Macrophage Migration Inhibitory Factor, the *Zelig* of Cytokines, Is a Chaperone for SOD1 in Non-Neuronal Cells

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Pathogenic properties of mutant SOD1 in ALS likely involve binding to mitochondrial and ER membranes. In this issue of *Neuron*, Israelson et al. (2015) show that motor neurons, selectively vulnerable in ALS, lack a chaperone that precludes mSOD1 binding intracellular membranes in other cells. This chaperone is identified as the pleiotropic cytokine macrophage migration inhibitory factor.

> That Zelig could be responsible for the behavior of each of the personalities he assumed means dozens of lawsuits. He is sued for bigamy, adultery, automobile accidents, plagiarism, household damages, negligence, property damages, and performing unnecessary dental extractions.
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> —*Zelig* (1983), written by Woody Allen

Mutant forms of cytoplasmic Cu/Zn superoxide dismutase (SOD1), a ubiquitously expressed protein, cause amyotrophic lateral sclerosis (ALS) with selective degeneration in the CNS motor system. ALS incident cases in the United States approximately equal those of multiple sclerosis (MS), but ALS prevalence is far lower due to its virulent lethality. More than 100 ALS-associated SOD1 mutations (collectively termed mSOD1), including many which leave catalytic function unaffected, have been described. Cellular phenotypes associated with mutant SOD1, which accounts for about 20% of familial ALS cases, have also been described in sporadic ALS, lending urgency to the deciphering of its pathogenic mechanisms.

Providing a potential clue to its relation to ALS pathogenesis, mutant SOD1 binds the cytosolic face of mitochondrial membranes and to ER selectively in neuronal cells and CNS tissue lysates, corresponding to the CNS-specific degeneration seen in patients. Israelson and coworkers (Israelson et al., 2015) addressed this neuronal specificity by asking the following question: is there an activity in neurons which drives SOD1 binding to mitochondria, or is a binding inhibitor present in non-neuronal cells? Their results indicated that non-neuronal cytosol contains a protein which inhibits SOD1 binding to mitochondria, and they identified this activity as macrophage migration inhibitory factor (MIF). This result will provoke curiosity in those who've never heard of MIF and a sigh of bemused, perhaps weary, recognition for those who've followed MIF's 40-year odyssey through biomedicine.

Characterized in 1966 as a factor secreted by lymphocytes (Bloom and Bennett, 1966; David, 1966), MIF was among the earliest-studied cytokines and has accumulated a bewildering variety of functions which account for its ability to appear in ever-changing guises in a seemingly endless series of biological contexts (Bucala, 2012). Interwoven cell-autonomous, autocrine, paracrine, and organism-wide activities consistently emerge in studies of MIF biology. In the immune system, MIF exhibits inflammatory cytokine activity and also enhances immunological processes through direct inhibitory effects on glucocorticoid action (Bernhagen et al., 1993). Surprisingly (but not for MIF), the anterior pituitary is a major source of MIF in the setting of endotoxemia (Bucala, 1996).

MIF homologs are widely expressed throughout biology with representative family members found in *C. elegans* and *Danio rerio* (Vermeire et al., 2008), where the MIF homolog is required for CNS organogenesis. Microbial species including *Rickettsia* also express MIF-like proteins (Calandra and Roger, 2003). In solution, MIF is a homotrimer, and this tertiary structure is stringently conserved for MIF homologs, while amino acid identities are not. Ketoenol tautomerase activity toward organic substrates such as phenylpyruvate is also conserved both in animal and bacterial MIF family proteins. Thiol reductase activity is considered important for MIF's cardioprotective action in acute myocardial infarction by reducing oxidative stress, thereby suppressing apoptotic signaling (Miller et al., 2008).

In its non-cell-autonomous functions, MIF mediates receptor-dependent cytokine-characteristic paracrine and autocrine signaling, binding homotrimeric CD74. Binding is, however, insufficient for cellular responses, as the ligated receptor must complex with CD44 to activate Src upstream of Raf-1, leading to Erk1 phosphorylation and transcription of MAP kinase pathway genes (Shi et al., 2006). In parallel, MIF inhibits c-Jun N-terminal activation domain-binding protein-1 (JAB-1), suppressing Jnk signaling and prolonging activation of phospho-Erk1 (Kleemann et al., 2000). Much of MIF's receptor-mediated action relies on cAMP-dependent protein kinase (PKA), making it somewhat paradoxical that a counter-regulatory enzyme AMP-activated kinase (AMPK) is considered a key effector of MIF's cardioprotective function (Miller et al., 2008). One cell's cytoprotection is of course another cell's release from physiological growth control.

The same pathways which support cardiac myocyte survival in the ischemic heart have been found to promote leukemogenesis in B cell lymphoma cells (Shachar and Haran, 2011).

The film *Zelig* recounts the fictional saga of a man who takes on the appearance and behavior of those around him, leading to his nickname "The Human Chameleon." Given its array of intracellular, intercellular, and systemic effects in host defense, inflammatory disease, ischemia, cancer, and (now) neurodegeneration, one might nominate MIF to be the "Zelig of cytokines."

In the present study, Israelson et al. (2015) proceeded to demonstrate that MIF suppresses binding to mitochondria and ER membranes in neuron-like NSC-34 cells, and this assay was used to demonstrate that MIF thiol reductase activity was dispensable for inhibiting mSOD1 binding to mitochondria, through examination of a MIF point mutant. As misfolded mSOD1 can be monitored in vitro using a conformation-dependent antibody DSE2, further studies could be done in cell-free systems to demonstrate that MIF reduces accumulation of DSE2 immunoreactivity. In this assay, the activity of purified MIF was equivalent to that of liver cytosol, the input material used to show that non-neuronal cells suppress the binding of mSOD1 to mitochondrial and ER membranes. Both catalytically active and inactive forms of mSOD1 were used in these studies. The possibility that MIF served as a chaperone for client mSOD1 was addressed by coimmunoprecipitation experiments showing that MIF and SOD1 were physically associated in cells, with an apparent Kd of approximately 350 nM. Overexpression of MIF (by lentiviral transduction) protected against the accelerated cell death for iPSC-derived motor neurons expressing mSOD1.

Why are motor neurons nearly devoid of MIF protein by tissue immunostaining, which readily detects MIF in glia and in other neurons? The mRNA is plentiful in CNS neurons, macroglia, microglia, and endothelial cells (http://web.stanford.edu/group/barres_lab/cgi-bin/igv_cgi_2.py?lname=Mif). Perhaps motor neurons fail to transcribe, accumulate, or translate MIF message. All three possibilities were addressed directly in experiments using choline acetyltransferase (ChAT)-bacTRAP mice to enable affinity isolation and analysis of actively translated mRNAs in motor neurons. Quite unexpectedly, it was shown that ChAT-positive motor neurons abundantly produced MIF message and protein in vivo. Near absence of cytosolic MIF protein in motor neurons must then reflect either or both of two processes, MIF secretion or MIF degradation, and these possibilities remain to be discriminated. Also, it is unexplained how lentiviral transduction, but not endogenous MIF gene, maintains cytoprotective levels of MIF protein.

In the present studies, SOD1 is a client for MIF's chaperone activity. Previous research demonstrated that MIF also provides chaperone function for insulin: when secreted from *Mif*$^{-/-}$ cells, insulin shows reduced competence to drive glucose uptake by hepatocytes (Vujicic et al., 2014). Placing the potential roles of MIF for ALS pathogenesis in context of biology from other systems, it's not certain which functions beyond cell-autonomous SOD1 chaperone activity may also be implicated. While MIF is exuberantly expressed in the CNS, the localization of MIF-responsive receptors remains uncertain: CD74 is strongly and exclusively present on microglia, while CD44 is mainly restricted to astrocytes. MIF has been reported to induce matrix metalloproteinase (MMP)-9, another ALS suspect component in motor neurons (Kaplan et al., 2014). In cancer cells, inhibiting heat-shock protein (HSP)-90 leads to rapid degradation of MIF protein (Schulz and Moll, 2014), so that it may be worthwhile to examine whether a comparable pathway is involved in possible destabilization of MIF protein in motor neurons. Although it's hard to predict where this story will go from here, it can be confidently anticipated that we'll hear much more from this protean molecule.

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