A Disease Associated Conformation of Macrophage Migration Inhibitory Factor as a Novel Drug Target in Alzheimer's Disease

Funding: Prosetta Biosciences, Inc, USA

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Abstract

Background:
While the analysis of familial Alzheimer's disease (AD) has led to the understanding of key cellular neuropathological pathways, the origins of sporadic AD are still obscure despite many epidemiological hints to systemic risk factors such as infections, metabilic disorders etc.

Rationale:
In order to identify molecular interfaces that connect systemic disturbances with the key pathways in AD such as the amyloid beta cascade and tau hyperphosphorylation, we investigated the molecular components mediating herpes virus (HSV-1) infection which has been linked to accelerated sporadic AD. One assumption was that these molecular interfaces might also be relevant for non-viral causes of sporadic AD.

Results:
• A preselected pharmaceutical library of antiviral compounds targeting host proteins aiding in virus replication rather than the virus itself was probed for anti-HSV-1 effects, and a lead compound 7.25 identified to potently inhibit HSV-1 infection (EC_{50} = 35 nM) in human neuronal cell lines and human brain organoids.

• Using drug-affinity chromatography (DRAC), a disease-associated proinflammatory isoform of macrophage migration inhibitory factor (MIF), termed oxMIF, was identified as a target.

• NMR spectroscopy confirmed a unique and novel allosteric binding site of 7.25 between two adjacent subunits of the MIF trimer.

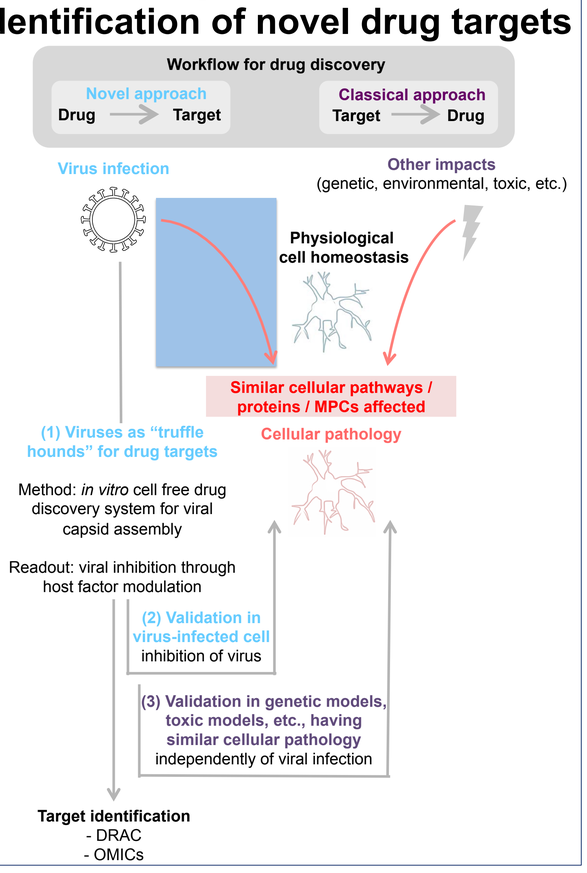
• AD patients' *post mortem* brains contained increased levels of oxMIF.

• 7.25 inhibited tau phosphorylation at critical residues both in the presence or absence of HSV-1 infection in a MIF-dependent manner.

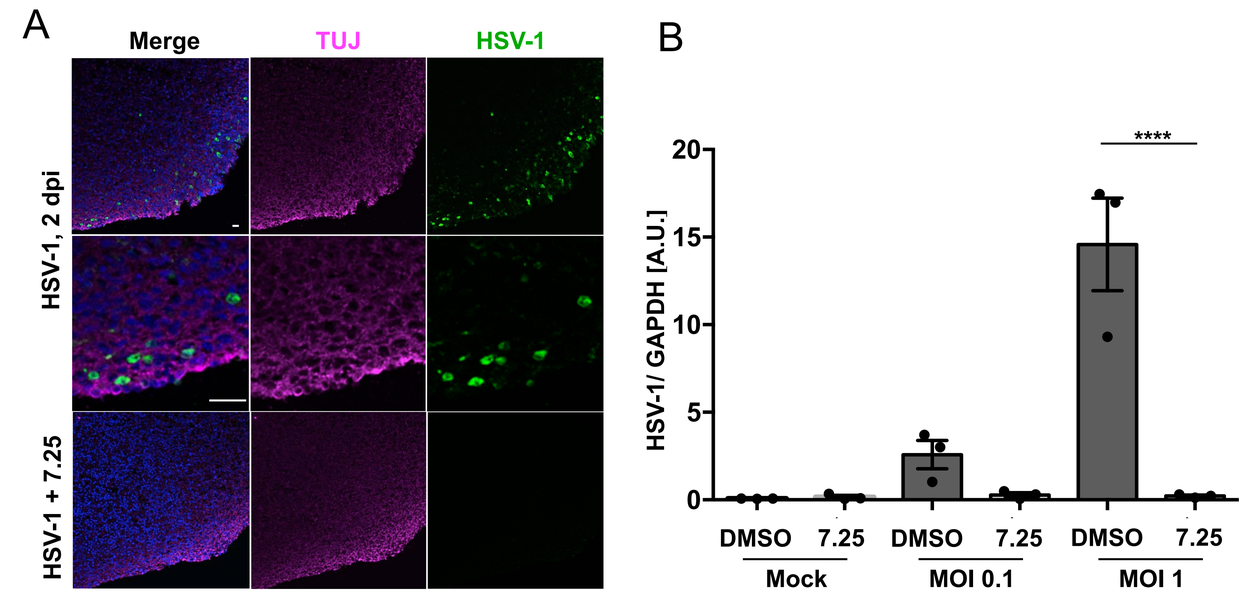
• A recombinant surrogate of oxMIF (MIF-C81W) induced tau phosphorylation.

Conclusions:
MIF is a molecular interface upstream of and able to modulate AD-related tau hypophosphorylation. Compound 7.25 targeting MIF may therefore be a novel kind of compound for treating sporadic AD.

Utilizing viruses for the identification of novel drug targets

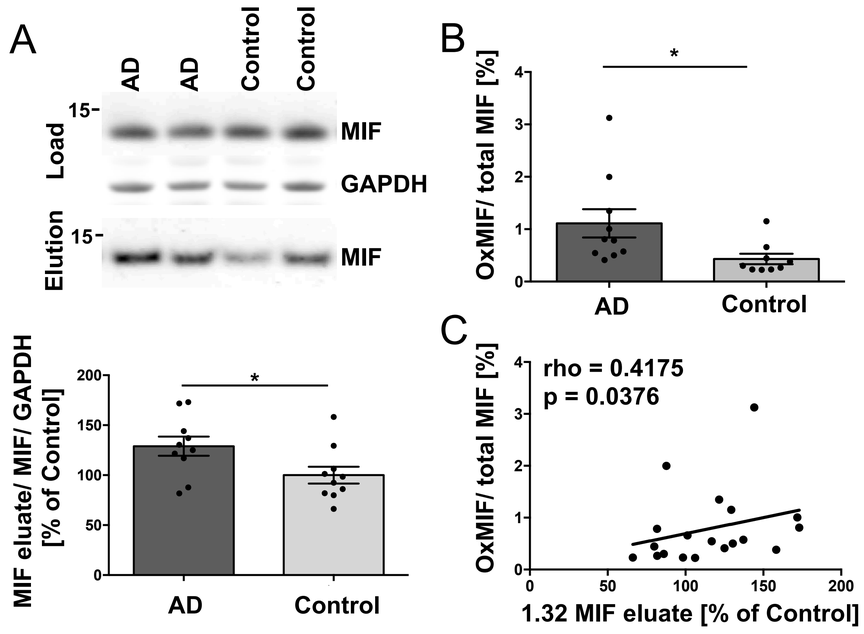


7.25 is an efficient inhibitor of HSV-1 replication in neurons



A: Staining of 60d human brain organoids that were infected with HSV-1 (MOI=1). HSV-1 (green) targeted TUJ-1-positive neurons (magenta) in the outer layer of the organoids. Infection with HSV-1 was completely abolished when 7.25 (250 nM) was present (lower panel). (bars = 30 µm)
B: Quantification of HSV-1 antigen by Western Blot (WB). The diagram represents the results from three infected organoids that were analyzed in three (n=3) independent WB.

7.25 specifically binds to a conformation of MIF in brain homogenates of AD patients that correlates with oxMIF

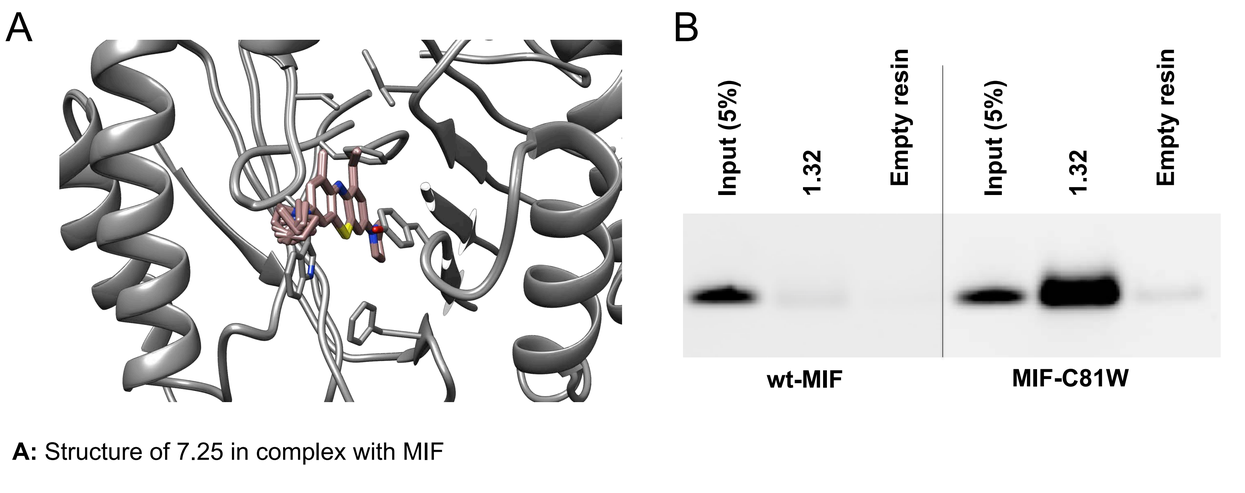


A: DRAC analysis of 20 brain samples from AD patients and age-matched healthy controls. Top: WB (representative section). Below: Significantly more MIF was precipitated from AD brain samples by compound resin and eluted with free 7.25.

B: An oxMIF specific Sandwich ELISA revealed increased levels of oxMIF in AD brain samples.

C: The oxMIF levels positively correlated with the amounts of MIF species eluted from 1.32 resin.

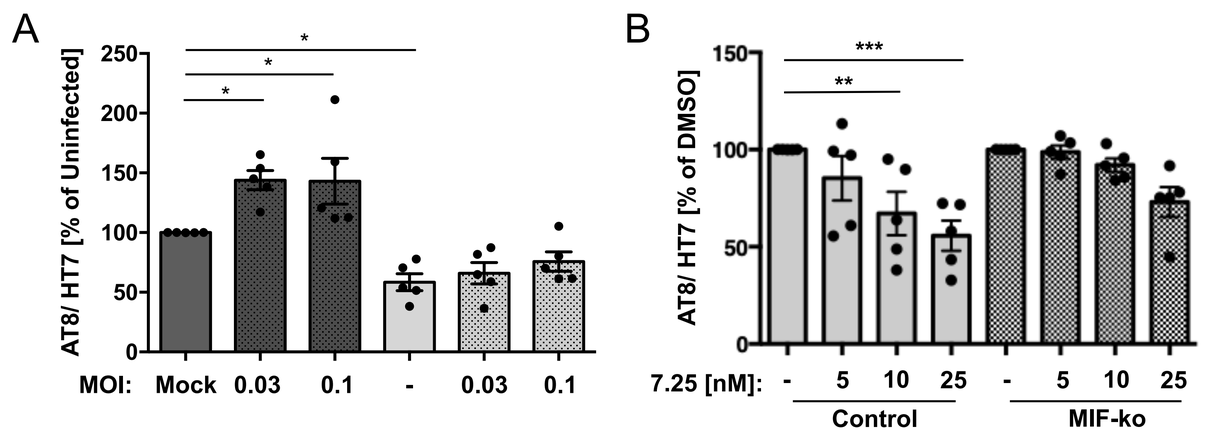
A disease associated conformation of MIF (oxMIF) was identified as a direct binding partner for 7.25



A: Structure of 7.25 in complex with MIF

B: Analysis of MIF binding by DRAC. A structural and functional analog of 7.25 (1.32) has been covalently coupled to resin, that was then incubated with recombinat human wildtype MIF (wt-MIF) or MIF-C81W, that mimicks oxMIF. Urea eluted proteins were separated by SDS-PAGE and detected by WB. 7.25 weakly bound to wt-MIF but much stronger to MIF-C81W (oxMIF).

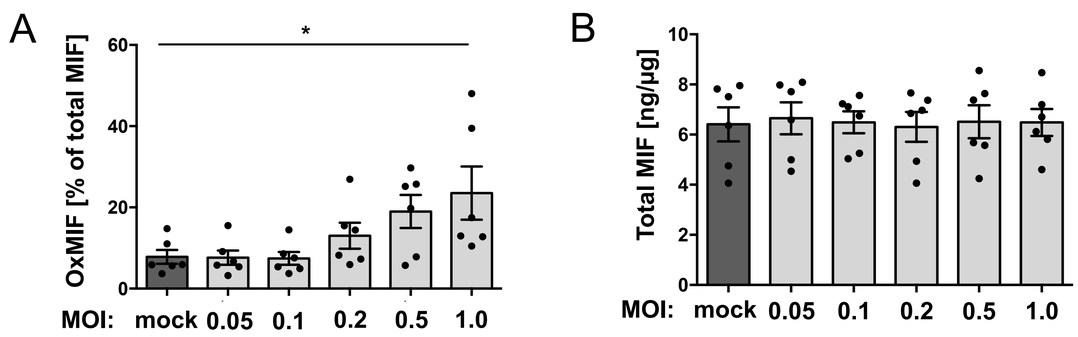
7.25 inhibits tau phosphorylation in a MIF-dependent manner



A: Infection of SH-SY5Y-tau-P301S cells with HSV-1 increased tau phosphorylation (AT8) 6h p.i. This increase was absent in the presence of 7.25 (20 nM). AT8 signals were normalized to total tau (HT7).

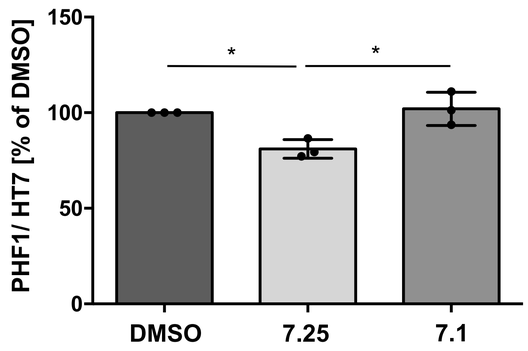
B: Tau phosphorylation (AT8) by 7.25 was reduced also in the absence of infection but the effect of 7.25 was impaired in SH-SY5Y-tau-P301S-MIF-ko cells.

OxMIF but not total MIF is induced by HSV-1 infection of neurons



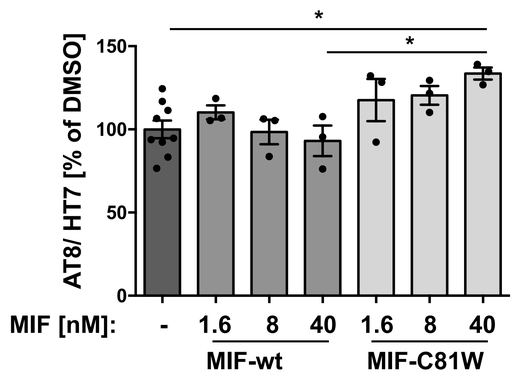
A: Induction of oxMIF in differentiated human neurons (LUHMES) upon infection with HSV-1 as measured by sandwich ELISA. The results were normalized to total MIF levels, that were not changed (**B**).

7.25 inhibits tau phosphorylation in differentiated iPSCs



7.25 (50 nM) but not a dead analog (7.1) reduced tau phosphorylation (PHF-1) in differentiated neurons derived from human iPSCs of a patient carrying the MAPT IVS10+16 mutation (V97).

MIF-C81W but not wt-MIF induces tau phosphorylation



Recombinant wt-MIF or MIF-C81W were applied to SH-SY5Y-tau-P301S cells and incubated for 6h. Only MIF-C81W significantly induced tau phosphorylation.