TREM2 and CD163 ameliorate microglia-mediated inflammatory environment in the aging brain

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Abstract

Aging declines cognitive functions, especially learning and memory. Microglia-mediated neuroinflammation occurs in age-related neurodegenerative diseases. Our study obtained the expression profile of GSE11882 from the Gene Expression Omnibus database and used microarray data to explore the expression of age-related genes in the hippocampus. A total of 120 differentially expressed genes (DEGs) were screened out and subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. A protein-protein interaction (PPI) network was constructed. In Cytoscape software, 18 key genes were identified by the plugin cytoHubba. Two genes with positive impact on cognition during aging were teased out, including triggering receptor expressed on myeloid cells 2 (TREM2) and scavenger receptor (CD163). Finally, reverse transcription quantitative polymerase chain reaction (RT-qPCR) and western blot (WB) verified that the mRNA expression of two genes was significantly up-regulated in the Aged group. Moreover, the inflammatory factor IL-1β and IL-6 significantly increased. The up-regulation of TREM2 and CD163 may exert a compensatory effect on neurodegeneration by mitigating the inflammatory environment in the aging brain.

Introduction

Aging leads to physiological neurodegeneration in the brain. Their common features, such as accumulation of phosphoTau and amyloid peptide, release of pro-inflammatory mediator and presence of oxidative stress (Mecca et al.,, 2018, Teissier et al.,, 2020), can also be observed in the early stage of pathological neurodegeneration, such as Alzheimer's disease (AD). Studies have found that the annual incidence of AD increases sharply with age (Schrijvers et al.,, 2012, 2011). Therefore, efficient tools should be developed to diagnose pathological aging in the brain.

The cerebral cortex undergoes significant changes between ages 60 and 70, a critical time for the brain to degenerate (Berchtold et al.,, 2008). In the case of cognitive dysfunction, age-related genes are dysregulated in the frontal and temporal regions. Previous studies focus on those genes in the prefrontal/frontal cortex (Irizarry et al.,, 2003). But their expression profiles in other brain regions remain unclear. The hippocampus is responsible for cognitive functions, such as spatial learning and memory (Joaqu¨¨ªn et al.,, 2017). Therefore, the expression of age-related genes in the hippocampus may provide new information about neurodegenerative diseases.

Bioinformatics has been widely used to analyze pathogeneses of diseases. Berchtold NC et al used microarray analysis to obtain RNA expression profiles of genes in different brain regions in postmortem young with normal function and aged cases (Berchtold et al.,, 2008). In the present study, we used the microarray data to grope for the expression of age-related genes in the hippocampal region.

Materials And Methods

Microarray data

In the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.gov/geo/), the expression profile of GSE11882 was obtained. The microarray data consisted of brain samples of postmortem young and aged cases in the ADRC bank. We assessed gene expression profiles in the hippocampus in 43 cognitively intact individuals aged 20–99 years. The Young group (20–59 years) comprised 18 samples, and the Aged group (60–99 year) 25 samples.

Data preprocessing

R package Affy was used to read the raw data, and then a Robust Multichip Average (RMA) algorithm to normalize the data between samples (Irizarry et al.,, 2003). Then, the annotation package in R was used to convert the probe name to the gene name. When a gene corresponded to multiple probes, the median expression value of multiple probe IDs was taken.

Identification of differentially expressed genes (DEGs)

The Limma(Ritchie et al.,, 2015) software package in R was utilized to screen the DEGs between two groups. The DEGs were defined with $P < 0.01$, $q < 0.05$, and log2 |Fold Change|>0.9), Then, the volcano plot and heat map were constructed in R (http://cran.r-project.org/package).

Enrichment analysis of DEGs

The Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were performed via the Database for Annotation, Visualization and Integrated Discovery (DAVID,version 6.8) (https://david.ncifcrf.gov/). The threshold of $P < 0.05$ and enrichment counts > 2 were set as significant, the enrichment results were displayed in tables.

Analysis of the PPI network

PPI network analysis of DEGs was performed on the Search Tool for the Retrieval of Interacting Genes (STRING; version 11.0) database(https://string-db.org/) (Szklarczyk et al.,, 2017). Then Cytoscape software (Shannon et al.,, 2003) was used to visualize the network. The key genes were screened out via the plugin cytoHubba. The top 30 genes were ranked through the intersection of four algorithms: Maximal Clique Centrality (MCC), Maximum Neighborhood Component (MNC), Density of Maximum Neighborhood Component (DMNC) Degree(Lin et al.,, 2008) (Rajendran and Paolicelli, 2018). Then, 18 key genes were identified to have compensatory effects on neuroinflammation.

Animal experiments

Male C57BL/6J (weight, 20–25 g and 40-45g; n = 8) mice aged 3 were assigned to the Young group, 18 months to the Aged group, and housed in a constant milieu (temperature of 22 ± 2°C and a humidity of 50–70%), with a light/dark cycle of 12 hours, 2 weeks of adaptation, and free access to food and water. All animal experiments were approved by the Experimental Animals Welfare and Ethical Inspection of Nanjing Drum Tower Hospital (Nanjing, China) (No. 2019AE01077).

RNA extraction

The mice in two groups were decapitated, and the brain was hemi-dissected along the midline to collect hippocampal tissue. Total RNA was extracted from the hippocampus using RNA extraction kit (Bioteke, Beijing, China). UV spectrophotometry NanoDrop (NanoDrop Technologies; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to determine RNA concentration.

RT-qPCR

The Takara PrimeScript RT master mix (Takara Biotechnology Co, RR036A-1) was used to reversely transcribe RNA into cDNA. RT-qPCR was performed using ABI Step One Plus real-time PCR system (Thermo Fisher Scientific) and SYBR premixed Ex Taq (Takara Biotechnology Co., Ltd.) with 10 ul of reaction system. In addition, the reaction system (10 μl) contained SYBR-Green mixture (5 μl), forward primers (10 μM; 0.2 μl), reverse primers (10 μM; 0.2 μl), RNase-free water (3.6 μl) and cDNA (1 μl). Thermal cycles: initial denaturation, 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. Values were normalized to that of housekeeping gene-actin named β-actin. The expression level of genes was calculated by $2^{-\triangle\triangle Ct}$ method(Livak and Schmittgen, 2001). The following gene-specific primer sequences: β-actin forward, 5’-CTGTCCCTGTATGCCTCTG-3’ and reverse 5’-ATGTCACGATTTCC-3’. TREM2 forward,5’-CTGGAACCGTCACCATCACTC-3’and reverse 5’-CGAAACTCGATGACTCCTCGG-3’.CD163 forward, 5’-TACAATGGAGCTTGGGGCAG-3’and reverse5’- TGCCTCGATGGTGTCTTGTC – 3’.

Western blot analysis

The hippocampal tissue was weighted and washed with pre-cold PBS before lysis in RIPA buffer (no. P0013B, Beyotime, China) containing proteinase and phosphatase inhibitors (Sigma, USA), then homogenized using an ultrasonic crusher. The lysates were centrifuged at 12000rpm and 4 ℃ for 20 min. Bicinchoninic acid (BSA) method was performed to detect the protein concentration. The protein (30 ug) was separated by electrophoresis in 10% SDS-PAGE gels (KayGen Biotech, Co., Ltd), then transferred to polyvinylidene difluoride (PVDF; Bio-Rad Laboratories, USA) membranes. The PVDF membranes were incubated with 5% nonfat milk for 1 h for blocking nonspecific binding reactions. These primary antibodies were used for incubating membranes overnight at 4°C: TREM2 (13483-1-AP, proteintech,1:1000), CD163(ab182422,abcam,1:1000),IL-1β(ab9722,abcam,1:1000),IL-6(ab229381,abcam,1:1000),actin (bs-0061R, Bioss, 1:20000). On the next day, the membranes were washed with TBST for three times and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h. Finally, the protein bands were detected using a chemiluminescence kit (ECL; Pierce, Illinois, USA) and analyzed via ImageJ.

Statistical analysis

All experimental data were expressed as mean ± standard deviation. SPSS software version 24.0 (IBM Corporation, New York, USA) was used to perform t test. The number of experimental animals was

indicated by 'n'. The differences were considered statistically significant at the level of $P < 0.05$, and GraphPad Prism 7.0 was used to draw the graph.

Results

Identification of DEGs

With P value < 0.01, adjusted P (q) value < 0.05 and log2 | Fold Change |> 0.9, a total of 120 DEGs were identified between two groups, with 116 up-regulated and 4 down-regulated, as shown in the volcanic plot (Fig. 1A). The heatmap was constructed to perform clusters analysis (Fig. 1B).

GO and KEGG pathway analyses

Gene Ontology (GO) (Table I) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses (Table II) were preformed. The DEGs were mainly enriched in the plasma membrane (GO: 0005886) and extracellular space (GO:0005615) in the aspect of cellular component (CC). In the aspect of biological process (BP), the DEGs were mainly involved in regulation of inflammatory response (GO:0006954), phagocytosis (GO:0006909), immune response (GO:0006955). KEGG pathway enrichment analysis showed that the DEGs were enriched in Phagosome (hsa04145), complement and coagulation cascades (hsa04610) and cell adhesion molecules (CAMs) (hsa04514).

PPI network of DEGs

In our study, the PPI network was constructed based on the STRING database. After deleting disconnected nodes in the network, clusters of 97 genes were displayed by MCODE tool in Cytoscape(Fig. 2).

Identification of key genes

In Cytoscape, the top 30 most involved genes were ranked out with four algorithms (MCC, DMNC, MNC, Degree), and the intersection was considered as key genes (Table III, Fig. 3A). Finally, 18 genes were figured out. Based on Table IV, LY86 C3AR1 CD14 were found related to inflammatory response, and HLA-DRA HLA-DPA1 C1QC CD74 were related to immune response (Table IV, Fig. 3B). Interestingly, it was found that two of the key genes, TREM2 (Triggering receptor expressed on myeloid cells 2) and CD163 (scavenger receptor), could ameliorate inflammation in the normal aging brain.

RT-qPCR and Western Blot validation

RT-qPCR and WB were used to verify the reliability and accuracy of bioinformatics analysis. The expression levels of TREM2, CD163 and inflammatory cytokines IL-1β and IL-6 in the Aged group were significantly up-regulated, compared with those in the Young group ($P < 0.01$, $P < 0.05$) (Figure.4, Figure.5).

Discussion

Cognitive function declines as the brain ages, especially its hippocampus. In age-related diseases, neurogenesis is inhibited in the hippocampal dentate gyrus and the microglia are activated(Morel et al.,, 2015). Furthermore, aging exerts negative effects on neuronal growth and function through dysregulating the expression of genes in the hippocampus (Yan et al.,, 2015). In the present study, we explored age-dependent gene expression in the hippocampus and its functional changes. After bioinformatics analysis, TREM2 and CD163 were identified to express in an age-dependent manner. RT-qPCR and WB results were consistent with those of bioinformatics analysis.

We found a wide distribution in gene expression levels calculated by binary logarithm of fold-change (log2(FC)). Based on microarray data, we screened out 120 DEGs between two groups, with 4 down-regulated and 116 up-regulated, as shown in the volcanic plot (Fig. 1A) and heatmap (Fig. 1B). GO enrichment analysis indicated that these DEGs were mainly enriched in plasma membrane and cell surface (CC), immune response, inflammatory response, phagocytosis (BP) (Table I), which were consistent with the results of KEGG analysis (Table II). The PPI network was constructed using STRING database and visualized by Cytoscape software (Fig. 2). Among the 18 hub genes screened by the intersection of four algorithms in Cytoscape MCODE (Table III. Figure 3), most of the up-regulated genes were related to immune and inflammatory responses, which was further validated by the high-level IL-1β and IL-6 in the aging brain (Figure.5ADE). TREM2 and CD163 up-regulation can promote phagocytosis and the release anti-inflammatory mediators from the microglia. Therefore, we hypothesized that the TREM2 and CD163 may counter microglia-mediated neuroinflammation, thus mitigating age-related cognitive decline. Our RT-qPCR and WB experiments also confirmed the up-regulation of TREM2, CD163 and high-level IL-1β and IL-6 in the hippocampus of aged mice (Fig. 4, Figure.5).

The aged brain demonstrate accumulation of endogenous factors (such as fibrillar Aβ, pro-inflammatory cytokines) and up-regulation of complement components, inflammasomes and toll-like receptors (David et al.,, 2012). Microglia protect against neurodegenerative disorders by phagocytosing debris, removing protein aggregates, and assisting neural repair (Puigdell¨¨avol et al.,, 2020). Microglial activation is a hallmark of aged brain; meanwhile, their neuroprotective effects are impaired (Soto et al.,, 2015, Michelle et al.,, 2007). Microglia show a moderately activated phenotype in the normal human brain. In addition, reactive microglia are indispensable for neuroinflammation in AD, posing a high risk of cognitive decline (Azam et al.,, 2021, Hui-Ming et al.,, 2003, Mandrekar-Colucci and Landreth, 2010).

The triggering receptor expressed on myeloid cells 2 (TREM2), an innate immune receptor of the immunoglobulin superfamily, is mainly expressed in the myeloid cells and uniquely expressed by microglia in the central nervous system (Frank et al.,, 2008). The cytoplasmic portion of TREM2 associates with DNAX-activating protein of 12 kDa (DAP12, also called TYROBP), which is a type I transmembrane adaptor protein, forming a molecular complex for its signaling and functions (Daws et al.,, 2001, Humphrey et al.,, 2006). Studies have found that activated TREM2 can regulate the functions of microglia, including stimulating phagocytosis and suppressing cytokine production. Recent evidence also proves the crucial role of TREM2 in maintaining the expansion and survival of microglia(Kawabori et al.,, 2015, Zhong et al.,, 2015). Previous genome-wide association studies have shown that the rare mutation

(R47H) of TREM2 is a risk for AD (Colonna and Wang, 2016, Guerreiro et al.,, 2013, Tanzi, 2015); in addition, the silence of TREM2 in microglia impairs their capacity to phagocytize cell membrane debris, and increases the production of pro-inflammatory cytokines (Hsieh et al.,, 2009, Kleinberger et al.,, 2014). Microglia are also activated in the brain of cognitively normal elderly (Takahashi and K, 2005). Previous research has found the expression levels of TREM2 in the brain of AD patients and AD mice are positively correlated with age, and higher than those in the normal controls of the same age(Zhao, 2019, Piccio et al.,, 2016).

In our study, we found that the expression level of TREM2 was significantly up-regulated in the hippocampus of the Aged group, compared with the Young group (Fig. 4A, Fig. 5AB). This result can be explained by that the up-regulation of TREM2 compensatively enhances the ability of microglia to clear debris, reduce the stimulating effect of endogenous debris, and suppress the generation of pro-inflammatory factor. It also promotes the accumulation of microglia and strengthens their response to the breakdown of neurons astrocytes, oligodendrocytes and myelin, thus mitigating the inflammation in the aging brain. Based on this evidence, we hypothesize that TREM2 protects from age-related cognitive degeneration.

CD163, a member of scavenger receptor cysteine-rich (SRCR) family of class B, is expressed in macrophages, monocytes and microglia (Fabriek et al.,, 2005, Moestrup and M?ller, 2004). CD163 has a homeostatic capacity, and the soluble CD163 generated via the shedding of the outer domain can exert anti-inflammatory effects (Pey et al.,, 2014). Endogenous pro-inflammatory cytokines, such as IFN-γ and tumor necrosis factor-alpha (TNF-α) classic activation macrophages/microglia, can decrease the expression of CD163; while glucocorticoids, IL-6 and IL-10 can increase CD163 expression (Christa et al.,, 2000). In recent years, CD163 has been considered as a specific marker with strong anti-inflammatory properties in microglia (Gorp et al.,, 2010), monocytes and macrophages. CD163 is the haemoglobin-haptoglobin (HbHp) complex receptor, whereas its metabolites, such as bilirubin, free iron and CO released by HbHp degradation upon the action of HO-1 enzyme, have strong anti-oxidative and anti-inflammatory effects (Otterbein et al.,, 2003, Soares Miguel and Bach Fritz, 2009). CD163 not only acts as an anti-inflammatory marker, but also binds to its ligands to transduce signals, leading to the release of anti-inflammatory mediators, such as interleukin-10 (IL-10). The IL-10 further up-regulates the expression of CD163 and HO-1 in an autocrine or paracrine manner, forming a positive feedback loop involved in the clearance of haemoglobin (Hb) and prevention of extracellular Hb triggering inflammatory responses. In addition, the increases of CD163 immunoreactive microglia is a specific immune response to AD neuropathology. In the present study, we found a significant difference in CD163 expression between the Aged and Young groups ($P < 0.05$) (Fig. 4B, Figure.5AC). The upregulation of CD163 and its positive feedback loop with IL-10 are prominent in aged people, suggesting that the protection of CD163 against Hb-induced inflammation is associated not only with the scavenging of HbHp complexes from the extracellular milieu, but also with the active release of IL-10 and haem metabolites.

With age, more microglia are activated and their ability is reduced to engulf dead neurons, neuron residues or β-amyloid protein, thereby inducing oxidative stress and the overproduction of pro-

inflammatory mediators. In the aged, the up-regulation of TREM2 and CD163 may pose a compensatory effect on the pro-inflammatory environment in hippocampal microglial cells, which curbs the decline of cognitive function. The molecular mechanisms of TREM2 and CD163 in regulating microglia-mediated inflammatory milieu should be further explored, in order that appropriate interventions can be designed to counter age-related neuroinflammation and reverse age-related cognitive degeneration.

Abbreviations

TREM2
triggering receptor expressed on myeloid cells 2 CD163:scavenger receptor
GEO
Gene Expression Omnibus DEGs:differentially expressed genes
GO
Gene Ontology KEGG:Kyoto Encyclopedia of Genes and Genomes
PPI
protein-protein interaction AD:Alzheimer's disease
DAP12
DNAX-activating protein of 12 kDa

Declarations

Ethical Statemate:

Ethics approval and consent to participate

All animal experiments were approved by the Experimental Animals Welfare and Ethical Inspection of Nanjing Drum Tower Hospital (Nanjing, China). (approval no. 2019AE01077).

Consent for publication

All authors consent for publication in Journal of Molecular Neuroscience.

Availability of data and materials

The analysis of datasets generated during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that there are no conflicts of interest in this work.

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Authors' Contributions

Acquisition and analysis of data: Xue Han

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Citical revision of the manuscript for important intellectual content:Yujia Liu, Tianjiao Xia, Xiaoping Gu.

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Tables

Table I. Functional enrichment analysis for the DEGs

GO category	GO number	GO term	Number of differentially expressed genes	P-Value
CC	GO:0005886	plasma membrane	54	7.03E-09
CC	GO:0005615	extracellular space	27	1.25E-07
CC	GO:0005887	integral component of plasma membrane	27	3.27E-07
CC	GO:0009986	cell surface	24	1.91E-13
CC	GO:0070062	extracellular exosome	37	7.28E-06
CC	GO:0016021	integral component of membrane	47	0.002482099
BP	GO:0006955	immune response	17	1.48E-08
BP	GO:0045087	innate immune response	16	1.33E-07
BP	GO:0006954	inflammatory response	18	4.08E-10
BP	GO:0008284	positive regulation of cell proliferation	11	9.70E-04
BP	GO:0042130	negative regulation of T cell proliferation	4	0.001807211
BP	GO:0007165	signal transduction	17	0.00362507
BP	GO:0006909	phagocytosis	4	0.00381852
MF	GO:0005515	protein binding	69	0.00558168

CC, cellular component; BP, biological process; MF, molecular function; GO, Gene Ontology.

Table II. KEGG pathway enrichment analysis for the DEGs

KEGG term	Number of differentially expressed genes	KEGG number	P-Value
hsa05150	Staphylococcus aureus infection	15	5.51E-18
hsa05152	Tuberculosis	14	2.01E-09
hsa05140	Leishmaniasis	10	6.58E-09
hsa05322	Systemic lupus erythematosus	12	1.28E-08
hsa04145	Phagosome	12	4.18E-08
hsa05323	Rheumatoid arthritis	9	6.77E-07
hsa04610	Complement and coagulation cascades	8	1.69E-06
hsa05133	Pertussis	8	2.99E-06
hsa05168	Herpes simplex infection	9	1.50E-04
hsa04514	Cell adhesion molecules (CAMs)	8	1.92E-04
hsa05166	HTLV-I infection	9	0.001340516
KEGG, Kyoto Encyclopedia of Genes and Genomes.			

Table III. Top 30 genes and the intersection in the cytoHubba.

MCC, Maximal Clique Centrality. DMNC, Density of Maximum Neighborhood Component. MNC, Maximum Neighborhood Component.

Table IV. Intersection of DEGs (inflammatory response and immune response) with key genes. BP, biological process.

Figures

		Hub genes
Algorithms	**MCC**	ITGB2 CD53 FCER1G VSIG4 CD163 PTPRC SRGN HLA-DRA CD74 CD93 C1QC C1QB RNASE6 C1QA LAPTM5 CD86 TREM2 TYROBP FCGR2A HLA-DPA1 ALOX5AP CLEC7A LY86 CLEC5A CD14 GS1 TLR2 FPR1 C3AR1 HCLS1
	MNC	ITGB2 CD53 FCER1G VSIG4 CD163 PTPRC SRGN CD44 HLA-DRA CD74 CD93 C1QC C1QB C1QA LAPTM5 CD86 C3 TREM2 TYROBP FCGR2A HLA-DPA1 ALOX5AP CLEC7A LY86 CLEC5A CD14 TLR2 FPR1 C3AR1 HCLS1
	DMNC	C1R CD53 FCER1G CD163 GPR34 C1orf162 HLA-DRA TMEM176B CD74 APLNR CD93 S1PR3 C1QC MS4A7 C1QB RNASE6 C1QA LAPTM5 SLC2A5 TREM2 FCGR2A HLA-DPA1 ALOX5AP CLEC7A LY86 CLEC5A CD14 RGS1 C3AR1 HCLS1
	Degree	ITGB2 CD53 FCER1G VSIG4 CD163 PTPRC SRGN CD44 HLA-DRA CD74 CD93 C1QC C1QB C1QA LAPTM5 CD86 C3 TREM2 TYROBP FCGR2A HLA-DPA1 ALOX5AP LY86 CLEC5A CD14 RGS1 TLR2 FPR1 C3AR1 TIMP1
	Intersection	TREM2 LAPTM5 HLA-DRA HLA-DPA1 C1QA CD53 CD163 LY86 C3AR1 ALOX5AP CD93 C1QB FCER1G FCGR2A CLEC5A CD14 C1QC CD74

Inflammatory response (GO:0006954)	BCL6 CLEC7A CD14 FAS S100A8 ANXA1 CHI3L1 C3 C3AR1 CRH FPR1 ITGB2 LY86 LY96 SERPINA3 S1PR3 TLR2 TGFB1
Immune response (GO:0006955)	CD74 CD86 FAS FCGR1B GEM C1QC C1R C3 IFITM2 IL1R HLA-DMA HLA-DPA1 HLA-DPB1 HLA-DRA HLA-DRB4 RGS1 TLR2
Key genes	TREM2 LAPTM5 HLA-DRA HLA-DPA1 C1QA CD53 CD163 LY86 C3AR1 ALOX5AP CD93 C1QB FCER1G FCGR2A CLEC5A CD14 C1QC CD74
BP-inflammatory response VS Key genes	LY86 C3AR1 CD14
BP-immune response VS Key genes	HLA-DPA1 HLA-DRA C1QC CD74

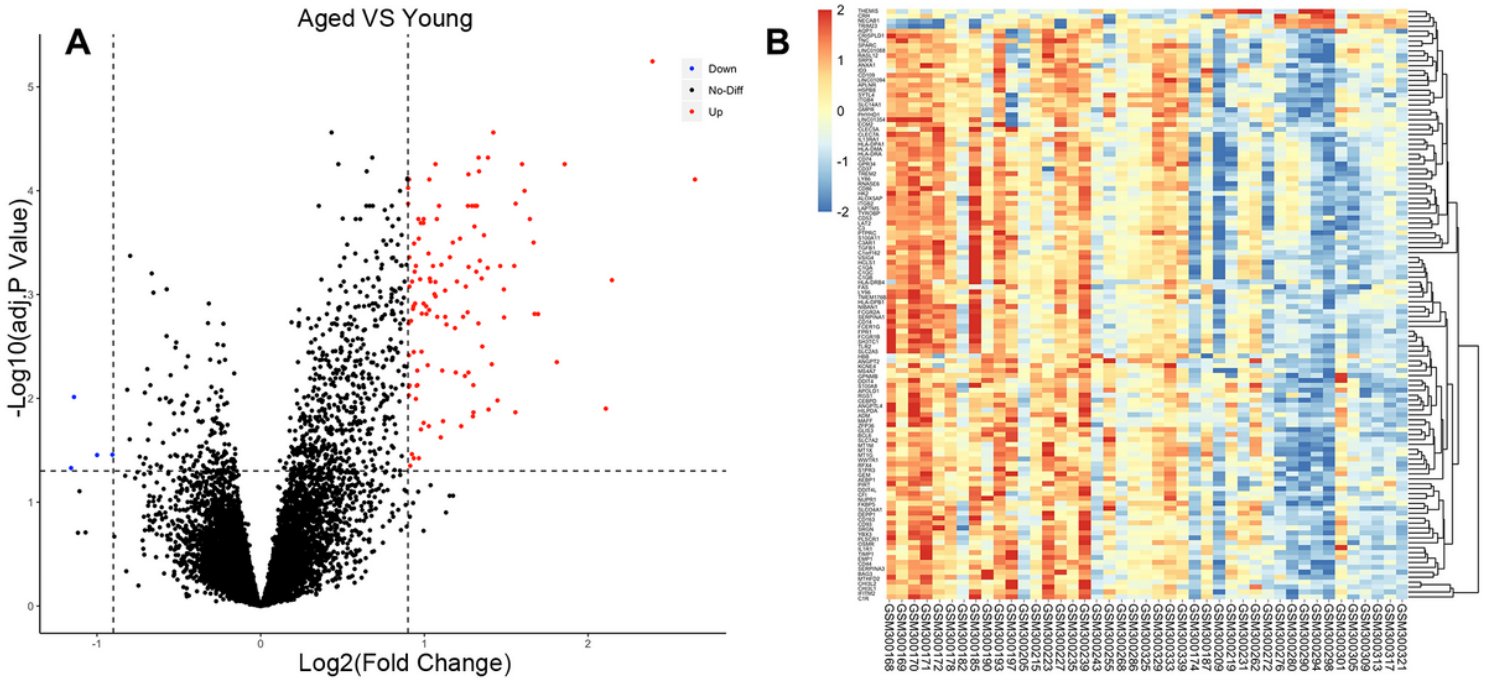

Figure 1

Volcanic plot and heat map analysis of identified DEGs. (Fig.1A) Volcanic plot of the expression of DEGs for the Aged and Young groups in the gene profiles, significantly up-regulated genes are indicated in red, down-regulated in blue, black indicates no significant change. (Fig.1B) The clustering pattern and heat map concerning the common DEGs. the upregulations of genes expression are indicated in red, the downregulations in blue.

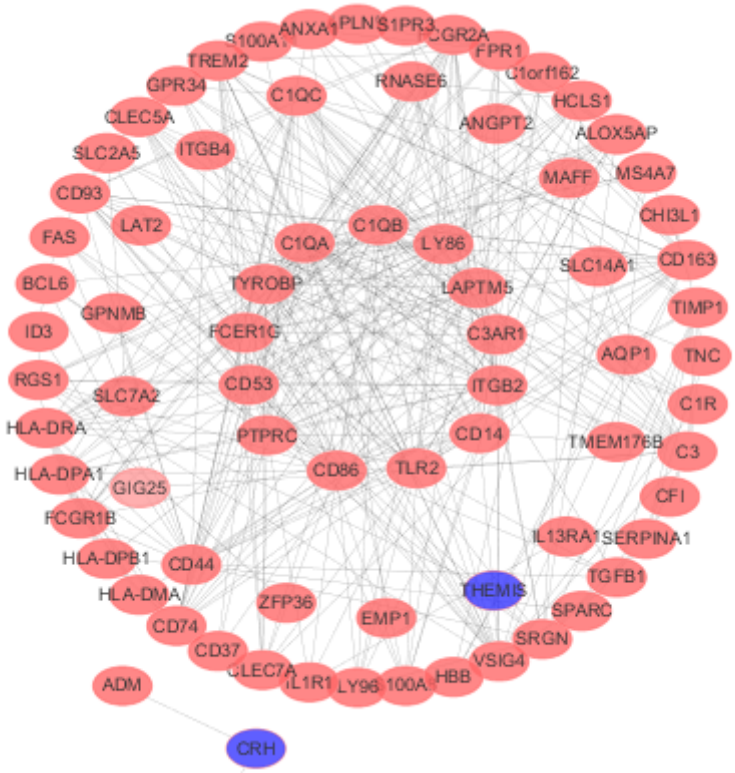

Figure 2

PPI network and clusters of DEGs. DEGs in protein-protein interaction network. Red nodes indicate genes that are up-regulated and blue nodes indicate genes that are down-regulated (Fig.2). the external and internal circles indicate different clusters in the whole PPI calculated by MCODE tool.

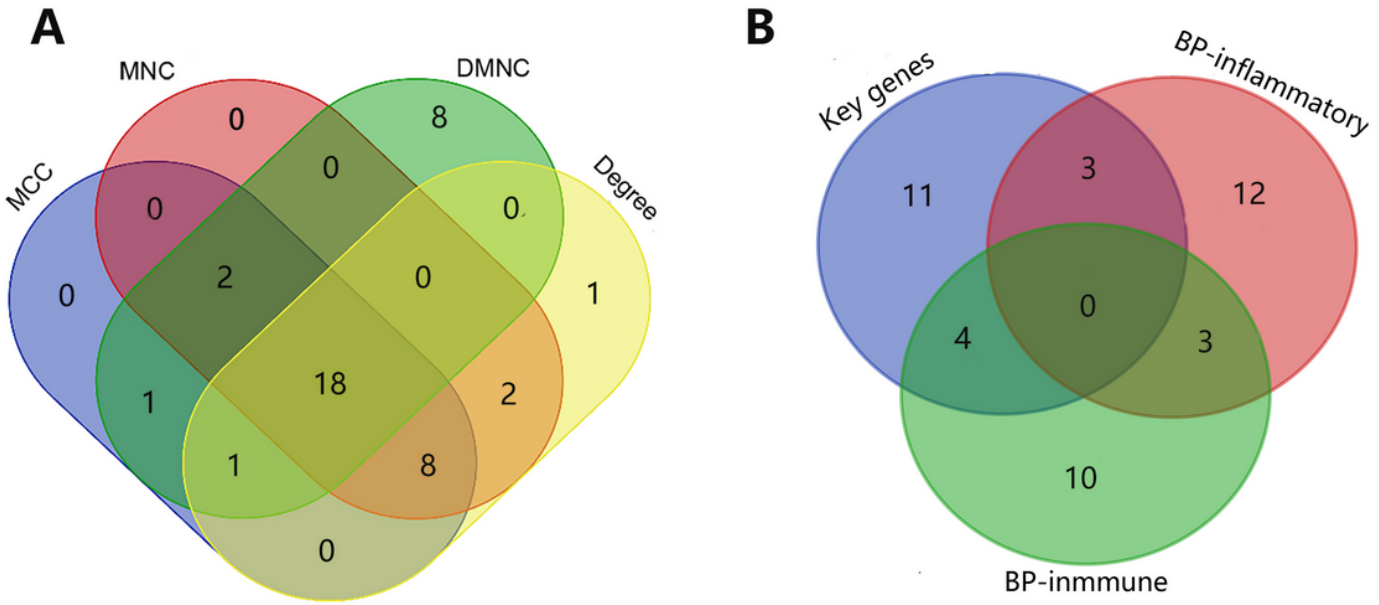

Figure 3

Venn diagrams for identification of key genes (Fig.3). 18 key genes were identified via the intersection of four algorithms (Figure,3A). Intersection of key genes and immune-inflammatory response in BP terminology to obtain immune-inflammation-related genes (Figure,3B).

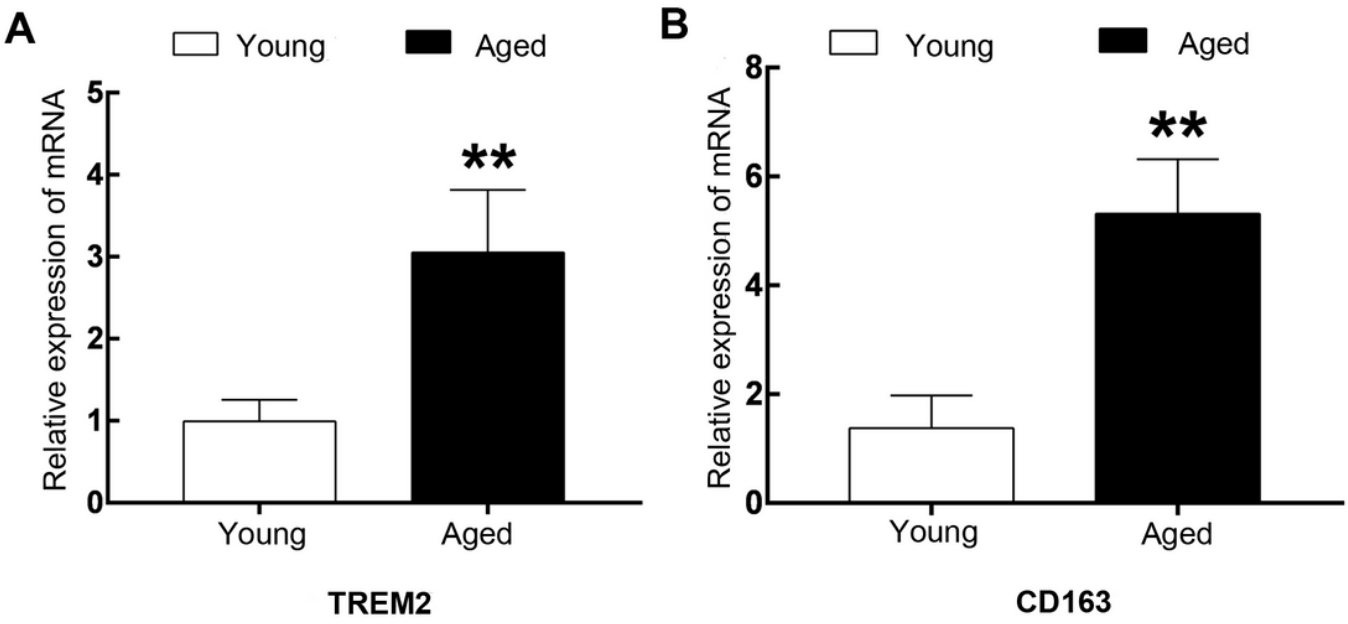

Figure 4

The key genes are expressed in both young and aged mice. The expression of TREM2 and CD163 mRNA were detected by reverse transcriptional quantitative polymerase chain reaction. TREM2: Comparison between Young group and Aged group, P<0.01(Fig.4A); CD163: Comparison between Young group and Aged group, P<0.01(Fig.4B).

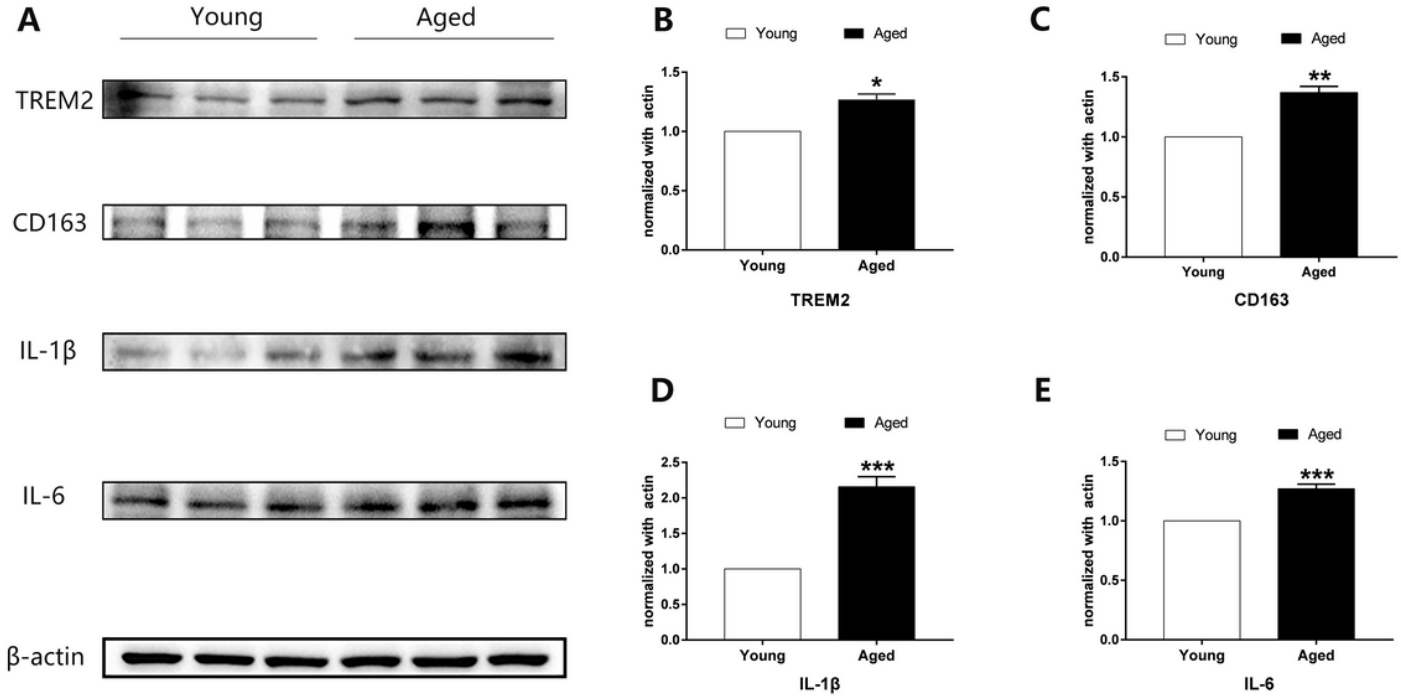

Figure 5

The key genes expression of TREM2 and CD163 and inflammatory factors IL-1β and IL-6 were assessed through western blot, and in the Aged group was significantly up-regulated, compared with the young group (Figure,5)