



Examination of Ligand-Receptor Interactions amongst Different Multiple Sclerosis Lesion Types

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Abstract

Multiple sclerosis represents a debilitating disease. It has many different forms and warrants further investigation. The purpose of this research paper is to look at the available RNA sequencing for white matter lesion disorders in the brain. We performed a comprehensive systematic review to correlate RNA sequence to white matter lesion accuracy. In particular, we looked at ligand receptor scoring. The results are highlighted in a series of tables showing key significant findings. The paper will serve as a catalyst for further scientific development.

Keywords: White matter lesions; RNA sequencing; Ligand binding; Clinical improvements

Introduction

Multiple sclerosis (MS) represents a disabling autoimmune disease of the central nervous system characterized by predominantly white matter lesions, or distinct areas of myelin loss and axonal injury [1]. MS is distinguished from neurodegenerative mimics by primary demyelination (with oligodendrocyte loss) and marked perivascular infiltration [2,3]. At the time of writing, best estimates suggest that north of 2.5 million persons worldwide (400,000+ in United States alone) live with active MS and prevalence is on the rise [4,5]. MS disproportionately affects females (3:1) and individuals of African-American and European descent [4,6]. Owing to its heterogeneous presentation, MS is clinically divided into multiple disease phenotypes (e.g., relapsing-remitting disease and progressive disease) and lesion types (e.g., active and chronic lesions), both of which are becoming increasingly subdivided [7–10]. Approximately 85-90% of initial MS diagnoses are relapsing-remitting MS, which presents clinically as periods of neurological deficit bridged by stretches of remission [11].

Interestingly, however, a smattering of evidence suggests that MS disability accumulation is an age-dependent process, independent of disease phenotype at initial diagnosis (and related relapses) [12,13]. Novel investigation of the variable MS lesion environment therefore represents a worthwhile pursuit.

The hallmark lesioning contributes to variable patterns of gliosis and inflammation [14]. Available science recommends that non-mixed MS lesions be classified as follows (note: additional lesion types have been proposed, but these are beyond the focus of the present study). Active type lesions host significant immune cell infiltrates that have moved into the lesion from the blood [15]. Active lesions are characterized by a wealth of T- and B-cells, macrophages, and microglia permeating the entire lesion area [10]. Chronic active type lesions are slow-growing which evidence greater accumulation of peripheral microglia and/or macrophages (compared to active type lesions), consistent with greater differentially expressed gene counts [9,16]. Inactive type lesions observe complete loss of oligodendrocytes and a hypocellular lesion center which limits macrophage and microglia populations to the lesion edge, attributing to negligible counts of

each [10,15]. Normal-appearing white matter (NAWM) type lesions are MRI-based lesions characterized by their comparative indistinguishability in MRI imaging from normal white matter tissue, save early reduction in T1/T2 ratios of specific tissues [17,18]. Remyelinating type lesions are a distinct active lesion subtype featuring thin myelin sheaths, shorter internodal lengths, and oligodendrocyte recruitment [19]. The deleterious action of specific ligand-receptor pairs in the context of MS pathophysiology coupled with its described complexity supports lesion type-centric study of ligand-receptor interaction [20,21]. Advances in spatial transcriptomic and single-cell technologies have allowed for high resolution of the molecular environment of numerous malignancies by understanding gene expression at the lesion level [22–24]. However, less attention has been focused on multiple sclerosis. Although bulk transcriptomics may not provide the resolution of single-cell sequencing, this data can still provide valuable insight into interactions in the lesion microenvironment. Bulk lesions are composed of infiltrating immune and stromal components and this characteristic is reflected in resulting gene expression data [25–28]. The present study uses a novel computational technique that leverages this property to deconvolute the bulk gene expression data into theoretical stromal and lesion compartments and estimates the relative strength of ligand-receptor interactions within the multiple sclerosis lesion types. Since it is now known that the microenvironment may play a critical role in pathogenesis, it is crucial to elucidate any interactions amongst these stromal and lesion compartments to guide future experiments and improve treatment options. Therefore, the present *in silico* exploratory study nominated novel and testable ligand-receptor interactions between stromal and lesion compartments underlying different multiple sclerosis molecular subtypes using pre-existing bulk gene expression data.

Methods

Data acquisition

RNA-sequencing data for different lesion types in brain white matter in patients with progressive multiple sclerosis were used for gene expression deconvolution [16]. The lesion types examined included chronic active (n = 17), active (n = 16), inactive (n= 14), NAWM (n= 21), and remyelinating lesions (n= 5). White matter (n= 25) was used as normal controls. The corresponding raw count matrix was retrieved from Gene Expression Omnibus GSE138614. A total of 98 samples that had complete data for RNA-sequencing were used. Raw count data was converted to TPM space before downstream analyses.

Lesion purity estimation

The ESTIMATE algorithm was used to estimate lesion purity (defined broadly as the proportion of lesion cells in the tissue sample). The ESTIMATE formula and pipeline uses known mRNA expression signatures of stromal and immune cells to infer lesion purity (Supplementary Figure 1) [29].

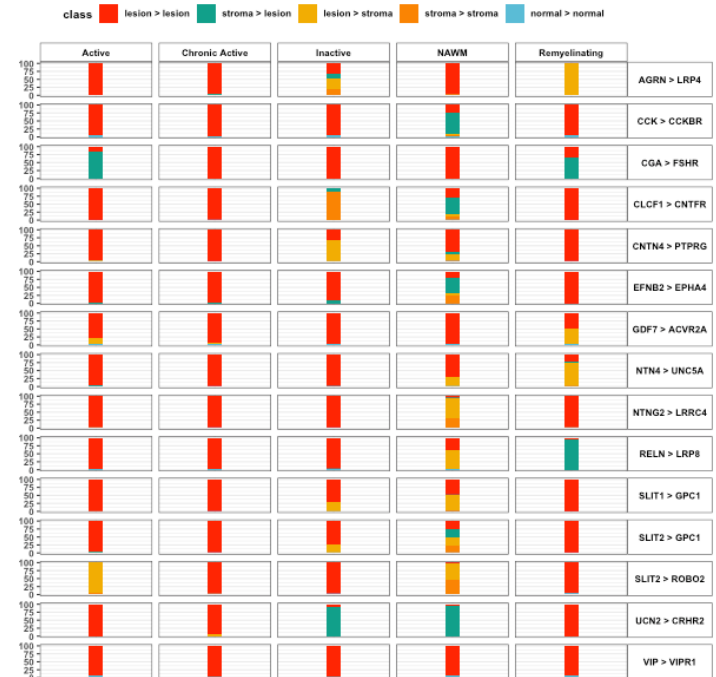


Figure 1: Top 15 ligand-receptor pairs (ligand > receptor) with highest median relative crosstalk scores for lesion-to-lesion (lesion > lesion) compartment interactions amongst the subtypes.

Lesion and stroma gene expression deconvolution

The total mRNA expression $e_{bulk,s}$ for a specific gene in a bulk lesion sample s can be modeled as follows where p_s represents the estimated lesion purity in sample s , \bar{e}_{lesion} represents the mean expression for the gene in the lesion compartment and \bar{e}_{stroma} denotes the mean expression for the gene in the stroma compartment [30]:

$$e_{bulk,s} = p_s \bar{e}_{lesion} + (1 - p_s) \bar{e}_{stroma}$$

Therefore, the stroma and lesion compartment expression levels were estimated using non-negative least-squares regression, assuming that these expression levels are constant across the lesion samples. Bootstrapping was used to derive 95% confidence intervals for the lesion and stromal point estimates. TPM RNA-sequencing data was \log_2 transformed before regression.

Ligand-receptor Interaction scoring

A combined database of 1380 ligand-receptor pairs, previously curated by Ramilowski et al. and Ghoshdastider et al. were used to annotate the inferred compartmental gene expression output [30,31].

To quantify the ligand-receptor interactions from the deconvolved data, the ligand-receptor crosstalk (RC) metric was used [31]. Thus, the molar concentration of ligand-receptor [LR] interaction complexes in equilibrium can be modeled with:

$$[LR] = [L][R]\kappa_D^{-1}$$

where [L] and [R] represent the molar concentrations of individual ligand L and receptor R, respectively, along with the dissociation constant κ_D^{-1} . As molar concentrations were not available, mRNA expression levels were treated as reasonable proxies. Thus, if the following conditions are assumed: (1) the inferred mRNA expression values are reasonable proxies for the ligand and receptor concentrations (2) ligand-receptor kinetics are constant across all samples (3) assumptions of Law of Mass Action are met, then the following Relative Crosstalk (RC) score can be applied (example given below for specific lesion-lesion ligand-receptor interaction):

$$RC_{T,T} = \frac{\bar{e}_{L,T}\bar{e}_{R,T}\kappa_D^{-1}}{\bar{e}_{L,T}\bar{e}_{R,T}\kappa_D^{-1} + \bar{e}_{L,T}\bar{e}_{R,S}\kappa_D^{-1} + \bar{e}_{L,S}\bar{e}_{R,T}\kappa_D^{-1} + \bar{e}_{L,S}\bar{e}_{R,S}\kappa_D^{-1} + \bar{e}_{L,N}\bar{e}_{R,N}\kappa_D^{-1}}$$

$RC_{T,T}$: relative crosstalk score for lesion-lesion ligand-receptor interaction

$\bar{e}_{L,T}\bar{e}_{R,T}$: interaction of lesion ligand and lesion receptor

$\bar{e}_{L,T}\bar{e}_{R,S}$: interaction of lesion ligand and stromal receptor

$\bar{e}_{L,S}\bar{e}_{R,S}$: interaction of stromal ligand and stromal receptor

$\bar{e}_{L,N}\bar{e}_{R,N}$: interaction of matched normal ligand and matched normal receptor

where the numerator represents the ligand receptor complex of interest and the denominator represents all possible directions of ligand-receptor interactions. This simplifies to:

$$= \frac{(\bar{e}_{L,T}\bar{e}_{R,T})\kappa_D^{-1}}{(\bar{e}_{L,T}\bar{e}_{R,T} + \bar{e}_{L,T}\bar{e}_{R,S} + \bar{e}_{L,S}\bar{e}_{R,T} + \bar{e}_{L,S}\bar{e}_{R,S} + \bar{e}_{L,N}\bar{e}_{R,N})\kappa_D^{-1}}$$

and since the dissociation constant is cancelled, it does not need to be accounted for in the downstream analysis. Therefore, the relative crosstalk score for an example lesion ligand and lesion receptor interaction can be modeled as follows:

$$RC_{T,T} = \frac{\bar{e}_{L,T}\bar{e}_{R,T}}{\bar{e}_{L,T}\bar{e}_{R,T} + \bar{e}_{L,T}\bar{e}_{R,S} + \bar{e}_{L,S}\bar{e}_{R,T} + \bar{e}_{L,S}\bar{e}_{R,S} + \bar{e}_{L,N}\bar{e}_{R,N}}$$

In summary, for a given ligand-receptor pair, this score estimates the relative changes of the unidirectional ligand-receptor binding complex between the compartments of interest compared to all other possible directions between the two compartments. Also, it accounts for interactions in matched normal white matter control tissue.

Results

The top-scoring ligand-receptor interactions across all lesion subtypes for each signaling direction were analyzed (Figures 1-5). For lesion-to-lesion signaling, active and chronic active lesion

types were enriched for AGRN interacting with LRP4 and CLCF1 interacting with CNTFR, among several others (Figure 1). Chronic active lesion types evidenced consistently strong lesion-to-lesion signaling in the set of top-scoring ligand-receptor pairs. Further, the VIP-VIPR1 ligand-receptor pair reported high median relative crosstalk scores for lesion-to-lesion signaling directionality across all lesion types. Unique results include stroma-to-lesion signaling between UCN2 and CRHR2 in inactive and NAWM lesion types and the reliance of CGA on stroma-to-lesion signaling to interact with FSHR in active and remyelinating lesions.

Ligand-receptor pairs with preference for stroma-to-lesion signaling in multiple lesion types include AGT-MAS1, HGF-MET, INHBB-ACVR2A, LTF-LRP11, TNFSF15-TNFRSF25, WNT5A-MCAM, and WNT5A-ROR1 (Figure 2).

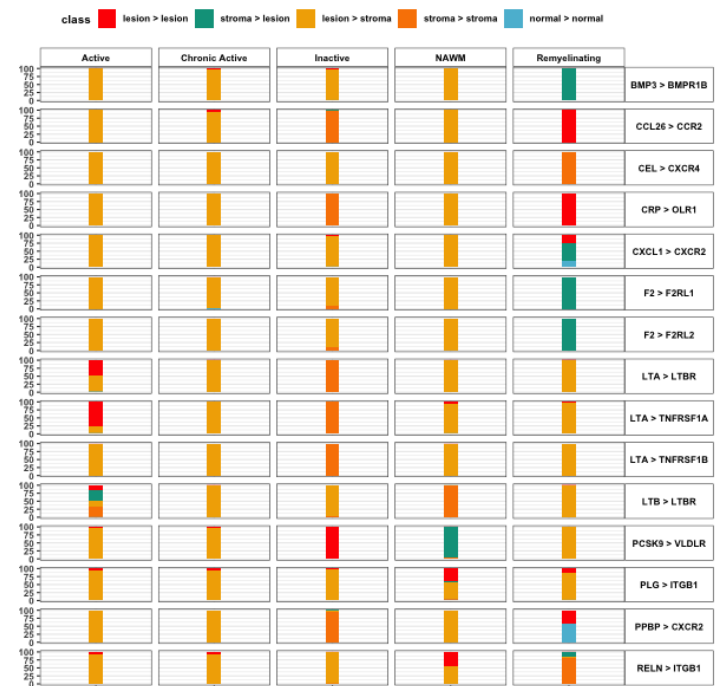


Figure 2: Top 15 ligand-receptor pairs (ligand > receptor) with highest median relative crosstalk scores for stroma-to-lesion (stroma > lesion) compartment interactions amongst the subtypes.

Ligands EFNA1, INHBB, and WNT5A are implicated in 9 of the 15 top-scoring ligand-receptor interactions, indicating their importance in stroma-to-lesion signaling. In chronic active lesions, the set of top-scoring ligand-receptor pairs again reported consistently high relative crosstalk scores for stromal ligand and lesion receptor interactions. Active and remyelinating lesions were uniquely enriched for PROK2 interacting with PROKR1 via normal-to-normal and stroma-to-stroma signal directionality.

Analysis of the top 15 ligand-receptor pairs by highest median relative crosstalk score for lesion-stroma compartment interactions highlighted cytokine ligands (namely LTA)

chemokine receptors (namely CXCR2) (Figure 3). Chronic active lesions displayed consistently relative preference for lesion-stroma signaling, which was not identified in other lesion types. Unique to the selected ligand-receptor pair set, PPBP predominantly interacted with CXCR2 via matched normal-to-normal ligand-receptor signaling. Remyelinating lesions reported distinct enrichment for stromal F2 and BMP3 ligand binding action with lesion receptors.

interacting with white matter PROKR1. Only remyelinating lesions enriched the ARTN-RET interaction for stroma-to-stroma signaling.

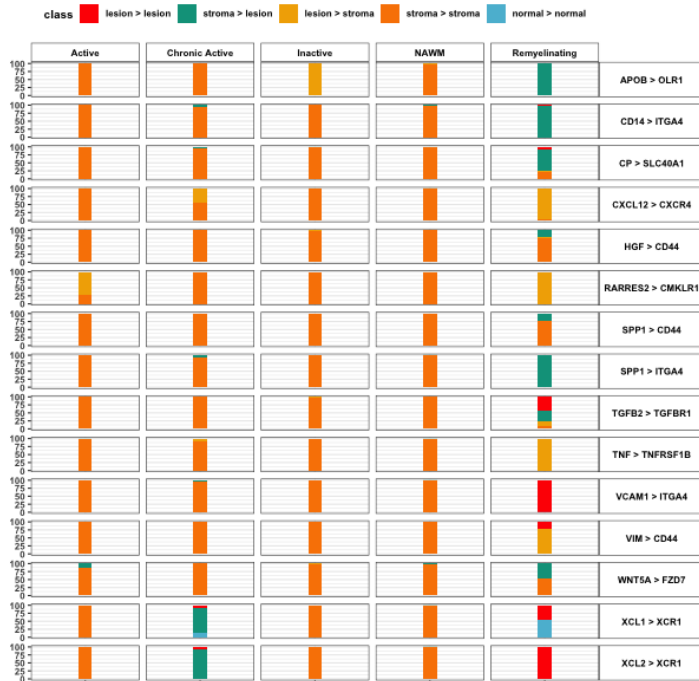


Figure 3: Top 15 ligand-receptor pairs (ligand > receptor) with highest median relative crosstalk scores for lesion-to-stroma (lesion > stroma) compartment interactions amongst the subtypes.

Interaction directionality between top-scoring stromal ligands and stromal receptor pairs were similarly decisive (Figure 4). NAWM lesions showed uniquely predominant enrichment for the entire set of stromal ligand-receptor pairs. Remyelinating lesions enriched only ligand-receptor pairs SPP1-CD44, WNT5A-FZD7, and HGF-CD44 for stroma-to-stroma signaling. CD44 stromal receptors were involved in 3 of the 15 top-scoring ligand-receptor interactions. Interestingly, CD44 stromal receptors interacted primarily with lesion ligands in remyelinating lesions, despite high interaction preference for stromal ligands in all other lesion types.

Few multiple sclerosis lesion types showed preferential enrichment for ligand-receptor pairs involved in matched normal-to-normal tissue signaling (Figure 5). Of the selected ligand-receptor pairs, active and chronic active lesions dedicated 73% (11/15) of compartment interactions to strictly lesion-to-lesion and normal-to-normal signaling. Inactive, NAWM, and remyelinating lesions were enriched for white matter PROK1

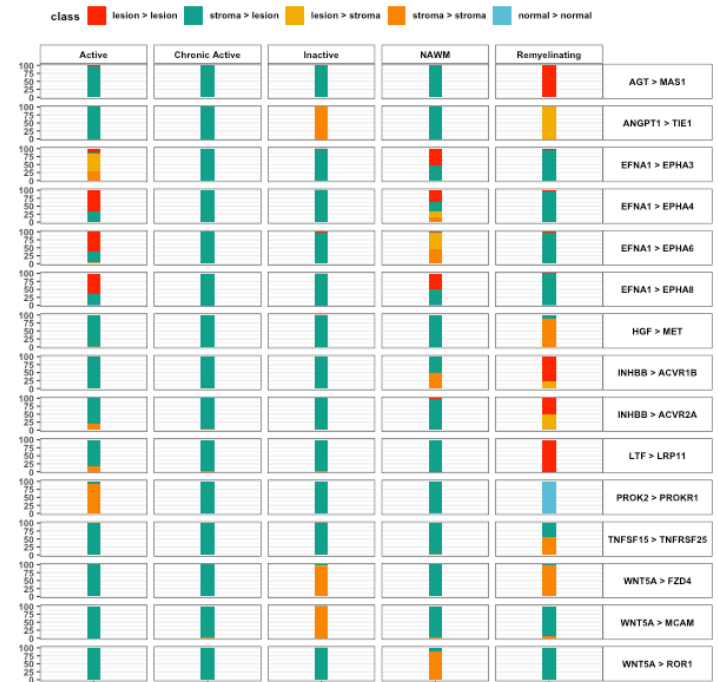


Figure 4: Top 15 ligand-receptor pairs (ligand > receptor) with highest median relative crosstalk scores for stroma-to-stroma (stroma > stroma) compartment interactions amongst the subtypes.

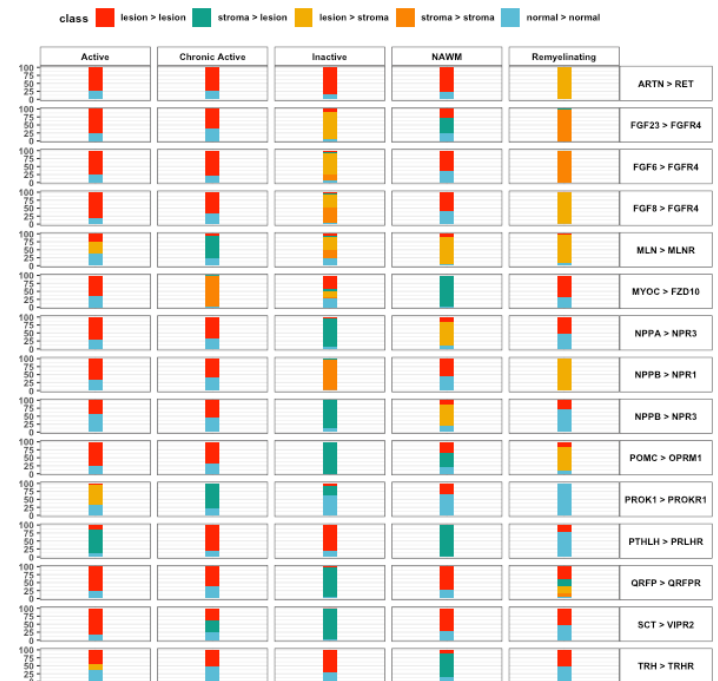


Figure 5: Top 15 ligand-receptor pairs (ligand > receptor) with highest median relative crosstalk scores for normal-to-normal (normal > normal) compartment interactions amongst the subtypes.

Discussion

For each signaling direction, interesting ligand-receptor interactions were identified within the interactions with the most crosstalk across lesion subtypes. Of the 15 top-scoring ligand-receptor interactions for lesion-to-lesion signaling, 3 involved SLIT1 or SLIT2 (Figure 1). SLIT proteins are known predominantly for their interactions with Robo receptors however there is growing evidence of SLIT proteins engaging in interactions related to tumor cell migration and metastasis as well as inflammatory cell and leukocyte chemotaxis [32,33]. This has potential implications in multiple sclerosis as the inflammatory disease is often defined by the axonal damage, demyelination, and inflammation occurring as a result of leukocyte infiltration [34].

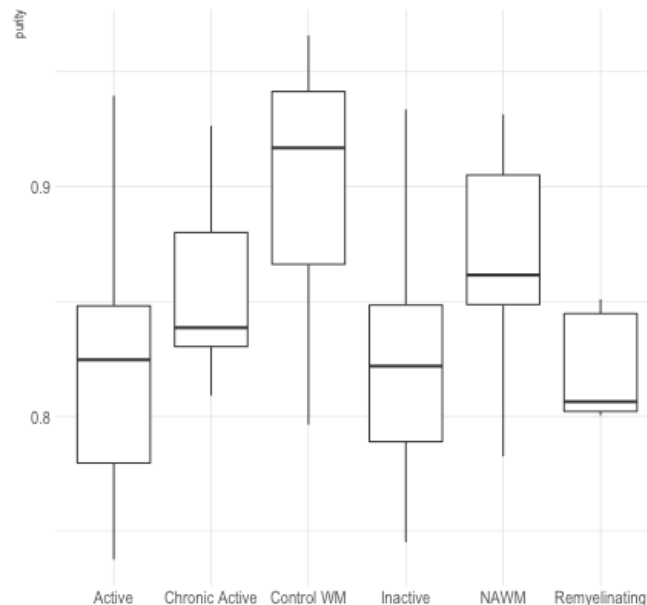
In stroma-to-lesion signaling, 3 out of the 15 top-scoring interactions between ligands and receptors involved WNT5A (Figure 2). WNT5A is of particular interest as it has been shown to have significant signaling alterations in amyotrophic lateral sclerosis (ALS) patients [35]. Furthermore, there is evidence that WNT5A is upregulated in the spinal cord dorsal horn of mice with experimental autoimmune encephalomyelitis (EAE) [36]. EAE is widely used to study neurological complications related to multiple sclerosis such as demyelination and motor impairments [36]. By better understanding the role of WNT5A in such neurological complications, treatment of these complications can be further researched.

For lesion-to-stroma signaling, 4 out of the top-scoring 15 ligand-receptor interactions involve either LTA or LTB (Figure 3). Both cytokine proteins are known for tumor proliferation regulation and immune regulations that could have serious implications for therapeutic treatment of multiple sclerosis [37]. Furthermore, studies show LTB as a considerable option for cancer therapies due to its abilities to regulate apoptosis and immune responses which could also be applied to multiple sclerosis [38].

Interestingly, 2 out of the 15 top-scoring ligand-receptor interactions for stroma-to-stroma signaling involve the SPP1 protein (Figure 4). Of note, osteopontin (SPP1) has been linked heavily to multiple sclerosis remissions and relapse in about two thirds of multiple sclerosis patients [39]. Osteopontin functions as a binding partner to the integrin primarily responsible for attracting lymphocytes to the brain causing multiple sclerosis. By inhibiting apoptosis of the integrin, osteopontin also has serious implications in multiple sclerosis. Interestingly, studies injecting osteopontin into EAE mice (used as a model for multiple sclerosis) causes relapse [39]. More research is warranted exploring the prominence of SPP1 in stroma-to-stroma signaling.

Finally, in normal-to-normal signaling, 3 out of 15 top-scoring ligand-receptor interactions involve fibroblast growth factor (FGF) signaling (Figure 5). FGF signaling has been shown to possibly regulate inflammation and myelination in multiple

sclerosis and is being researched further as a potential therapeutic option for induced remyelination and decreasing inflammation in EAE as well as multiple sclerosis [40] (Figure 6).



The prominence of FGF signaling in top-scoring normal-to-normal signaling further highlights the possibility of such a treatment option. If the mechanism of inflammation and myelination regulation from FGF as well as its relation to multiple sclerosis can be better understood, advances can be made in the treatment of the disease.

Conclusion

The above-described findings provide support for the heterogeneity of the MS plaque environment. Here we leveraged the composition properties of bulk lesions to estimate the relative strength of 1380 databased ligand-receptor interactions, using mean gene expression as a proxy for concentration. Our analysis theorized distinct lesion and stroma compartments to base ligand-receptor signaling directionality amongst a 98-sample set of active, chronic active, NWAM, and remyelinating lesions with matched WM controls. Relative crosstalk scoring of each compartment revealed highly variable signaling directionality between tested lesion-receptor pairs and between MS lesion types. Notable results include: the existence of 10-plus ligand-receptor pairs dedicated (~100%) for 4 of the 5 tested signaling paths in chronic active lesions; the unshared enrichment of VIP interacting with VIPR1 for lesion-to-lesion signaling in all lesion types; the importance of EFN family A ligands and Eph receptors in mediating stroma-to-lesion signaling in chronic active, inactive, and remyelinating MS lesions. The authors hope the present study serves to inspire future exploration of ligand-receptor interaction in the context of spatially heterogeneous disease environments.

Conflicts of Interest

The authors declare no conflicts of interest.

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