

Synergistic regulation of signaling pathways and transcription factors

In addition to the critical role of transcription factors, signaling pathways play an essential role in the fine-tuning of neuronal differentiation during SMN development. The differentiation of SMNs is dynamically fine-tuned by signaling pathways that engage in bidirectional regulatory circuits with transcription factors: (1) Notch signaling inhibits differentiation by maintaining neural progenitor cells in an undifferentiated state through *Hes1*-mediated suppression of *Neurog2*^[29]. Furthermore, RBP-J, a core component of the Notch pathway, is crucial for this process, and its knockout leads to premature differentiation of neural precursors^[30]. (2) Wnt/ β -catenin promotes differentiation; Wnt7a activates β -catenin via Dishevelled, directly upregulating *Ngn2* expression,

which subsequently induces the assembly of the Hb9/Lhx3 complex. In β -catenin conditional knockout models, the efficiency of SMN differentiation decreases by 68%^[5]. (3) Shh-FGF signaling is integrated; Shh sustains the ventral signaling environment, while FGF8 enhances the transcriptional activity of Isl1 through ERK phosphorylation, thereby forming a positive feedback loop between signaling and transcription factors^[31].

Migration and axonal outgrowth of SMNs

Spatiotemporal dynamics of migration

The migration of SMNs exhibits strict spatiotemporal specificity, which can be divided into two critical phases: (1) Early ventral migration involves progenitor cells traversing from the dorsal to the ventral neural tube through the basal lamina. This process relies on laminin-integrin interactions within the extracellular matrix (ECM) to provide mechanical support^[32,33]. (2) During late target-directed migration, guidance cues build upon the ECM foundation to direct SMNs along specific pathways, ensuring their precise arrival at target muscle tissues. For instance, mature neurons extend toward target myotomes along Netrin-1/Slit concentration gradients, with their path selection being precisely regulated by the chemoattractive-repulsive signals of these guidance cues^[34,35].

Bidirectional regulation by guidance cues

Netrin-1, a key guidance cue in nervous system development, is primarily secreted by the ventral floor plate and generally functions as a chemoattractive molecule. It binds to the DCC receptor, activating the FAK-Src pathway and inducing actin cytoskeletal reorganization (via F-actin and GTPase Rac1 activation) to precisely guide SMN migration. Conversely, signaling through the UNC5B receptor inhibits dorsal deviation by promoting RhoA-mediated contraction^[36]. In contrast, Slit, another crucial guidance factor mainly secreted by the dorsal neural tube, acts primarily as a repulsive cue. By binding to its Robo receptors, Slit activates Cofilin-mediated microfilament depolymerization, thereby inhibiting motor neuron migration and contributing to the organized development of the nervous system^[37]. The expression patterns of Netrin-1 and Slit are highly

dynamic during development. Their spatially and temporally regulated expression provides precise positional information for neuronal migration^[36]. The coordinated interplay of these attractive and repulsive signals ensures that SMNs migrate correctly to their predetermined locations and establish proper neural connections, which is fundamental to the structural integrity and function of the nervous system.

Several other guidance factors also play significant roles in the migration process of SMNs. For instance, Semaphorins, often expressed in specific tissues, function as repulsive signals that prevent neuronal migration into certain regions^[38]. Conversely, Ephrins, expressed in target muscle tissues, can provide attractive signals that guide neurons^[39]. Semaphorin3A (repulsive) and EphrinB1 (attractive) establish orthogonal signaling gradients and regulate the topographic specificity of migratory paths through their respective receptors, PlexinA2 and EphB2. This sophisticated regulatory mechanism is essential for proper neural circuit formation.

If the expression or function of guidance cues becomes aberrant, it may lead to failure in neuronal migration, consequently compromising normal muscle function. Gene knockout studies have demonstrated that Netrin-1 deficiency results in approximately 60% of motor neurons being abnormally retained in dorsal regions^[40], while Slit2 knockout causes erroneous dorsal migration in about 23% of neurons, confirming the necessity of spatiotemporal balance in guidance cues for proper neural circuit patterning^[41].

Abnormal development of SMNs and diseases

Types and clinical manifestations of motor neuron diseases

Motor neuron diseases (MNDs) represent a group of chronic, progressive neurodegenerative disorders characterized primarily by the degeneration of motor neurons. These diseases predominantly affect the anterior horn cells of the spinal cord, brainstem motor neurons, cortical pyramidal cells, as well as the corticospinal and corticobulbar tracts^[42]. This group of conditions primarily impacts the nerve cells controlling voluntary muscle movement, leading to the progressive loss of

muscular function. Based on the anatomical locations of the lesions, MNDs are classified into several types: hereditary spastic paraplegia, which involves only upper motor neurons; SMA, which involves only lower motor neurons; and ALS, which involves both upper and lower motor neurons. Among these, ALS is the most common adult-onset MND, characterized by progressive muscle weakness, atrophy, and spasticity. The symptoms gradually worsen as the disease progresses, causing significant suffering and disability for patients. SMA, on the other hand, is a common MND in children, presenting with symmetrical muscle weakness and atrophy^[43]. Research has found that developmental defects or functional loss of motor neurons may underlie various motor neuron diseases. UTS2 is upregulated in SMA, and its inhibition ameliorates cellular phenotypes^[44], whereas neuronal complex IV deficiency recapitulates ALS-like TDP-43 redistribution, selective motor neuron loss, and paralysis^[45].

The role of abnormal motor neuron development in disease

Dysregulated motor neuron development constitutes a key pathogenic mechanism in MNDs. During neurogenesis, impaired FGF/Shh signaling can compromise the generation of SMN progenitors^[46], while aberrant cell adhesion molecules disrupt precise neuronal positioning^[47]. Transcriptional dysregulation during differentiation prevents the acquisition of proper motor neuron identity, with Notch/Wnt pathway disruptions leading to erroneous cell fate decisions. Faulty migration, caused by defective guidance cue-receptor interactions, prevents target localization and disrupts network formation^[34]. At maturation stages, impaired ion channel function and synaptogenesis undermine neuronal excitability and circuit connectivity. Notably, ALS pathogenesis involves progressive synapse loss and structural alterations in spinal and cortical regions^[48].

Current treatment status and challenges

Currently, the exact etiology and pathogenesis of MNDs remain incompletely understood but may involve genetic factors, viral infections, immune dysfunction, heavy metal poisoning, nutritional and metabolic disturbances, and environmental factors. Furthermore, there is

currently a lack of pharmacological treatments that can effectively ameliorate the clinical symptoms.

Consequently, current treatment of MNDs remains largely symptomatic and multidisciplinary, encompassing respiratory support, nutritional management, and rehabilitation. Adjunctive approaches such as acupuncture and integrated Chinese-Western swallowing rehabilitation have been described in clinical experience and case reports^[49,50], but their efficacy needs to be confirmed by rigorous trials. Fallar shared ALS pathomechanisms, including RNA processing, proteostasis, metabolism, and inflammation, as targets for broadly applicable disease-modifying therapies^[51]. For SMA, disease-modifying therapies have substantially changed the clinical landscape; risdiplam has shown sustained two-year efficacy and safety in patients with type 2 or non-ambulant type 3 SMA^[52], although important limitations persist, including incomplete functional recovery, age-dependent responses, and high treatment costs. In ALS, edaravone can slow functional decline in selected patients, even those with reduced forced vital capacity^[53,54], and numerous investigational agents targeting shared pathomechanisms such as RNA processing, proteostasis, mitochondrial dysfunction, and neuroinflammation are being evaluated^[55]. Despite these advances, no available therapy can halt or reverse MND progression, and major challenges remain, including disease heterogeneity, delayed diagnosis, lack of robust biomarkers, limited blood-brain barrier penetration, and unequal access to care. Therefore, mechanism-based, personalized, and combination therapeutic strategies are urgently needed to improve clinical outcomes in MNDs.

Discussion

The development of SMNs is a multilayered and dynamically regulated process, encompassing key stages such as origin specification, differentiation, migration, and functional maturation. This process is precisely governed by multifaceted factors, including gene regulatory networks, intracellular signaling pathways (e.g., Shh, BMP, and Wnt), the physicochemical properties of the ECM, neurotrophic factors such as glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor, and the formation of NMJs. The spatiotemporal specificity

of axonal guidance and synaptogenesis further relies on the coordinated interplay of neuron-glia interactions, mechanical signaling (e.g., substrate stiffness), and bioelectrical activity. Notably, imbalances in the developmental microenvironment, such as aberrant ECM composition or dysregulation of ion channels, can contribute to neurodegenerative diseases like SMA and ALS, underscoring the clinical relevance of deciphering these developmental mechanisms.

Currently, the treatment of MNDs continues to face core challenges, including high etiological heterogeneity, poor blood-brain barrier permeability, and limited neuronal regenerative capacity. However, the integration of interdisciplinary technologies offers novel pathways to overcome these obstacles: (1) Multi-omics technologies, which integrate single-cell transcriptomics and spatial proteomics analyses, can reveal specific molecular signatures in motor neurons of ALS patients (e.g., pathways associated with TDP-43 aggregation)^[56]. (2) Artificial intelligence-driven approaches, such as deep learning-based virtual drug screening platforms (e.g., AlphaFold2), accelerate the development of small molecules targeting mutants of SOD1 or SMN1^[57]. (3) Precision medicine, which utilizes patient-derived induced pluripotent stem cells to construct spinal cord organoid models, enables the prediction of personalized treatment strategies^[58]. (4) Cross-barrier delivery systems based on engineered exosomes carrying CRISPR-Cas9 systems can cross the blood-brain barrier to precisely repair genetic defects in motor neurons^[59].

International collaborative innovation networks, such as the Answer ALS Consortium, have identified 17 potential therapeutic targets for ALS by integrating clinical databases and biobanks from 20 countries^[60]. Future research should further incorporate technologies like optogenetics and *in situ* metabolic imaging to dissect the molecular coupling mechanisms between developmental and degenerative processes at single-cell resolution, thereby promoting a leap forward from basic research to clinical translation.

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Authors' contributions

Lingling Li was the main author of this review, responsible for the collection and analysis of relevant literature and the writing of the first draft of the manuscript. Yulong Tang and Jinan Chen participated in the collation of literature. Youjia Wu served as the supervisor and corresponding author, participating in the conceptualization and guidance of the work. Yuqin Zheng participated in the English revision of the manuscript. All authors have read and approved the final version of the manuscript.

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Ethics approval and consent to participate

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The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in the preparation, analysis, or writing of this manuscript.

Competing interests

The authors declare no conflicts of interests.

Consent for publication

All the authors consent to the publication of identifiable details, which can include data within the text to be published by *Translational Neurology and Neurosurgery*.

Data availability statement

No datasets were generated or analyzed for this work.

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