Rare Diseases 3:1, e1061164; Published with license by Taylor & Francis Group, LLC

# Macrophage migration inhibitory factor as a component of selective vulnerability of motor neurons in ALS

Salah Abu-Hamad and Adrian Israelson*

Department of Physiology and Cell Biology; Faculty of Health Sciences; The Zlotowski Center for Neuroscience; Ben-Gurion University of the Negev; Beer Sheva, Israel

**Amyotrophic lateral sclerosis (ALS) is a progressive adult-onset neurodegenerative disorder characterized by the selective loss of upper and lower motor neurons. Mutations in superoxide dismutase (SOD1) cause about 20 percent of familial ALS which is accompanied by accumulation of misfolded SOD1 onto intracellular organelles. Recently, we identified the 12 kDa macrophage migration inhibitory factor (MIF) as a chaperone for mutant SOD1 which is abundant in non-neuronal tissues. Purified recombinant MIF was shown to directly inhibit mutant SOD1 misfolding and association with mitochondria and ER. Elevating MIF in neuronal cells inhibited the accumulation of misfolded SOD1 and its association with mitochondria and ER, and extended survival of mutant SOD1-expressing motor neurons. Our results revealed that the levels of MIF protein are very low in motor neurons, implicating low chaperone activity as a component of selective vulnerability of motor neurons to mutant SOD1 misfolding and toxicity.**

**Keywords:** ALS, SOD1, misfolded SOD1, MIF, chaperone activity



*Correspondence to: Adrian Israelson; Email: adriani@bgu.ac.il

Submitted: 04/27/2015

Revised: 06/04/2015

Accepted: 06/05/2015

http://dx.doi.org/10.1080/21675511.2015.1061164



Addendum to: Israelson A, Ditsworth D, Sun S, Song SW, Liang J, Hruska-Plochan M, McAlonis-Downes M, Abu-Hamad S, Zoltsman G, Shani T, et al. Macrophage migration inhibitory factor as a chaperone inhibiting accumulation of misfolded SOD1. Neuron 2015; 86:218–32.

Amyotrophic lateral sclerosis (ALS), also called Lou Gehrig's disease, is the most common form of motor neuron disease. It is a fatal adult-onset neurodegenerative disease with major characteristic symptoms including muscle weakness, spasticity, atrophy, paralysis and premature death. Motor neurons in the cortex, brain stem, and spinal cord gradually degenerate in ALS patients, and most ALS patients die within 2–5 y from disease onset due to respiratory failure. However, approximately 10% of patients survive more than 10 y. The typical age of onset is between 50 to 60 y for most forms of ALS. The disease significantly affects the patient's quality of life, being characterized by progressive muscle weakness, atrophy, and spasticity. ALS so far is incurable and there is only one FDA approved treatment for use in ALS, the drug riluzole. This drug delays the onset of ventilator-dependence and may extend life by up to 3 months.[1] Accordingly, there is an urgent need for therapeutics for ALS.

Most of the cases of ALS are sporadic (SALS) lacking any apparent genetic linkage, but 10% are inherited in a dominant manner (FALS). Twenty percent of these familial cases have been attributed to dominant mutations in the gene encoding cytoplasmic Cu/Zn superoxide dismutase (SOD1)[2] which is involved in the cellular detoxification of superoxide anions. Native SOD1 forms a very stable homodimer, but almost all ALS-linked SOD1 mutations are susceptible to partial unfolding at physiological pH and temperature. Furthermore, mutant SOD1-containing insoluble inclusions are highly accumulated within affected motor neurons of SOD1-related FALS patients.

Transgenic mice and rats constitutively expressing SOD1 harboring ALS-linked mutations develop late onset motor neuron death and muscle atrophy, similarly to that seen in ALS patients. Given the pathological similarities between sporadic and familial forms of ALS, it is generally accepted that these transgenic rodents are valuable models to study the pathogenic mechanisms. These studies have indicated that SOD1 mutants provoke selective loss of motor neurons through acquisition of one or more toxic effects (yet unidentified) and not through loss of dismutase activity.

SOD1-positive inclusions with ALS-like symptoms were reproduced in a FALS- mouse model expressing human SOD1 with a pathogenic mutation and were found to be stained by Thioflavin S, supporting the formation of amyloid-like, β-sheet-rich fibrils in mice.[3,4] Insoluble SOD1 aggregates were also successfully isolated from the spinal cords of affected FALS mice, and quite notably, those SOD1 aggregates exhibited seeding activity toward fibrillation of purified SOD1 proteins *in vitro*. Chia et al. have prepared homogenates of spinal cords from transgenic mice expressing human SOD1 containing the G93A mutation and showed that the homogenates triggered fibrillation of wild-type as well as mutant $SOD1^{G93A}$ proteins under *in vitro* conditions with acidic pH solution in the presence of guanidine hydrochloride.[5] While destabilization of SOD1 proteins under artificial conditions appears to be required for a seeded acceleration of fibrillar aggregation, inclusions containing mutant SOD1 would function as seeds and thereby contribute to propagation of pathological changes among contiguous motor neurons and then disease progression of SOD1-related FALS cases.

Currently, more than 171 mutations have been identified within SOD1 that are linked to ALS. Approximately 20–25% of FALS cases and 6% of all ALS cases are caused by mutations in *SOD1*. The majority of these mutations (>80%) result in amino acid substitutions, while the remaining lesions are a combination of insertions, polymorphisms, and deletions. FALS-linked mutations are not localized to one portion of SOD1, but rather span the entire protein. Moreover, relatively conservative amino acid substitutions within SOD1 can cause ALS, suggesting that even minor alterations severely affect SOD1 structure and/or function. Much effort has been focused on determining the common "toxic" feature within SOD1 that is induced by all of these ALS-linked mutations. Except for the mutations that directly interfere with metal coordination, as copper coordination is required for catalytic activity, many ALS-linked mutations have no effect on SOD1 dismutase activity.

The effect of ALS-linked mutations on SOD1 signaling activity remains largely unexplored. However, prior hypotheses for pathogenesis in SOD1-mediated ALS (reviewed by)[6] include aberrant glutamate handling from delayed synaptic glutamate recovery by astrocytes,[7] mutant damage in the extracellular space following aberrant co-secretion with chromogranin[8] and excessive production by microglia of extracellular superoxide following mutant SOD1 binding to the small G protein Rac1 and its subsequent stimulation of NAPDH oxidase.[9]

Intracellular targets for SOD1 toxicity include mitochondria, which are essential for many fundamental cellular processes including energy production, $Ca^{2+}$ homeostasis and the urea cycle, and the endoplasmic reticulum (ER), where misfolded mutant SOD1 association with derlin-1, a component of the endoplasmic reticulum-associated degradation (ERAD) pathway, has been implicated in induction of ER stress from disrupted removal of misfolded proteins.[10,11] Derlin-1 is bound by at least 132 of the ALS-linked SOD1 mutants, each of which exposes a derlin-1 binding domain buried in correctly folded SOD1.[10]

Damage to mitochondria has been reported not only in rodent models of ALS but also in inherited and sporadic human ALS, including altered respiratory chain function and ultrastructure abnormalities within motor neurons, and respiratory and metabolic defects in muscle mitochondria.[12-20] In addition, mitochondrial dysfunction(s) within mutant astrocytes have been recently reported to cause motor neuron death in astrocyte-motor neuron co-cultures.[21] and mutant SOD1 astrocytes were reported to cause mitochondrial dysfunction within motor neurons[22] In the rodent models of mutant SOD1, there is specific association of misfolded mutant SOD1 with spinal cord mitochondria.[20]

Most recently, using a combination of different approaches, including purified mitochondria and purified voltage-dependent anion channel-1 (VDAC1) and mutant SOD1 proteins, we have demonstrated that misfolded SOD1 binds to the mitochondrial channel protein, VDAC1. This binding leads to the reduction in adenine nucleotide permeability through the outer mitochondrial membrane and the obligate reduction in energy supply, making motor neurons more vulnerable to any of these additional stresses derived either from mutant SOD1 acting within motor neurons or their neighboring non-neuronal cells.[23]

In addition, SOD1-mutant mediated deficits in protein import that alter the mitochondrial protein composition have been described.[24] These effects are specific for spinal cord mitochondria and are not observed in non-affected tissues, such as liver, despite comparable mutant SOD1 abundance in the 2 tissues.[23]

A direct consequence of mutation-induced misfolding of SOD1 is aggregation, which refers to the irreversible assembly of misfolded SOD1 species into an insoluble structure. SOD1 aggregation has been extensively investigated *in vivo*, both in human post-mortem tissues of ALS patients and in mutant SOD1 transgenic mice. The enhanced aggregation propensities of FALS-linked SOD1 mutants have also been comprehensively examined in cell culture and in other *in vitro* assays.

Although it remains unclear whether SOD1 aggregation is a causative or protective factor in disease progression, several recent reports demonstrate that misfolded SOD1 species can spread from cell to cell in a prion-like fashion.[25] Munch et al demonstrated that the uptake of aggregated ALS-linked SOD1 mutants in cultured neuronal cells seeded aggregation of endogenous SOD1. These endogenous SOD1 aggregates persisted well after the original aggregates dissipated from cell division, consistent with a prion-like propagation of aggregated SOD1.[26]

With the ubiquitous expression of SOD1, one of the most important unsolved questions is about the structural features underlying the selectivity of mutant SOD1 misfolding and association with intracellular membranes. We have now established that mutant SOD1 misfolding onto mitochondria is blocked by a cytosolic factor present in liver cytosol. This factor was identified as the 12 $k_D$ macrophage migration inhibitory factor (MIF).[27] We showed that recombinant MIF can suppress both dismutase active

and inactive SOD1 mutant association with intracellular membranes and can inhibit misfolded SOD1 accumulation in neuronal cells. Furthermore, increased MIF, which normally accumulates only to low levels within the cell bodies of motor neurons, extends mutant SOD1-expressing motor neuron survival in culture.[27] The low level of MIF accumulated within motor neurons correlates with the accumulation of misfolded SOD1 species and their association with different intracellular organelles within those neurons.

While a proportion of MIF can be sequestered into vesicles and released extracellularly in response to a variety of signals, MIF is synthesized as a soluble, cytoplasmic protein. Moreover, MIF has previously been implicated in intracellular protein chaperone activity. When MIF switches from multimeric to monomeric forms, it exposes a hydrophobic surface that can provide ATP-independent chaperone activity.[28] Therefore, one possibility is that MIF function depends on its direct interaction with SOD1, although the affinity for such interaction seems to be relatively low. On the other hand, MIF has 2 enzymatic activities working as a tautomerase and an oxidoreductase enzyme. In our previous studies, in which we used a MIF mutant that has no thiol-oxidoreductase activity, we observed that this activity is not required for MIF chaperone-like function. However, the effect of MIF tautomerase activity on this function remains unknown and should be addressed as well.

Apparently unrelated to its chaperone-like function, MIF was one of the first cytokines to be described[29] and has a pivotal role in the immune response.[30] Historically, T cells were regarded as the major source of circulating MIF[31,32] but studies in the last 2 decades have shown that MIF is released from numerous other cell types from the lung, liver, heart, bowel, kidney, spleen, and skin[33,34] as well as in tissues of the endocrine system[35,36] and acts in an autocrine and paracrine manner. Cells that are activated by MIF include immune cells, epithelial and endothelial cells, various parenchymal cells, and cancer cells. In addition to induction by inflammatory stimuli (LPS or TNF-α), macrophages and T cells secrete MIF after stimulation with low doses of glucocorticoids, and MIF also counter-regulates their immunosuppressive effects.[37,38] Through this mechanism, MIF sustains inflammation. In the cytokine cascade, MIF is localized upstream of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interferon (IFN)-γ, and other effector cytokines, in large part because it is released initially from preformed cytoplasmic pools.[39] Its rapid release from damaged or necrotic cells suggests that it may subserve functions of alarmins or damage-associated molecular patterns (i.e. DAMPs).

The MIF cell surface receptor is CD74, which signals via regulated intramembrane cleavage or by co-activating CD44[40,41] and the chemokine receptors CXCR2 and CXCR4.[42,43] As its name indicates, MIF first was associated with inhibition of the random migration of macrophages.[32] it was later determined that MIF also inhibits its directed migration of monocytes toward chemokines, such as monocyte chemoattractant protein 1 (MCP-1).[44] However, MIF also promotes the recruitment of T cells, monocytes and neutrophils via the non-cognate receptors CXCR2 and CXCR4.[43]

For decades, any immune activity in the central nervous system (CNS) was considered detrimental. However, studies initiated more than a decade ago[45] have shown that CNS plasticity requires immune support. Specifically, it was shown that a local immune response, well controlled in time, place, and intensity by peripheral adaptive immune processes, is a critical requirement for CNS maintenance[46] and for post-traumatic neuronal survival and repair.[45] These results and others suggest that CD4+ T cells that recognize CNS autoantigens are needed for CNS maintenance and repair.

In the mouse model of ALS, it is becoming clear that the ability of the motor neurons to resist the spread of toxicity from the surrounding damaged cells is greatly influenced by peripheral T cells. Thus, for example, breeding mutant SOD1 mice with immunodeficient mice shortens their life span[47,48] and as a corollary, active vaccination in some strains of ALS mice[49] or passive transfer of activated T cells, are able to extend life expectancy.[50] Moreover, mutant SOD1 synthesized by astrocytes and microglia leads to neuroinflammation, driving rapid disease progression.[51–53] In addition, astrocytes expressing mutant SOD1 or generated from neural precursor cells (NPCs) isolated from spinal cords of sporadic ALS patients have been found to be toxic to co-cultured motor neurons.[54-57]

Of relevance to MIF's extracellular activities, we suggest that one of the manifestations of immune deficits in ALS could be a malfunction of MIF, bridging the gap between the chaperone and the immune systems. Moreover, in view of the potential for cell-to-cell spread of misfolded SOD1 as a means of disease propagation.[26,58,59] chaperone activity by extracellular MIF may act to limit such spreading. Interestingly, lipocalin 2 has been recently reported as an inducible factor to be secreted by reactive astrocytes, which is selectively toxic to neurons.[60] In this regard, it is possible that MIF could interfere with lipocalin 2 to prevent its toxic effect.

Now we showed that MIF accumulation is very minimal in the spinal motor neurons. Since we observed that MIF mRNA is abundant in these cells, the question is why is the protein level very low? There are 2 possible explanations: MIF can be either secreted or rapidly degraded in the motor neurons. Combined with the recognition that extracellular MIF is an inducer of metalloproteinase 9 (MMP9).[61] a component contributing to the selectivity of motor neuron vulnerability to SOD1 mutant-mediated death.[62] it may be possible that MIF is highly secreted from the most sensitive motor neurons, not only leading to reduced chaperone activity and accumulation of misfolded proteins in these cells, but also inducing extracellular MMP9, leading to further damage to the surrounded cells. This idea suggests that approaches to increase intracellular MIF by reducing its clearance from motor neurons and thus to inhibit MMP9 induction could be attractive therapeutic strategies.

## Conclusions

Elucidating the basic mechanisms causing neurodegeneration in ALS represents an urgent medical need that would allow for the development of new therapies. The

identification of MIF has the potential to yield important information about the toxic mechanism of protein misfolding and may lead to the discovery of new targets for therapies. The finding of this novel protective factor may have implications not only for ALS, but for other neurodegenerative diseases, in which intracellular targets are affected by one or more misfolded proteins.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Funding

A.I. has been supported by a career development grant from the Muscular Dystrophy Association (MDA) and a Marie Curie Career Integration Grant (CIG).

### References

1. Costa J, Gomes C, de Carvalho M. Diagnosis, pathogenesis and therapeutic targets in amyotrophic lateral sclerosis. CNS Neurol Disord Drug Targets 2010; 9:764-78; PMID:20942786; http://dx.doi.org/10.2174/187152710793237502
2. Rosen DR. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993 364:362; PMID:8332197; http://dx.doi.org/10.1038/364362c0
3. Furukawa Y, Kaneko K, Yamanaka K, O'Halloran TV, Nukina N. Complete loss of post-translational modifications triggers fibrillar aggregation of SOD1 in the familial form of amyotrophic lateral sclerosis. J Biol Chem 2008; 283:24167-76; PMID:18552350; http://dx.doi.org/10.1074/jbc.M802083200
4. Wang J, Xu G, Gonzales V, Coonfield M, Fromholt D, Copeland NG, Jenkins NA, Borchelt DR. Fibrillar inclusions and motor neuron degeneration in transgenic mice expressing superoxide dismutase 1 with a disrupted copper-binding site. Neurobiol Dis 2002; 10:128-38; PMID:12127151; http://dx.doi.org/10.1006/nbdi.2002.0498
5. Chia R, Tattum MH, Jones S, Collinge J, Fisher EM, Jackson GS. Superoxide dismutase 1 and tgSOD1 mouse spinal cord seed fibrils, suggesting a propagative cell death mechanism in amyotrophic lateral sclerosis. PLoS One 2010; 5:e10627; PMID:20498711; http://dx.doi.org/10.1371/journal.pone.0010627
6. Ilieva H, Polymenidou M, Cleveland DW. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. J Cell Biol 2009; 187:761-72; PMID:19951898; http://dx.doi.org/10.1083/jcb.200908164
7. Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW, Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol 1995; 38:73-84; PMID:7611729; http://dx.doi.org/10.1002/ana.410380114
8. Urushitani M, Sik A, Sakurai T, Nukina N, Takahashi R, Julien JP. Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis. Nat Neurosci 2006; 9:108-18; PMID:16369483; http://dx.doi.org/10.1038/nn1603.
9. Harraz MM, Marden JJ, Zhou W, Zhang Y, Williams A, Sharov VS, Nelson K, Luo M, Paulson H, Schoneich C. SOD1 mutations disrupt redox-sensitive Rac regulation of NADPH oxidase in a familial ALS model. J Clin Invest 2008; 118:659-70; PMID:18219391
10. Fujisawa T, Homma K, Yamaguchi N, Kadowaki H, Tsuburaya N, Naguro I, Matsuzawa A, Takeda K, Takahashi Y, Goto J, Tsuji S, Nishitoh H, Ichijo H. A novel monoclonal antibody reveals a conformational alteration shared by amyotrophic lateral sclerosis-linked SOD1 mutants. Ann Neurol 2012; 72:739-49; PMID:23280792; http://dx.doi.org/10.1002/ana.23668
11. Nishitoh H, Kadowaki H, Nagai A, Maruyama T, Yokota T, Fukutomi H, Noguchi T, Matsuzawa A, Takeda K, Ichijo H. ALS-linked mutant SOD1 induces ER stress- and ASK1-dependent motor neuron death by targeting Derlin-1. Genes Dev 2008; 22:1451-64; PMID:18519638; http://dx.doi.org/10.1101/gad.1640108
12. Beretta S, Sala G, Mattavelli L, Ceresa C, Casciati A, Ferri A, Carri MT, Ferrarese C, Mitochondrial dysfunction due to mutant copper/zinc superoxide dismutase associated with amyotrophic lateral sclerosis is reversed by N-acetylcysteine. Neurobiol Dis 2003; 13:213-21; PMID:12901835; http://dx.doi.org/10.1016/S0969-9961(03)00043-3
13. Carri MT, Ferri A, Battistoni A, Famhy L, Gabbianelli R, Poccia F, Rotilio G, Expression of a Cu,Zn superoxide dismutase typical of familial amyotrophic lateral sclerosis induces mitochondrial alteration and increase of cytosolic $Ca^{2+}$ concentration in transfected neuroblastoma SH-SY5Y cells. FEBS Lett 1997; 414:365-8; PMID:9315720; http://dx.doi.org/10.1016/S0014-5793(97)01051-X
14. Damiano M, Starkov AA, Petri S, Kipiani K, Kiaei M, Mattiazzi M, Flint Beal M, Manfredi G Neural mitochondrial $Ca^{2+}$ capacity impairment precedes the onset of motor symptoms in G93A Cu/Zn-superoxide dismutase mutant mice. J Neurochem 2006; 96:1349-61; PMID:16478527; http://dx.doi.org/10.1111/j.1471-4159.2006.03619.x
15. Hervias I, Beal MF, Manfredi G, Mitochondrial dysfunction and amyotrophic lateral sclerosis. Muscle Nerve 2006; 33:598-608; PMID:16372325; http://dx.doi.org/10.1002/mus.20489
16. Liu J, Lillo C, Jonsson PA, Vande Velde C, Ward CM, Miller TM, Subramaniam JR, Rothstein JD, Marklund S, Andersen PM, et.al. Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. Neuron 2004; 43:5-17; PMID:15233913; http://dx.doi.org/10.1016/j.neuron.2004.06.016
17. Manfredi G, Xu Z. Mitochondrial dysfunction and its role in motor neuron degeneration in ALS. Mitochondrion 2005; 5:77-87; PMID:16050975; http://dx.doi.org/10.1016/j.mito.2005.01.002
18. Mattiazzi M, D'Aurelio M, Gajewski CD, Martushova K, Kiaei M, Beal MF, Manfredi G. Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. J Biol Chem 2002; 277:29626-33; PMID:12050154; http://dx.doi.org/10.1074/jbc.M203065200
19. Menzies FM, Cookson MR, Taylor RW, Turnbull DM, Chrzanowska-Lightowlers ZM, Dong L, Figlewicz DA, Shaw PJ. Mitochondrial dysfunction in a cell culture model of familial amyotrophic lateral sclerosis. Brain 2002; 125:1522-33; PMID:12077002; http://dx.doi.org/10.1093/brain/awf167
20. Vande Velde C, Miller TM, Cashman NR, Cleveland DW, Selective association of misfolded ALS-linked mutant SOD1 with the cytoplasmic face of mitochondria. Proc Natl Acad Sci U S A 2008; 105:4022-7; PMID:18296640; http://dx.doi.org/10.1073/pnas.0712209105
21. Cassina P, Cassina A, Pehar M, Castellanos R, Gandelman M, de Leon A, Robinson KM, Mason RP, Beckman JS, Barbeito L, et.al. Mitochondrial dysfunction in SOD1G93A-bearing astrocytes promotes motor neuron degeneration: prevention by mitochondrial-targeted antioxidants. J Neurosci 2008; 28:4115-22; PMID:18417691; http://dx.doi.org/10.1523/JNEUROSCI.5308-07.2008
22. Bilsland LG, Nirmalananthan N, Yip J, Greensmith L, Duchen MR, Expression of mutant SOD1 in astrocytes induces functional deficits in motoneuron mitochondria. J Neurochem 2008; 107:1271-83; PMID:18808448; http://dx.doi.org/10.1111/j.1471-4159.2008.05699.x
23. Israelson A, Arbel N, Da Cruz S, Ilieva H, Yamanaka K, Shoshan-Barmatz V, Cleveland DW. Misfolded mutant SOD1 directly inhibits VDAC1 conductance in a mouse model of inherited ALS. Neuron 2010; 67:575-87; PMID:20797535; http://dx.doi.org/10.1016/j.neuron.2010.07.019
24. Li Q, Vande Velde C, Israelson A, Xie J, Bailey AO, Dong MQ, Chun SJ, Roy T, Winer L, Yates JR, et.al. ALS-linked mutant superoxide dismutase 1 (SOD1) alters mitochondrial protein composition and decreases protein import. Proc Natl Acad Sci U S A 2010; 107:21146-51; PMID:21078990; http://dx.doi.org/10.1073/pnas.1014862107
25. Grad LI, Cashman NR. Prion-like activity of Cu/Zn superoxide dismutase: implications for amyotrophic lateral sclerosis. Prion 2014; 8:33-41; PMID:24394345; http://dx.doi.org/10.4161/pri.27602
26. Munch C, O'Brien J, Bertolotti A. Prion-like propagation of mutant superoxide dismutase-1 misfolding in neuronal cells. Proc Natl Acad Sci U S A 2011; 108:3548-53; PMID:21321227; http://dx.doi.org/10.1073/pnas.1017275108
27. Israelson A, Ditsworth D, Sun S, Song S, Liang J, Hruska-Plochan M, McAlonis-Downes M, Abu-Hamad S, Zoltsman G, Shani T, et.al. Macrophage Migration Inhibitory Factor as a Chaperone Inhibiting Accumulation of Misfolded SOD1. Neuron 2015; 86:218-32; PMID:25801706; http://dx.doi.org/10.1016/j.neuron.2015.02.034
28. Cherepkova OA, Lyutova EM, Eronina TB, Gurvits BY. Chaperone-like activity of macrophage migration inhibitory factor. Int J Biochem Cell Biol 2006; 38:43-55; PMID:16099194; http://dx.doi.org/10.1016/j.biocel.2005.07.001
29. George M, Vaughan JH. In vitro cell migration as a model for delayed hypersensitivity. Proc Soc Exp Biol Med 1962; 111:514-21; PMID:13947220; http://dx.doi.org/10.3181/00379727-111-27841
30. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol 2003; 3:791-800; PMID:14502271; http://dx.doi.org/10.1038/nri1200
31. Bloom BR, Bennett B. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. Science 1966; 153:80-2; PMID:5938421; http://dx.doi.org/10.1126/science.153.3731.80
32. David JR. Delayed hypersensitivity in vitro: its mediation by cell-free substances formed by lymphoid cell-antigen interaction. Proc Natl Acad Sci U S A 1966; 56:72-7; PMID:5229858; http://dx.doi.org/10.1073/pnas.56.1.72
33. Merk M, Zierow S, Leng L, Das R, Du X, Schulte W, Fan J, Lue H, Chen Y, Xiong H, et.al. The D-dopachrome tautomerase (DDT) gene product is a cytokine and functional homolog of macrophage migration inhibitory factor (MIF). Proc Natl Acad Sci U S A 2011; 108:E577-85; PMID:21817065; http://dx.doi.org/10.1073/pnas.1102941108
34. Bacher M, Meinhardt A, Lan HY, Mu W, Metz CN, Chesney JA, Calandra T, Gemsa D, Donnelly T, Atkins RC, et.al. Migration inhibitory factor expression in experimentally induced endotoxemia. Am J Pathol 1997; 150:235-46; PMID:9006339
35. Bernhagen J, Calandra T, Mitchell RA, Martin SB, Tracey KJ, Voelter W, Manogue KR, Cerami A, Bucala R, MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. Nature 1993; 365:756-9; PMID:8413654; http://dx.doi.org/10.1038/365756a0

36. Fingerle-Rowson G, Koch P, Bikoff R, Lin X, Metz CN, Dhabhar FS, Meinhardt A, Bucala R. Regulation of macrophage migration inhibitory factor expression by glucocorticoids in vivo. Am J Pathol 2003; 162:47-56; PMID:12507889; http://dx.doi.org/10.1016/S0002-9440(10)63797-2
37. Calandra T, Bernhagen J, Metz CN, Spiegel LA, Bacher M, Donnelly T, Cerami A, Bucala R. MIF as a glucocorticoid-induced modulator of cytokine production. Nature 1995; 377:68-71; PMID:7659164; http://dx.doi.org/10.1038/377068a0
38. Leng L, Wang W, Roger T, Merk M, Wuttke M, Calandra T, Bucala R. Glucocorticoid-induced MIF expression by human CEM T cells. Cytokine 2009; 48:177-85; PMID:19646897; http://dx.doi.org/10.1016/j.cyto.2009.07.002
39. Merk M, Baugh J, Zierow S, Leng L, Pal U, Lee SJ, Ebert AD, Mizue Y, Trent JO, Mitchell R, et.al. The Golgi-associated protein p115 mediates the secretion of macrophage migration inhibitory factor. J Immunol 2009; 182:6896-906; PMID:19454686; http://dx.doi.org/10.4049/jimmunol.0803710
40. Leng L, Metz CN, Fang Y, Xu J, Donnelly S, Baugh J, Delohery T, Chen Y, Mitchell RA, Bucala R. MIF signal transduction initiated by binding to CD74. J Exp Med 2003; 197:1467-76; PMID:12782713; http://dx.doi.org/10.1084/jem.20030286
41. Shi X, Leng L, Wang T, Wang W, Du X, Li J, McDonald C, Chen Z, Murphy JW, Lolis E, et.al. CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex. Immunity 2006; 25:595-606; PMID:17045821; http://dx.doi.org/10.1016/j.immuni.2006.08.020
42. Weber C, Kraemer S, Drechsler M, Lue H, Koenen RR, Kapurniotu A, Zernecke A, Bernhagen J. Structural determinants of MIF functions in CXCR2-mediated inflammatory and atherogenic leukocyte recruitment. Proc Natl Acad Sci U S A 2008; 105:16278-83; PMID:18852457; http://dx.doi.org/10.1073/pnas.0804017105
43. Bernhagen J, Krohn R, Lue H, Gregory JL, Zernecke A, Koenen RR, Dewor M, Georgiev I, Schober A, Leng L, et.al. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. Nat Med 2007; 13:587-96; PMID:17435771; http://dx.doi.org/10.1038/nm1567
44. Hermanowski-Vosatka A, Mundt SS, Ayala JM, Goyal S, Hanlon WA, Czerwinski RM, Wright SD, Whitman CP. Enzymatically inactive macrophage migration inhibitory factor inhibits monocyte chemotaxis and random migration. Biochemistry 1999; 38:12841-9; PMID:10504254; http://dx.doi.org/10.1021/bi991352p
45. Moalem G, Leibowitz-Amit R, Yoles E, Mor F, Cohen IR, Schwartz M. Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. Nat Med 1999; 5:49-55; PMID:9883839; http://dx.doi.org/10.1038/4734
46. Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, Cohen H, Kipnis J, Schwartz M Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. Nat Neurosci 2006; 9:268-75; PMID:16415867; http://dx.doi.org/10.1038/nn1629
47. Beers DR, Henkel JS, Zhao W, Wang J, Appel SH. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. Proc Natl Acad Sci U S A 2008; 105:15558-63; PMID:18809917; http://dx.doi.org/10.1073/pnas.0807419105
48. Chiu IM, Chen A, Zheng Y, Kosaras B, Tsiftsoglou SA, Vartanian TK, Brown RH, Jr, Carroll MC. T lymphocytes potentiate endogenous neuroprotective inflammation in a mouse model of ALS. Proc Natl Acad Sci U S A 2008; 105:17913-8; http://dx.doi.org/10.1073/pnas.0804610105
49. Angelov DN, Waibel S, Guntinas-Lichius O, Lenzen M, Neiss WF, Tomov TL, Yoles E, Kipnis J, Schori H, Reuter A, et.al. Therapeutic vaccine for acute and chronic motor neuron diseases: implications for amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 2003; 100:4790-5; PMID:12668759; http://dx.doi.org/10.1073/pnas.0530191100
50. Beers DR, Henkel JS, Zhao W, Wang J, Huang A, Wen S, Liao B, Appel SH. Endogenous regulatory T lymphocytes ameliorate amyotrophic lateral sclerosis in mice and correlate with disease progression in patients with amyotrophic lateral sclerosis. Brain 2011; 134:1293-314; PMID:21596768; http://dx.doi.org/10.1093/brain/awr074
51. Beers DR, Henkel JS, Xiao Q, Zhao W, Wang J, Yen AA, Siklos L, McKercher SR, Appel SH. Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 2006; 103:16021-6; PMID:17043238; http://dx.doi.org/10.1073/pnas.0607423103
52. Boillee S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, Kollias G, Cleveland DW. Onset and progression in inherited ALS determined by motor neurons and microglia. Science2006; 312:1389-92; PMID:16741123; http://dx.doi.org/10.1126/science.1123511
53. Yamanaka K, Boillee S, Roberts EA, Garcia ML, McAlonis-Downes M, Mikse OR, Cleveland DW, Goldstein LS. Mutant SOD1 in cell types other than motor neurons and oligodendrocytes accelerates onset of disease in ALS mice. Proc Natl Acad Sci U S A 2008; 105:7594-9; PMID:18492803; http://dx.doi.org/10.1073/pnas.0802556105
54. Haidet-Phillips AM, Hester ME, Miranda CJ, Meyer K, Braun L, Frakes A, Song S, Likhite S, Murtha MJ, Foust KD, et.al. Astrocytes from familial and sporadic ALS patients are toxic to motor neurons. Nat Biotechnol 2011; 29:824-8; PMID:21832997; http://dx.doi.org/10.1038/nbt.1957.
55. Di Giorgio FP, Carrasco MA, Siao MC, Maniatis T, Eggan K. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. Nat Neurosci 2007; 10:608-14; PMID:17435754; http://dx.doi.org/10.1038/nn1885
56. Marchetto MC, Muotri AR, Mu Y, Smith AM, Cezar GG, Gage FH, Non-cell-autonomous effect of human SOD1 G37R astrocytes on motor neurons derived from human embryonic stem cells. Cell Stem Cell 2008; 3:649-57; PMID:19041781; http://dx.doi.org/10.1016/j.stem.2008.10.001
57. Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. Nat Neurosci 2007; 10:615-22; PMID:17435755; http://dx.doi.org/10.1038/nn1876
58. Grad LI, Cashman NR. Prion-like activity of Cu/Zn superoxide dismutase: Implications for amyotrophic lateral sclerosis. Prion .2014; 8; 33-41
59. Grad LI, Guest WC, Yanai A, Pokrishevsky E, O'Neill MA, Gibbs E, Semenchenko V, Yousefi M, Wishart DS, Plotkin SS, Cashman NR. Intermolecular transmission of superoxide dismutase 1 misfolding in living cells. Proc Natl Acad Sci U S A 2011; 108:16398-403; PMID:21930926; http://dx.doi.org/10.1073/pnas.1102645108
60. Bi F, Huang C, Tong J, Qiu G, Huang B, Wu Q, Li F, Xu Z, Bowser R, Xia XG, Zhou H. Reactive astrocytes secrete lcn2 to promote neuron death. Proc Natl Acad Sci U S A 2013110:4069-74; PMID:23431168; http://dx.doi.org/10.1073/pnas.1218497110
61. Yu X, Lin SG, Huang XR, Bacher M, Leng L, Bucala R, Lan HY. Macrophage migration inhibitory factor induces MMP-9 expression in macrophages via the MEK-ERK MAP kinase pathway. J Interferon Cytokine Res 2007; 27:103-9; PMID:17316137; http://dx.doi.org/10.1089/jir.2006.0054
62. Kaplan A, Spiller KJ, Towne C, Kanning KC, Choe GT, Geber A, Akay T, Aebischer P, Henderson CE. Neuronal matrix metalloproteinase-9 is a determinant of selective neurodegeneration. Neuron 2014; 81:333-48; PMID:24462097; http://dx.doi.org/10.1016/j.neuron.2013.12.009