Potential Novel Therapeutic Targets in Epilepsy from The View of Intestinal Comorbidities

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Abstract

Background: Epilepsy is a complicated neurological disorder with almost 30% refractory. Recent years, several studies showed that epilepsy is associated with its comorbidities by shared molecular mechanisms. However, the association of epilepsy and digestive comorbidities are still unclear. In this study, we aim to explore the association between inflammatory bowel disease (IBD) and epilepsy, and to find promising therapeutic targets for refractory epilepsy.

Methods: Two gene expression profiles (GSE134697 and GSE59071) were selected from Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were identified by GEO2R and the DESeq2 package. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of intersection DEGs and Gene Set Enrichment Analysis (GSEA) were conducted by clusterProfiler package. The protein-protein interactions (PPI) network was established by using STRING and visualized by Cytoscape. Genes in the most significant module identified by MCODE plug-in were considered as candidate hub genes. Validation of hub genes were performed by using the GSE143272 dataset.

Results: *CXCL8*, *CXCR4* and *ITGAX* were identified as the hub genes.

Conclusions: *CXCL8*, *CXCR4* and *ITGAX* were the shared molecular mechanism of epilepsy and IBD, which were potentially novel therapeutic targets in epilepsy.

1. Introduction

Epilepsy is a brain disorder characterized by an enduring predisposition to generate epileptic seizures [1]. Over 70 million people worldwide are affected by epilepsy and suffering various negative consequences from epileptic seizures, including disability and mortality [2, 3]. Although 70% of seizures could be controlled by using antiseizure medications (ASMs), 30% of were refractory that make epilepsy a burdensome disease [2-4]. Therefore, it is essential to explore novel therapy targets for epilepsy, especially for drug-resistant epilepsy [3, 5].

Recent studies showed that several diseases of different systems were found more common in people with epilepsy than in the general population, including neuropsychiatric, cardiovascular, digestive, and immune system diseases [6]. This is owing to a bidirectional factor between epilepsy and its comorbidities in mechanism, including genetic, environmental, structural, and physiological factors [6, 7]. It was found that epileptic seizures could be controlled by treating comorbidities [6, 8, 9], which potentially provided a novel treating idea for refractory epilepsy. Therefore, to explore shared molecular mechanisms of epilepsy and comorbid conditions is undoubtedly a fast and effective method to develop novel treatments for epilepsy [6]. Until now, some shared molecular mechanisms of epilepsy and neuropsychiatric comorbidities have been discovered [10-12], but the association of epilepsy and intestinal comorbidities are still unclear.

Inflammatory bowel disease (IBD) is a common intestinal comorbid condition of epilepsy, which are characterized by a chronic and recurrent gastrointestinal condition including ulcerative colitis (UC) and Crohn's disease (CD) [6, 13, 14]. IBD and epilepsy were found to be involved in the shared mechanisms in inflammatory, hypercoagulable, and genetic factors [13, 15]. However, the certain molecules acting in the shared mechanisms are unknown. In this study, we conducted a combined transcriptome analysis of epilepsy and IBD patients from Gene Expression Omnibus (GEO) database to identify the shared molecules mechanisms and determine the potential pathway by which develop novel therapeutic targets for epilepsy.

2. Materials And Methods

2.1. Data Collection

All microarray datasets were downloaded from the GEO database. We searched GEO database for microarray datasets using the keywords “epilepsy” and “IBD” {including “Crohn’s disease (CD)” and “ulcerative colitis (UC)”}. Datasets were recruited by the following criteria: (1) transcriptome data; (2) samples were derived from homo sapiens; (3) the count of samples was > 15; (4) including control groups. Finally, gene expression microarray datasets GSE134697 and GSE59071 were qualified to be selected. According to the IBD classification, GSE59071 was separated into two parts: GSE59071-UC and GSE59071-CD. The two datasets were based on the platforms of GPL16791 [Illumina HiSeq 2500 (Homo sapiens)], and GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]}, respectively.

The dataset GSE134697 contained 36 samples, including 17 hippocampal samples (the case group) obtained from patients with drug-resistant temporal lobe epilepsy, and 19 neocortex samples (the control group) obtained from 17 patients with drug-resistant temporal lobe epilepsy and 2 healthy individuals. The dataset GSE59071 contained 116 samples, including 105 affected intestinal mucosal samples (the case group) obtained from patients with IBD (consisting of 97 UC and 8 CD), and 11 intestinal mucosal samples (the control group) obtained from healthy individuals in the control group.

2.2. Identification of Differentially Expressed Genes (DEGs)

DEGs screenings of the two datasets GSE134697 and GSE59071 were conducted by the GEO2R online analysis tool (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>) and the R package “DESeq2” [16], respectively. *P*-values were adjusted by using the false discovery rate (FDR) method. Genes with adjusted *P* < 0.05 and |log2 fold change (FC)| > 1 were considered as DEGs. We used Venn diagram web tool (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) to find the DEGs shared by both datasets.

2.3. Function Enrichment Analysis of DEGs

To explore the enriched biological pathways and annotations, we utilized the RStudio v4.0.5 to conduct the Gene Ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis by clusterProfiler package [17]. Adjust P -values < 0.05 were regarded as the cut-off criteria.

2.4. Gene Set Enrichment Analysis (GSEA) of Two Expression Profiles

To further reveal the common pathway between epilepsy and IBD, GSEA was performed based on each single expression matrix. R package “clusterProfiler” [17] was utilized to perform GSEA [18]. Enrichment analyses were conducted to determine whether a series of a priori-defined biological processes was enriched. The genes of two datasets were ranked according to the false discovery rate (FDR). $P < 0.05$ were considered statistically significant.

2.5. Protein-protein Interaction Network Construction, Significant Module Analysis and Candidate Hub Gene Identification

The protein-protein interactions (PPI) network of DEGs was established by using STRING v11.0 (<https://string-db.org>). A PPI network of the DEGs with combined score > 0.4 in STRING was considered as a functional link and was visualized by Cytoscape software v3.7.2. The Cytoscape's plug-in molecular complex detection technology (MCODE) was used to identify closely connected modules from the PPI network with MCODE score > 4.5. The GO enrichment analysis of the module genes was conducted by using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 (<https://david.abcc.ncifcrf.gov>). Genes in the most significant module identified by MCODE plug-in were selected as candidate hub genes.

2.6. Validation of Candidate Hub Genes

We used GEO2R to identify the DEGs in the GSE143272 dataset [epilepsy response to valproate (VPA) treatment vs. healthy control]. The candidate hub genes that downregulated in this dataset were identified as hub genes.

3. Results

3.1. Identification of DEGs in Epilepsy and IBD

In the GSE59071 dataset, 1243 DEGs (UC tissues vs. control) and 697 DEGs (CD tissues vs. control) were identified. In the GSE134697 dataset, 1846 DEGs (hippocampus vs. control) were identified. The shared DEGs of datasets GSE134697 and GSE59071 were 78 genes, including 56 upregulated and 22 downregulated genes (Figure 1, Table 1).

3.2. GO Enrichment Analysis of DEGs

GO enrichment analysis showed that various DEGs were upregulated in the pathways of biological processes (BP), molecular functions (MF), and cellular components (CC). In BP, upregulated DEGs were significantly enriched in the “regulation of vasculature development”, “response to molecule of bacterial origin”, “response to lipopolysaccharide”, “positive regulation of cytokine production”, and “positive regulation of cell-cell adhesion” (Figure 2a, Table 2). In MF, upregulated DEGs were enriched in the “glycosaminoglycan binding”, “cytokine activity”, “receptor ligand activity”, and “signaling receptor activator activity” (Figure 2b, Table 2). In CC, upregulated DEGs were significantly enriched in the “secretory granule membrane” and “collagen-containing extracellular” (Figure 2c, Table 2). There also various downregulated DEGs enriched in MF (Figure 2d, Table 2). More detailed GO enrichment results are shown in Table 2. The number of upregulated genes involved in the different processes of BP, CC, and MF was much more than that of downregulated genes. These results suggest that epilepsy and IBD may undergo common metabolic activities involving in cytokine associated inflammatory response and other complex biological process.

3.3. KEGG Enrichment Analysis of DEGs

KEGG enrichment analysis showed that upregulated genes were enriched in 26 pathways, such as rheumatoid arthritis, graft-versus-host disease, type I diabetes mellitus, hematopoietic cell lineage, leishmaniasis, intestinal immune network for IgA production, toll-like receptor signaling pathway, inflammatory bowel disease, and cytokine-cytokine receptor interaction pathway and so on (Table 3, Figure 3). These pathways are mainly involved in inflammatory or proinflammatory response. The downregulated genes were not enriched in any pathway (Table 3).

3.4. GSEA

GSEA showed that the dataset GSE134697 (epilepsy) is enriched in the pathways of antigen processing and presentation, cytokine-cytokine receptor interaction, graft-versus-host disease, hematopoietic cell lineage, and leishmaniasis infection. As for dataset GSE59071, the expression profiles of IBD samples were enriched in cell adhesion molecules, chemokine signaling pathway, complement and coagulation cascades, cytokine-cytokine receptor interaction, and ECM receptor interaction. The results indicated that the cytokine-cytokine receptor interaction pathway is the communal pathway between epilepsy and IBD (Figure 4a, 4b).

3.5. PPI Network Construction, Significant Module Analysis and Candidate Hub Gene Identification

We developed a PPI network containing 51 nodes and 162 edges and visualized by using Cytoscape software (Figure 5a). Nodes were drawn in different colors, representing the node degree (up or down). There were 56 upregulated genes and 22 downregulated genes. The most significant module with the highest score (module score: 9.111) was shown in Figure 5b, containing 10 genes in a network with 41 edges. GO analysis revealed that these genes were involved in chemokine-mediated signaling pathway, inflammatory response, and the other biological process were shown in Table 4. Genes in the most significant module were selected as candidate hub genes. These genes were all upregulated genes, as follows: *CXCL8*, *CXCR4*, *ITGAX*, *CD80*, *SELL*, *CD44*, *CD69*, *IL1B*, *CD38*, and *CXCL2*.

3.6. Validation of Candidate Hub genes

We finally identified three hub genes *CXCL8*, *CXCR4* and *ITGAX*, which validated by using GSE143272 dataset [epilepsy response to VPA treatment vs. healthy control]. In the group of epilepsy to VPA treatment, *CXCL8* was significantly downregulated (adjusted $P < 0.05$ and $\log_2FC = -1.2911696$), *CXCR4* and *ITGAX* were slightly downregulated. The three genes are significantly upregulated in the case groups (untreated) of GSE134697 and GSE59071 datasets.

4. Discussion

Although previous studies pointed out that epilepsy and IBD are related, the mechanism of their association is not addressed [19-21]. In this study, we conducted a transcriptome analysis to explore the association of epilepsy and IBD, aiming to discover promising therapeutic targets for refractory epilepsy.

A total of 78 overlapping DEGs were identified based on GSE134697 and GSE59071, of which 56 genes were upregulated and 22 genes were downregulated. GO analysis revealed that DEGs were enriched in the pathways of regulation of vasculature development, response to molecule of bacterial origin, response to lipopolysaccharide, and positive regulation of cytokine production. KEGG analysis showed that the DEGs enriched in the pathways of rheumatoid arthritis, intestinal immune network for IgA production, and toll-like receptor signaling. GSEA showed that the gene expression profile of epilepsy was enriched in cytokine-cytokine receptor interaction pathway that was same as IBD. Previous study showed that central/peripheral inflammation played an important role in triggering or sustaining epileptic activity [8, 22-25]. Therefore, IBD may induce or aggravate seizures through the communal pathway and other inflammation/proinflammatory process. We identified 3 hub genes of *CXCL8*, *CXCR4*, and *ITGAX*, which were all upregulated in both epilepsy and IBD datasets. Additionally, *CXCL8*, *CXCR4*, and *ITGAX* were all downregulated in epilepsy under VPA treatment (dataset GSE143272), in which *CXCL8* was downregulated most obviously. These findings suggested that the three hub genes, especially *CXCL8*, may be promising therapeutic targets for epilepsy.

Interleukin-8 (IL-8: *CXCL8*) is a chemotactic and inflammatory cytokine that involves in central nervous system (CNS) inflammation [26, 27]. Chemokines are small (8–12 kDa) chemotaxis proteins which play an important physiological role in immune system, mainly being responsible for guiding leukocyte to move to inflammatory sites [28]. Increased *CXCL8* expression has been widely demonstrated in colonic mucosa of UC compared with normal control subjects, and upregulated *CXCL8* concentration is correlated with the degree of inflammation [28]. There also some studies showed that the levels of *CXCL8* transcripts were significantly higher in total epileptic patients compared with healthy subjects [29]. Elevated levels for *CXCL8*/IL-8 were reported in several different epilepsy etiologies and media [30], such as febrile infection-related epilepsy syndrome, and epileptic temporal lobe [31]. Thus, we supposed that *CXCL8* may cause epilepsy by ways of immunoregulatory.

The chemokine CXC motif receptor 4 (*CXCR4*) is cognate with chemokine *CXCL12*. It is known as stromal cell derived factor-1 (SDF-1), which is highly expressed in the CNS and plays a crucial role in brain architecture [32-34]. Several studies indicated that the chemokine receptors were upregulated expressed in epilepsy and the active stage of UC [35-37], which was consistent with this study. Additionally, AMD3100, the antagonist of *CXCR4*, could reverse the neurogenesis promoted by enriched environment and suppresses long-term seizure activity [38, 39]. Animal experiments also showed that AMD-3100 can reduce colon injury in mice and *CXCL12*/*CXCR4* blocking was beneficial in improving experimental colitis in rodent models [40-43].

ITGAX encodes the integrin alpha X chain protein (CD11c), which is a pan dendritic cells (DCs) marker. While normally residing in the meninges and choroid plexus, perivascular and intraparenchymal DCs can be detected in brain under several inflammatory conditions. The level of CD11c protein was higher after status epilepticus (SE), while there was no detectable expression of CD11c in control group [44].

The evidence above supported that the three hub genes (*CXCL8*, *CXCR4* and *ITGAX*) found in this study were potentially the shared molecular mechanism of epilepsy and IBD. *CXCL8*, *CXCR4* and *ITGAX* were discovered as potentially novel therapeutic targets in epilepsy from the view of intestinal comorbidities in this study. More details in mechanism warrant further experimental validation.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests for this research.

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Author Contributions

Conceptualization, M.J. and Z.S.; Methodology, M.J. and J.L.; Software, M.J.; Validation, M.J., Z.S., and J.L.; Investigation, M.J. and Z.S.; Writing – Original Draft Preparation, M.J.; Writing – Review & Editing, M.J., Z.S., and J.L.; Visualization, M.J.; Supervision, Z.S.; Project Administration, Z.S.; Funding Acquisition, Z.S.

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References

1. Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J, Jr.: **Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE).** *Epilepsia* 2005, **46**(4):470-472.
2. Collaborators GBDN: **Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016.** *The Lancet Neurology* 2019, **18**(5):459-480.
3. Löscher W, Potschka H, Sisodiya SM, Vezzani A: **Drug Resistance in Epilepsy: Clinical Impact, Potential Mechanisms, and New Innovative Treatment Options.** *Pharmacol Rev* 2020, **72**(3):606-638.
4. Perucca P, Mula M: **Antiepileptic drug effects on mood and behavior: molecular targets.** *Epilepsy Behav* 2013, **26**(3):440-449.
5. Kelley MS, Jacobs MP, Lowenstein DH: **The NINDS epilepsy research benchmarks.** *Epilepsia* 2009, **50**(3):579-582.
6. Keezer MR, Sisodiya SM, Sander JW: **Comorbidities of epilepsy: current concepts and future perspectives.** *The Lancet Neurology* 2016, **15**(1):106-115.
7. Gaitatzis A, Sisodiya SM, Sander JW: **The somatic comorbidity of epilepsy: a weighty but often unrecognized burden.** *Epilepsia* 2012, **53**(8):1282-1293.
8. Ong MS, Kohane IS, Cai T, Gorman MP, Mandl KD: **Population-level evidence for an autoimmune etiology of epilepsy.** *JAMA Neurol* 2014, **71**(5):569-574.
9. Vincent A, Bien CG, Irani SR, Waters P: **Autoantibodies associated with diseases of the CNS: new developments and future challenges.** *The Lancet Neurology* 2011, **10**(8):759-772.
10. Fan C, Gao Y, Liang G, Huang L, Wang J, Yang X, Shi Y, Dräger UC, Zhong M, Gao TM *et al.*: **Transcriptomics of Gabra4 knockout mice reveals common NMDAR pathways underlying autism, memory, and epilepsy.** *Molecular autism* 2020, **11**(1):13.
11. Jiang X, Lu H, Liu W, Wu Z, Wu Q, Li X, Xu Z, Hui F, Zhao Q: **The overlap between Alzheimer's disease and epilepsy uncovered by transcriptome sequencing.** *Clinical and translational medicine* 2020, **10**(5):e169.

12. Xi XJ, Tang JH, Zhang BB, Xiao X, Hu XY, Wan Y, Zhou C, Lin H: **Dlg4 and Vamp2 are involved in comorbid epilepsy and attention-deficit hyperactivity disorder: A microarray data study.** *Epilepsy Behav* 2020, **110**:107192.
13. Devinsky O, Schein A, Najjar S: **Epilepsy associated with systemic autoimmune disorders.** *Epilepsy currents* 2013, **13**(2):62-68.
14. Guan Q: **A Comprehensive Review and Update on the Pathogenesis of Inflammatory Bowel Disease.** *J Immunol Res* 2019, **2019**:7247238.
15. Akbarian S, Sucher NJ, Bradley D, Tafazzoli A, Trinh D, Hetrick WP, Potkin SG, Sandman CA, Bunney WE, Jr., Jones EG: **Selective alterations in gene expression for NMDA receptor subunits in prefrontal cortex of schizophrenics.** *The Journal of neuroscience : the official journal of the Society for Neuroscience* 1996, **16**(1):19-30.
16. Love MI, Huber W, Anders S: **Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2.** *Genome biology* 2014, **15**(12):550.
17. Yu G, Wang LG, Han Y, He QY: **clusterProfiler: an R package for comparing biological themes among gene clusters.** *Omics : a journal of integrative biology* 2012, **16**(5):284-287.
18. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES *et al*: **Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles.** *Proceedings of the National Academy of Sciences of the United States of America* 2005, **102**(43):15545-15550.
19. Benavente L, Morís G: **Neurologic disorders associated with inflammatory bowel disease.** *Eur J Neurol* 2011, **18**(1):138-143.
20. Elsehety A, Bertorini TE: **Neurologic and neuropsychiatric complications of Crohn's disease.** *Southern medical journal* 1997, **90**(6):606-610.
21. Lossos A, River Y, Eliakim A, Steiner I: **Neurologic aspects of inflammatory bowel disease.** *Neurology* 1995, **45**(3 Pt 1):416-421.
22. Vezzani A, Granata T: **Brain inflammation in epilepsy: experimental and clinical evidence.** *Epilepsia* 2005, **46**(11):1724-1743.
23. Choi J, Nordli DR, Jr., Alden TD, DiPatri A, Jr., Laux L, Kelley K, Rosenow J, Schuele SU, Rajaram V, Koh S: **Cellular injury and neuroinflammation in children with chronic intractable epilepsy.** *Journal of neuroinflammation* 2009, **6**:38.
24. Ho YH, Lin YT, Wu CW, Chao YM, Chang AY, Chan JY: **Peripheral inflammation increases seizure susceptibility via the induction of neuroinflammation and oxidative stress in the hippocampus.** *Journal of biomedical science* 2015, **22**(1):46.
25. Lin Z, Si Q, Xiaoyi Z: **Association between epilepsy and systemic autoimmune diseases: A meta-analysis.** *Seizure* 2016, **41**:160-166.
26. Uddin J, Garcia HH, Gilman RH, Gonzalez AE, Friedland JS: **Monocyte-astrocyte networks and the regulation of chemokine secretion in neurocysticercosis.** *J Immunol* 2005, **175**(5):3273-3281.
27. Baggiolini M, Clark-Lewis I: **Interleukin-8, a chemotactic and inflammatory cytokine.** *FEBS letters* 1992, **307**(1):97-101.
28. Zhu Y, Yang S, Zhao N, Liu C, Zhang F, Guo Y, Liu H: **CXCL8 chemokine in ulcerative colitis.** *Biomed Pharmacother* 2021, **138**:111427.
29. Mazdeh M, Omrani MD, Sayad A, Komaki A, Arsang-Jang S, Taheri M, Ghafouri-Fard S: **Expression analysis of cytokine coding genes in epileptic patients.** *Cytokine* 2018, **110**:284-287.
30. de Vries EE, van den Munckhof B, Braun KP, van Royen-Kerkhof A, de Jager W, Jansen FE: **Inflammatory mediators in human epilepsy: A systematic review and meta-analysis.** *Neurosci Biobehav Rev* 2016, **63**:177-190.
31. Farias-Moeller R, LaFrance-Corey R, Bartolini L, Wells EM, Baker M, Doslea A, Suslovic W, Greenberg J, Carpenter JL, Howe CL: **Fueling the FIRES: Hemophagocytic lymphohistiocytosis in febrile infection-related epilepsy syndrome.** *Epilepsia* 2018, **59**(9):1753-1763.
32. Tham TN, Lazarini F, Franceschini IA, Lachapelle F, Amara A, Dubois-Dalcq M: **Developmental pattern of expression of the alpha chemokine stromal cell-derived factor 1 in the rat central nervous system.** *The European journal of neuroscience* 2001, **13**(5):845-856.
33. Tissir F, Wang CE, Goffinet AM: **Expression of the chemokine receptor Cxcr4 mRNA during mouse brain development.** *Brain research Developmental brain research* 2004, **149**(1):63-71.
34. McGrath KE, Koniski AD, Maltby KM, McGann JK, Palis J: **Embryonic expression and function of the chemokine SDF-1 and its receptor, CXCR4.** *Developmental biology* 1999, **213**(2):442-456.
35. Lin X, Wang H, Li Y, Yang J, Yang R, Wei D, Zhang J, Yang D, Wang B, Ren X *et al*: **Functional characterization of CXCR4 in mediating the expression of protein C system in experimental ulcerative colitis.** *American journal of translational research* 2017, **9**(11):4821-4835.
36. Hosomi S, Oshitani N, Kamata N, Sogawa M, Okazaki H, Tanigawa T, Yamagami H, Watanabe K, Tominaga K, Watanabe T *et al*: **Increased numbers of immature plasma cells in peripheral blood specifically overexpress chemokine receptor CXCR3 and CXCR4 in patients with ulcerative colitis.** *Clinical and experimental immunology* 2011, **163**(2):215-224.

37. Zhang X, Liu T, Zhou Z, Mu X, Song C, Xiao T, Zhao M, Zhao C: **Enriched Environment Altered Aberrant Hippocampal Neurogenesis and Improved Long-Term Consequences After Temporal Lobe Epilepsy in Adult Rats.** *J Mol Neurosci* 2015, **56**(2):409-421.
38. Song C, Xu W, Zhang X, Wang S, Zhu G, Xiao T, Zhao M, Zhao C: **CXCR4 Antagonist AMD3100 Suppresses the Long-Term Abnormal Structural Changes of Newborn Neurons in the Intraventricular Kainic Acid Model of Epilepsy.** *Mol Neurobiol* 2016, **53**(3):1518-1532.
39. Zhou Z, Liu T, Sun X, Mu X, Zhu G, Xiao T, Zhao M, Zhao C: **CXCR4 antagonist AMD3100 reverses the neurogenesis promoted by enriched environment and suppresses long-term seizure activity in adult rats of temporal lobe epilepsy.** *Behav Brain Res* 2017, **322**(Pt A):83-91.
40. Li X, Lan X, Zhao Y, Wang G, Shi G, Li H, Hu Y, Xu X, Zhang B, Ye K *et al*: **SDF-1/CXCR4 axis enhances the immunomodulation of human endometrial regenerative cells in alleviating experimental colitis.** *Stem Cell Res Ther* 2019, **10**(1):204.
41. Xia XM, Wang FY, Xu WA, Wang ZK, Liu J, Lu YK, Jin XX, Lu H, Shen YZ: **CXCR4 antagonist AMD3100 attenuates colonic damage in mice with experimental colitis.** *World J Gastroenterol* 2010, **16**(23):2873-2880.
42. Xia XM, Wang FY, Zhou J, Hu KF, Li SW, Zou BB: **CXCR4 antagonist AMD3100 modulates claudin expression and intestinal barrier function in experimental colitis.** *PLoS One* 2011, **6**(11):e27282.
43. Mikami S, Nakase H, Yamamoto S, Takeda Y, Yoshino T, Kasahara K, Ueno S, Uza N, Oishi S, Fujii N *et al*: **Blockade of CXCL12/CXCR4 axis ameliorates murine experimental colitis.** *The Journal of pharmacology and experimental therapeutics* 2008, **327**(2):383-392.
44. Li XW, Yang F, Wang YG, Wang JC, Ma L, Jiang W: **Brain recruitment of dendritic cells following Li-pilocarpine induced status epilepticus in adult rats.** *Brain Res Bull* 2013, **91**:8-13.

Tables

Table 1. Co-expression of differential genes

Group	Gene symbol
Upregulated	PADI2 IFI44L CAPN13 FPR1 HLA-DPB1 THBS2 ADGRE2 EGR1 HLA-DPA1 TREM1 STX11 PRRX1 ITGAX CXCL2 PDK4 ABCA12 ANGPTL2 ADM SCIN MT1A CXCR4 VCAN RASGRP3 ENPP2 THEMIS2 IL1A FGF7 SGK2 PDPN ZBP1 ADAMTS9 SPP1 DMBT1 SELL NPSR1 APLNR BCL2A1 SPARC SLC51A SLC11A1 CHI3L2 FOS CD80 CD38 PCK1 CD69 GBA3 IL1B SERPINE1 LIPG CD44 CXCL8 RGS1 TNC OSM SERPINA3
Downregulated	HSD17B2 SATB2-AS1 MUC5B MT1H SLC38A4 PTGDR MEDAG FAM20A GLRA2 TRHDE CEMIP SLC04C1 CNTN3 COL3A1 OLFM4 CNTN4 ADGRL2 STRIP2 SLC9A2 NOS2 THRB MGP

Table 2. GO enrichment analysis of DEGs

Table 2. GO enrichment analysis of DEGs (continued)

Category		Term	Description	Gene ID
Upregulated	BP	GO:1901342	regulation of vasculature development	CXCR4/IL1A/ENPP2/ITGAX/ADM/IL1B/ADAMTS9/APLNR/CXCL8/EGR1/THBS2/SERPINE1/S
		GO:0002237	response to molecule of bacterial origin	IL1A/SLC11A1/CXCL2/ADM/IL1B/CXCL8/PCK1/CD80/DMBT1/FOS/SERPINE1/SPARC
		GO:0045765	regulation of angiogenesis	CXCR4/IL1A/ENPP2/ITGAX/ADM/IL1B/ADAMTS9/APLNR/CXCL8/THBS2/SERPINE1/SPARC
		GO:0032496	response to lipopolysaccharide	IL1A/SLC11A1/CXCL2/ADM/IL1B/CXCL8/PCK1/CD80/FOS/SERPINE1/SPARC
		GO:1904018	positive regulation of vasculature development	CXCR4/IL1A/ITGAX/ADM/IL1B/APLNR/CXCL8/EGR1/SERPINE1
		GO:0045766	positive regulation of angiogenesis	CXCR4/IL1A/ITGAX/ADM/IL1B/APLNR/CXCL8/SERPINE1
		GO:0001819	positive regulation of cytokine production	IL1A/OSM/SLC11A1/HLA-DPB1/IL1B/EGR1/HLA-DPA1/ZBP1/CD80/SERPINE1
		GO:0022409	positive regulation of cell-cell adhesion	CD44/IL1A/HLA-DPB1/IL1B/HLA-DPA1/PCK1/PDPN/CD80
		GO:0030198	extracellular matrix organization	CD44/SPP1/ITGAX/VCAN/ADAMTS9/PDPN/TNC/SERPINE1/SPARC
		GO:0043062	extracellular structure organization	CD44/SPP1/ITGAX/VCAN/ADAMTS9/PDPN/TNC/SERPINE1/SPARC
	MF	GO:0005539	glycosaminoglycan binding	CD44/SELL/ADGRE2/LIPG/VCAN/FGF7/THBS2
		GO:0005125	cytokine activity	IL1A/SPP1/OSM/CXCL2/IL1B/CXCL8
		GO:0048018	receptor ligand activity	IL1A/SPP1/OSM/CXCL2/ADM/IL1B/CXCL8/FGF7
		GO:0030546	signaling receptor activator activity	IL1A/SPP1/OSM/CXCL2/ADM/IL1B/CXCL8/FGF7
		GO:0050840	extracellular matrix binding	SPP1/DMBT1/SPARC
		GO:0140375	immune receptor activity	CD44/CXCR4/FPR1/HLA-DPA1
		GO:0005149	interleukin-1 receptor binding	IL1A/IL1B
		GO:0008528	G protein-coupled peptide receptor activity	CXCR4/FPR1/APLNR/NPSR1
		GO:0001653	peptide receptor activity	CXCR4/FPR1/APLNR/NPSR1
		GO:1901681	sulfur compound binding	SELL/ADGRE2/LIPG/FGF7/THBS2
	CC	GO:0030667	secretory granule membrane	CD44/SELL/CD38/SLC11A1/FPR1/ITGAX/DMBT1/SPARC
		GO:0062023	collagen-containing extracellular matrix	VCAN/SERPINA3/ADAMTS9/TNC/THBS2/SERPINE1/ANGPTL2/SPARC

Category		Term	Description	Gene ID	Count	P-value
Upregulated	CC	GO:0031091	platelet alpha granule	SERPINA3/THBS2/SERPINE1/SPARC	4	1.34E-04
		GO:0101003	ficolin-1-rich granule membrane	SLC11A1/FPR1/ITGAX	3	7.11E-04
		GO:0031093	platelet alpha granule lumen	SERPINA3/SERPINE1/SPARC	3	9.35E-04
		GO:0042613	MHC class II protein complex	HLA-DPB1/HLA-DPA1	2	9.42E-04
		GO:0030666	endocytic vesicle membrane	SLC11A1/HLA-DPB1/HLA-DPA1/DMBT1	4	1.25E-03
		GO:0031258	lamellipodium membrane	CD44/PDPN	2	1.79E-03
		GO:0030139	endocytic vesicle	SLC11A1/HLA-DPB1/HLA-DPA1/DMBT1/SPARC	5	1.99E-03
		GO:0042611	MHC protein complex	HLA-DPB1/HLA-DPA1	2	2.32E-03
Downregulated	MF	GO:0008509	anion transmembrane transporter activity	SLC38A4/SLC9A2/GLRA2/SLCO4C1	4	5.33E-04
		GO:0031406	carboxylic acid binding	NOS2/CEMIP/GLRA2	3	1.50E-03
		GO:0043177	organic acid binding	NOS2/CEMIP/GLRA2	3	1.75E-03
		GO:0016597	amino acid binding	NOS2/GLRA2	2	1.86E-03
		GO:0015291	secondary active transmembrane transporter activity	SLC38A4/SLC9A2/SLCO4C1	3	2.04E-03
		GO:0008509	anion transmembrane transporter activity	SLC38A4/SLC9A2/GLRA2/SLCO4C1	4	5.33E-04

note:GO, Gene Ontology; DEGs, differentially expressed genes; BP, biological processes; MF, molecular functions; CC, cellular components

Table 3. The KEGG enrichment analysis of DEGs (top 10 terms according to *P*-value)

ID	Description	Genes	Count	P-value
hsa05323	Rheumatoid arthritis	IL1A/CXCL2/HLA-DPB1/IL1B/CXCL8/HLA-DPA1/CD80/FOS	8	1.26E-08
hsa05332	Graft-versus-host disease	IL1A/HLA-DPB1/IL1B/HLA-DPA1/CD80	5	1.68E-06
hsa04940	Type I diabetes mellitus	IL1A/HLA-DPB1/IL1B/HLA-DPA1/CD80	5	1.89E-06
hsa04640	Hematopoietic cell lineage	CD44/CD38/HLA-DPA1/IL1A/HLA-DPB1	6	7.81E-06
hsa05140	Leishmaniasis	IL1A/HLA-DPB1/IL1B/HLA-DPA1/FOS	5	3.44E-05
hsa04672	Intestinal immune network for IgA production	CXCR4/HLA-DPB1/HLA-DPA1	4	9.17E-05
hsa04933	AGE-RAGE signaling pathway in diabetic complications	IL1A/IL1B/CXCL8/EGR1/SERPINE1	5	1.21E-04
hsa04620	Toll-like receptor signaling pathway	SPP1/IL1B/CXCL8/HLA-DPA1/FOS	5	1.45E-04
hsa05321	Inflammatory bowel disease	IL1A/HLA-DPB1/IL1B/HLA-DPA1	4	2.77E-04
hsa05133	Pertussis	IL1A/IL1B/CXCL8/FOS	4	5.05E-04

note: KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes

Table 4. The GO analysis of genes in the significant module (top 5 terms according to *P*-value)

Category	Term	Description	Gene ID	Count	P-value
BP	GO:0070098	chemokine-mediated signaling pathway	CXCL12, CXCL8, CXCR4	3	6.23E-04
	GO:0006954	inflammatory response	CXCL12, CXCL8, IL1B, CXCR4	4	8.66E-04
	GO:0007155	cell adhesion	CXCL12, SELL, ITGAX, CD44	3	0.001507293
CC	GO:0009897	external side of plasma membrane	CXCL12, SELL, CD80, ITGAX, CD69	5	2.18E-06
	GO:0009986	cell surface	CD80, ITGAX, CD38, CXCR4, CD44	5	8.66E-05

note: GO, Gene Ontology; BP, biological processes; CC, cellular components

Figures

Figure 1

Overlapping DEGs between epilepsy and inflammatory bowel disease (IBD).

A total of 78 overlapping DEGs of the epilepsy dataset (GSE134697) and IBD (UC and CD) dataset (GSE59071) were identified by the Venn web tool. DEG, differentially expression genes; UC, ulcerative colitis; CD, Crohn's disease.

Figure 2

GO enrichment analysis of DEGs.

(a) BP GO terms for upregulated DEGs; **(b)** MF GO terms for upregulated differentially expressed genes (DEGs); **(c)** CC GO terms for upregulated DEGs; **(d)** MF GO terms for downregulated DEGs. GO, Gene Ontology; DEGs, differentially expression genes; BP, biological process; MF, molecular function; CC, cellular component.

Figure 3
KEGG pathway enrichment analysis of DEGs.
 The significant KEGG pathways for upregulated DEGs were shown. KEGG, Kyoto Encyclopedia of Genes and Genomes; DEG, differentially expression genes.

Figure 4
Gene Set Enrichment Analysis (GSEA).
 (a) KEGG pathways enrichment in the gene expression profile of epilepsy (GSE134697), ranking by P -value; (b) KEGG pathways enrichment in the gene expression profile of IBD (GSE59071) ranking by P -value; KEGG, Kyoto Encyclopedia of Genes and Genomes; IBD, inflammatory bowel disease.

Figure 5

Constructed PPI networks of the DGEs and identified candidate hub genes.

(a) The PPI network of DEGs was constructed using Cytoscape. The nodes meant proteins; the edges meant the interaction of proteins. Upregulated genes are marked in light red; downregulated genes are marked in blue. **(b)** The most significant module of the PPI network was identified using molecular complex detection technology (MCODE) plug-in. Candidate hub genes were *CXCL8*, *CXCR4*, *ITGAX*, *CD80*, *SELL*, *CD44*, *CD69*, *IL1B*, *CD38*, and *CXCL2*. PPI, protein–protein interaction; DEGs, differentially expressed genes.