Analysis of common gene expression profiles from three vascular dementia rodent studies indicating neuroinflammation and microglia specific enrichment

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Abstract

Vascular dementia is characterized by deterioration in an individual's cognitive capacities, such as memory deficits, decreased cognitive processing speed and difficulties in sustaining attention, which necessitate a thorough understanding of its molecular mechanisms. Here, we attempt to investigate pathways, brain-specific mechanisms and predicted drugs using three different transcriptome studies of the BCAS (Bilateral Carotid Artery Stenosis) mice model of VaD. We used the NCBI GEO database for gene expression analysis from three RNA-Seq datasets across the hippocampal and cortex regions of the brains of BCAS mice models and identified differentially expressed genes (DEGs). We further performed functional gene enrichment using DAVID and brain cell type enrichment using Enrichr. Furthermore, a drug prediction was conducted using DGIdb. From the three datasets selected, we identified 980 DEGs (GSE223580), 1,236 DEGs (GSE210666) and 1,231 DEGs (Sang-Ha Baik et al) after filtering the significant LogFc. After filtering them by applying a cutoff of ± 0.4, we found 170 common DEGs.

The common DEGs were involved in pathways associated with neuroinflammation, metabolism and cell death related pathways. These DEGs are also significantly enriched in microglial cell type, affirming neuroinflammatory response in the BCAS model. The hub genes associated with the common DEGs model network revealed CD44, TLR2 and ICAM-1also having role in neuroinflammation. The top 2 drugs predicted in this condition include disulfiram and sulfasal. Current analysis reveals a role of neuroinflammation and microglia in vascular dementia pathogenesis and reveals multiple targets that can be experimentally tested.

Keywords: Bilateral Carotid Artery Stenosis, Vascular Dementia, Bioinformatic Analysis, Neuroinflammation.

Introduction

Vascular dementia (VaD) is the second most prevalent form of dementia after Alzheimer's disease (AD). It is remarkable that change in hemodynamics in the brain is a common node in several neurodegenerative diseases like aging, ischemia, AD, VaD disease etc., thus molecular insight in this area will have widespread implication[11,19,25]. Vascular dementia is primarily caused by reduced cerebral blood flow due to vascular abnormalities. It is clinically characterized by cognitive impairments including memory deficits, reduced cognitive processing speed and difficulties in maintaining attention, which are often associated with vascular dysfunction[27]. The underlying vascular issues include conditions such as atherosclerosis and hemorrhagic stroke[24].

Several comorbidities, such as hypertension, stroke, hypercholesterolemia and smoking, significantly increase the risk of developing vascular disease[1]. The etiopathogenesis of VaD predominantly involves ischemic or hemorrhagic events, such as stroke and chronic hypoperfusion, alongside genetic factors that play crucial roles[10,28]. Being multifactorial, VaD is characterized by processes including inflammation, oxidative stress, hypoxia and blood-brain barrier (BBB) dysfunction[22]. At the molecular level, vascular dementia is often associated with extensive reprogramming of gene expression in the brain[2,37].

Such widespread alterations in cognitive and neural functions are presumably not caused by the perturbation of a single pathway but rather result from the cumulative dysregulation of multiple signaling modules[23]. These include pathways such as PI3-Akt signaling, HIF-1 signaling, MAPK, RAP-1 signaling and others, which collectively contribute to neurovascular dysfunction and impaired synaptic plasticity[13,21].

Various studies have aimed to elucidate the role of specific genes and pathways in VaD pathophysiology. While these investigations provide valuable insights, they often yield limited evidence and fail to capture the complex interplay among multiple genes and proteins[20,36]. Consequently, there is a critical need for innovative approaches to decipher the intricate gene and pathway alterations in complex pathological conditions like VaD. A comprehensive, multifaceted strategy is essential for deepening our understanding of VaD's complexity and developing effective therapeutic interventions.

RNA sequencing (RNA-Seq) has emerged as a powerful tool for exploring biological mechanisms, identifying novel drug targets and evaluating therapeutic responses[30]. RNA-Seq facilitates the detection of a wide array of differentially expressed genes and rare transcripts, enabling transcriptome-wide analysis in a single assay[8]. Effective integration of RNA-Seq data is crucial for advancing our

understanding of cellular mechanisms and their variations under different conditions or treatments[14,34].

In the context of VaD, brain regions like the hippocampus, prefrontal cortex and cerebellum exhibit distinct pathological changes that serve as key clinical manifestations and markers of disease activity[9,10]. A popular model for researching to investigate chronic cerebral hypoperfusion and its pathophysiological implications, such as white matter injury and cognitive impairment, is the Bilateral Common Carotid Artery Stenosis (BCAS) model.

Originally designed to simulate human situations such as chronic ischemia and vascular dementia, the BCAS model uses micro coils to cause partial stenosis in the common carotid arteries, which lowers cerebral blood flow (CBF) without resulting in total ischemia. In rodents, usually mice, the BCAS model entails wrapping both common carotid arteries with micro coils of a particular internal diameter. Over weeks to months, this causes a slow and persistent decrease in CBF which culminates in gliosis, white matter lesions and cognitive impairments.

Because BCAS prevents acute ischemic damage, unlike other models of global ischemia, researchers may concentrate on the long-term consequences of hypoperfusion. In the current study we used publicly available BCAS transcriptomic dataset and re-analyzed to understand molecular mechanism of Vascular dementia.

Material and Methods

Retrieval and Identification of differentially expressed genes in BCAS data sets from the NCBI GEO database: In this study, three high-throughput sequencing datasets related to the BCAS model were retrieved from the NCBI Gene Expression Omnibus (GEO): GSE210666[38,39], GSE223580 and Baik[3] dataset. These are all RNA seq datasets and belong to the organism Mus Musculus.GSE210666. In this cortex specific gene expression is performed from RNA Seq data using 8 replicates of sham and BCAS. Analysis of the hippocampal gene expression profile was carried out using information from the RNA-Seq of three biological replicates in BCAS.

The resulting datasets were filtered and assessed by thoroughly examining their metadata and descriptions to validate their relevance. After identifying suitable datasets, they were downloaded in appropriate formats provided by GEO such as FTP, TXT, or TAR. Subsequently, the downloaded datasets underwent preprocessing steps that included normalization, quality control and transformation using bioinformatics tools to prepare the data for further analysis. This systematic approach enabled the acquisition of high-quality datasets conducive to robust analysis and valuable biological insights.

Furthermore, we utilized the Deseq 2 package of R to identify the differentially expressed genes based on their log2fold change and the third dataset we retrieved from supplementary files of Sang-Ha Baik et al[3].

Finding of common genes across BACS data sets via BioVenn: We used BioVenn, a web-based tool that helps to find common genes among multiple sets of data. The tool highlights common and distinct genes across various situations or datasets by using Venn diagrams to illustrate the overlap of gene sets. With the help of BioVenn, we compared our three datasets, GSE210666, GSE223580 and Sang-Ha Baik et al and found the common genes that overlap between them.

Functional annotation and PPI network construction and analysis: Pathway enrichment analysis in GSE210666, GSE223580 and Sang-Ha-Baik[3] was performed with DAVID (version 6.7; http://david.abcc.ncifcrf.gov/). The number of genes corresponding to each significantly enriched pathway was termed gene count. GO term and pathway groups were considered significant if the p-value <.01. The data sets were further subjected to another analysis tool called STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) 9.1 database. A network was constructed based on direct and indirect protein-protein interactions. Only a high level of confidence interaction based on co-expression was considered to extract the PPI network. The constructed network is imported in Cytoscape *v 3.0* for visualization. Further, the topological property of the network of degree is obtained employing Cytoscape plug-in *"Network Analyzer"*. The nodes with a high degree of degree are considered hubgene. The top ten hub genes are colored in green as illustrated in fig. 3A.

Cell type enrichment and Drug-gene interaction analysis: We conducted cell-type enrichment of common genes across three datasets using Enrichr[12] employing the cell marker 2024 (database). The significant cell types were identified based on their combined score. The drug gene interaction database (DGIdb, version 3.0; https://dgidb.org/search_interactions) was utilized to identify potential drug that can have therapeutic potential based on gene expression.

Results

Identification of common Differentially Expressed gene across three independent BCAS mice model of vascular dementia: To investigate the molecular mechanisms underlying vascular dementia, we curated RNA sequence based differential gene expression analyses in BCAS model in three independent study and retrieved data from the NCBI gene expression omnibus (GEO). Data included are GSE210666, GSE223580 and Sang-Ha Baik[3]. A schematic work flow of the analysis presentation of the experiment pipeline adopted in current study is shown in figure 1A. These three data sets were subjected to preprocessing and analyzed using R, applying a bioinformatics workflow to identify differentially expressed genes (DEGs).

The analysis pipeline included normalization, statistical modeling and stringent filtering to isolate DEGs with biological and statistical significance. Post analysis we found 1,855, 2,555 and 23,871 DEG in GSE223580, GSE210666 and Sang-Ha Baik RNA seq data of BCAS model respectively. We then applied a cutoff of ±2 for GSE223580 and GSE210666 and ±0.4 for the dataset by Sang-Ha Baik et al. Following the application of these cutoffs, we identified 980 DEGs in GSE223580 (804 upregulated, 176 downregulated), 1,236 DEGs in GSE210666 (1,010 upregulated, 226 downregulated) and 1,231 DEGs in the dataset (709 upregulated, 522 downregulated), as illustrated in figure 1B. Common DEGs across the datasets were identified using BioVenn, an online tool for Venn diagram visualization.

This step facilitated the identification of overlapping molecular signatures that may play pivotal roles in the pathophysiology of vascular dementia. We found that 170 genes were commonly expressed across all three datasets, as illustrated in figure 1C.

This analysis provided a conserved DEG across three studied, offering a foundation for understanding the molecular etiology of vascular dementia and identifying potential therapeutic targets.

Signal pathway enrichment analysis and PPI network analysis of conserved BCAS Differentially Expressed gene: After performing differential expression analysis and identifying DEGs, we performed functional enrichment analysis using DAVID bioinformatics to identify biological pathways and extracted key biological pathways (KEGG) that were significantly ($p < 0.1$) enriched. Enriched pathways were classified into six major categories: Neuroinflammation, Cell death, Metabolism, Neuronal Sertogenic, Endothelial and other inflammatory diseases.

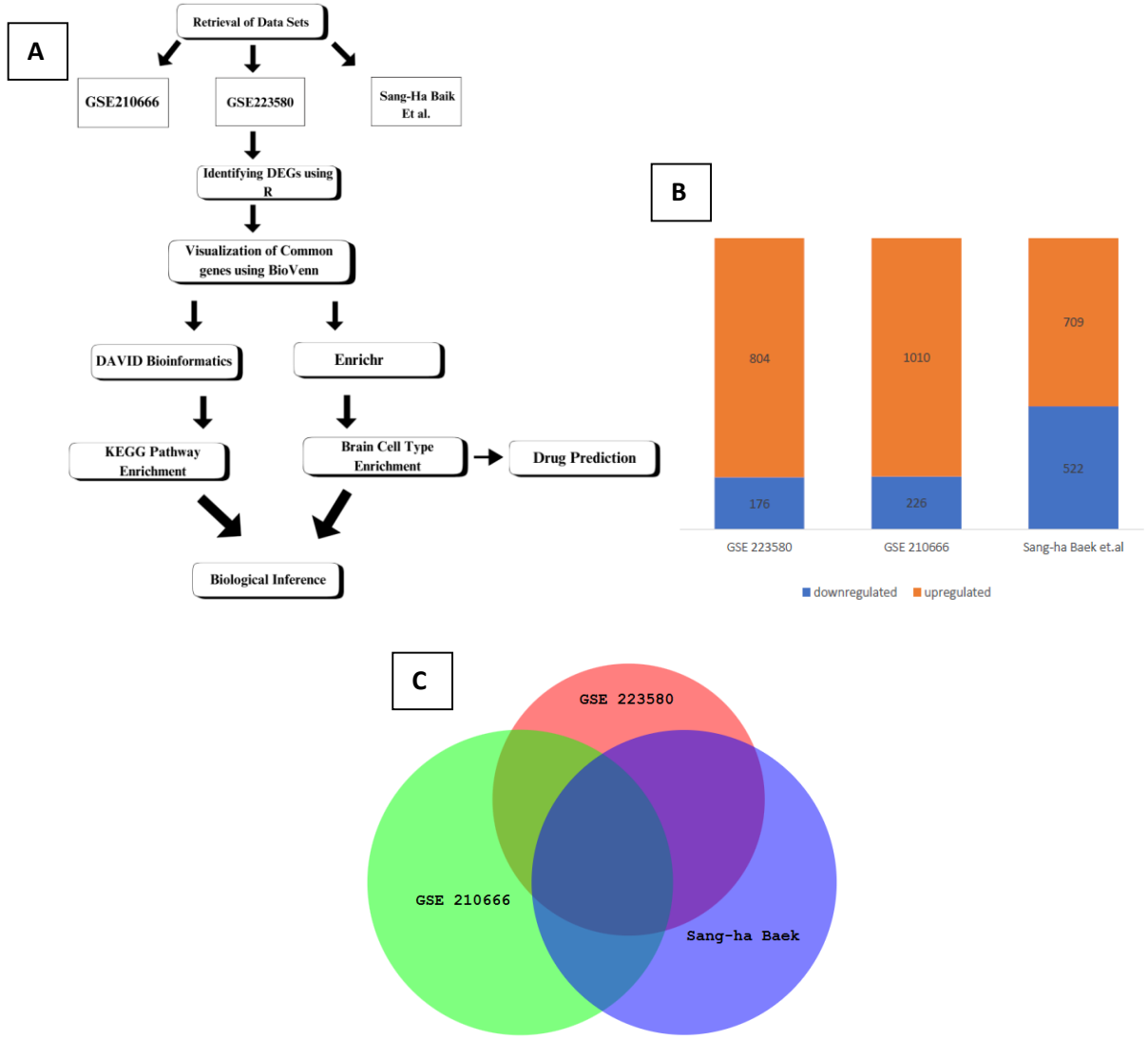


Figure 1: A) Schematic workflow for gene expression analysis B) Venn diagram illustrating the overlap of common genes identified among the three datasets: GSE210666, GSE223580 and Sang-Ha Baik et al. using BioVenn. C) Bar graph showing the distribution of upregulated and downregulated genes across the three datasets, using a cutoff of ±2 for GSE210666 and GSE223580 and ±0.4

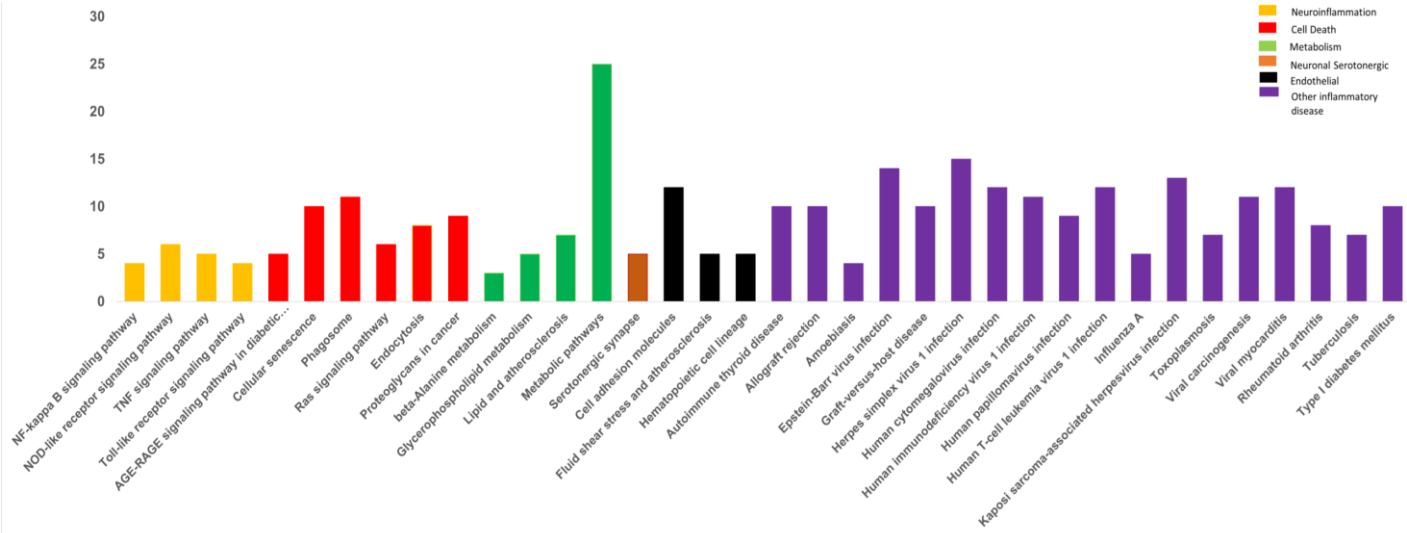


Figure 2: KEGG pathways significantly enriched after multiple testing adjustments (p < 0.1) represented as Bar graph indicating Pathways under the same group (Neuroinflammation, cell death, metabolism, Neuronal Sertogenic, Endothelial and other inflammatory diseases are clustered together and marked by the same color.

Pathways under the same group are clustered together and are marked by the same color as shown in figure 2. The number of genes modulated in each group are shown in Y-axis as gene number next to the respective group names.

Among the pathways associated with neuroinflammation were the NF-kappa B signaling pathway (4 genes), TNF signaling pathway (5 genes), NOD-like receptor signaling pathway (6 genes) and TLR signaling pathway, among others (4 genes). Pathways related to metabolism were beta-alanine metabolism (3 genes), glycerophospholipid metabolism (5 genes), lipid and atherosclerosis (7 genes) and metabolic pathways (25 genes).

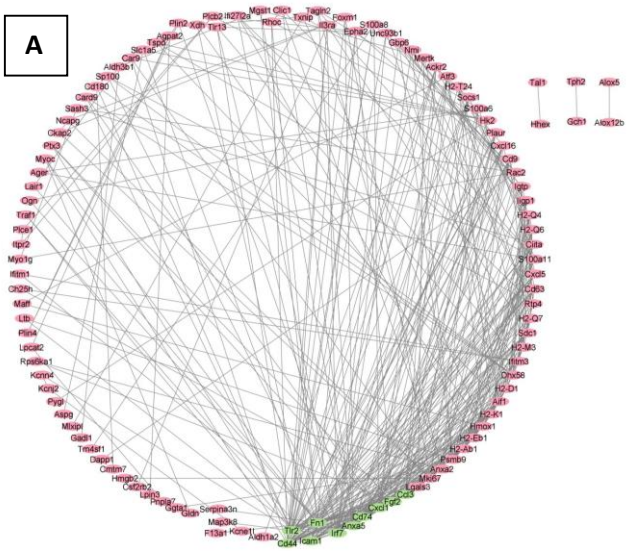
Other inflammatory diseases involve the majority of these associated biological pathways such as Autoimmune thyroid disease (10 genes), Allograft rejection (10 genes), Amoebiasis (4 genes), Epstein-Barr virus infection (14 genes), Graft-versus-host disease (10 genes), Herpes simplex virus 1 infection (15 genes), Human cytomegalovirus infection (12 genes), Human immunodeficiency virus 1 infection (11 genes), Human papillomavirus infection (9 genes), Human T-cell leukemia virus 1 infection (12 genes), Influenza A (5 genes), Kaposi sarcoma-associated herpesvirus infection (13 genes), Toxoplasmosis (7 genes), Viral carcinogenesis (11 genes), Viral myocarditis (12 genes), Rheumatoid arthritis (8 genes), Tuberculosis (7 genes) and Type I diabetes mellitus (10 genes).

We next applied a widely used protein-protein interaction network tool to mine STRING 12.0 database to find gene networks. The interaction from this database helped in creating network based on the functional connection between proteins. The network thus derived was visualized and analyzed utilizing visualization software Cytoscape 3.0.2 as shown in figure 3A. As network obtained from the string and cytoscape analysis is complex, we employed cytoscape plug-in network Analyzer‖ to evaluate degree, a topological feature gene network which indicated number of direct partners of gene.

This analysis revealed CD44 node occupied the centre of network, having the largest degree, which suggests that CD44 could be considered as a hub gene. Other top hub genes which are in top 10 degree of network include Tlr2, Icam1, Fn1, Anxa5, Irf7, Cxcl1, Cd74, Ccl3, Fgf2 as shown in figure 3 B. Out of these hub genes Tlr2 is one of the gene out of 170 common DEGs that show upregulation in all three datasets.

Though, PPI network clustering is independent approach and involves different sophisticated mathematical analysis. The function of hub genes thus obtained included same biological themes as revealed by KEGG pathway analysis related to neuroinflammation.

Cell type enrichment and Drug-gene interaction analysis of conserved BCAS Differentially Expressed gene: As the brain is heterogeneous tissue composed of various cell types, we next ask what are brain cell type enrichment of 170 common genes across three independent BACS RNA seq study. We performed cell type enrichment analysis using cell marker 2024. We found enrichment of top 10 cell types based on significant p-value using cell marker (2024) database. Using combined score we plotted a bar graph illustrated in figure 4 A showing the highest upregulation of microglia in the case of VaD. Out of prevalence of 36 genes (TLR2, RAC2, METRK, LGALS3 and ALF1 are some notable once), TLR2 is upregulated. The results thus obtained shows microglia enrichment, a neuroinflammatory associated cell in brain corroborated with the previous analysis and reaffirms its major role in neuropath physiology associated with vascular dementia.



B

Sr.n	Gene	Degree	Cell type
1	Cd44	32	Astrocytes
2	Tlr2	31	Microglia
3	Icam1	27	Endothelial
4	Fn1	25	Endothelial
5	Anxa5	23	Endothelial
6	Irf7	23	Endothelial
7	Cxcl1	22	Endothelial
8	Cd74	22	Microglia
9	Ccl3	19	Microglia
10	Fgf2	19	Astrocytes

Figure 3: A) STRING PPI network constructed from Cytoscape from common genes in BCAS. Nodes having a high degree are represented in green color. B) The right panel shows the top 10 nodes in PPI network arranged by descending degree value.

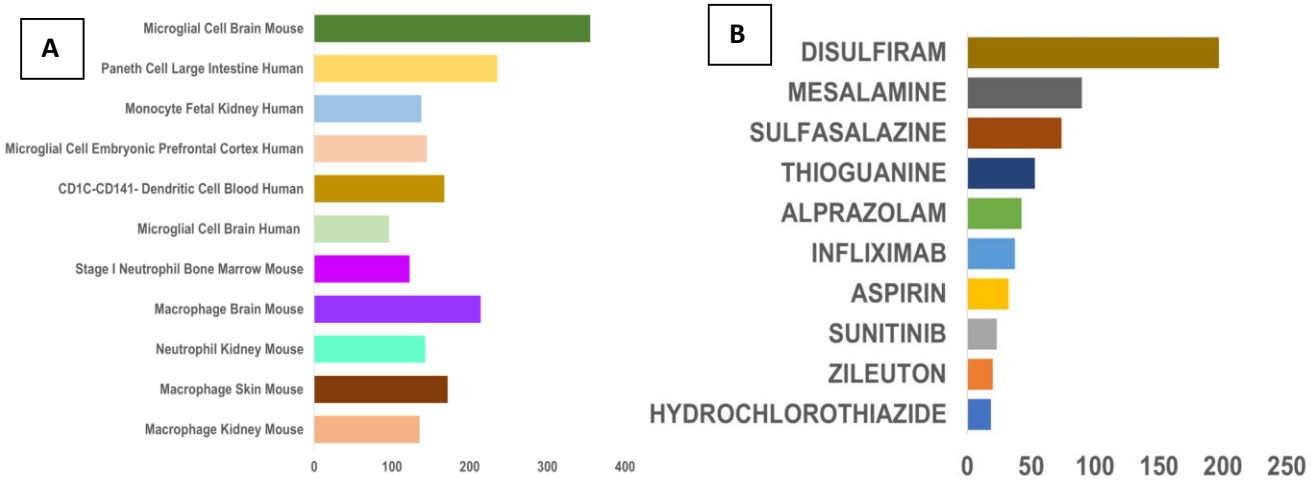


Figure 4: A) Bar graph represents cell type enrichment on the basis of comb ined score B) Bar graph representing top 10 drug targets using DGIdb database on the basis of p-value

We next predicted drug that having potential therapeutic effect in conserved common DEG three studies using DGIdb database of gene-drug interactions. As shown in figure 4 B, a list of top 10 genes was compiled based on significant p value. The top drug is Disulfiram, which is recently used widely. Disulfiram may reduce neuroinflammation and oxidative stress, acts as a proteasome inhibitor, inhibits nuclear factor kappa B (NF-κB). The other drugs are hydrochlorothiazide, zileuton, sunitinib, aspirin, infliximab, alprazolam, thioguanine, sulfasalazine and mesalamine. Common uses of these drugs are: hypertension, chronic asthma, treatment of cancers, anti-inflammatory effects, autoimmune diseases, vascular disease treatment, immune function regulation and hormone regulation.

Discussion

Our result showed perturbation of multiple immune and inflammatory related pathways. Like NF-kappa B signaling pathway, TNF signaling pathway and TLR signaling pathway are the most significant immune/inflammation pathways deregulated in BACS mouse model of vascular dementia, suggesting that these processes play a crucial role in the neuronal vulnerabilities in vascular dementia. Inflammatory cytokines such as TNF-α, IL-1β and IL-6 are upregulated[6] in our data sets and contribute to neuronal dysfunction[35] and synaptic loss[16]. These cytokines disrupt neurovascular coupling[4,15], impairing the regulation of cerebral blood flow[4,15,29] and exacerbating neuronal injury. This impairment is strongly linked to cognitive deficits observed in VaD patients.

These results are consistent with previous studies showing Toll-like receptors (TLRs) activate[32,33] and signal their downstream pathways to induce NF-κB and pro-IL-1β, both of which are implicated in neuroinflammation and are associated with the etiology of various neurological disorders[7,33].

Canonical signaling pathways also revealed modulation of metabolic pathways and cell death pathways[15]. Both biological phenomena are involved in VaD progression and engaged in its pathogenesis. Alterations in immunological responses and oxidative stress were indicated by metabolic pathways showing decreased mitochondrial function and energy metabolism, which are also found in other neurodegenerative conditions like Alzheimer disease. Our analysis also found AGE-RAGE pathways in common genes across dataset. Notably this pathway is involved in various pathological processes contributing to neurodegeneration including blood-brain barrier disruption, neuroinflammation and extracellular matrix remodelling.

Our study also provides the microglia associated genes enrichment in our data sets notably. Chronic microglial activation perpetuates a cycle of inflammation and neurodegeneration, exacerbating cognitive decline. Modulation of microglial genes such as, RAC2, METRK, LGALS3 and ALF1 is associated with the development and/or susceptibility to dementia. These genes expressed by microglia could serve as promising therapeutic targets for restoring neuronal function.

In our analysis, we identified 10 hub genes, among which Toll-like receptor 2 (TLR2) and CD44 emerged as two critical molecules implicated in the pathophysiology of neuroinflammation in vascular dementia (VaD). TLR2, a member of the pattern recognition receptor (PRR) family, plays a crucial role in detecting both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). CD44, a cell surface glycoprotein, is involved in cell-cell interactions, cell migration and tissue remodeling. Both TLR2 and CD44 are activated in response to cerebrovascular insults and their involvement in neuroinflammation contributes significantly to the progression of VaD. The activation of TLR2 leads to the recruitment of microglia and other immune cells to the site of injury where they release additional inflammatory mediators, resulting in exacerbated neuronal damage and contributing to cognitive decline.

Top drug candidate in our analysis showed disulfiram interestingly, beyond its established role in alcohol aversion therapy. Disulfiram has gained attention for its potential therapeutic applications in neurodegenerative diseases, owing to its ability to modulate oxidative stress, inflammation and protein aggregation. This dual action, attenuation of inflammation and promotion of proteostasis, positions disulfiram as a promising candidate for repurposing in the treatment of neurodegenerative disorders. Further investigations into its mechanisms and clinical efficacy are warranted to fully elucidate its therapeutic potential in VaD.

Conclusion

This study employed three independent transcriptomic datasets of BCAS mouse model of Vascular Dementia to investigate common gene expression profile to deepen our understanding of mechanistic basis of Vascular Dementia and to identify potential drug targets[5,31,32]. Neuroinflammation has emerged as a central mechanism in the development and progression of VaD, serving as a critical link between vascular damage, blood-brain barrier (BBB) dysfunction and cognitive decline[26]. It is imperative that future studies should provide valid functional and clinical experimental evidences of candidate gene and drugs produced in this study have role in VaD pathophysiology.

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