The effect of GPNMB on muscular atrophy caused by ALS

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Amyotrophic lateral sclerosis (ALS) is a devastating disease characterized by the degeneration of motor neurons and subsequent muscular defects. Recent findings have highlighted the contribution of intrinsic skeletal muscle defects to ALS. Research focusing on skeletal muscle disorders might therefore offer alternative routes to the development of new therapeutic options for the treatment of ALS. Glycoprotein non-metastatic melanoma protein B (GPNMB) is a transmembrane protein also known as osteoactivin (OA) and dendritic cell-heparin integrin ligand (DC-HIL). GPNMB exerts a protective effect on the central nervous system, and it has been shown to improve memory and to help recovery from reperfusion injury following brain ischemia. In ALS model mice, overexpression of GPNMB prevents motor neuron death and reduces denervation of neuromuscular junctions and atrophy of the skeletal muscles, resulting in a delayed onset of ALS and a longer life span of the animals. When directly injected into skeletal muscle, GPNMB also helps prevent injury of myofibers. These data indicate that GPNMB has a dual site of action against the central nervous system and directly skeletal muscle. Here, we review and highlight recent findings on the effect of GPNMB against ALS, and we discuss remaining challenges of the field and the possible therapeutic applications of GPNMB.

Keywords: ALS; GPNMB; Skeletal muscle

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impairs limb movements in patients, who in the late stage of the pathology, are forced to be bedridden and to use a ventilator. Patients with severe ALS communicate through eye movements or using a computer. Their intelligence, consciousness, and self-awareness are normal until the end of pathology, contributing to deepen the misery of the disease [2].

The pathological hallmarks of ALS are the degeneration and loss of motor neurones and the presence of intraneuronal inclusions in degenerating neurones and glia. Bunina bodies, ubiquitinated inclusions, skein-like inclusions, and hyaline conglomerate inclusions are ALS typical histopathological features [2]. It has been proposed that defects in skeletal muscle, leading to muscle cell dysfunction, also contribute to the motor neuron pathology via a dying-back phenomenon [18, 19].

Twenty percent of patients with autosomal-dominant familial ALS show mutations in the copper-zinc superoxide dismutase 1 (SOD1) gene [20]. Transgenic animals expressing SOD1G93A (glycine residue 93 mutated to alanine), SOD1G37R (glycine residue 37 mutated to arginine), SOD1G85R (glycine residue 85 mutated to arginine), and SOD1H46R (histidine residue 46 mutated to arginine) experience motor neuron loss leading to limb paralysis and display other physiological effects typical of the ALS pathology [21]; thus, they are widely used in ALS study [22, 23, 24, 25, 26]. The exact mechanism causing motor neuronal degeneration in sporadic ALS is still unknown but there are several hypotheses. For example, some studies have linked ALS to excitotoxicity, the neuronal injury induced by excessive stimulation of the postsynaptic glutamate receptors (such as the cell surface N-methyl-D-aspartate NMDA and the α-amino-3-hydroxy-5-methylisoxazole-4-propionate AMPA receptors), which may cause neuronal toxicity [27, 28]. In other studies, the transactive response (TAR)-DNA-binding protein 43 (TDP-43) has been associated with both familial and sporadic ALS [29, 30, 31]. TDP-43 is a transcriptional repressor that binds to chromosomally integrated TAR DNA and represses human immunodeficiency virus-1 (HIV-1) transcription [32]. Endoplasmic reticulum (ER) stress, oxidative stress, impaired axonal transport, deficits in neurotrophic factors, inflammatory dysfunction, and protein aggregation are also possible causes of ALS [2].

Currently, patients have only few therapeutic options and heavily rely on supportive and palliative cares. Riluzole is a drug that prevents glutamate release from presynaptic terminals and that can prolong patients’ lifespan, although by only 2-3 months [2, 33]. Edaravone, a free-radical scavenger, was approved for clinical use in Japan in 2015, and ameliorates ALS functional rating scale, but its effect on life span is not clear [34, 35, 36]. This highlights the need for further research and drug discovery projects that could lead to the development of more effective medicines.

**GPNMB**

Glycoprotein nonmetastatic melanoma protein B (GPNMB, SWISS-PROT accession number Q14956) is a type I transmembrane protein [37]. Its cDNA was initially cloned from low-metastatic human melanoma cell lines as a regulator of tumor growth [38]. In its mature form, the protein is glycosylated and is involved in a wide variety of processes [39]. It is involved in osteoblasts differentiation and function [40, 41], in osteoclasts differentiation and activity [42, 43], in the inhibition of T-lymphocyte activation and in the regulation of autoimmune responses [44, 45]. GPNMB is also related with invasion and metastasis of several cancers and sarcomas, including breast cancer, melanoma, hepatoma, and glioblastoma [46, 47, 48, 49, 50, 51, 52]. It is a potential oncogene, highly expressed in melanoma and breast cancer. As previously reported, the levels of GPNMB are significantly higher in the human epidermal growth factor receptor 2 (HER2)-rich subtype of breast cancer patients than in other subtypes [53]; GPNMB might be therefore be used as a marker for breast cancer and may crossstalk with the HER2 signal pathway.

In a proteolytic step called shedding, GPNMB is cleaved into two functional fragments by matrix metalloproteinases (MMPs), including a disintegrin and metalloproteinase (ADAM) 10 and 12 [54, 55, 56]. The N-terminal fragment activates the extracellular signal-regulated kinase 1 and 2 (ERK1/2) pathway and the protein kinase B (Akt) pathway [25, 54, 57], while the C-terminal fragment is involved in the regulation of pre-mRNA splicing [58]. Mutations of the C-terminal fragment are linked to ALS [59].

**Effect of GPNMB on central nervous system**

We evaluated the effect of GPNMB on diseases affecting the central nervous system. Memory and learning skills were tested by the Morris water maze test and by the novel object recognition test. These analyses revealed that intracerebroventricular administration of GPNMB increases the hippocampal expression of the AMPA receptor subunit, GluA1, which improves memory [60]. Hence, GPNMB may be a novel target for research on higher order brain functions. Moreover, GPNMB is reported to protect neurons against brain ischemia-reperfusion injury via activation of the extracellular signal-regulated kinase 1 and 2 (ERK1/2) pathway and of the protein kinase B (Akt) pathway [57]. Focal ischemia-reperfusion injury was induced via filament middle cerebral artery occlusion (MCAO) followed by reperfusion...
upon withdrawal of the filament. We observed that the expression levels of GPNMB increased after ischemia-reperfusion injury and that overexpression of the protein significantly ameliorated infarct volume [57] (Table 1).

### Relationship between GPNMB and ALS

We previously reported that expression of GPNMB in SOD1<sup>G93A</sup> mice model of ALS is highly increased compared to wild-type (WT) mice [23, 25], suggesting a link between GPNMB and ALS. This was supported by the observation that GPNMB expression in the spinal cords of ALS model mice significantly increases with the disease progression. Besides, patients suffering from sporadic ALS contains a much higher level of protein in their cerebrospinal fluid, their sera, and their spinal cord compared to patients with non-neurological diseases and patients with Alzheimer or Parkinson diseases [25]. It was shown that GPNMB rescued the motor neuronal cell (NSC34 cell) death induced by SOD1<sup>G93A</sup> and serum deprivation stress, and that its overexpression prolonged the survival rate and improved motor performance on the rotarod test of SOD1<sup>G93A</sup> mice [25]. This is the first report showing the relationship between ALS and GPNMB and the protective effect that GPNMB exerts against ALS (Table 1).

### Importance of preventing skeletal muscle defects in neuromuscular disease

Muscular atrophy is caused by different diseases such as ALS, spinal muscular atrophy (SMA), spinal and bulbar muscular atrophy (SBMA), and muscular dystrophy (MD). ALS, SMA, and SBMA are classified as neuropathies, while MD is classified as a myopathy. Research on neurodegenerative disease, including ALS, SMA, and SBMA, have mainly focused on preventing the spinal motor neuron disorder. However, recent findings highlighted the contribution of intrinsic skeletal muscle defects to motor neuron diseases, and showed that muscle defects occur prior to and independently from motor neuron degeneration in motor neuron diseases [61]. It is also reported that

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### Table 1. Effect of GPNMB targets on central nerve system

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Models</th>
<th>Experimental findings</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>ALS</td>
<td>SOD1&lt;sup&gt;G93A&lt;/sup&gt; mice, SOD1&lt;sup&gt;H46R&lt;/sup&gt; rat, ALS patients, and motor neuron that transfected mutant SOD1 gene (in vitro)</td>
<td>GPMBM prevented the motor neuronal cell death induced by transfection of mutant SOD1, and GPNMB prolonged the survival rate and delayed the onset of SOD1&lt;sup&gt;G93A&lt;/sup&gt; mice.</td>
<td>[25]</td>
</tr>
<tr>
<td>Memory</td>
<td>Morris water maze test, and novel object recognition test.</td>
<td>Memory was improved in GPNMB transgenic mice or intracerebroventricular administration of GPNMB. It revealed that GPNMB may be a novel target for research on higher order brain functions.</td>
<td>[60]</td>
</tr>
<tr>
<td>Cerebral ischemia/reperfusion injury</td>
<td>Middle cerebral artery occlusion (MCAO) and reperfusion.</td>
<td>Expression of GPNMB was increased after ischemia/reperfusion injury, and that genomic overexpression of GPNMB significantly ameliorated infarct volume.</td>
<td>[57]</td>
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### Table 2. Denervation-induced muscle atrophy observed earlier than spinal motor neuronal disorders in ALS

<table>
<thead>
<tr>
<th>Models</th>
<th>Experimental outlines</th>
<th>Experimental findings</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>SOD1&lt;sup&gt;G93A&lt;/sup&gt; mice, and Sporadic ALS patient</td>
<td>Quantitative pathological analysis were performed in mice at multiple ages at neuromuscular junctions, ventral roots, and spinal cord. ALS patient who died unexpectedly was examined at autopsy.</td>
<td>Motor neuron pathology begins at the distal axon and proceeds in a dying back pattern.</td>
<td>[67]</td>
</tr>
<tr>
<td>SOD1&lt;sup&gt;G37R&lt;/sup&gt; mice</td>
<td>Evaluation were performed using presymptomatic stage of ALS model mice. Neuromuscular junction and perisynaptic schwann cells were evaluated.</td>
<td>The impairments of perisynaptic schwann cells functions may contribute to neuromuscular junction dysfunction and ALS pathogenesis.</td>
<td>[66]</td>
</tr>
<tr>
<td>SOD1&lt;sup&gt;G93A&lt;/sup&gt; mice, Motoneuron degeneration mice, and Progressive motoneuropathy mice</td>
<td>Using three genetic mouse models of motoneuron disease, they proposed a model of selective weakening, dying-back, and denervation progression in motor neuronal diseases</td>
<td>Observed a selective loss of synaptic connections that begun long before the onset of clinical deficits.</td>
<td>[68]</td>
</tr>
<tr>
<td>SOD1&lt;sup&gt;G93A&lt;/sup&gt;-GFP zebrafish</td>
<td>Evaluated neuromuscular junction, neuronal pathology, and motor neuronal cell death throughout the disease course.</td>
<td>Denervation at the neuromuscular junction around 20 weeks, subsequent alterations in innervation patterns at 30 weeks, and loss of motor neuron around 40 weeks of age.</td>
<td>[73]</td>
</tr>
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</table>
dysfunctions of the muscle are not only a result of motor neuronal cell death, but a direct consequence of the muscle toxicity [62, 63]. Muscle-restricted SOD1 mutations also recapitulate the hallmark signs of ALS [19, 64, 65]. Thus, skeletal muscle may be a primary site of pathogenesis in ALS that triggers motor neuronal degeneration [65].

In the case of ALS, studies carried out in patients and animal models showed that denervation-induced muscle atrophy occurs earlier than motor neuronal degeneration; moreover, muscle-restricted SOD1 mutations recapitulated the hallmark signs of ALS. Quantitative pathological analyses performed at neuromuscular junctions, ventral roots, and spinal cord in SOD1G93A mice at multiple ages, revealed that the motor neuron pathology begins at the distal axon and proceeds in a dying back pattern (Table 2) [18, 66, 67, 68, 69, 70, 71, 72, 73, 74]. Impairment of perisynaptic Schwann cell functions may contribute to neuromuscular junction dysfunction and to ALS pathogenesis [66]. Another study carried out on mice models showed that the selective loss of synaptic connections begun long before the onset of clinical deficits [68]. The SOD1G93A zebrafish ALS model displays early defects in motor neurons outgrowth and axonal branching [73]; denervation at the neuromuscular junction was observed at 20 weeks of age, with subsequent alterations in innervation patterns at 30 weeks of age, and loss of motor neurons at 40 weeks of age [73]. Longitudinal MRI study revealed that muscle volume reduced in SOD1G93A mice at 8 weeks of age, 4 weeks prior to the onset. In contrast, neurodegeneration of brain observed at 10-18 weeks of age [75]. Therefore, muscle atrophy precedes cerebral neuronal degeneration in SOD1G93A mice [75]. The ER stress response, autophagy and the ubiquitin-proteasome degradation pathway are activated in the skeletal muscle of SOD1G93A mice [19, 76, 77, 78, 79, 80]. Mitochondrial dysfunction in skeletal muscle occurs early in the course of ALS, and contributes to the pathogenesis and progression of ALS [81, 82]. These stresses may be a trigger of the progression of ALS.

SMA is a childhood neurodegenerative disease similar to ALS, which leads to paralysis due to a progressive disorder of the spinal motor neurons and to the degeneration of skeletal muscles. SMA is caused by diminished levels of the full-length survival of motor neuron (SMN) protein which is fundamental for survival of the motor neurons [83]. Here as well, a study performed on a mouse model of SMA, indicated that the damage of the skeletal muscle DNA precedes the one of the spinal motor neuron DNA [84]. This suggests that DNA damage and cell death in the skeletal muscle may represent therapeutic targets for SMA [84].

Finally, SBMA is a neurodegenerative disease caused by the expansion of a trinucleotide (CAG) repeat in the androgen receptor gene [85]. Overexpression of the insulin-like growth factor 1 (IGF-1) in the skeletal muscle attenuates the severity of the disease and improves the motor neuron pathology of SBMA model mice [86].

The results reported above indicate that the study of intrinsic skeletal muscle defects might be crucial for understanding the pathophysiology of neurodegenerative diseases and might reveal new therapeutic options for the treatment of motor neuron diseases [61]. While scientists have put a lot of effort into designing therapeutics that target the motor neuron disorder in ALS and in other neurodegenerative diseases, many candidate compounds were not successful. Discovering new medicine against these diseases is an urgent issue, and the development of drugs that have a completely different mechanism of action might be a breakthrough in this area.

Potential targets against skeletal muscle defects induced by ALS

Research studies that focused on the skeletal muscle defects derived from ALS have identified several molecules that can be exploited as potential therapeutic targets against skeletal muscle atrophy [65, 87, 88, 89, 90].

The peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α) induces multiple effects on muscle, including increased mitochondrial mass and activity [87]. The effect of GPNMB against skeletal muscle atrophy in SOD1G37R mice was evaluated using MCK-PGC-1α (muscle creatine kinase PGC-1α) mice, which overexpressed PGC1-α in skeletal muscle. Selective increase of the PGC 1-α levels in the muscles of ALS mice models resulted in reduction of the muscle atrophy and in a significant improvement of the muscle endurance even at a late stage of the disease, although survival was not extended [87].

Heat shock protein 70 (HSP70) protects against ALS in SOD1G93A mice by acting on the hindlimb skeletal muscle [69, 89]. Studies showed that HSP70 injected intraperitoneally was not detected in the central nervous system but was localized to the skeletal muscle. Animals that were administered the injection had increased life span, delayed symptom onset, preserved motor functions, and prolonged survival of motor neurons [69, 89].

Another report indicated that transgene glial cell line-derived neurotrophic factor (GDNF) from muscle is neuroprotective in ALS model mice [90]. Overexpression of GDNF in the skeletal muscle, using Myo-GDNF (myogenin-GDNF) mice, significantly delayed the onset of the disease and increased the life span of SOD1G93A mice. On
the muscle wasting of SOD1G93A mice [88].

Neuromuscular junctions, delayed the onset, and attenuated SOD1G93A rats [92].

The survival rate of motor neuron and its function of cells (hMSCs) which engineered to secrete GDNF improved intramuscular transplantation of human mesenchymal stem cells (hMSCs) which engineered to secrete GDNF improved neuroprotective effects [90, 91]. Furthermore, it is reported that the other hand, overexpression of GDNF in astrocytes in the central nervous system in while searching for a cure for ALS [88].

It is also reported that muscle specific expression of IGF-1 protects motor neurons in the ALS mouse model [88]. Muscle-specific expression of Igf-1 stabilized the neuromuscular junctions, delayed the onset, and attenuated the muscle wasting of SOD1G93A mice [88].

These reports indicate that targeting the regeneration of skeletal muscle might be just as important as targeting the central nervous system in while searching for a cure for ALS (Table 3).

**GPNMB and skeletal muscle**

Several reports indicate that GPNMB may have a protective effect against skeletal muscle atrophy (Table 4). The GPNMB levels in the skeletal muscle of rats that stayed in space for 16 days in the space-shuttle Columbia is higher compared with those of ground control rats [93]. Following space flights, muscle atrophy caused by disuse can arise, with skeletal muscles being particularly vulnerable to remarkable atrophy induced by microgravity. This suggests a link between GPNMB and muscular atrophy. Other research showed that the expression levels of GPNMB are higher than normal in the denervated skeletal muscle of mice that underwent removal of a 5 mm sciatic nerve section of 5 mm [94]. Using mice that suffered sciatic neurectomy, mouse embryonic fibroblast cell line (NIH-3T3), and mouse myoblast cell line (C2C12), the authors of the study suggested that GPNMB might function as an activator for fibroblasts infiltrated into denervated skeletal muscles [94]. Others observed that overexpression of GPNMB protects the skeletal muscle from severe degeneration in the portion of the sciatic nerve in the mid-thigh area [95]. They proposed that GPNMB-mediated increase of MMP-3 and MMP-9 in the skeletal muscle might be useful for protecting injured muscle from severe degeneration.

**Table 3. Protective effect for which targets skeletal muscle of ALS model mice**

<table>
<thead>
<tr>
<th>Materials</th>
<th>Models</th>
<th>Overexpression Methods</th>
<th>Experimental findings</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>PGC-1α</td>
<td>SOD1G93A mice</td>
<td>MCK-PGC-1α mice were used, which heterozygous for PGC-1α cDNA under the control of muscle creatine kinase promoter.</td>
<td>Delayed muscle atrophy, and significantly improved muscle endurance and muscle function even at late disease stages.</td>
<td>[87]</td>
</tr>
<tr>
<td>HSP70</td>
<td>SOD1G93A mice</td>
<td>HSP70 were intraperitoneally injected, but injected HSP70 localized to skeletal muscle and was not detected in the central nerve system.</td>
<td>Increasing lifespan, delaying symptom onset, preserving motor function and prolonging survival of motor neurons.</td>
<td>[69, 89]</td>
</tr>
<tr>
<td>GDNF</td>
<td>SOD1G93A mice</td>
<td>Myo-GDNF mice were used, that overexpress GDNF under a muscle-specific (myogenin) promoter.</td>
<td>Delayed the onset of disease, increased life span, and improved locomotor performance.</td>
<td>[90, 91]</td>
</tr>
<tr>
<td>Igf-1</td>
<td>SOD1G93A mice</td>
<td>MLC/mlgf-1 mice (muscle restricted mlgf-1 transgene) were used.</td>
<td>Delayed the onset of disease, stabilized the neuromuscular junctions, and attenuated the muscle wasting.</td>
<td>[88]</td>
</tr>
<tr>
<td>GPNMB</td>
<td>SOD1G93A mice</td>
<td>GPNMB plasmid DNAs were injected into gastrocnemius muscle. GPNMB were overexpressed in muscle and not detected in spinal cord.</td>
<td>Atrophy of myofiber and reduction of the number of myofiber were improved in the skeletal muscle.</td>
<td>[98]</td>
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**Table 4. GPNMB and skeletal muscle**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Species</th>
<th>Experimental findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space-flight</td>
<td>Rats</td>
<td>Expression level of GPNMB was increased in the skeletal muscle of space-flew rat.</td>
<td>[93]</td>
</tr>
<tr>
<td>Denervation (sciatic neurectomy)</td>
<td>Mice, and NIH-3T3 fibroblasts and C2C12 myoblasts</td>
<td>Expression of GPNMB was increased in the skeletal muscle which suffered denervation. GPNMB might be an activator for fibroblasts infiltrated into denervated skeletal muscles.</td>
<td>[94]</td>
</tr>
<tr>
<td>Denervation (sciatic neurectomy)</td>
<td>Mice</td>
<td>GPNMB mediated increase in metalloprotease (MMP)-3 and MMP-9 in skeletal muscle might be useful for protecting injured muscle from fibrosis, leading to full regeneration after denervation.</td>
<td>[95]</td>
</tr>
<tr>
<td>Genetic heart failure</td>
<td>Mice (Desmin-deficient mice)</td>
<td>Expression of GPNMB was increased in the myocardium of genetic heart failure model and it revealed that GPNMB regulates myocardial remodelling and its functions.</td>
<td>[96]</td>
</tr>
<tr>
<td>Distraction osteogenesis</td>
<td>Mice</td>
<td>GPNMB increased the expression of MMPs, and GPNMB attenuated the skeletal muscle fibrosis caused by distraction osteogenesis.</td>
<td>[97]</td>
</tr>
<tr>
<td>ALS</td>
<td>Mice (SOD1G93A mice)</td>
<td>Expression level of C-terminal of GPNMB was increased in the skeletal muscle of ALS model mice and ALS patients. GPNMB improved injury of myofiber of ALS model mice.</td>
<td>[98]</td>
</tr>
</tbody>
</table>
muscle from fibrosis, leading to full regeneration after denervation [95]. Finally, GPNMB attenuates skeletal muscle fibrosis caused by distraction osteogenesis [96] and regulates muscle regeneration in desmin-deficient cardiomyocyte, which represents a model of genetic heart failure [97].

**GPNMB and skeletal muscle atrophy caused by ALS**

We observed that the expression levels of the C-terminal fragment of GPNMB are significantly higher in the skeletal muscle of SOD1^{G93A} mice compared with that of WT mice. Similarly, they were sensibly higher in the skeletal muscle of sporadic ALS patients compared with patients affected by other diseases, such as corticobasal degeneration, familial amyloid polyneuropathy, HTLV (human T-lymphotropic virus) 1-associated myelopathy, hypertrophic pachymeningitis, multiple system atrophy, Parkinson’s disease, and progressive supranuclear palsy [98]. We therefore hypothesized that GPNMB might be specifically related to skeletal muscle atrophy caused by ALS. Atrophy of skeletal muscles and myofiber symptoms were significantly decreased and denervation of neuromuscular junctions was improved in SOD1^{G93A}/GPNMB double-transgenic mice compared with SOD1^{G93A} mice (Fig. 1A) [98]. Such a remarkable improvement of the muscular defects indicates that GPNMB

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**Figure 1. Summary of reported effects of GPNMB against ALS pathology.** (A) Experimental outline and summary of the research of Nagahara et al., which focused on the effects of GPNMB on skeletal muscle in ALS models. Skeletal muscle atrophy, myofiber injury, and denervation of neuromuscular junction improved in SOD1^{G93A}/GPNMB mice than in SOD1^{G93A} mice model of ALS. Myofiber injury was slightly improved by direct injection of the GPNMB plasmid. These figures were reproduced from Nagahara et al. [98]. (B) Effects of GPNMB against ALS pathology. GPNMB prevents motor neuronal cell death in the central nervous system of ALS models as reported by Tanaka et al. [25]. GPNMB also prevents skeletal muscle disorder and denervation of neuromuscular junction by protecting against skeletal muscle injuries in ALS models [98]. GPNMB may act on dual sites: the central nervous system and skeletal muscle.
may be important not only for the central nervous system but also for the integrity of the skeletal muscle. To evaluate the effect of GPNMB on the skeletal muscle of SOD1G93A mice, GPNMB plasmid DNA was directly injected into the gastrocnemius muscle. This is a widely adopted and relatively simple method to overexpress a gene in the skeletal muscle [99, 100, 101, 102, 103, 104, 105] and it has been used for example to study the effect of human hepatocyte growth factor (HGF) on critical limb ischemia [99, 106, 107], and intramuscular injection of the heart muscle ameliorates myocardial infarction [108, 109]. We observed that overexpression of GPNMB in the skeletal muscle reduced myofibers atrophy and rescued myofibers reduction (Fig. 1A) [98]. Our results showed for the first time that GPNMB is not only active within the central nervous system, but that it also directly affects skeletal muscles, preventing muscular pathology (Fig. 1B).

Mechanism of the protective effect of GPNMB against ALS derived muscle atrophy

Although the exact mechanism by which GPNMB exerts its effect on skeletal muscle atrophy is still unknown, there are several hypotheses and we will discuss seven of here. The first hypothesis is that GPNMB prevents muscular atrophy by activating the ERK1/2 signaling pathway, a system involved in many cellular programs such as cell proliferation, differentiation, motility, and death. Activation of the ERK1/2 pathway by GPNMB is key to improving brain ischemia-reperfusion injury and to protecting ALS animal models from motor neurons cell death [25,57]. Addition of recombinant GPNMB increases the levels of phosphorylated ERK1/2 in C2C12 cells [54], and GPNMB stimulates the expression of phosphorylated ERK1/2 by activating the fibroblast growth factors (FGF) receptor in human umbilical vein endothelial cells (HUVEC) [110]. Owing to these findings, it seems reasonable that GPNMB may also up-regulate the levels of phosphorylated ERK1/2 in the skeletal muscle of SOD1G93A mice. The second hypothesis is that GPNMB alleviates muscular atrophy through activation of the Akt signaling pathway, which regulates the balance between cell survival and apoptosis through phosphorylation [111]. Few studies indicate that this mechanism already plays an important role in the recovery from brain ischemia-reperfusion injury and in rescuing motor neuronal cell death in in vitro ALS models [25, 57]. Interestingly, the expression levels of phosphorylated Akt are lower in the skeletal muscles of ALS patients respect to healthy subjects; activation of the Akt pathway may therefore represent a specific therapeutic target for ALS [112]. The third hypothesis is that GPNMB prevents ER stress and subsequent muscular atrophy. ER stress is triggered by accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum and recent evidence indicates that it plays a central role in ALS pathogenesis [113]. We also revealed that ER stress apoptotic mediator, CAAT-enhancer-binding protein homologous protein (CHOP), is involves sporadic ALS disease [114]. Moreover, a recent study showed that the ER stress response is activated in the skeletal muscles of ALS mice models [19,78] and we found that GPNMB modulates the ER stress response (unpublished data), supporting this hypothesis. The fourth possible mechanism by which GPNMB might prevent skeletal muscle injury is through increase of MMP-3 and MMP-9 in the skeletal muscle. This protects injured muscle from fibrosis, leading to full regeneration after denervation [95] and improves skeletal muscle fibrosis caused by distraction osteogenesis [96]. The fifth hypothesis is that the C-terminal fragment of GPNMB produced by shedding may have a role in the progress of muscular atrophy. C-terminal fragment of GPNMB is involved with the regulation of pre-mRNA splicing [58] and mutation of which causes ALS [59]. The concentration of the C-terminal fragment of GPNMB is increased in the skeletal muscle of ALS mice model and of sporadic ALS patients [98]. Mature glycosylated GPNMB is highly overexpressed in the skeletal muscle of SOD1G93A/GPNMB mice, and in the skeletal muscle of mice injected with the GPNMB plasmid DNA [98]. Therefore, cleaving GPNMB and producing C-terminal fragment of GPNMB may be the bad to muscular symptoms. Although further analyses are needed, it is possible that muscular disorders are improved by overexpression of GPNMB because the mature isoform of GPNMB is relatively increased and effects of cleaving and producing C-terminal fragments of GPNMB may be offset. The sixth hypothesis is that the action of GPNMB is related to calcium signaling. In fact it has been recently reported that muscle-bone crosstalk is important in ALS [115], and GPNMB is involved in osteoblasts and osteoclasts differentiation [40, 41, 42, 43]. It is also reported that change of calcium signaling in skeletal muscle is related to the progression of muscle atrophy in ALS [116, 117]. Finally, the last hypothesis is that GPNMB prevents autophagy and ubiquitin-proteasome degradation. Autophagy and the ubiquitin-proteasome degradation system are activated in the skeletal muscle of SOD1G93A mice during disease progression [77] and polyubiquitinated GPNMB is increased in SOD1G93A expressing NSC34 cells [25]. Therefore, it is possible that GPNMB affects the ubiquitin-proteasome degradation system in ALS.

Future perspective

Injection of GPNMB plasmid DNA in the skeletal muscle significantly rescued injury of the myofibers but had no effect on muscle weight and on the denervation of the neuromuscular junction [98]. We speculate that these
differences may be linked to the expression levels and to the lifetime of GPNMB in the skeletal muscles. We think that further evaluation using different overexpression methods is needed, for example using virus vectors or transgenic mice specifically overexpressing GPNMB in skeletal muscles. The survival rate, onset, and motor functions must be evaluated. In addition, measuring the concentrations of each GPNMB fragments and deciphering their role within the skeletal muscle will be fundamental to understand GPNMB mechanism of action against skeletal muscle atrophy. We also plan to evaluate the effect of GPNMB against other muscular diseases, such as SMA, SBMA, and muscular dystrophy. GPNMB shows protective effect against familial ALS induced by SOD1G93A in vivo and in vitro models. However, it is still unclear whether GPNMB has a protective effect against ALS independent of SOD1 mutation. TDP-43 is a transcriptional repressor that binds to chromosomally integrated TAR DNA and represses human immunodeficiency virus-1 (HIV-1) transcription [32]. In 2006, it was reported that TDP-43 is associated with ALS and with frontotemporal lobar degeneration (FTLD) [118]. Moreover mutations of TDP-43 and abnormal intracellular aggregates are observed in familial ALS and sporadic ALS [119, 120, 121]. Therefore, the evaluation of the effect of GPNMB against mutat-TDP-43 induced ALS model is necessary.

Despite the need for further research on GPNMB functions and mechanisms, we think that the protein should represent a therapeutic target for ALS, especially if its association with sporadic ALS is confirmed.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgements

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Abbreviation

ALS: amyotrophic lateral sclerosis; GPNMB: glycoprotein non-metastatic melanoma protein B; SOD1: superoxide dismutase 1; TDP-43: transactive response DNA binding protein 43; ER stress: endoplasmic reticulum stress; MMP: matrix metalloproteinase; ERK1/2: extracellular signal-regulated kinase 1 and 2; Akt: protein kinase B; SMA: spinal muscular atrophy; SBMA: spinal and bulbar muscular atrophy.

References

5. Song P. The Ice Bucket Challenge: The public sector should get ready to promptly promote the sustained development of a system of medical care for and research into rare diseases. Intractable Rare Dis Res 2014; 3: 94-96.


