Amyotrophic Lateral Sclerosis as a Non-Cell-Autonomous Disease: Multiple Roles of Transforming Growth Factor Beta

Subjects: Pathology
Contributor: Mariarita Galbiati

Transforming growth factor beta (TGFB) is a pleiotropic cytokine known to be dysregulated in many neurodegenerative disorders, including in amyotrophic lateral sclerosis (ALS). TGFB and its signaling pathway play multiple physiological roles in the various cell types, which are affected in ALS pathogenesis. Data from literature and from our group also demonstrated a crucial role of TGFB in the etiology and progression of ALS, leading us to hypothesize that an imbalance of TGFB signaling, diminished at the pre-symptomatic stage and then increased with time, could be linked to ALS progression. A reduced stimulation of the TGFB pathway at the beginning of the disease blocks its neuroprotective effects and promotes glutamate excitotoxicity. At later disease stages, the persistent activation of the TGFB pathway promotes an excessive microglial activation and strengthens muscular dysfunctions.

The article has been published on 10.3390/ijms21124291 (https://doi.org/10.3390/ijms21124291)

Keywords: ALS ; TGFB ; motor neuron ; glial cells ; skeletal muscle ; SMAD

This article is related to the review paper “Multiple Roles of Transforming Growth Factor Beta in Amyotrophic Lateral Sclerosis” that has been published in *International Journal of Molecular Sciences (10.3390/ijms21124291)*

Amyotrophic Lateral Sclerosis as a Non-Cell-Autonomous Disease

ALS is a disease affecting upper and lower motor neurons, with an incidence of 1–2/100,000 per year, and mean survival of 3–5 years after diagnosis [1]. It is characterized by a progressive loss of motor neurons, but the precise pathological mechanisms involved are not fully establishedas their complex interplay with neighboring and target cells. ALS is primarily caused by the death of upper and lower motor neurons. Nevertheless, in the last 15 years, besides the main classical “neuron-centric” view of ALS, a number of research studies evidenced that ALS could also be a non-cell-autonomous disease [2,3]. Data have been mostly obtained using ALS mouse models, but they may also be linked to sporadic ALS cases [4]. Glial and skeletal muscle cells demonstrated their ability to trigger or modulate ALS. The analysis of chimeric mice indicated that the restricted expression of human mutant SOD1 (mutSOD1) in motor neuron is not sufficient to induce a cell-autonomous degeneration of motor neurons [5]. Moreover, utilizing floxed mutSOD1 gene, it has been demonstrated that the damaging process starts in motor neurons and determines the disease onset, with little influence on its progression [2]. Conversely, mutSOD1 activates glial cells exacerbating the disease progression, while motor neuronal mutSOD1 has little influence on the progression of ALS [2]. Astrocytes, microglia, oligodendrocytes, and Schwann cells are all able to modulate ALS pathology, and gliosis is a hallmark of ALS (see, for review [6,7]). Activated and proliferating astrocytes may no longer provide the metabolic support to motor neurons, and also become neurotoxic by secreting cytokines or other toxic factors (among which is the TGFB) that are critical for determining the rate of disease progression [8,9]. Furthermore, activated astrocytes reduce the expression of the excitatory amino acid transporter-2 (EAAT-2), that is mandatory for glutamate re-uptake from the synaptic cleft into astrocyte, leading to excitotoxicity in motor neurons [10].The extent of microglia activation correlates with the severity of the upper motor neuron involvement [11]. Whether microglial cells are beneficial or detrimental to motor neurons is already an open question. In addition to neighboring cells, motor neurons can also be influenced by their target, the skeletal muscle cells. It has been shown, at least in familial ALS, a direct muscular toxicity and/or a functional impairment that has denervation and motor neuron death as a consequence [12,13,14]. A contribution to the initiation and progression of muscle atrophy is given by altered ALS satellite cell properties [15,16]. In addition, our previous works have indicated a dysfunctional protein quality control system in ALS muscle cells, which seem more protected than motor neurons against the presence of accumulating misfolded proteins [17,18,19].

TGFB Plasma Levels in ALS Patients

TGFB1 plasma concentration in ALS patients is significantly higher than in the healthy controls, and it positively correlates with the disease [20], but whether TGFB1 plasma level is a biomarker of ALS or not is still an open question.

TGFB and ALS-Nervous System

TGFBs have multiple functions in the CNS. They enhance synapse formation and synaptic transmission [21,22], regulate synaptic plasticity and memory [23], increase the number and length of neurites [24], control neuronal migration [25], and cerebral cortex angiogenesis [26]. CNS-TGFB1-deficient mice have reduced brain weight and loss of neurons in the CA1 hippocampal region. These mice show a reduction of dendritic spine density, impaired long-term potentiation, and facilitated long-term depression in the hippocampus, in addition to the loss of the astrocyte glutamate transporters GLT-1 (EAAT2) and GLAST (EAAT1), and decreased glutamate uptake, resulting in a higher sensibility to glutamate excitotoxicity, that is one of the possible pathogenic mechanism in ALS [27]. Even if the comparative analysis of familial ALS and sporadic ALS tissues indicates the existence of common and distinct biological mechanisms driving the different forms of the pathology, an altered regulation of the TGFB1 pathway has been reported in motor neurons of most ALS models and patients. Reduced *Tgfb1* mRNA levels in the spinal cord of pre-symptomatic mutSOD1 mice could indicate a lack of the TGFB neuroprotective effect in the early stages of the disease [28]. Indeed, levels of pSMAD2 in the nuclei of lumbar motor neurons are significantly decreased at the pre-symptomatic stage, leading to the hypothesis of an aberrant nucleo/cytoplasm transport [29,30]. The role of glia-derived TGFB1 in the spinal cord of ALS patients and mice has been studied by Endo and colleagues [8]. They determined that astrocyte-derived TGFB1 accelerates disease progression in ALS mice, preventing neuroprotective responses mediated by the microglia and T cells [8].

TGFB pathway in ALS skeletal muscle

In skeletal muscle, the expression of TGFB is related to normal processes such as growth, differentiation, regeneration, and stress response. However, continuously elevated levels of TGFB are linked to impaired regeneration and atrophy. TGFB blocks myogenic responses and stimulates fibrosis [31]. It inhibits the activation of MyoD and myogenin (two transcription factors regulating muscle cell differentiation) through the signaling of SMAD3 or by inactivating cyclin-dependent kinases [32,33]. Satellite cell activation is also prevented in the presence of TGFB, and muscle overexpression of TGFB leads to muscle weakness and atrophy [34,35]. ALS muscle tissue is also characterized by alterations of the TGFB pathway. We reported increased levels of the *Tgfb1* mRNA in the muscle of mice expressing mutSOD1[36]. Notably, these changes are gender-related, since male mice present an increased TGFB expression in muscle already at the pre-symptomatic stage, while in female animals, TGFB increases only at the symptomatic stage [28]. *Tgfb* mRNA levels are further increased with the administration of an anabolic/androgenic steroid (nandrolone decanoate), indicating that, at least at the muscular level, these molecules might exert a detrimental role in ALS, since it might exacerbate some of the alterations induced by mutSOD1 [36,37]. Evidence in human confirmed the involvement of TGFB1 since we reported an increased *TGFB1* expression in muscle of female and male sporadic ALS patients with a significant gender effect [28], and other authors also reported the increase of *TGFB1*, *2*, and *3* in ALS patient muscles [38,39]. It must also be highlighted that *TGFB1* and *TGFB3* mRNA show a negative correlation with muscle strength in ALS patients [39]. In the same manner, the increase of TGFB1 correlates with disease progression in mutSOD1 mice [36]. It has also been proposed that excessive oxidative processes may be a mechanism of activation of latent TGFB pool in ALS, as in other neurodegenerative diseases, leading to an increased TGFB1 release from the complex [40].

TGFB and Neuro-Muscular Junction in ALS

Since the first histological studies, recurrent denervation and reinnervation have been observed in the NMJs of ALS patients [41]. Because of that, it has been proposed to consider ALS also as a distal axonopathy, with pathological changes occurring at the NMJs prior to motor neuron degeneration and muscle fiber atrophy (see, for review [42]). TGFB pathway regulates the formation and stability of the NMJs. TGFB1 is capable of doubling the size of acetylcholine receptor clusters increasing the percentage of nerve–muscle contacts. It has also been demonstrated that this synaptogenic effect of TGFB1 might be ascribed to its ability to induce neuronal agrin expression [43]. Agrin is a proteoglycan important for the maintenance of the architecture of the postsynaptic membrane and known to be down-regulated in the muscle of ALS mice expressing mutSOD1 [13]. TGFB1 is highly concentrated at NMJs of pre-symptomatic mutSOD1 mice, and represses the expression of FGFBP1 (a factor that might potentiate the bioactivity of FGF family members during reinnervation), indicating TGFB1 pathway as a potential target for preventing NMJ dismantling in ALS mice [44].

TGFB as a target for ALS treatment

The therapeutic potential of TGFB has been investigated. SB-431542, a selective inhibitor of TGFBRI kinase activity, has been proven to extend the survival of mutSOD1 expressing mouse, even if administered after disease onset [8]. Moreover, the intraperitoneal injection of TGFB2 in the same mouse model is able to reverse initial muscle weakness, permitting a better performance at rotarod test, probably through a marked trophic action on motor neurons, as can be

inferred by motor neuron nuclei and axonal enlargement. Unfortunately, this amelioration is transient, leading to an even more rapid progression of the disease [45]. Antibodies neutralizing myostatin delayed the onset and the progression of the disease in ALS mice, even if without extending their survival [46,47].

Conclusions

The imbalance of TGFB signaling has been linked to ALS progression and may have selective impact on different body districts. In the CNS, there is a lack of the neuroprotective effects of TGFB at the first stages of the disease; later, the strong increase of TGFB levels due to microglial stimulation shifts the CNS milieu toward a proinflammatory and neurotoxic environment. In the skeletal muscle, the chronically increased TGFB signaling facilitates the development of atrophy and fibrosis in skeletal muscle fiber, and the process of NMJ dismantling.

References

1. Marin, B.; Boumediene, F.; Logroscino, G.; Couratier, P.; Babron, M.C.; Leutenegger, A.L.; Copetti, M.; Preux, P.M.; Beghi, E., Variation in worldwide incidence of amyotrophic lateral sclerosis: A meta-analysis. *J. Epidemiol.* **2017**, *46*, 57–74.
2. Boillee, S.; Vande Velde, C.; Cleveland, D.W. ALS: A disease of motor neurons and their nonneuronal neighbors. *Neuron* **2006**, *52*, 39–59.
3. Ilieva, H.; Polymenidou, M.; Cleveland, D.W. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *Cell Biol.* **2009**, 187, 761–772.
4. Haidet-Phillips, A.M.; Hester, M.E.; Miranda, C.J.; Meyer, K.; Braun, L.; Frakes, A.; Song, S.; Likhite, S.; Murtha, M.J.; Foust, K.D.; et al. Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. *Biotechnol.* **2011**, *29*, 824–828.
5. Clement, A.M.; Nguyen, M.D.; Roberts, E.A.; Garcia, M.L.; Boillee, S.; Rule, M.; McMahon, A.P.; Doucette, W.; Siwek, D.; Ferrante, R.J.; et al. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* **2003**, *302*, 113–117.
6. Yamanaka, K.; Komine, O. The multi-dimensional roles of astrocytes in ALS. *Res.* **2018**, *126*, 31–38.
7. Yamanaka, K; Chun, SJ; Boillee, S; Fuijimori-Tonou, N; Yamashita, H; Gutmann, DH; Takahashi, R; Misawa, H; Cleveland, DW. Astrocytes as Determinants of Disease Progression in Inherited Amyotrophic Lateral Sclerosis. Nat Neurosci 2008, 11(3), 251-253.
8. Endo, F.; Komine, O.; Fujimori-Tonou, N.; Katsuno, M.; Jin, S.; Watanabe, S.; Sobue, G.; Dezawa, M.; Wyss-Coray, T.; Yamanaka, K. Astrocyte-derived TGF-beta1 accelerates disease progression in ALS mice by interfering with the neuroprotective functions of microglia and T cells. *Cell Rep.* **2015**, *11*, 592–604.
9. Ferraiuolo, L.; Higginbottom, A.; Heath, P.R.; Barber, S.; Greenald, D.; Kirby, J.; Shaw, P.J. Dysregulation of astrocyte-motoneuron cross-talk in mutant superoxide dismutase 1-related amyotrophic lateral sclerosis. *Brain* **2011**, *134*, 2627–2641.
10. Rosenblum, L.T.; Trotti, D. EAAT2 and the molecular signature of amyotrophic lateral sclerosis. *Neurobiol.* **2017**, *16*, 117–136.
11. Alexianu, M.E.; Kozovska, M.; Appel, S.H. Immune reactivity in a mouse model of familial ALS correlates with disease progression. *Neurology* **2001**, *57*, 1282–1289.
12. Dobrowolny, G.; Aucello, M.; Rizzuto, E.; Beccafico, S.; Mammucari, C.; Boncompagni, S.; Belia, S.; Wannenes, F.; Nicoletti, C.; Del Prete, Z.; et al. Skeletal muscle is a primary target of SOD1G93A-mediated toxicity. *Cell Metab.* **2008**, *8*, 425–436.
13. Dobrowolny, G.; Giacinti, C.; Pelosi, L.; Nicoletti, C.; Winn, N.; Barberi, L.; Molinaro, M.; Rosenthal, N.; Musaro, A. Muscle expression of a local Igf-1 isoform protects motor neurons in an ALS mouse model. *Cell Biol.* **2005**, *168*, 193–199.
14. Wong, M.; Martin, L.J. Skeletal muscle-restricted expression of human SOD1 causes motor neuron degeneration in transgenic mice. *Mol. Genet* **2010**, *19*, 2284–2302.
15. Manzano, R.; Toivonen, J.M.; Olivan, S.; Calvo, A.C.; Moreno-Igoa, M.; Munoz, M.J.; Zaragoza, P.; Garcia-Redondo, A.; Osta, R. Altered expression of myogenic regulatory factors in the mouse model of amyotrophic lateral sclerosis. *Dis.* **2011**, *8*, 386–396.
16. Pradat, P.F.; Barani, A.; Wanschitz, J.; Dubourg, O.; Lombes, A.; Bigot, A.; Mouly, V.; Bruneteau, G.; Salachas, F.; Lenglet, T.; et al. Abnormalities of satellite cells function in amyotrophic lateral sclerosis. *Lateral Scler.* **2011**, *12*, 264–271.
17. Cicardi, M.E.; Cristofani, R.; Rusmini, P.; Meroni, M.; Ferrari, V.; Vezzoli, G.; Tedesco, B.; Piccolella, M.; Messi, E.; Galbiati, M.; et al. Tdp-25 Routing to autophagy and proteasome ameliorates its aggregation in amyotrophic lateral sclerosis target cells. *Rep.* **2018**, *8*, 12390.

18. Crippa, V.; Galbiati, M.; Boncoraglio, A.; Rusmini, P.; Onesto, E.; Giorgetti, E.; Cristofani, R.; Zito, A.; Poletti, A. Motoneuronal and muscle-selective removal of ALS-related misfolded proteins. *Soc. Trans.* **2013**, *41*, 1598–1604.
19. Onesto, E.; Rusmini, P.; Crippa, V.; Ferri, N.; Zito, A.; Galbiati, M.; Poletti, A. Muscle cells and motoneurons differentially remove mutant SOD1 causing familial amyotrophic lateral sclerosis. *Neurochem.* **2011**, *118*, 266–280.
20. Houi, K.; Kobayashi, T.; Kato, S.; Mochio, S.; Inoue, K. Increased plasma TGF-beta1 in patients with amyotrophic lateral sclerosis. *Acta Neurol. Scand.* **2002**, *106*, 299–301.
21. Fukushima, T.; Liu, R.Y.; Byrne, J.H. Transforming growth factor-beta2 modulates synaptic efficacy and plasticity and induces phosphorylation of CREB in hippocampal neurons. *Hippocampus* **2007**, *17*, 5–9.
22. Poon, V.Y.; Choi, S.; Park, M. Growth factors in synaptic function. *Synaptic Neurosci.* **2013**, *5*, 6.
23. Diniz, L.P.; Matias, I.; Siqueira, M.; Stipursky, J.; Gomes, F.C.A. Astrocytes and the TGF-beta1 Pathway in the Healthy and Diseased Brain: A Double-Edged Sword. *Neurobiol.* **2019**, *56*, 4653–4679.
24. Bottner, M.; Krieglstein, K.; Unsicker, K. The transforming growth factor-betas: Structure, signaling, and roles in nervous system development and functions. *Neurochem.* **2000**, *75*, 2227–2240.
25. Miller, M.W. Expression of transforming growth factor-beta in developing rat cerebral cortex: Effects of prenatal exposure to ethanol. *Comp. Neurol.* **2003**, *460*, 410–424.
26. Siqueira, M.; Francis, D.; Gisbert, D.; Gomes, F.C.A.; Stipursky, J. Radial glia cells control angiogenesis in the developing cerebral cortex through TGF-beta1 signaling. *Neurobiol.* **2018**, *55*, 3660–3675.
27. Koeglsperger, T.; Li, S.; Brenneis, C.; Saulnier, J.L.; Mayo, L.; Carrier, Y.; Selkoe, D.J.; Weiner, H.L. Impaired glutamate recycling and GluN2B-mediated neuronal calcium overload in mice lacking TGF-beta1 in the CNS. *Glia* **2013**, *61*, 985–1002.
28. Meroni, M.; Crippa, V.; Cristofani, R.; Rusmini, P.; Cicardi, M.E.; Messi, E.; Piccolella, M.; Tedesco, B.; Ferrari, V.; Soraru, G.; et al. Transforming growth factor beta 1 signaling is altered in the spinal cord and muscle of amyotrophic lateral sclerosis mice and patients. *Aging* **2019**, *82*, 48–59.
29. Nakamura, M.; Ito, H.; Wate, R.; Nakano, S.; Hirano, A.; Kusaka, H. Phosphorylated Smad2/3 immunoreactivity in sporadic and familial amyotrophic lateral sclerosis and its mouse model. *Acta Neuropathol.* **2008**, *115*, 327–334.
30. Zhang, J.; Ito, H.; Wate, R.; Ohnishi, S.; Nakano, S.; Kusaka, H. Altered distributions of nucleocytoplasmic transport-related proteins in the spinal cord of a mouse model of amyotrophic lateral sclerosis. *Acta Neuropathol.* **2006**, *112*, 673–680.
31. Kim, M.S.; Hwang, N.S.; Lee, J.; Kim, T.K.; Leong, K.; Shamblott, M.J.; Gearhart, J.; Elisseeff, J. Musculoskeletal differentiation of cells derived from human embryonic germ cells. *Stem Cells* **2005**, *23*, 113–123.
32. Gumucio, J.P.; Sugg, K.B.; Mendias, C.L. TGF-beta superfamily signaling in muscle and tendon adaptation to resistance exercise. *Sport Sci. Rev.* **2015**, *43*, 93–99.
33. Liu, D.; Black, B.L.; Derynck, R. TGF-beta inhibits muscle differentiation through functional repression of myogenic transcription factors by Smad3. *Genes Dev.* **2001**, *15*, 2950–2966.
34. Mendias, C.L.; Gumucio, J.P.; Davis, M.E.; Bromley, C.W.; Davis, C.S.; Brooks, S.V. Transforming growth factor-beta induces skeletal muscle atrophy and fibrosis through the induction of atrogin-1 and scleraxis. *Muscle Nerve* **2012**, *45*, 55–59.
35. Narola, J.; Pandey, S.N.; Glick, A.; Chen, Y.W. Conditional expression of TGF-beta1 in skeletal muscles causes endomysial fibrosis and myofibers atrophy. *PLoS ONE* **2013**, *8*, e79356.
36. Galbiati, M.; Onesto, E.; Zito, A.; Crippa, V.; Rusmini, P.; Mariotti, R.; Bentivoglio, M.; Bendotti, C.; Poletti, A. The anabolic/androgenic steroid nandrolone exacerbates gene expression modifications induced by mutant SOD1 in muscles of mice models of amyotrophic lateral sclerosis. *Res.* **2012**, *65*, 221–230.
37. Aggarwal, T.; Polanco, M.J.; Scaramuzzino, C.; Rocchi, A.; Milioto, C.; Emionite, L.; Ognio, E.; Sambataro, F.; Galbiati, M.; Poletti, A.; et al. Androgens affect muscle, motor neuron, and survival in a mouse model of SOD1-related amyotrophic lateral sclerosis. *Aging* **2014**, *35*, 1929–1938.
38. Pradat, P.F.; Dubourg, O.; de Tapia, M.; di Scala, F.; Dupuis, L.; Lenglet, T.; Bruneteau, G.; Salachas, F.; Lacomblez, L.; Corvol, J.C.; et al. Muscle gene expression is a marker of amyotrophic lateral sclerosis severity. *Dis.* **2012**, *9*, 38–52.
39. Si, Y.; Kim, S.; Cui, X.; Zheng, L.; Oh, S.J.; Anderson, T.; AlSharabati, M.; Kazamel, M.; Volpicelli-Daley, L.; Bamman, M.M.; et al. Transforming growth factor beta (TGF-beta) is a muscle biomarker of disease progression in ALS and correlates with smad expression. *PLoS ONE* **2015**, *10*, e0138425.
40. Barcellos-Hoff, M.H.; Dix, T.A. Redox-mediated activation of latent transforming growth factor-beta 1. *Endocrinol.* **1996**, *10*, 1077–1083.
41. Tsujihata, M.; Hazama, R.; Yoshimura, T.; Satoh, A.; Mori, M.; Nagataki, S. The motor end-plate fine structure and ultrastructural localization of acetylcholine receptors in amyotrophic lateral sclerosis. *Muscle Nerve* **1984**, *7*, 243–249.
42. Moloney, E.B.; de Winter, F.; Verhaagen, J. ALS as a distal axonopathy: Molecular mechanisms affecting neuromuscular junction stability in the presymptomatic stages of the disease. *Neurosci.* **2014**, *8*, 252.

43. Feng, Z.; Ko, C.P. Schwann cells promote synaptogenesis at the neuromuscular junction via transforming growth factor-beta1. *Neurosci.* **2008**, *28*, 9599–9609.
44. Taetzsch, T.; Tenga, M.J.; Valdez, G. Muscle fibers secrete FGFBP1 to slow degeneration of neuromuscular synapses during aging and progression of ALS. *Neurosci.* **2017**, *37*, 70–82.
45. Day, W.A.; Koishi, K.; Nukuda, H.; McLennan, I.S. Transforming growth factor-beta 2 causes an acute improvement in the motor performance of transgenic ALS mice. *Dis.* **2005**, *19*, 323–330.
46. Holzbaur, E.L.; Howland, D.S.; Weber, N.; Wallace, K.; She, Y.; Kwak, S.; Tchistiakova, L.A.; Murphy, E.; Hinson, J.; Karim, R.; et al. Myostatin inhibition slows muscle atrophy in rodent models of amyotrophic lateral sclerosis. *Dis.* **2006**, *23*, 697–707.
47. Morrison, B.M.; Lachey, J.L.; Warsing, L.C.; Ting, B.L.; Pullen, A.E.; Underwood, K.W.; Kumar, R.; Sako, D.; Grinberg, A.; Wong, V.; et al. A soluble activin type IIB receptor improves function in a mouse model of amyotrophic lateral sclerosis. *Neurol.* **2009**, *217*, 258–268.