MIF inhibits the formation of misfolded SOD1 amyloid aggregates: Implications for familial ALS

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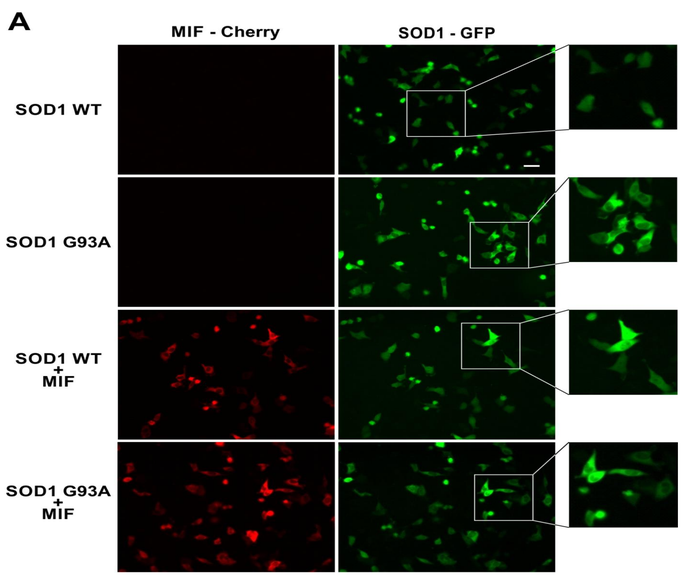
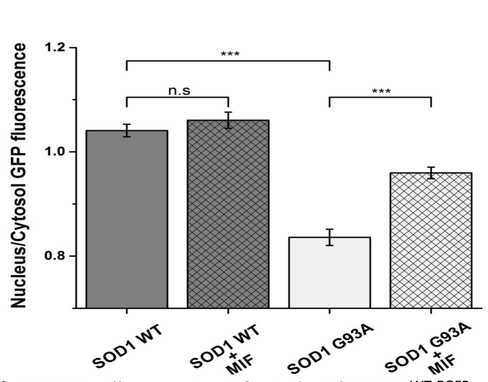
Abstract

Mutations in superoxide dismutase (SOD1) cause amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease caused by the progressive loss of motor neurons in the brain and spinal cord. It has been suggested that toxicity of mutant SOD1 results from its misfolding, however, it is yet unclear why misfolded SOD1 accumulates specifically within motor neurons. We recently demonstrated that macrophage migration inhibitory factor (MIF)—a multifunctional protein with cytokine/chemokine activity and cytosolic chaperone-like properties—inhibits the accumulation of misfolded SOD1. Here, we show that MIF inhibits mutant SOD1 nuclear clearance when overexpressed in motor neuron-like NSC-34 cells. In addition, MIF alters the typical SOD1 amyloid aggregation pathway in vitro, and, instead, promotes the formation of disordered aggregates, as measured by Thioflavin T (ThT) assay and transmission electron microscopy (TEM) imaging. Moreover, we report that MIF reduces the toxicity of misfolded SOD1 by directly interacting with it, and that the chaperone function and protective effect of MIF in neuronal cultures do not require its intrinsic catalytic activities. Importantly, we report that the locked-trimeric MIFN110C mutant, which exhibits strongly impaired CD74-mediated cytokine functions, has strong chaperone activity, dissociating, for the first time, these two cellular functions. Altogether, our study implicates MIF as a potential therapeutic candidate in the treatment of ALS.

Results

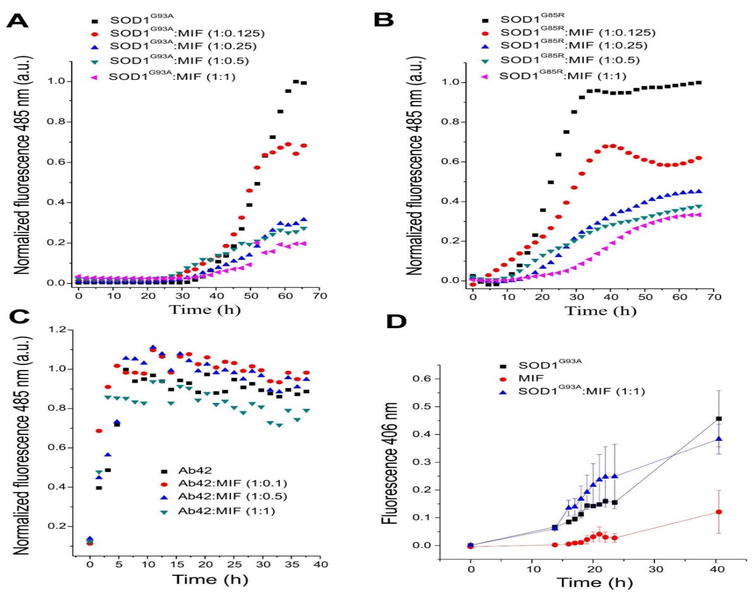
1 MIF inhibits misfolded SOD1 nuclear export in NSC-34 cells





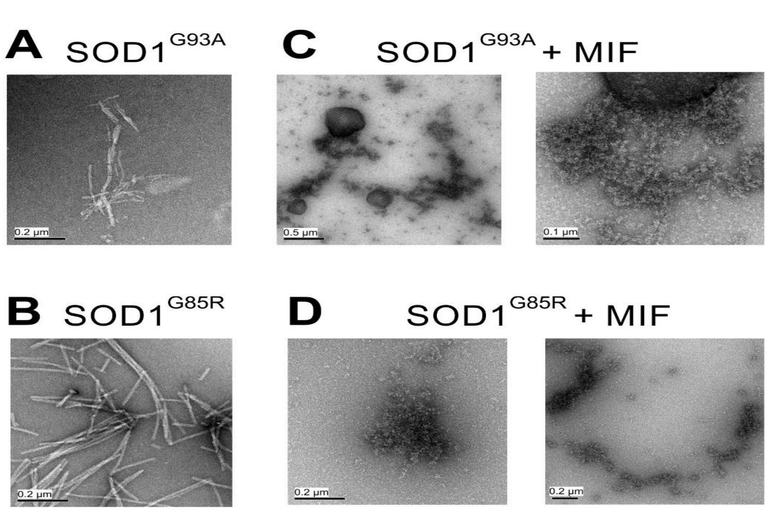
(A) NSC-34 cells were transfected with SOD1$^{WT\text{-}EGFP}$ or SOD1$^{G93A\text{-}EGFP}$ plasmid with or without co-transfection of MIF-Cherry. 24h after transfection, between 57-60 images were recorded for each group. Scale bar, 25 µm. **(B)** GFP fluorescence levels in the nucleus and in the cytoplasm were quantified separately and the ratio for each cell were taken for analysis. One-Way ANOVA test was applied on the average of the different groups, all normal distributed, followed by Tukey post hoc test. ***p<0.001

2 Recombinant MIF specifically suppresses mutant SOD1 amyloid fibril formation in a dose-dependent manner



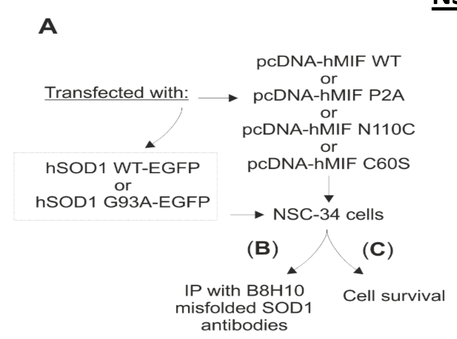
ThT fluorescence was monitored during the co-incubation of SOD1^{G93A} (50 µM) **(A)** or SOD1^{G85R} (50 µM) **(B)** with recombinant MIF at different molar concentrations. **(C)** ThT fluorescence of the β-amyloid peptide (Ab42), incubated alone or with increasing concentrations of recombinant MIF, under the same conditions as in **(A)** and **(B)**. **(D)** An increase in the turbidity measured at 406 nm indicates the formation of SOD1^{G93A} aggregates in solution in the absence (black) or presence (blue) of 50 µM MIF. The turbidity of MIF alone (50 µM; red) is also shown as a control.

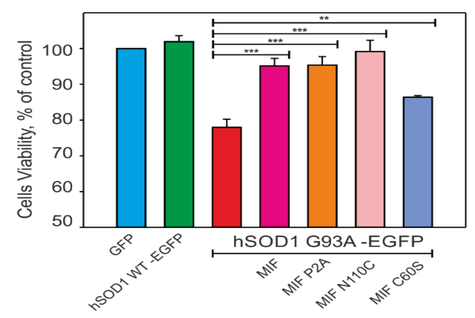
3 Recombinant MIF alters the morphology of SOD1^{G93A} and SOD1^{G85R} aggregates

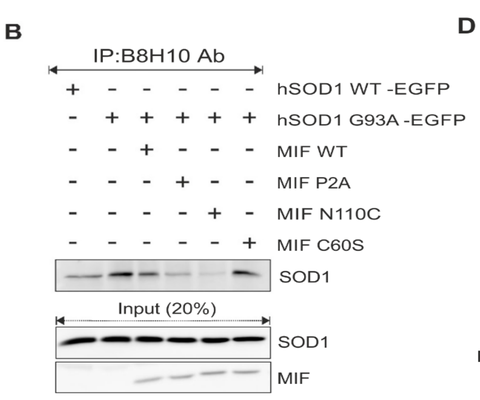


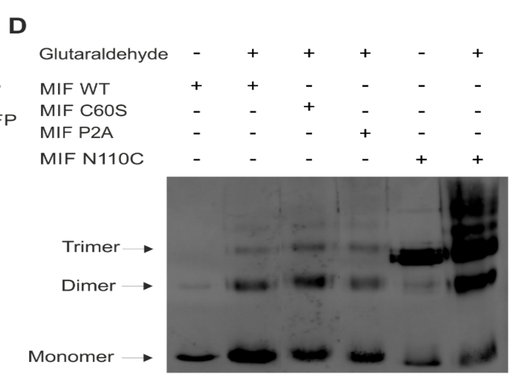
TEM images of a SOD1^{G93A} or SOD1^{G85R} (both at 50 µM) solution after a 64-h incubation at 37 ºC with continuous shaking. SOD1^{G93A} and SOD1^{G85R} were incubated either alone (**A, B,** respectively) or in the presence of recombinant MIF at a molar ratio of 1:1 (**C, D**, respectively).

4 The catalytic activities of MIF and its normal oligomeric states are not necessary for its chaperone activity and its protective effect against mutant SOD1 toxicity in NSC-34 cells



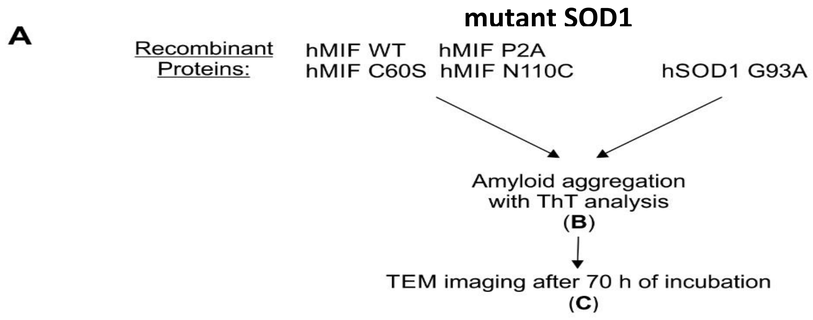


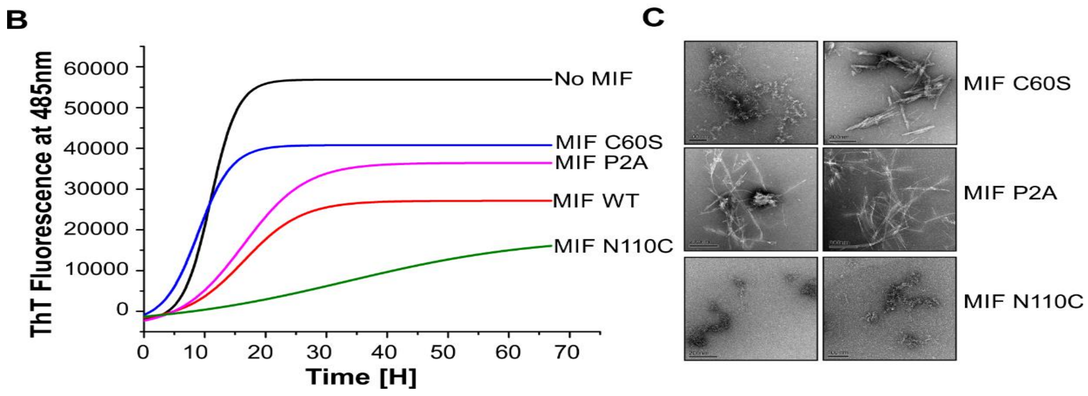




(A) A schematic representation of the experimental protocol. MIF-dependent inhibition of misfolded SOD1 accumulation and toxicity was tested in motor neuron-like NSC-34 cells, which were transfected to express the human wild-type SOD1 (SOD1^{WT}–GFP) or the human mutant SOD1^{G93A}–GFP with wild-type MIF (MIF^{WT}), MIF^{C60S} (which lacks oxidoreductase activity), MIF^{P2A} (which lacks tautomerase activity), or MIF^{N110C} (which is locked into a trimeric conformation). The cells were then subjected to an immunoprecipitation assay **(B)** or to a cell-survival assay **(C)**. **(B)** EGFP-tagged wild-type or mutant human SOD1 levels were determined by immunoblotting in the initial cytosolic fractions. **(C)** A cell-survival analysis was performed with the CellTiter 96 AQueous one-solution cell proliferation assay with ELISA at 490 nm. **(D)** Recombinant MIFWT and mutant proteins at a concentration of 10 µM were crosslinked with 1% glutaraldehyde and samples electrophoresed as indicated. Wild-type MIF and non-crosslinked samples were analyzed for comparison. The monomer, dimer and trimer oligomers are indicated. +, sample crosslinked prior to electrophoresis; -, non-crosslinked control.

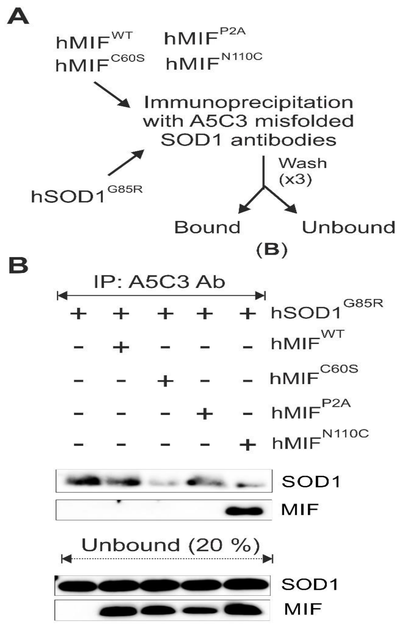
5 The locked-trimeric mutant MIF^{N110C} suppresses amyloid fibril formation of the mutant SOD1

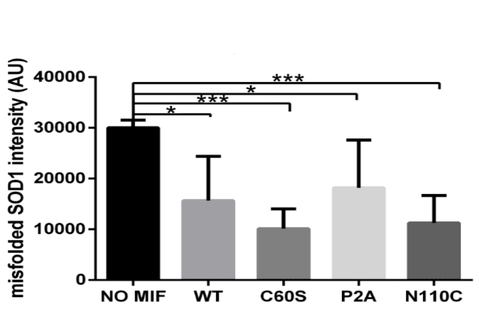




(A) Experimental protocol used for determining whether purified recombinant MIF^{WT} or MIF mutants suppress the amyloid aggregation (left panel) and accumulation of misfolded SOD1 (right panel). **(B)** ThT fluorescence was monitored during the incubation of mutant SOD1^{G93A} (50 µM), either without (green) or with recombinant MIF^{WT} (black), MIF^{C60S} (red), MIF^{P2A} (blue), or MIF^{N110C} (pink), all at a 50 µM concentration. **(C)** The accumulation of misfolded SOD1 was determined by immunoprecipitation with the A5C3 antibody after incubating recombinant hSOD1^{G85R} (4 µg) with (+) or without (–) recombinant MIF^{WT}, MIF^{C60S}, MIF^{P2A} or MIF^{N110C} (all at 2 µg). One representative experiment out of 4 performed showing a decrease in misfolded SOD1 in the recombinant MIF bound samples with distinct presence of MIF^{N110C} in its bound sample.

6 MIF inhibition of mutant SOD1 misfolding is independent of its enzymatic activities or its normal oligomeric structure





The accumulation of misfolded SOD1 was determined by immunoblotting of immunoprecipitates with the A5C3 antibody after incubating recombinant hSOD1G85R (4 µg) with (+) or without (–) recombinant MIFWT, MIFC60S, MIFP2A or MIFN110C (all at 2 µg). Immunoblotting was used to determine the levels of SOD1 and MIF that remained in the unbound fraction of each immunoprecipitation assay.

Conclusion

Our findings suggest that MIF directly interacts with misfolded SOD1 to inhibit its toxic amyloid aggregation by inducing the formation of disordered aggregates with lower toxicity. These findings provide new insights regarding the potential therapeutic role of MIF in suppressing the selective accumulation of misfolded SOD1 in ALS.

Funding

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