LPA1 Expression in CNS Fibroblasts During Multiple Sclerosis

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Background and Objectives

Multiple sclerosis (MS) is an inflammatory, demyelinating disease that drives neurodegeneration. Lysophosphatidic acid (LPA), a pro-inflammatory lipid elevated in MS plasma and cerebrospinal fluid (CSF), activates the LPA1 receptor in the central nervous system (CNS), inducing microglial activation and cytokine release. These processes promote demyelination and impair remyelination. Although LPA1 antagonism is a promising strategy for promoting remyelination in MS, its role in peripheral fibrosis is better established. We hypothesize that LPA1-driven CNS fibrosis contributes to remyelination failure in MS.

We aim to investigate LPA1 expression in MS fibroblasts and clarify the link between CNS fibrosis and MS pathology. We posit that reducing CNS fibrosis may enhance reparative processes, such as remyelination, in MS therapies.

Methods

Human MS tissue and CSF for FISH (fluorescent *in situ* hybridization), immunostaining, and biomarkers were obtained from the Netherlands Brain Bank. Human meningeal fibroblasts (IxCells, San Diego, CA) were cultured, activated, and immunostained. These fibroblasts were quality controlled to confirm expression of fibronectin, but are negative for GFAP, α-smooth muscle actin, and Thy-1. FISH studies were done by Amsterdam UMC. CSF biomarker studies were done by Nordic Biosciences.

Results

LPA receptors are expressed on cultured human leptomeningeal fibroblasts

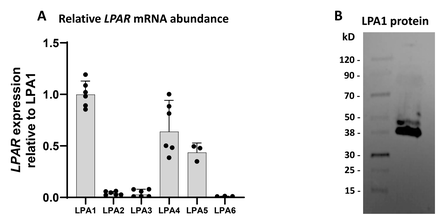


Figure 1. LPA1 is highly expressed on human brain leptomeningeal fibroblasts. A. Relative transcript expression of various *LPAR* subtypes by qPCR, B. Western blot confirmation of protein expression on meningeal fibroblasts

LPA pathway, TNFα, IFNγ, and TGFβ mRNAs are expressed on mouse meningeal fibroblasts

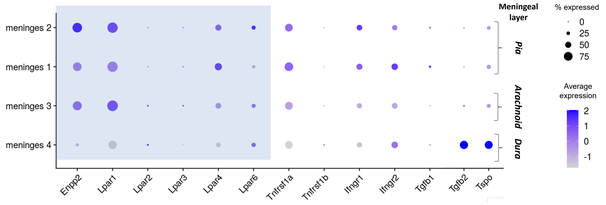
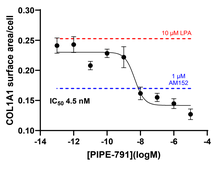


Figure 2. RNA-seq data showing expression of genes of interest in mouse meningeal layers. Autotaxin (*Enpp2*), *Lpar1*, *2*, *3*, *4*, *6*, and receptors for TNFα (*TNFRSF1a* and *b*), IFNγ (*Ifngr1* and *2*), and TGFβ (*Tgfb*1 and 2), and *TSPO*. Clusters *meninges 1* and *2* represent pia mater, *3* is arachnoid and *4*, dura. Leptomeningeal fibroblasts represent pia and arachnoid layers. *Adapted from DeSisto et al., 2020.*

LPA activates human fibroblasts and LPA1-dependent COL1A1 expression



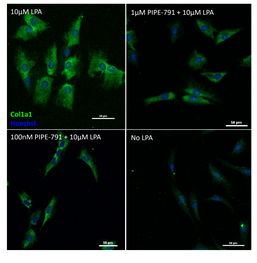
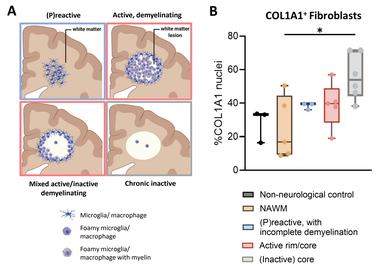


Figure 3. LPA (via LPA1) induces meningeal fibroblast activation. Primary human meningeal fibroblasts were treated with an LPA1-selective antagonist PIPE-791 (6 h) then with 10 µM LPA and immunostained for COL1A1 (green), Hoechst counterstain (blue) (error bars SEM, n=4). Poon et al., 2024.

LPA1 expressing fibroblasts and oligodendrocyte lineage cells are most prevalent around pre-active and active MS lesions



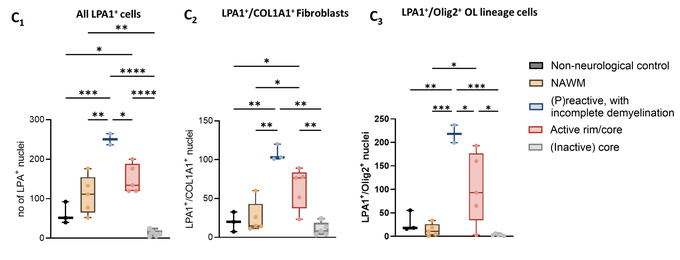
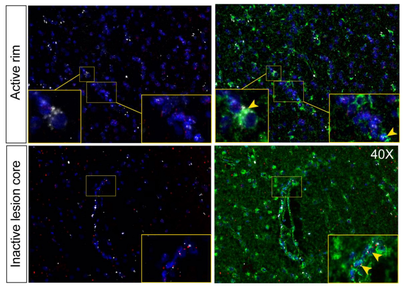


Figure 4. LPA1⁺ cells are prevalent around (P)re-active and active MS lesions. A. Diagram from Morgan et al., 2021 delineating lesion status/types; B, By RNAScope, *COL1A1*⁺ fibroblasts are present in all lesion types and most abundant in inactive lesions. C_1, Significant increase in total *LPA1*⁺ nuclei in (P)re/-active lesions with only sparse labeling in inactive lesions. C_2, In contrast to all *COL1A1*⁺ fibroblasts, *LPA1*⁺/*COL1A1*⁺ fibroblasts are prevalent in pre/-active lesions, but sparse in inactive lesions. C_3, In line with Poon et al 2024, a preponderance of *LPA1*⁺/*Olig2*⁺ cells are also found around active and scarce in inactive lesions.

LPA1⁺/*COL1A1*⁺ fibroblasts express TSPO, a marker for active inflammatory lesions



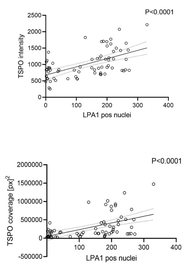
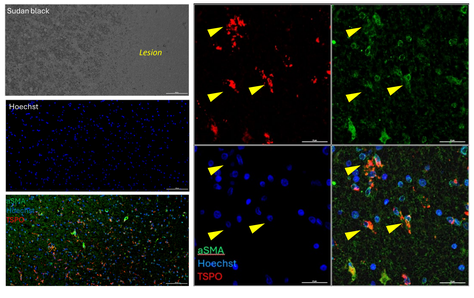


Figure 5. *LPA1* co-localizes with *COL1A1* and TSPO⁺ cells in MS tissue. Left, *LPA1* (red) and *COL1A1 (white)* were detected using RNAscope *in situ* hybridization and immunostained against TSPO (green). Cells positive for all three markers were found in both active and inactive lesions. Right, TSPO intensity (top) and area coverage (bottom) positively correlates with *LPA1*⁺ nuclei.

Activated fibroblast marker α-SMA (alpha smooth muscle actin), co-localizes with TSPO protein at lesion rim

Figure 6. Activated fibroblasts express TSPO at active lesions rims. Brain tissue containing an active lesion was immunostained with antibodies against α-SMA (a marker of activated fibroblasts) and TSPO. Sudan black and Hoechst were used as counterstains for myelin and total nuclei, respectively. Images on left, scale bar 100 µm. Magnified images (right), 25 µm.



LPA1 and TSPO are elevated and co-localize at lesion rims in MS tissue

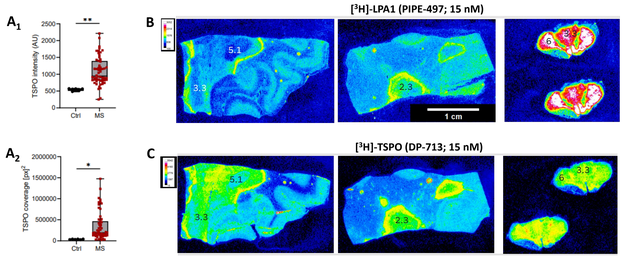


Figure 7. LPA1 and TSPO protein are elevated around active lesion rims. A_1, TSPO intensity and A_2, TSPO area were significantly higher in MS cases compared to non-neurological controls (ctrl). B, [³H]-TSPO (DP-713) and C, [³H]-LPA1 (PIPE-497) binding distribution is similar in active (2.3) and mixed active/inactive (Chronic active, 3.3) lesions. *Left and middle:* brain, *right:* spinal cord.

TSPO is induced by LPA and inhibited *in vitro* by an LPA1 selective antagonist, PIPE-791

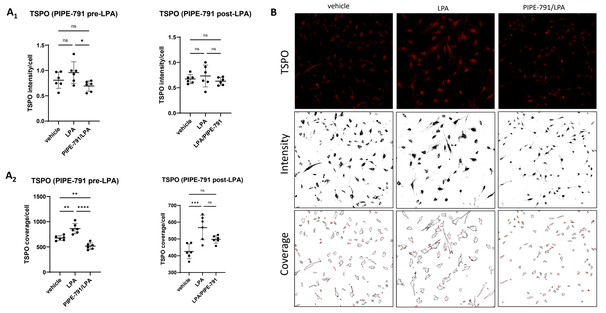


Figure 8. LPA1 mediates TSPO expression in human meningeal fibroblasts. Cultured fibroblasts were treated with 300 nM PIPE-791 before (pre-LPA) or after 10 µM LPA (post-LPA). Cells were then immunostained for TSPO and either area coverage or intensity quantified. A_1 Graphs of TSPO intensity with PIPE-791 (300 nM) added 24 h prior to LPA (10 µM) addition for 24 h, or LPA for 24 h followed by addition of PIPE-791 for 24 h. A_2, Graphs of TSPO area coverage. B, Representative images of TSPO, and images thresholded for intensity and coverage (* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001, n=6, 1-way ANOVA).

α-SMA and TSPO expression are induced by LPA, TNFα/IFNγ, and TGFβ

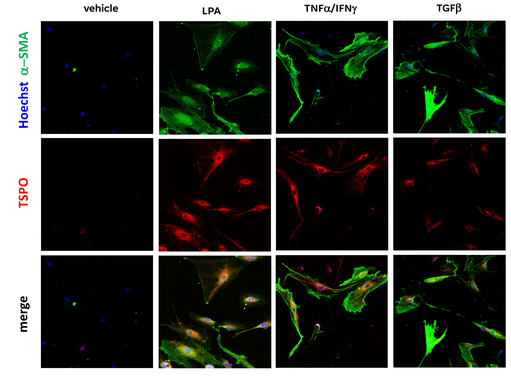


Figure 9. TSPO is induced by TNFα/IFNγ, TGFβ as well as LPA. Fibroblasts were cultured and treated for 24 h with insults indicated above, then immunostained for the activated fibroblast marker α-SMA (green) and TSPO (red); counterstained with Hoechst (blue).

LPA1 expression is increased by direct addition of LPA or TNFα/IFNγ

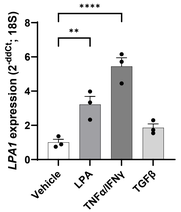
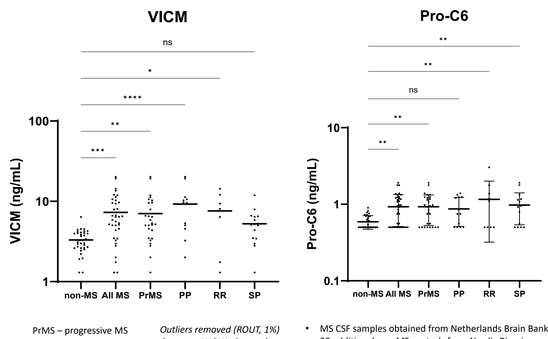


Figure 10. *LPAR1* expression increases in response to direct application of LPA, TNFα/IFNγ *in vitro*. Cultured human meningeal fibroblasts were treated for 24 h with 10 µM LPA, TNFα/IFNγ (10 ng/mL; 10 ng/mL), or TGFβ (20ng/mL). Cells were harvested and RNA isolated for qPCR analysis (** p < 0.01, **** p< 0.0001, 1-way ANOVA, error bars SEM, n=3)

VICM and Pro-C6 are potential MS-CSF biomarkers for fibrosis



PrMS – progressive MS
PP – primary PrMS
RR – relapse/remitting MS
SP – Secondary PrMS

Outliers removed (ROUT, 1%)
One way ANOVA, Dunnet's
(+) mean average
(__) median average

- MS CSF samples obtained from Netherlands Brain Bank
- 30 additional non-MS controls from Nordic Biosciences
- 14 primary PrMS, 9, RRMS, 17 secondary PrMS (PIRA), 39 non-MS controls

Figure 11. Citrullinated vimentin and pro-collagen C6 are significantly elevated in MS CSF. MS samples were subcategorized as PrMS: progressive MS and PP: primary progressive MS, RR: relapsing/remitting MS, and SP: secondary progressive MS. MS CSF samples were obtained from the Netherlands Brain Bank, with 30 additional non-MS controls from Nordic Biosciences. In total 14 PrMS, 9, RRMS and 17 PIRA with 39 non-MS controls were submitted to Nordic Biosciences for analysis.

Conclusions

- Antagonism of the LPA1 receptor has been well-characterized as an inhibitor of fibroblast activation outside the CNS
- LPA1 expression is high in pre-active and active lesion rims, lower in chronic inactive lesions, and are comprised of *COL1A1*⁺ fibroblasts and *OLIG2*⁺ oligodendrocyte-lineage cells
- TSPO, generally thought of as a marker of activated microglia, is high at the pre/-active lesion rim, but also co-localizes with LPA1 and activated fibroblasts (*COL1A1* and α–SMA).
- In addition to LPA, TNFα/IFNγ (two cytokines elevated in MS), and TGFβ (a well-known fibroblast activator) can also induce TSPO in meningeal fibroblasts
- Fibrosis biomarkers, VICM (citrullinated vimentin) and Pro-C6 (collagen VI), were higher in MS donor CSF samples compared to non-MS donors, and may serve as putative biomarkers of MS fibrosis.

Acknowledgements

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