Formyl peptide as a potential biomarker for neurodegeneration in multiple sclerosis

Zichun Xiao[1], Yulin Li[1,2], Zhiguo Li[1,2], Nan Yao[1], Pei Zheng[2], Hans-Gustaf Ljunggren[3], Friedemann Paul[4], Luc Van Kaer[5], Alexei Verkhratsky[6], and Fu-Dong Shi[1,2,*]

[1]Department of Neurology, Tianjin Neurological Institute, Tianjin Medical University General Hospital, Tianjin, China.
[2]Center for Neurological Diseases, China National Clinical Research Canter for Neurological Disease, Beijing Tiantan Hospital, Capital Medical University, Beijing, China.
[3]Center for Infectious Medicine, Department of Medicine Huddinge, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden.
[4]Charité - Universitätsmedizin Berlin, Department of Neurology, Berlin, Germany.
[5]Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, TN, USA.
[6]Faculty of Biology, Medicine and Health, the University of Manchester, Manchester, UK.

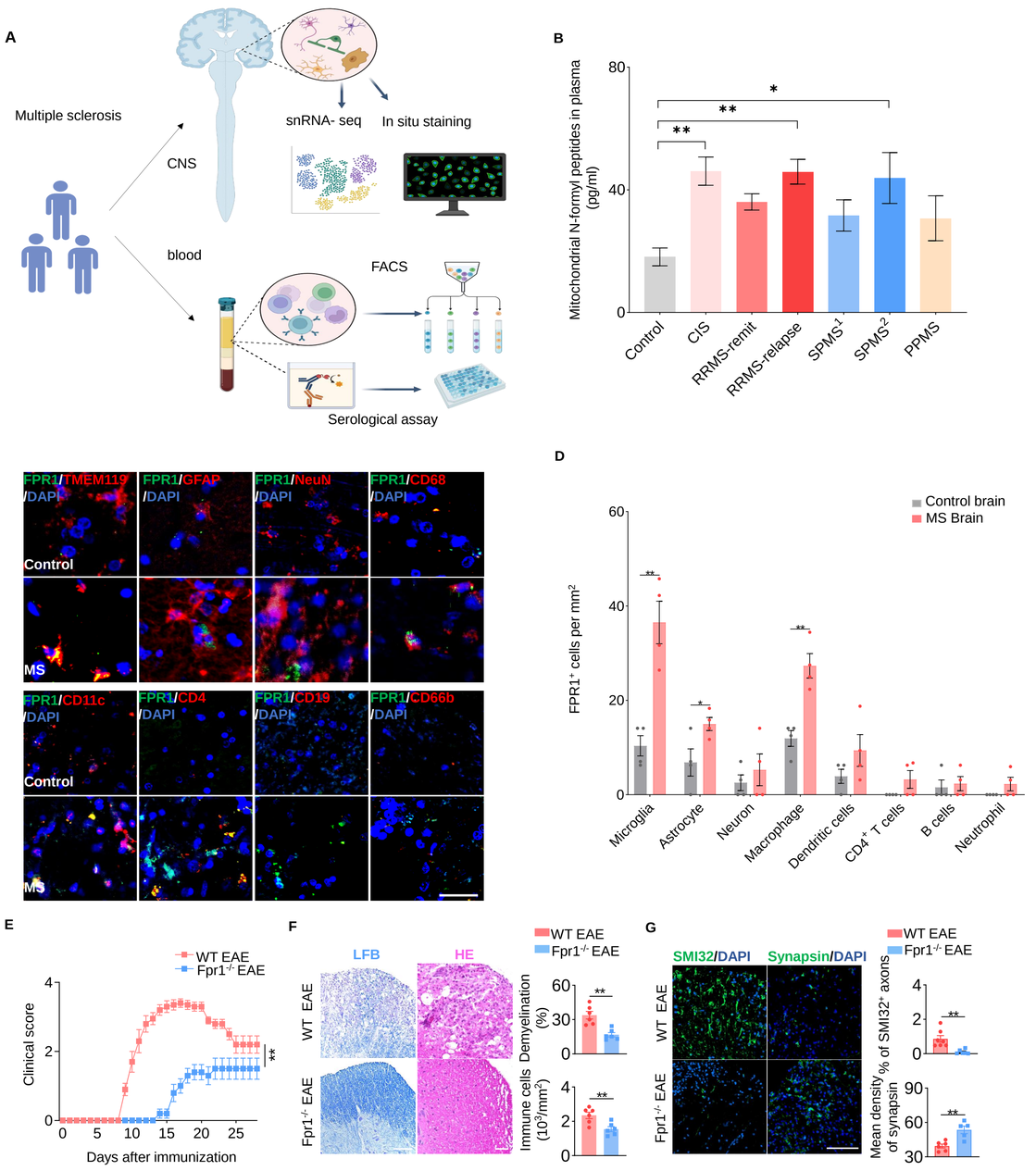
*Corresponding author: Prof. Fu-Donmail: fshi@tmu.edu.cn

Abstract

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) and a major cause of disability among young adults. Neurodegeneration is a driving force on the progression of MS, and its monitoring or treatment is an unmet need in clinic. In this study, we found that the neurodegeneration is accompanied with cell necrosis in the CNS lesion of MS patients, leading to an increased levels of necrotic cells derived mitochondrial formyl peptide (mtFP) in the peripheral blood and cerebrospinal fluid in patients. Meanwhile, the mtFP receptor-formyl peptide receptor 1 (FPR1) -is specifically upregulated in the CNS of MS, but not in parallel controls or Alzheimer's disease, another kind of neurodegeneration disease. By further assessing the potential link between mtFP/FPR1 and MS progression and neurodegeneration, we found that MS patients display significantly elevated levels of mtFP in blood both during acute attacks (n = 77, 42.68±3.64 vs. 16.05±2.4 pg/ml, P < 0.0001) and disease remission (n = 86, 38.45±2.51 vs. 16.05±2.4 pg/ml, P < 0.0001). In the experimental autoimmune encephalomyelitis (EAE) mouse model of MS, genetic elimination of FPR1 reduced the production of reactive oxygen species and the antigen presentation capacity of microglia and macrophages, attenuating neurodegeneration within the CNS. Collectively, our study has uncovered mtFP is as a potential new biomarker predicting the neurodegeneration in MS.

Result

1. The formyl peptide receptor 1 (FPR1) is upregulated in multiple sclerosis (MS) patients and its ligand is associated with MS status



2. FPR1 signaling-induced neuronal and oligodendrocyte death accelerates neurodegeneration

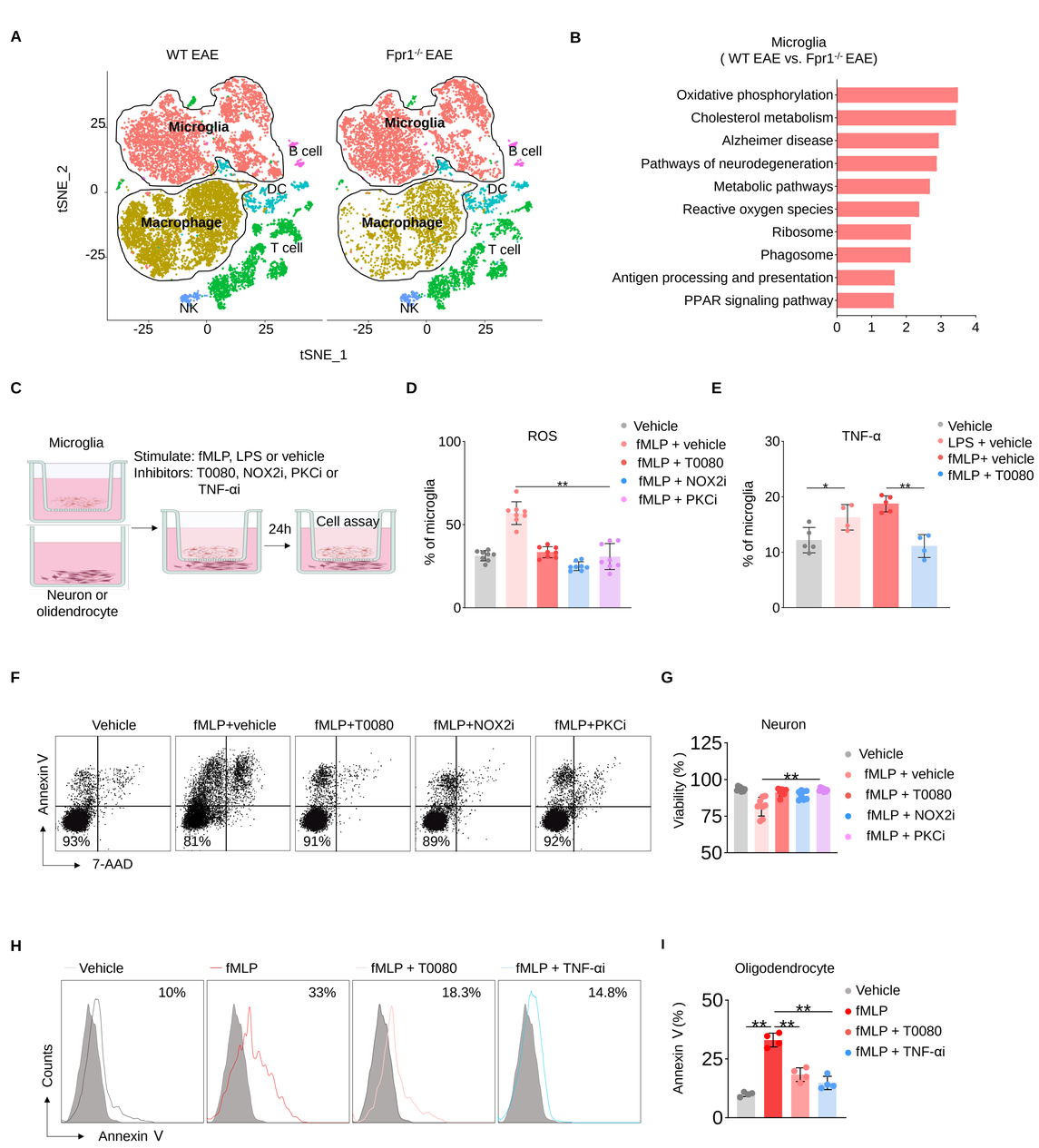


Figure 1. Probing formyl peptide receptor (FPR)1 and its ligand mitochondrial N-formyl peptide in patients with MS, and Fpr1$^{-/-}$ mice displayed attenuated EAE. (A) Schematic depiction of profiling FPR1 in blood and brain from patients with MS. **(B)** Quantification of blood mitochondrial N-formyl peptide (mtFP, FPR1 agonist) in healthy controls (n = 102), patients with clinically isolated syndrome (CIS, n = 7), relapsing-remitting MS (RRMS) with remit (n = 78), RRMS with relapse (n = 68), secondary progressive MS[1] (SPMS, n = 5), SPMS[2] (n = 6), primary progressive MS (PPMS, n = 2). Data are representative of three independent experiments. One-way ANOVA. Means ± SEM, **P < 0.01, *P<0.05. **(C)-(D)** Representative confocal images and quantification of FPR1 expression on microglia (TMEM119$^+$ cells), astrocytes (GFAP$^+$ cells), neurons (NeuN$^+$ cells), macrophages (CD68$^+$ cells), dendritic cells (CD11c$^+$ cells), CD4 T cells (CD4$^+$ cells), B cells (CD19$^+$ cells) and neutrophils (CD66b$^+$ cells) in brain tissue from healthy controls and patients with MS (n = 4/group). Data are representative of three independent experiments. Scale bar, 40 μm. **(E)** Clinical scores of WT and Fpr1-/- EAE mice (n =10 /group). Data are representative of three independent experiments. Two-way ANOVA. Means ± SEM, **P < 0.01, *P<0.05. [1]without enhanced MRI lesion, [2]with enhanced MRI lesion. **(F)** Luxol Fast blue and H&E staining of spinal cord sections from WT and Fpr1-/- mice at day 17 after EAE induction. Graphs show the percentage of demyelination and counts of infiltrated immune cells (n=6/group). Data are representative of three independent experiments. Scale bars, 100 μm. Two-tailed unpaired Student's t-test. **(G)** Immunostaining and quantification of neurofilament protein SMI 32 (green) and synapsin (green) in spinal cord sections from WT and Fpr1$^{-/-}$ mice at day 17 after EAE induction (n = 5-7/group). Data are representative of three independent experiments. Scale bar, 100 μm. Two-tailed unpaired Student's t-test.

Figure 2. FPR1-bearing microglia are neurotoxic to neural axon and oligodendrocyte cells. Using MOG35-55 induced EAE model in wild type (WT) and Fpr1 knock (Fpr1$^{-/-}$) mice. **(A)** t-SNE plot of cell clustering of the spinal cord from WT and Fpr1-/- mice at day 17 after EAE induction through scRNA-seq. Based on marker genes, cell clusters were recombined to 6 clusters, and each colour represents a cell-type cluster. Data are representative of three independent experiments. **(B)** Top 10 KEGG pathway enrichment of differentially expressed genes (DEGs) in the microglia. **(C)** Schematic diagram of mouse cell co-culture experimental design. **(D)** Quantification of reactive oxygen species (ROS) assessed by flow cytometry for culturing microglia after treatment with fMLP, T0080, NOX2 inhibitor, PKC inhibitor, or vehicle (n = 8/group). Data are representative of three independent experiments. One-way ANOVA. **(E)** The quantification of TNF-α assessed by flow cytometry for culturing microglia after treatment with fMLP, LPS, T0080 or vehicle (n = 5/group). LPS stimulation was employed as a positive control. Data are representative of three independent experiments. One-way ANOVA. **(F, G)** Gating strategy and quantification of viable neurons (Annexin V- 7-AAD-) after co-culture with microglia (C) (n = 8/group). Data are representative of three independent experiments. One-way ANOVA. **(H, I)** Gating strategy and quantification of apoptosis oligodendrocytes (Annexin V+) after co-culture with microglia (C) (n = 4/group). Data are representative of three independent experiments. One-Way ANOVA. Means ± SEM, **P < 0.01, *P<0.05.

Result

In this study, we found FPR1 upregulation by CNS-resident microglia and macrophage of MS patients, accompanied by elevated levels of circulating mtFPs, an endogenous agonist of FPR1. The level of mtFPs is associated with different stages of MS. Patients experiencing disease attacks or relapses, as well as those with disease progression and enhanced MRI lesions, have higher serum mtFP levels. Concurrently, FPR1 enhanced microglia production of cytotoxic factors such as ROS and TNF-α, contributing to neurodegeneration. Thus, our findings suggest that mtFPs are associated with disease activity and contribute to microglia activation in MS, and they could potentially serve as novel biomarkers for disease monitoring and prognosis in MS, as well as for investigating microglia-mediated neurodegeneration in the disease.