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Prefrontal cortex transcriptomic deconvolution implicates monocyte infiltration in Parkinson's disease

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Abstract

Introduction: Although not considered a primary cause, neuroinflammation is associated with many neurodegenerative disorders, including Parkinson's disease (PD).

Methods: To elucidate potential immune involvement in PD, the present study imputed immune cell abundances from bulk RNA-sequencing transcriptomic data of PD postmortem prefrontal cortices. CIBERSORTx, an RNA deconvolution algorithm that implements support vector regression, was used to measure the relative abundances of immune cells from a previously published gene expression dataset. Through this machine learning approach, relative proportions of 22 immune cell subtypes present in the original brain tissue were estimated.

Results: Prefrontal cortices from PD patients exhibited significantly higher relative abundances of monocytes compared to neuropathologically normal controls (p-value = 0.0005). The relative proportions of the other 21 immune subtypes showed no significant differences between control and PD samples.

Conclusion/Discussion: The findings corroborate previous reports and suggest monocytes may be involved in PD pathogenesis.

Keywords

neuroinflammation; Parkinson's disease; monocytes; transcriptome

Introduction

Parkinson's disease (PD) is distinguished by dopaminergic denervation of the striatum along with death of dopaminergic neurons within the substantia nigra *pars compacta* [1].

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Author Contributions

SB formulated design and analyzed data. Results were interpreted by SB. Manuscript was written and edited by SB. All authors read and approved final manuscript

Statement of Ethics

The data used in the present article had been generated through a previous study that was designated exempt (Protocol # H-28974) by the Boston University School of Medicine Institutional Review Board as no human participants were studied and all data derived from postmortem specimens. Since the data is publicly available, approval to conduct the present study by an institutional review board was not required.

Disclosure Statement

The authors have no conflicts of interest to declare.

Accumulating evidence suggests involvement of infiltrating immune cells PD and the observed neurodegeneration [2–6]. In order to elucidate potential immune cell subtypes involved in PD, the present study leveraged an unbiased computational approach to investigate the immunological composition of prefrontal cortices in patients with PD.

Materials and Methods

CIBERSORTx, an established *in silico* RNA deconvolution approach, was used to provide an estimation of the relative abundances of immune cells from a previously published gene expression dataset [7]. The dataset consists of postmortem prefrontal cortex gene expression data for 29 PD patients and 49 neuropathological controls [8]. The samples were sequenced using Illumina HiSeq2000 and the raw read counts were downloaded from the repository stated in the original publication [9]. The read counts were gene length normalized in TPM (transcripts per million) space prior to analysis with CIBERSORTx.

CIBERSORTx implements support vector regression, a machine learning approach that improves deconvolution ability through feature selection and allows for resolution of closely related subsets [7]. Furthermore, the algorithm accurately quantifies the relative fractions of distinct cell types within a complex gene expression mixture given *a priori* cell type-specific gene signatures. Specifically, the algorithm requires (1) a matrix of bulk tissue gene expression profiles to be deconvoluted and (2) a signature matrix containing signature gene expression profiles for cell subsets of interest. To estimate the proportion of a specific cell type, the algorithm assesses the relative expression changes of signature genes related to the cell type of interest compared with the expression of all other genes in the sample [7].

For the present study, a validated microarray-derived signature matrix containing 547 genes for distinguishing 22 human hematopoietic cell subsets, termed ‘LM22’, was used to deconvolute the bulk tissue gene mixture matrix [7]. The CIBERSORTx algorithm was run with bulk-mode batch correction without quantile normalization with 500 permutations in relative mode. Deconvoluted samples were deemed significant if CIBERSORTx p-value < 0.05, which represents the significance of the deconvolution results across all cell subsets for goodness-of-fit. This P-value is determined for each sample using Monte Carlo sampling, effectively providing a confidence measure in the results [7].

After CIBERSORTx, data was downloaded and analyzed with R programming language. Normality was not assumed and no values were excluded, thus the non-parametric Mann Whitney U test was used for comparisons. P-value < 0.05 was deemed significant.

Results

After RNA deconvolution for immune cell subset identification, all 78 samples were significant at the deconvolution p-value < 0.05 cutoffs and considered acceptable for further analysis. When comparing fractions of immune cell subsets between control and PD samples, prefrontal cortices from PD patients showed a significantly higher relative abundance of monocytes (p-value = 0.0005) (shown in Fig. 1). The relative proportions of the other 21 immune subtypes showed no significant differences between control and PD samples.

Discussion/Conclusion

Through the digital dissection of heterogeneous expression profiles, the work presented here has implicated monocytes as putative infiltrates of prefrontal cortices in PD. Previous studies have also reported significant infiltration in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of dopaminergic neurodegeneration [10–12]. However, many of these findings have come under question due to the use of artifact-prone techniques and the technical challenge of distinguishing between infiltrating monocytes becoming macrophages and the resident microglia themselves [13–14]. Contrarily, the machine-learning algorithm used in the present study is able to infer cell-type-specific gene expression profiles without conventional cytometric techniques. Indeed, the results shown here corroborate a more recent and impartial study confirming monocyte infiltration in an MPTP mouse model [2].

The prefrontal cortex may be more permissive for peripheral monocyte infiltration due to the heterogeneous nature of the blood-brain barrier, which may be more permeable near the prefrontal cortex [15–16]. It is also important to consider the possibility that disruptions in the blood-brain barrier integrity may be amplified near the prefrontal cortex in PD, allowing for an abnormal influx of proinflammatory substances, such as immune cells, from peripheral blood into brain parenchyma [17].

To our knowledge, this study is the first to utilize an unbiased computational approach to resolve monocytes as potential immune infiltrates in the brains of PD patients. Further studies are warranted to elucidate the neuroinflammatory role of monocytes in this neurodegenerative disease.

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Fig. 1. Violin plot showing relative abundances of monocytes. Overlaid boxplot represents upper and lower quartiles, with black notch depicting the median. Whiskers depict 1.5 times IQR. Asterisks (***) indicates significance at p-value < 0.001.