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MiR-18a-LncRNA NONRATG-022419 pairs targeted PRG-1 regulates diabetic induced cognitive impairment by regulating NGF\BDNF-Trkb signaling pathway

Qiong Xiang[1], Hu Lin[2†], Jia-Sheng Tao[1†], Chuan-Jun Fu[1†], Li-Ni Liu[1], Jing Deng[1] and Xian-Hui Li[2*]

Abstract

Background Diabetic encephalopathy (DE) is considered as one of the complications of diabetes,which is associated with cognitive impairment in the pathological process of development. Up to now, phospholipid phosphatase related 4 (Plppr4), also known as plasticity related gene 1 (PRG-1) has been revealed its important role in neuroplasticity. However, the underlying mechanisms of Plppr4 on the basis of diabetic-induced cognitive dysfunction (DCD) are still unknown. The aim of current study was to provide insight into molecular mechanism and cellular heterogeneity underlying DCD, and investigate the functional role of PRG-1 involved in this process.

Methods Combined Single-cell RNA sequencing (scRNA-seq) and RNA transcriptome analysis, the distinct subpopulations, functional heterogeneity as well as potential enriched signaling pathways of hippocampal cells could be elucidated.

Results We identified the sub-cluster of type I spiral ganglion neurons expressed marker gene as Amigo2 in cluster8 and Cnr1 in cluster 9 of hippocampal cells from DCD and the effect of those on neuronal cells interaction. We also found that PRG-1 was involved in the synaptic plasticity regulation of hippocampus via NGF\BDNF-Trkb signaling pathway. In high glucose induced HT22 cells injury model in vitro, we investigated that down-regulated PRG-1 along with down-regulated BDNF and also decreased expression of synapsin-1, PSD-95, SYN which are related to synaptic plasticity; Meanwhile, the *Prg-1* targeted miR-18a-LncRNA NONRATG-022419 pairs related with significantly down-regulated expression of PRG-1.

Conclusion This study revealed the synaptic plasticity regulation of PRG-1 in DCD, and might provide the therapeutic target and potential biomarkers for early interventions in DCD patients.

Keywords Diabetic encephalopathy, Diabetic induced cognitive dysfunction, Hippocampus, Synaptic plasticity regulation, Type I spiral ganglion neurons, PRG-1

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Introduction

Diabetes encephalopathy is known as the impairment of the function in brain, which is associated with changes of morphological structure, synaptic plasticity as well as neuronal signaling transduction [1–3]. Diabetic-induced cognitive dysfunction (DCD), one of the symptom of diabetes encephalopathy, is received considerable attentions [4], including learning and

memory deficits, impairs in problem-concern and resolve as well as information processing slows down [5]. In addition, lots of clinical research have demonstrated that DCD is more close to Alzheimer's disease (AD) and vascular dementia [6, 7]. A great deal studies have indicated that altered neuronal synaptic plasticity, changes of vascular integrity, neurogenesis and inflammation that might play a critical role in the pathological process of DCD [8]. As we known, hippocampus has an important functional role in learning and memory, and its dysfunction is related to DCD [9]. However, the more detailed mechanism of cognitive impairment induced by diabetes have not been clarified clearly. Because the cognitive impairment caused by hyperglycemia is irreversible, the hippocampal microenvironment and condition will not recover when it occurs, so it should be prevented and treated as soon as possible [10].

Comprehensive analysis of heterogeneity in hippocampus is s suitable way to discover the changes of DCD in the pathological progress [11], and the scRNA-seq has the advantage of integrated analysis of single cell transcriptome, and cells can be divided into different subgroups or sub clusters according to cell characteristics [12]. Combined with the whole RNA transcriptome and integrative enrichment analysis, showing the transcriptional and heterogeneous signature, we could have an opportunity to identify different cell types and different genes expression in hippocampals cells from DCD and control groups, and further investigate the regulatory mechanisms to clarify the heterogeneity and function [13].

Some studies have found that phospholipid phosphatase related 4(Plppr4), also known as Lppr4, plasticity related gene 1 (PRG-1), a neuron membrane protein located at the postsynaptic density of glutamatergic synapses has role of plasticity in central nervous system [14, 15]. Unichenko et al. reported that prg-1 affects mouse barrel cortex function via strengthening of glutamatergic thalamocortical transmission [16], and PRG-1 deficiency increases the probability of presynaptic glutamate release, leading to excessive neuronal excitation [17]. Our current study also investigated that PRG-1 was down-regulated expression in hippocampals of both GK diabetic and db diabetic animals with cognitive impairments. And then further analysis and validation experiments were performed to explore the details on PRG-1 involved in the development of DCD. These findings might provide the potential targets for DCD in clinic.

Materials and methods

Animals

All the animal experiments were studied under the requirements of the National Laboratory Animal Use Act of China and approved by the Laboratory Animal Use Committee of the Medical Research Institute of Jishou University (NO:JSDX-2021–0004). The animals were obtained from Changzhou Kawensi Laboratory Animal Company (Changzhou, China). They are kept in a temperature-controlled room (23 ± 1 °C) in a 12 h light/dark cycle and allow free to food and water.

New object recognition (NOR) tests

NOR tests the ability of animals to recognize and remember new objects. This experiment is divided into three stages: adaptation period, familiarity period and testing period.The day before the experiment, the animals are placed in a testing box to familiarize themselves with and adapt to the environment for 5–10 min. After the adaptation period ends, 24 h are needed to prepare three objects for testing. Two of them are blocks of the same color, and the other is a triangular block. These three objects are tasteless, and a familiarization period test is conducted, Place two identical blocks in the adjacent wall corners of the experimental box, with the animal facing its back towards the object and placing it in the box. Ensure that the animal is equidistant from the two block objects, and then allow the animal to explore freely for 5 min. After the familiarization period ends for 1 h, enter the testing period. Replace one of the blocks with a third new object of the same color but with a different shape (the triangular block), observe and record how long the animals recognized the new object, record how long the animals explored the new and familiar (old) object during the test, and use the recognition index to assess the animals' ability to recognize and remember the new object. Recognition index = recognition time of new things/(recognition time of new things + recognition time of old things).

Tissue prepared and RNA-transcript Sequencing

After the NOR tests, hippocampal tissues were dissected from both $GK^{-/-}$ diabetic rats with cognitive impairment ($n = 3$) and WT($n = 3$). Total RNA from the each sample was extracted and quantified. Then 1ug of the total RNA was for library preparation. Next-generation sequencing library preparations were constructed. Then the cDNA was synthesized by kits and purified by beads and fragments of approximate 400 bp were recovered. Each sample was then amplified by PCR and

the libraries with different indexes were multiplexed and sequenced by Illumina NovaSeq 6,000 instrument according to manufacturer's instructions.

scRNA-seq and data analysis

Following as the NOR tests, combined single-cell suspensions from DCD ($n=3$) and control mice ($n=3$) were transformed into a Chromium single-cell controller instrument (10×Genomics, Shenzhen, BGI, Inc.) to prepare droplets. The scRNA-seq library was prepared with chromium single cell 3 'reagent V3 kit and single cell chip kit. After droplet encapsulation, emulsion rupture, mrna capture bead collection, reverse transcription, cDNA amplification and purification. According to the manufacturer's protocol, the sequencing libraries were constructed and quantified on the Illumina HiSeq X Ten system (BGI Shenzhen) and sequenced via the DNBSEQTM platform. The raw sequencing data and cell barcodes were demultiplexed by using the Cell Ranger software pipeline and STAR comparison. To produce a matrix of gene counts and cells, the sample needs to be read down to produce normalized aggregated data between samples. The Unique Molecular identifier (UMI) count matrix is processed by using R package Seurat. The gene expression matrix from the sample was filtered and identified to remove low-quality cells and multiple chromosomes, as well as cells with > 200 genes and < 8,000 genes; 400 UMI and < 20% mitochondrial RNA (mtRNA) were retained. Principal component analysis (PCA) was performed on the highly variable genes, and select the top principal component (PC) for cell clustering. The clustering results are visualized by Unified Manifold Approximation and Projection (UMAP). Compared with typical markers of cell types, 9 cell types distributed in 16 distinct cell clusters. To explore changes of cellular communication in hippocampal cells during DCI, CellPhoneDB was performed to identify the relevant ligand -receptor pairs (21). *P* value < 0.05 and |log2foldchange| > 0.5 were set as the differential expression thresholds. Differentially gene expression (DEGs) was subjected to enrichment analysis(GO and KEGG pathway), and the PPI network of DEGs was constructed by STRING (https://string-db.org/).

Quantitative RT-PCR and western blot

All the samples of the hippocampus were immediately isolated from the the brain on ice, for quantitative RT-PCR, total RNA was extracted using the Trizol protocol (Invitrogen, San Diego, CA, USA) and the amount of cDNA was

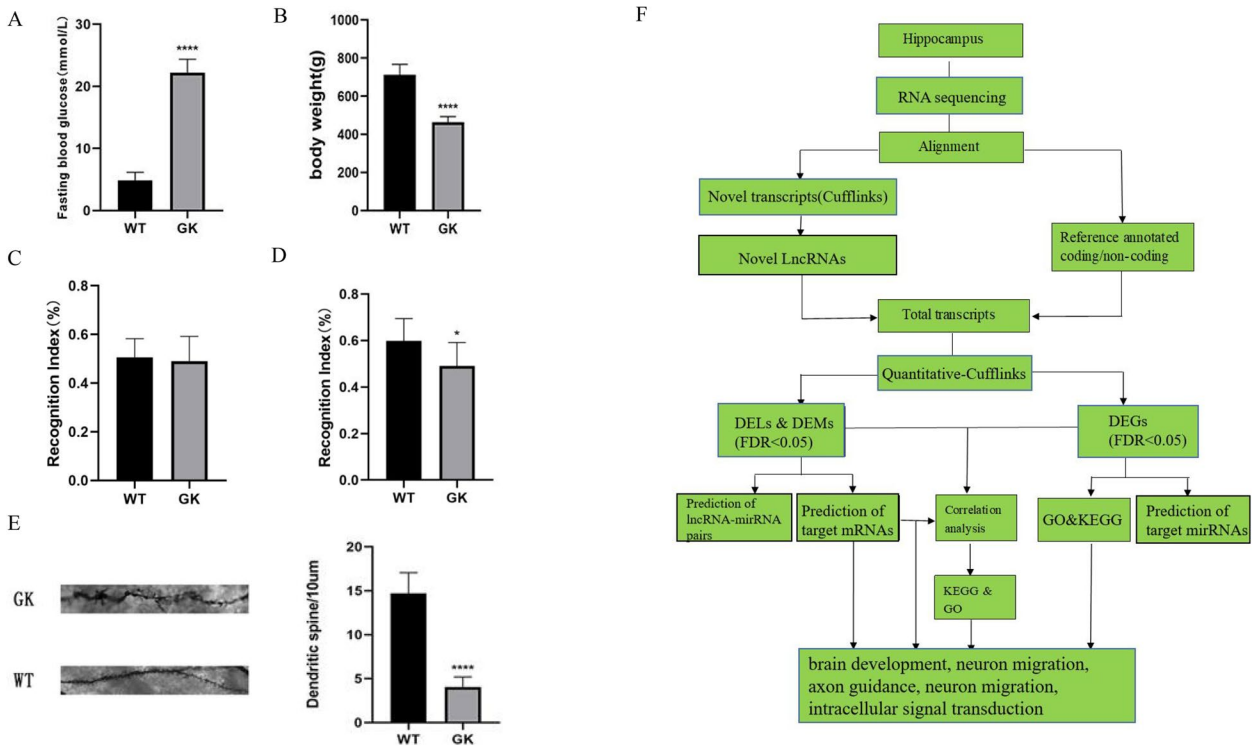

Fig. 1 Cognitive impairments in diabetic $GK^{-/-}$ rats and the workflow of full transcriptome analysis. **A-D** The new object recognition(NOR) tests were performed to detect recognition of new objects and memory ability in animals. The blood glucose and body weight were tested before experiments (**A, B**). During the training phase, the recognition index showed no significant difference between groups (**C**); And the recognition index of $GK^{-/-}$ rats declined significantly in testing phase, compared to WT group (**D**). **E** Golgi staining showed the density of dendritic spine in the $GK^{-/-}$ group compared to the WT group. **F** The workflow of full transcriptome analysis

quantified by real-time PCR. Gapdh cDNA levels were analyzed for normalization. Ct values from each sample were obtained. The amplification was performed by PCR reaction. For western blot, the samples were lysis in RIA buffer with protease inhibitor cocktail for 30 min on ice and then centrifuged. The total protein was collected and performed for SDS-PAGE and then transferred to PVDF membranes. The membranes were blocked and incubated with primary antibodies overnight at 4 °C and followed by the incubation of secondary antibodies for 1 h at RT. The bands were detected and the intensity of bands were quantified. Primary antibodies included rabbit anti-PRG-1 (1:500; Abcam); rabbit anti-BDNF (1:1000; Abcam); rabbit anti-PSD-95 (1:1000; Cell signaling); rabbit anti-SYN1 (1:1000; proteintech); rabbit anti-SYN (1:1000; proteintech); rabbit anti-Akt (1:1000; Cell signaling); rabbit anti-pAkt (ser473)(1:1000; Cell signaling);rabbit anti-ERK (1:1000; Cell signaling); rabbit anti-pERK(Thr202/Tyr204) (1:1000; Cell signaling); rabbit anti-GAPDH (1:5000; Abcam);rabbit anti-β-Actin (1:5000; proteintech);rabbit anti-Tublin (1:5000; proteintech). Secondary antibodies included goat anti-rabbit IgG(1:5000, Beijing bioss).

Statistical analysis

Statistical analysis was performed by GraphPad Prism Software. The two-sample comparisons was used by Student's T tests, and the one-way ANOVA or Tukey post hoc test was selected for multiple comparisons. The P value < 0.05 was accepted for significance.

Results

Cognitive impairments in diabetic $GK^{-/-}$ rats

The new object recognition(NOR) tests were performed to detect recognition of new objects and memory ability in animals. The blood glucose and body weight were tested before experiments (Fig. 1A-B). During the training phase, the recognition index showed no significant difference between groups (Fig. 1C); Meanwhile, the recognition index of $GK^{-/-}$ rats declined significantly in testing phase, compared to WT group (Fig. 1D). Interestingly, the density of dendritic spine in the $GK^{-/-}$ group presented significantly low compared to the WT group (Fig. 1E). The representative images of dendrite by Golgi staining were illustrated in Fig. 1E. In a word, both behavioral and morphological results showed that $GK^{-/-}$ rats developed cognitive deficits concomitantly. And then, we performed full transcriptome analysis of hippocampal tissues from WT and $GK^{-/-}$ rats.

DEGs analysis in $GK^{-/-}$ rats with cognitive impairment vs WT rats

The workflow of full transcriptome analysis was showed in Fig. 1F; Based on differentially expressed lncRNAs (DELs) and miRNAs (DEMs) in hippocampus of $GK^{-/-}$ rats with cognitive impairment compared with control WT rats (Fig. 2A-B); the differentially expressed genes (DEGs) was showed in Fig. 2C, and *Prg-1* is one of the down-regulated expressed genes; And further, the functional KEGG pathway enrichment (Fig. 2D) and GO enrichment were found in Fig. 2E-F, including axon regeneration, Wnt signaling pathway, PI3K-Akt signaling pathway and cAMP signaling pathway; brain development, axon guidance, phosphorylation, GTPase activator, glutamatergic synapse, postsynaptic density and neuronal cell body etc.; The PPI networks were constructed by the up-regulated expressed genes (Fig. 2G) and down-regulated expressed genes (Fig. 2H). To be noted, PRG-1 is down-regulated

Table 1 Functional annotation information of PRG-1

Description		
Molecular Function	GO:0005515	protein binding
	GO:0042577	lipid phosphatase activity
	GO:0008195	phosphatidate phosphatase activity
	GO:0052642	lysophosphatidic acid phosphatase activity
Cellular Component	GO:0005886	plasma membrane
	GO:0045202	synapse
	GO:0045211	postsynaptic membrane
	GO:0098839	postsynaptic density membrane
	GO:0098978	glutamatergic synapse
Biological Process	GO:0007186	G protein-coupled receptor signaling pathway
	GO:0007409	axonogenesis
	GO:0051966	regulation of synaptic transmission, glutamatergic
	GO:0050804	modulation of chemical synaptic transmission
	GO:0007165	signal transduction
Interactions	BioGRID:201851	Nrxn1
	BioGRID:199230	Dlg4 also known as PSD-95
	BioGRID:201403	Met also known as HGF
	BioGRID:201869	Ntrk2 also known as Trkb
	BioGRID:202370	Prkcsh
	BioGRID:200059	Gria2
	BioGRID:200069	Grin2b

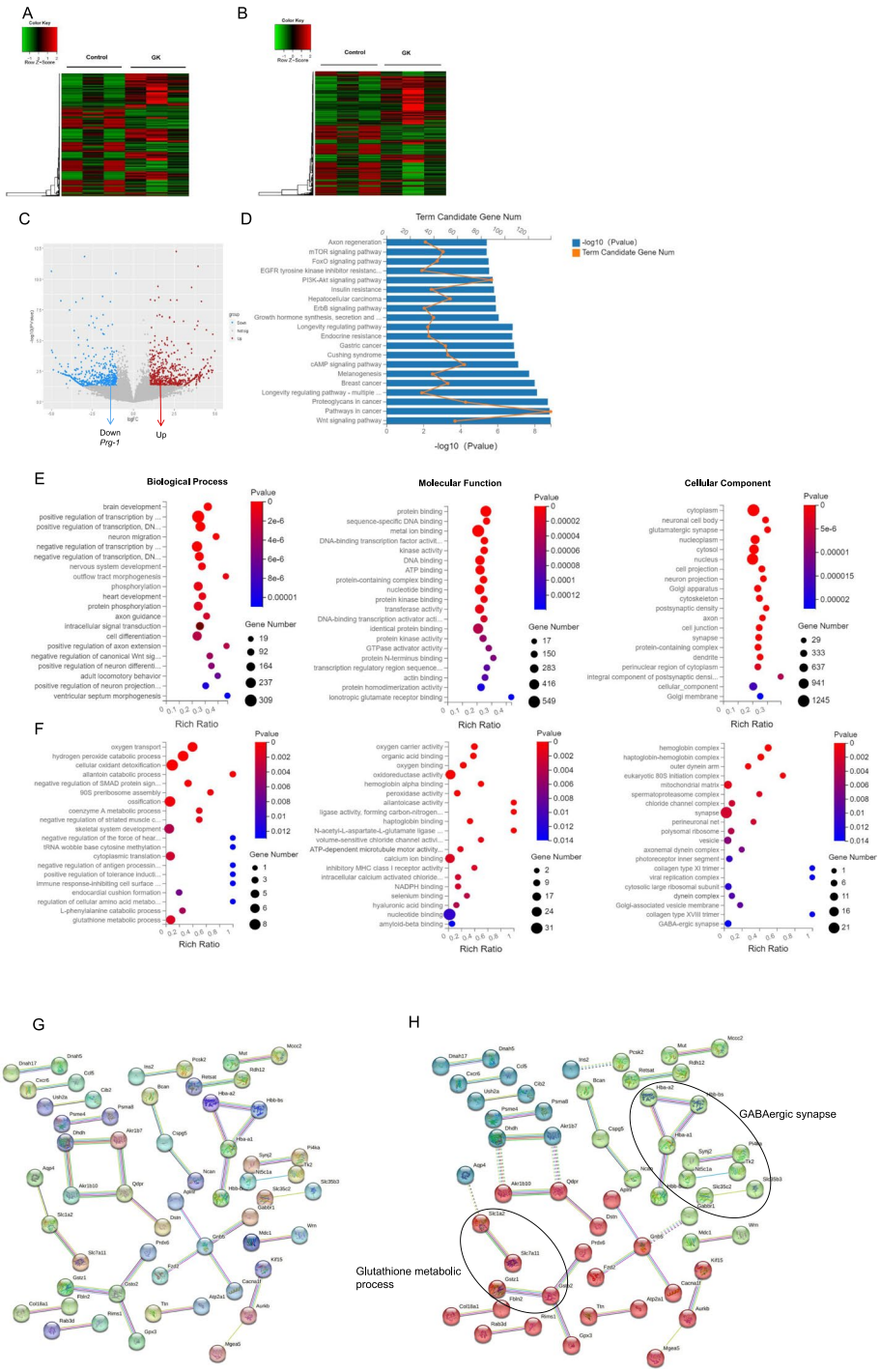

Fig. 2 The differentially expressed lncRNAs and miRNAs in hippocampus of GK$^{-/-}$ rats compared with control WT rats. **A** Heat map of differentially expressed lncRNAs (DELs) in hippocampus of GK$^{-/-}$ rats compared with WT rats. **B** Heat map of differentially expressed miRNAs (DEMs) in hippocampus of GK$^{-/-}$ rats compared with WT rats. **C** Volcanic scatter diagram of differentially expressed genes (DEGs) in hippocampus of GK.$^{-/-}$ rats compared with WT control rats (Blue are down-regulated genes and Red are up-regulated genes). And the functional KEGG pathway enrichment and GO enrichment were found in (**D-F**), including axon regeneration, Wnt signaling pathway, PI3K-Akt signaling pathway and cAMP signaling pathway; brain development, axon guidance, phosphorylation, GTPase activator, glutamatergic synapse, postsynaptic density and neuronal cell body etc.; The PPI networks were constructed by the up-regulated expressed genes (**G**) and down-regulated expressed genes (**H**)

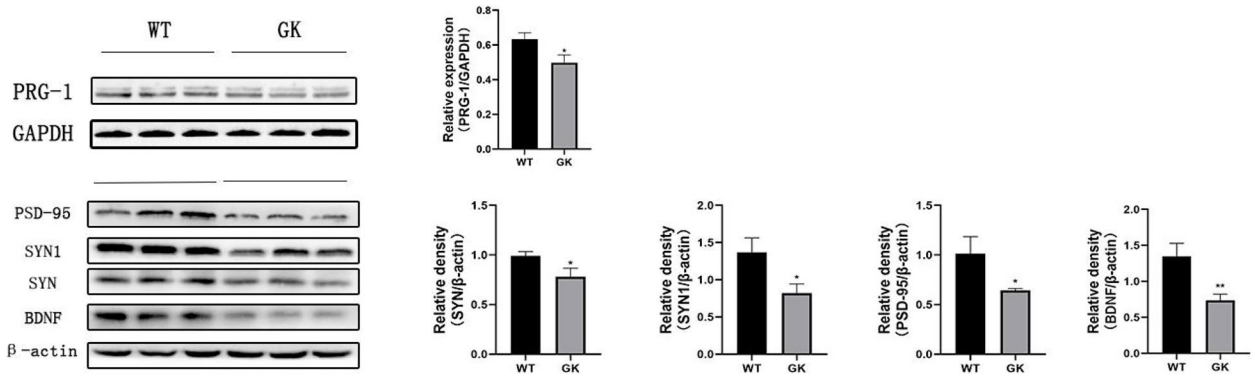

Fig. 3 Expression of PRG-1 and synaptic ralated protein in WT and GK with DCD

expressed, located in postsynaptic density membrane, interacted with Nrxn1, PSD-95, HGF, Trkb, Plc, Gria2, Grin2b; which has participated in axonogenesis, regulation of synaptic transmission and glutamatergic synapse etc. (Table 1). In addition, western blot were used to analyze the expression of BDNF (BNDF/Trkb pathway),which has protective role in nervous system [19, 20]. Down-regulated expressed synaptic plasticity associated protein (PSD-95, Syn and Syn-1) and p-Akt (PI3k-Akt pathway) in $GK^{-/-}$ rats hippocampus compared to the WT group rats, suggesting the altered synaptic plasticity in the $GK^{-/-}$ rats (Fig. 3).

scRNA-seq analysis in $db^{-/-}$ mice with cognitive dysfunction(DCD) vs WT mice

In order to detect and analyse heterogeneity of hippocampal cells between DCD and normal WT mice, scRNA-sep analysis was performed. Dissected hippocampus from DCD and WT mice were dissolved to single cell suspensions. The workflow is shown in Fig. 4A. The PCA and UMAP analysis was applied after quality control, the heat map showed top15PC in DCD vs WT(Fig. 4B) And *Plpp3* is also expressed significantly between WT and DCD groups. According to the well-expressed markers, the cells were divided into 9 distinct cell types and distributed in 16 distinct clusters (Fig. 4C); The 9 distinct cell types were annotated as followed: Microglial cell, Endothelial cell, type II spiral ganglion neuron, Oligodendrocyte, type IC spiral ganglion neuron, type I spiral ganglion neuron, Astrocyte, Oligodendrocyte precursor cell, Chandeller cell. Figure 4D showed the relative abundance of each cell type in DCD and WT. The scRNA-Seq data in this paper, compared with WT, the number of genes in type I spiral ganglion neuron and type IC spiral ganglion neuron in DCD mice are more abundant compared to any other cell types (Fig. 4E); In addition, cluster8(expressed marker gene as Amgo2) and cluster9 (expressed marker gene as Cnr1), which are belongs to type I spiral ganglion neuron (Fig. 4F), However, cluster 6 could not be definitely annotated as type I spiral ganglion neuron and was eliminated in further analysis according to the expression feature of marker genes in each cluster in Fig. 4G. Meanwhile, the time series analysis of cluster 8 and cluster9 development in DCD mice compared to WT, which presented at the late period of time series (Fig. 4H).

Functional enrichment analysis of cluster8 and cluster9 between DCD and WT

Compared to WT, the $db^{-/-}$ mice microglia, 16 genes were differentially expressed in cluster8 of the DCD mice, of which 6 genes were up-regulated and 10 genes were down-regulated; As seen in cluster9, which 17 genes were up-regulated and 18 genes were down-regulated. GO term analysis of DEGs cluster8 and cluster9 showed enrichment of biological processes, molecular function and cellular component including G-protein coupled glutamate receptor signaling pathway,synaptic signaling via neuropeptides, synaptic vesicle targeting, canonical Wnt signaling pathway involved in positive regulation of cell–cell adhesion and migration, calcitonin receptor binding,structural constituent of presynaptic active zone,neuronal cell body,axonal spine,synaptic vesicle lumen and dendritic microtubule etc.,which were more associated with synaptic plasticity in cognitive deficits induced by diabetics (Fig. 5A-B). Further, PPI networks were constructed by DEGs of cluster8 and cluster9, and their interaction items related to neuroactive ligand-receptor interaction, positive regulation of synaptic transmission, neuron projection development, and positive regulation of glutamatergic synapse (Fig. 5C, Table 2). Interestingly, as shown in PPI network of cluster8, Plpp3 has interacted with Ngfr and could be involved in regulation of neuroactive ligand-receptor mediated signaling pathway. Since Plpp3 and PRG-1 belongs to the phospholipid phosphatase family, which might provide important clue to the functional analysis of PRG-1 in further study.

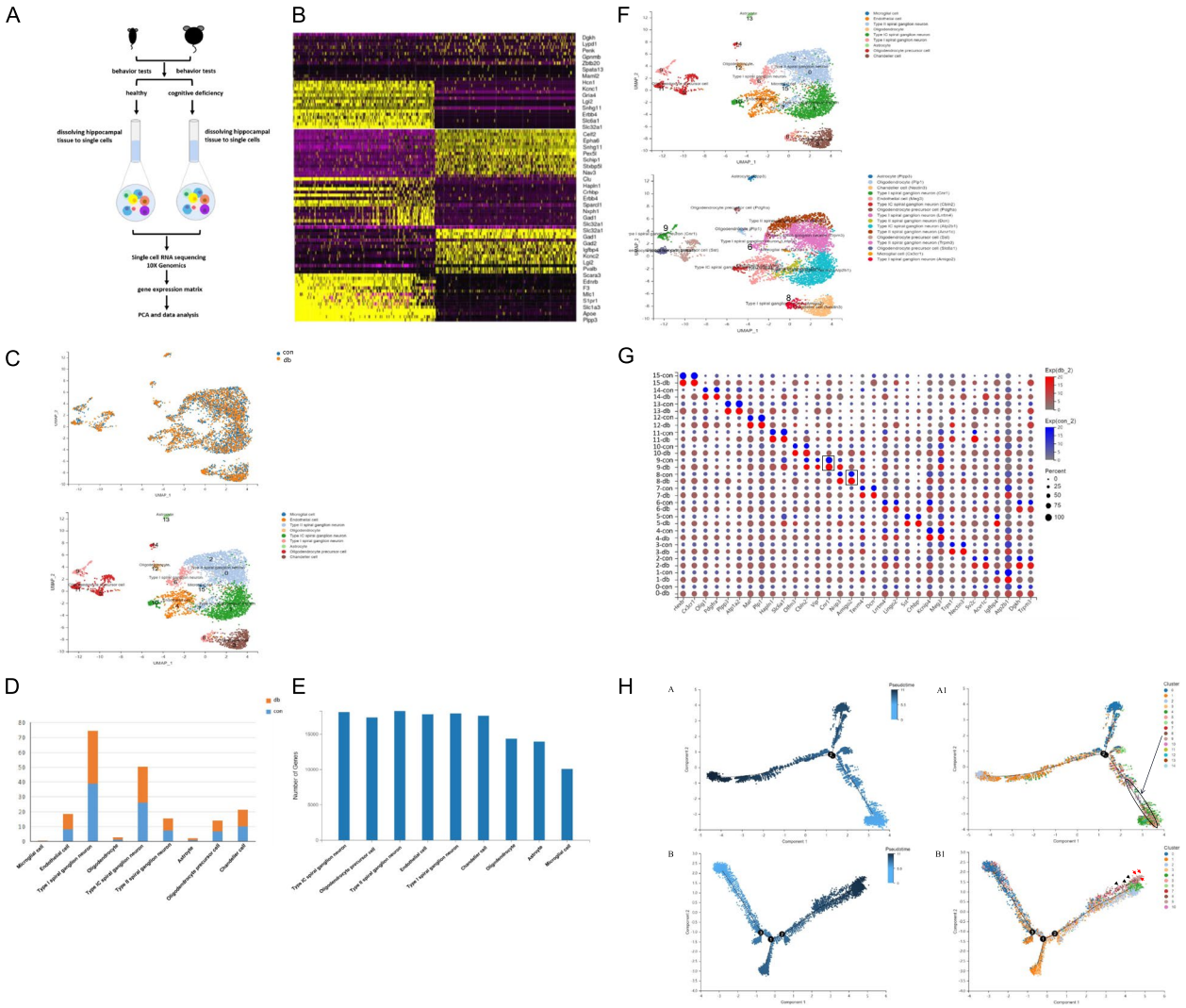

Fig. 4 The scRNA-seq analysis of hippocampal cells based on DCD mice compared to normal control mice. The experimental workflow is show in (**A**) and (**B**) heat map showed top15PC in DCD vs WT after PCA analysis; Yellow indicates high expression,purple indicates low expression,as the number of PC increases,we can see the feature is becoming less and less obvious.) (**C**) UMAP plot of hippocampal cells from DCD mice and normal control mice, showing16 clusters and 9 colored cell types that were identified. (D)The relative abundance of cell types between DCD and WT and number of genes in each cell type in DCD vs WT (**E**). **F** Subclustering of type I spiral ganglion neurons. UMAP plot showing type I spiral ganglion neurons subclusters expressed specific markers. such as cluster8 with marker gene as Amigo2, cluster9 with marker gene as Cnr1 and cluster6 with marker gene as Lrrtm4, however, cluster 6 could not be definitely annotated as type I spiral ganglion neuron and was eliminated in further analysis according to the expression feature of marker genes in each cluster in (**G**). **G** The clot plots of expression feature of marker genes in each cluster

Type I spiral ganglion neurons communication between DCD and WT

The CellPhoneDB was performed to identify the differential communications and potential interaction pairs of type I spiral ganglion neurons between DCD and control groups. The heat map of ligand-receptor interaction showed the interaction signatures between DCD and control groups of the different cell types expressed specific marker genes (Fig. 6A); And the bitmap of ligand-receptor intereacion in Fig. 6B, suggesting that markedly expressed ligand-receptor interaction pairs in type I spiral ganglion neuron (cluster8 and cluster9) are NTF3-NTRK2, FGFR1-NCAM and BDNF-TRK2, which might be associated with the regulation of synaptic transmission and glutamatergic synapse considering the above functional enrichment analysis. Moreover, the communication circos plot of interaction expression signature between DCD and WT mice (control) was investigated

Table 2 Prominent signature gene in type I spiral ganglion neurons subclusters based on GO and KEGG enrichment analysis of DEGs between DCD mice and control mice

Clusters&pathway	genes
Cluster 8	
neuronal cell body	*Phax,Pclo,Akap5,Kif5c*
postsynaptic recycling endosome	*Akap5*
dendrite	*Akap5,Kif5c,Pclo*
trans-Golgi network	*Plpp3,Pclo*
synaptic vesicle targeting	*Pclo*
regulation of sphingolipid mediated signaling pathway	*Plpp3*
Cluster 9	
positive regulation of neuron projection development	*Wnt5a, Serpine, serpini1, Tenm3, Reln, Cnr1, Ndnf, Epha3 Ntrk2, Ptprz1*
learning and memory	*Vip, Egfr, Crh, Aff2, Cnr1, Ptprz1, Reln, Ntrk2, Slc6a1, Cntnap2, Sorcs3*
synaptic transmission, glutamatergic	*Grid2, Grin3a, Gria4, Grik1, Slc17a8*
positive regulation of synaptic transmission, glutamatergic	*Reln, Ntrk2, Egfr, Gria4*
glutamatergic synapse	*Eps8, Cadps2, Cacng4, Rgs7bp, Wnt5a, Grik1, Cbln2, Gria4, P2ry1, Htr3a, Grip1, Pcdh17, Grin3a, Cnr1, Adra1a, Pclo, Ntrk2, Erbb4, Sorcs3, Fxyd6, Ptprz1, Grid2*
GABA-ergic synapse	*Nrx3, Erbb4, Pclo, Gabrg3, Slc6a1, Slc32a1, Cnr1,Cntnap4 Pcdh17, Adra1a, Epha3*
Neuroactive ligand-receptor interaction	*Grm8,Calca,Penk,Adra1a*
AMPK signaling pathway	*Adra1a*
Rap1 signaling pathway	*Fgf22*

(Fig. 6C), showing that the ligand Nlgn1 in cluster8 has a unique receptor Nrxn1 in cluster9 (Fig. 6D). Indeed, Nrxn1 can also interact with PRG-1 mentioned above, which formed neuroactive ligand-receptor complex with Ntrk2 and PSD-95 as well (Table 1). Additionally, western blot was also used to detect the expression of PRG-1, BDNF, PSD-95 as well as other synaptic plasticity related proteins, which has affected beneficially on cognitive impairment [21, 22]. As shown in Fig. 7A, the mice with cognitive impairment presented significantly lower levels of PRG-1, p-Akt and p-ERK in the hippocampus compared to WT, suggesting that the neuroprotective axis in type I spiral ganglion neurons was damaged. Interestingly, LncRNA NONRATG022419 could bind to miR-18a, which targeted Prg-1after double luciferase reporter genes experiments in HEK293cells, and the down-regulated expression of LncRNA NONRATG022419 and *Prg-1*, as well as up-regulated expression of miR-18a were confirmed in WT and GK with DCD groups (Fig. 7B). Above all, down-regulated synaptic plasticity related proteins such as PRG-1, PSD-95, SYN and SYN-1 in type I spiral ganglion neurons of DCD associated with glutamatergic/GABA-ergic synapse and synaptic transmission.

Analysis of type IC spiral ganglion neurons between DCD and WT

As noted above, compared to WT, the number of genes in type I spiral ganglion neuron and type IC spiral ganglion neuron in DCD mice are more abundant compared to any other cell types (Fig. 4D). To obtain a full view on heterogeneity and diversity, type IC spiral ganglion neurons were further investigated. There were two subpopulations of type IC spiral ganglion neurons that were identified based on UMAP analysis: type IC spiral ganglion neurons with marker gene as Atp2b1 expressed on cluster1 and with marker gene as Cbln2 expressed on cluster10 (Fig. 8A). Compared to WT, the relative proportion of the type IC spiral ganglion neuron with Chln2 marker gene expressed on cluster10 is significantly high in DCD group mice (Fig. 8B).

Go and KEGG pathway enrichment analysis of DEGs in cluster10 were performed. The top 20 enriched KEGG pathways were presented in cluster10, which suggested that compared to the WT mice type IC spiral ganglion neuron, the enrichment items of DCD mice were mostly concentrated in endocytosis and neuroactive ligand-receptor interaction pathways (Fig. 8C). Consistent with the findings above in type I spiral ganglion neurons, spiral ganglion neurons have a critical role in synaptic plasticity in diabetic induced learning and memory deficits, as well as other neurodegenerative diseases; and that PRG-1 could be involved in this process (Fig. 7 and Fig. 9).

Discussion

Cognitive deficits is prominent symptom of patients with diabetic encephalopathy and may reflect brain changes caused by diabetes [23]. Several researches revealed the relationship between AD induced cognitive dysfunction and diabetics, including chronic hyperglycemia resulting in excited neurotransmitters inducing neurotoxicity in neuronal cells and promote pathological process [24]. In view of multiple factors in process of diabetic encephalopathy still not clear, the diabetic rodents such

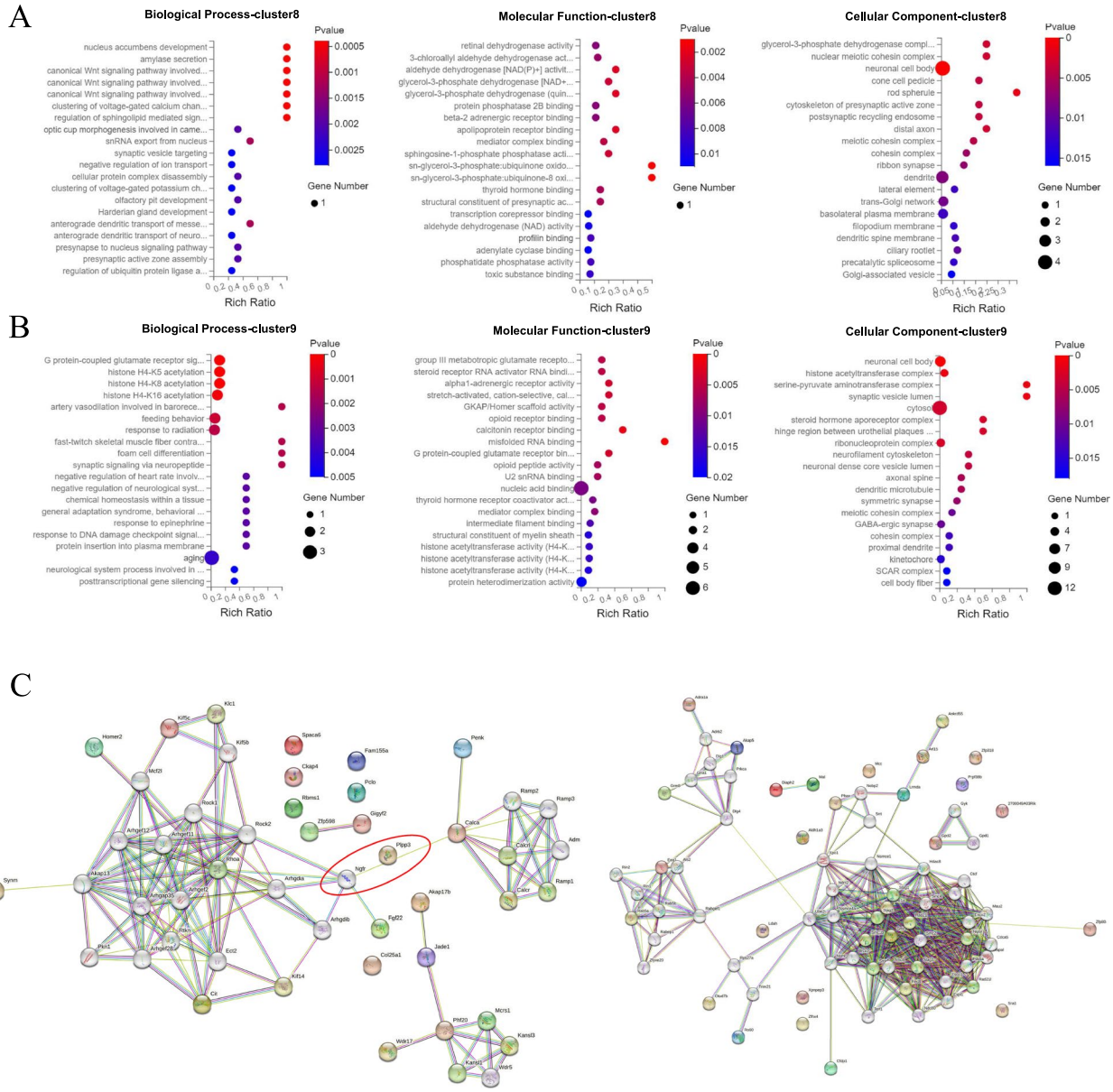

Fig. 5 Functional enrichment analysis of cluster8 and cluster9 between DCD and WT. **A**,**B** GO term analysis of DEGs cluster8 and cluster9 between DCD and WT showed enrichment of biological processes, molecular function and cellular component including G-protein coupled glutamate receptor signaling pathway,synaptic signaling via neuropeptides, synaptic vesicle targeting, canonical Wnt signaling pathway involved in positive regulation of cell–cell adhesion and migration, calcitonin receptor binding,structural constituent of presynaptic active zone,neuronal cell body,axonal spine,synaptic vesicle lumen and dendritic microtubule etc. **C** PPI networks were constructed by DEGs of cluster8 and cluster9, and their interaction items related to neuroactive ligand-receptor interaction, positive regulation of synaptic transmission, neuron projection development, and positive regulation of glutamatergic synapse

as $GK^{-/-}$ rats and $db^{-/-}$ mice, which are the ideal animal models to present and develop diabetes and complications [25, 26]; And then their show some behavioral deficits and cognitive impairment to mimic the symptom of diabetic patient [27, 28]. In the current study, the NOR testing results showed that both $GK^{-/-}$ and $db^{-/-}$ diabetic animals suffering impaired learning ability and memory function [29, 30]. In addition,the reduced dendritic spine of hippocampus in diabetic animals confirmed their dysfunction in cognition [31, 32]. Further, we dissected hippocampal tissue and performed full RNA transcriptome analysis and scRNA-seq, which showing their specific transcriptional features and distinctive cell types. In summary, the observations and findings

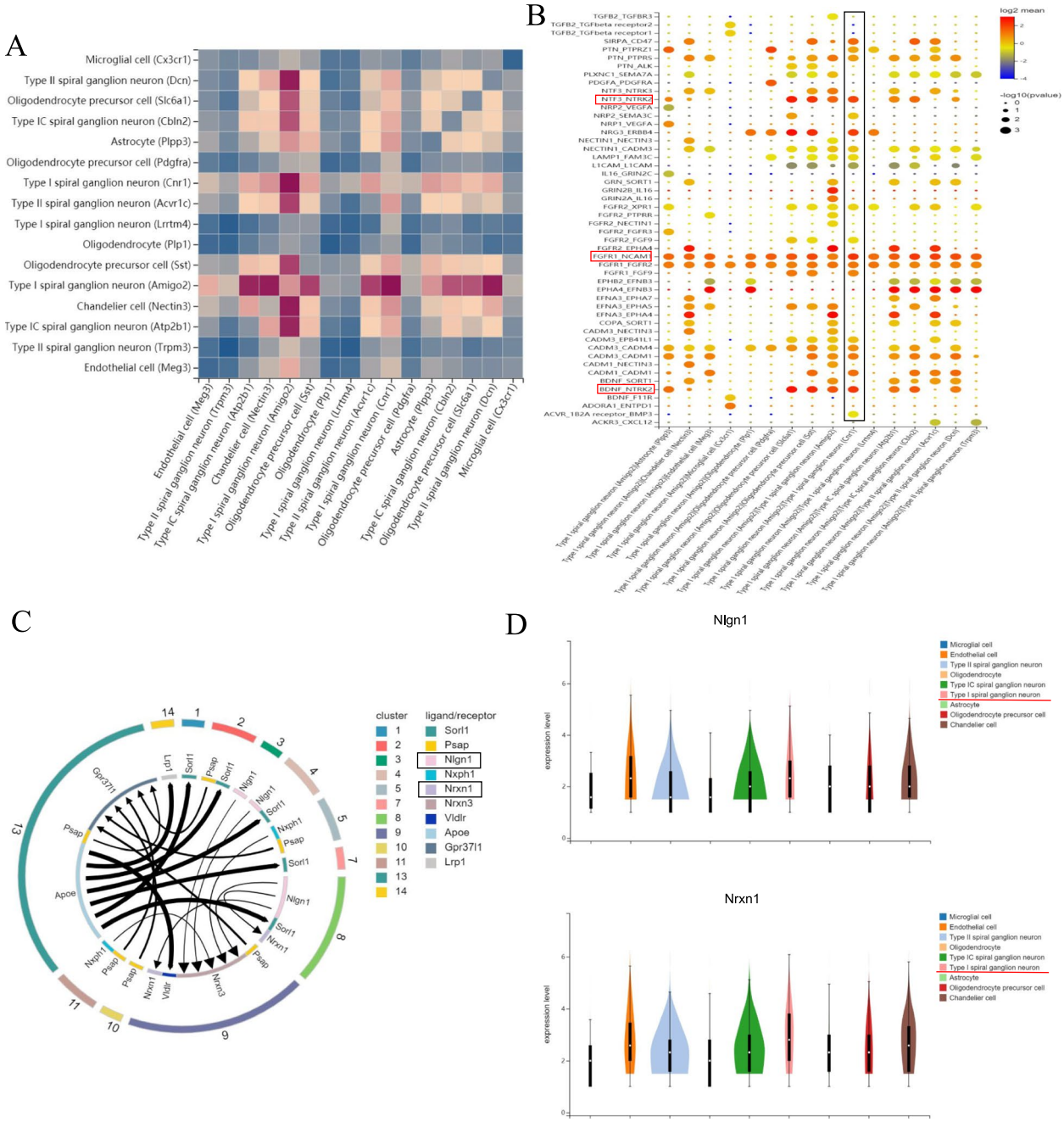

Fig. 6 Type I spiral ganglion neurons communication between DCD and WT. Heat map of ligand-receptor intereacion showing in **A**. horizontal axis: cell type;vertical axis: cell type; The color of rectangle in the plot represents the number of receptor ligands between cells. Bitmap of ligand-receptor intereacion showing in **B**, horizontal axis: Cell type comparison group,marker genes are in the brackets;vertical axis: ligand receptor pair;The color (log2 mean)of circle reprsents the log of average expression level,the size of circle represents P value,a larger point is more reliable. **C** The communication circos plot of interaction expression signature between DCD and WT mice(control) was investigated, showing that the ligand Nlgn1 in cluster8 has a unique receptor Nrxn1 in cluster9. From the outside to the inside,the first circle represents the category of the cluster,the second circle represents the ligand receptor pair,the third circle represents the relationship between the ligand and receptor;the gene pointed by the arrow(end point)is the receptor,and the gene at the other end(start point)is the ligand,a thick line indicates the ligand is highly expressed,a thin line indicates the ligand is lowly expressed;a wide arrow means the receptor is highly expressed,a narrow arrow means the receptor is lowly expressed. **D** The violin plots showing the expression level of Nlgn1and Nrxn1 in different cell type between DCD and control groups

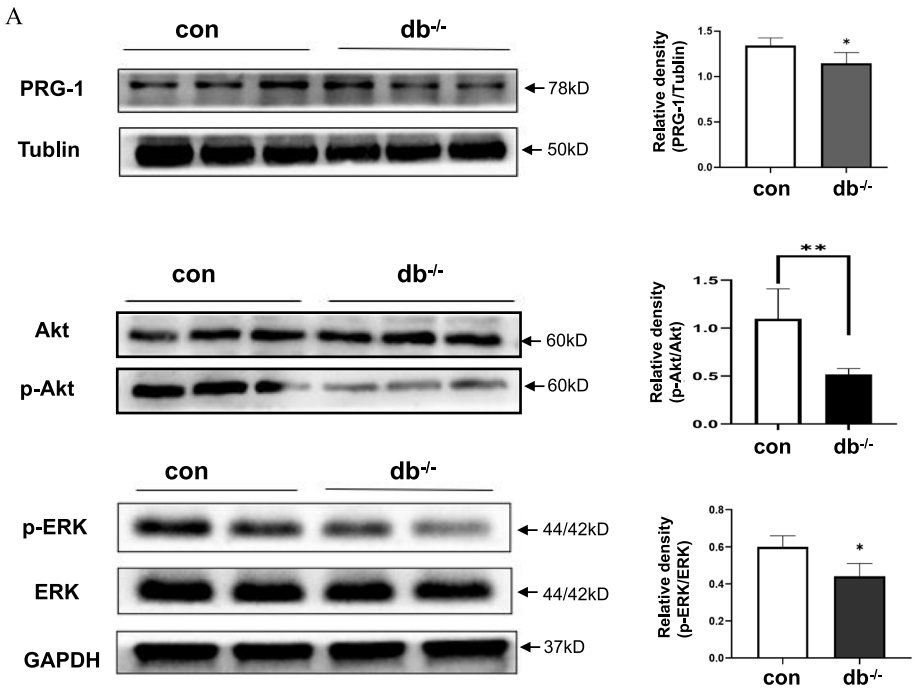

Fig. 7 Expression of PRG-1, p-Akt and p-ERK in WT and db with DCD (**A**) and (**B**) LncRNA NONRATG-022419 bind to miR-18a, which directly targets *prg-1* using double luciferase reporter gene assay in HEK293 cells

revealed that hippocampal cells appear heterogeneity in DCD. DEGs from control and DCD groups exhibited that gene transcription in different cell types under the cellular and molecular changes in DCD.

Up to now, the underlying mechanism of DCD is still less well understood, among which it is more clear that the changes of synaptic neuroplasticity and cellular heterogeneity are more related to DCD [33, 34]. Integrated analysis showed that ganglion neurons in DCD, including type I and IC spiral ganglion neurons, which has a crucial role in regulation of neuroplasticity [35, 36]. Several researches have already suggested that PRG-1 has the functional role of plasticity in central nervous system [15, 37]. PRG-1 is a neuron membrane protein, located at the postsynaptic density of synapses, interacted with PSD-95, Nrxn1, Ntrk2(Trkb), Prkcsh(PKC) etc., mainly involved in axonogenesis, G protein-coupled receptor signaling pathway, regulation of synaptic transmission, and modulation of chemical synaptic transmission as well. Petzold S et al. have reported that PRG-1, a novel synaptic molecule, highly expressed during development and regeneration processes at the postsynaptic density, modulates synaptic lysophosphatidic acid (LPA) levels, and revealed its relationship with plasticity-related neurotrophins, such as NTF, BDNF and NT-3; the possible mechanism related to hippocampal network activity and possibly exerting a protective effect against over-excitability [38]. Meanwhile, Bräuer AU et al. showed that PRG-1 facilitates axonal outgrowth during development and regenerative sprouting and specifically expressed in neurons, located in the membranes of outgrowing axons [39]. Further, it has already been reported that BDNF/Trkb signaling pathway contributed to the survival, differentiation, growth and apoptosis of neurons, which was primarily mediated by NTF, BDNF as well as other neurotrophic factors [40]. Tropomyosin receptor kinase b (Trkb) has its ligand as BDNF that mediates a series of signaling pathways correlate to neural plasticity development, survival and regeneration of damaged neurons [41]. PRG-1 can bind to the Trkb, our previous work confirmed that knockdown of Prg-1 improved BDNF and PSD-95 expression based on high glucose induced injury model in vitro (data not shown).

The state of spiral ganglion neurons changed rapidly along with the process of DCD, however, its cellular characteristics and molecular function are not known clearly [42]. The increasing evidence showed that the plasticity of ganglion neurons in hippocampus is heterogeneous, depending on the coordination of neuron-neuron interactions and neurons interacting with other different types of cells [43]. In current study, compared to WT, the relative abundance of type I spiral ganglion neurons in DCD mice changed significantly, and then the functional enrichment analysis

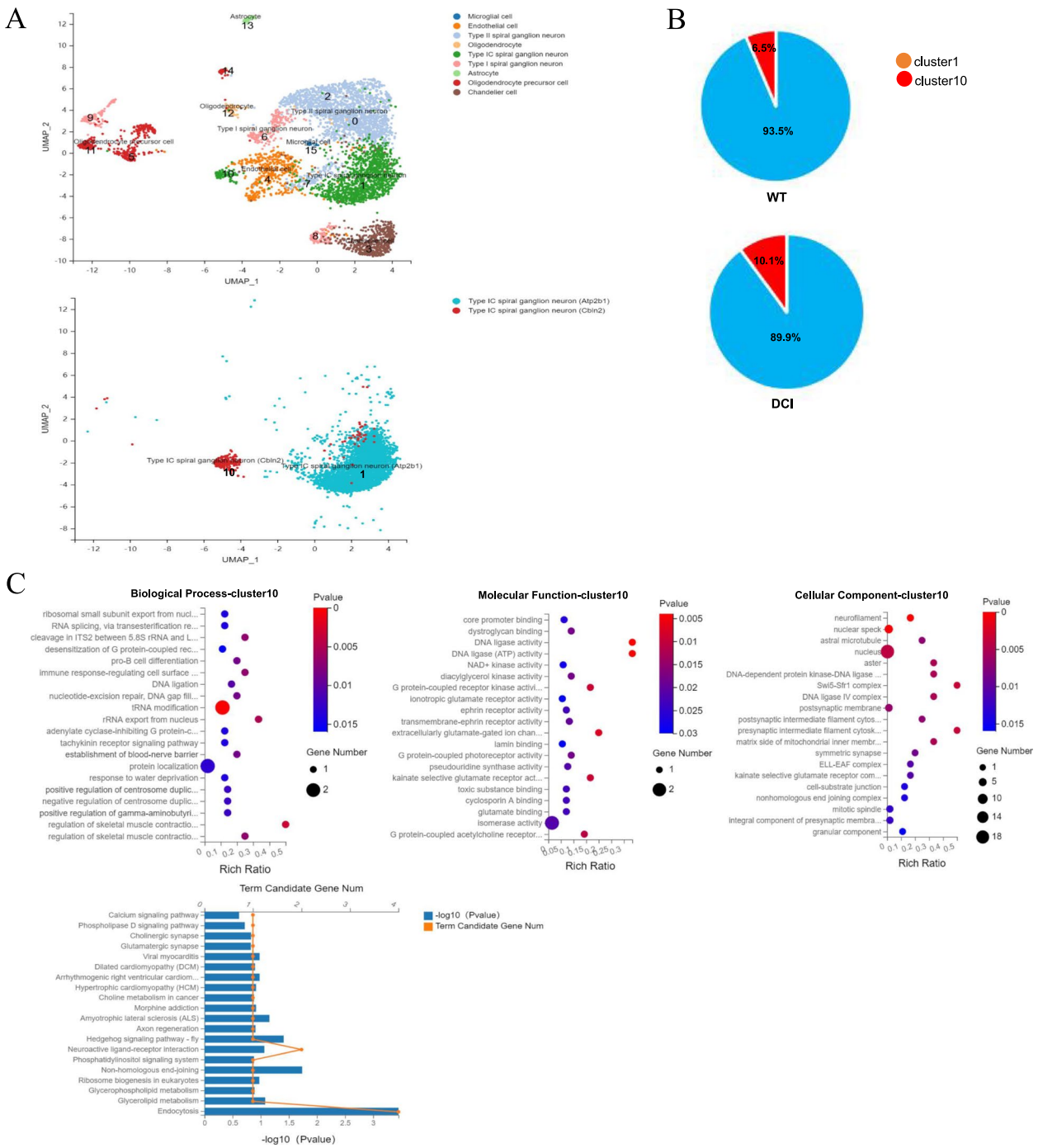

Fig. 8 Subclustering and annotation of immune cells. **A** UMAP analysis showing two sub-populations of type IC spiral ganglion neurons distributed on cluster 1 and cluster10. **B** Pie charts showing the proportion (percentage) of these two types of type IC spiral ganglion neurons in the WT and DCD (Red is types of type IC spiral ganglion neuron in cluster10 and bule is on cluster1). **C** Go and KEGG pathway enrichment analysis of DEGs in cluster10

of DEGs showed that the synaptic transmission/glutamatergic synapse related genes were mostly enriched in DCD type I spiral ganglion neurons, which guiding to speculate that these ganglion neurons participated in the regulation of synaptic plasticity. Further, two type I spiral ganglion neurons sub-populations with differently expressed marker genes were identified, which presented distinctive molecular features in the

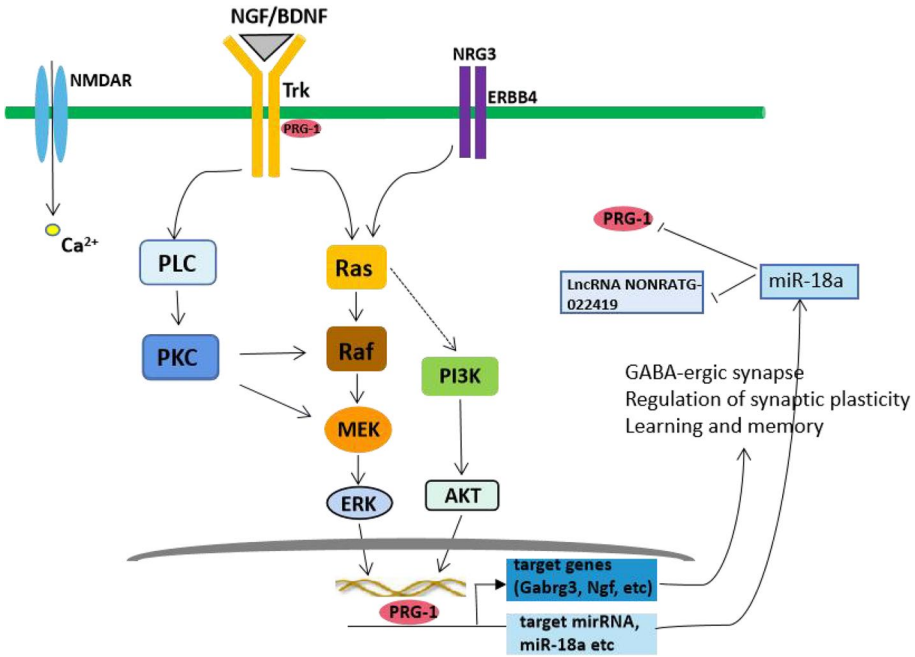

Fig. 9 The potential mechanism of PRG-1 in the synaptic plasticity regulation of hyperglycaemia induced cognitive dysfunction

state of DCD. We found that the highly expressed level of Nrxn1 in cluster 8 and cluster9, which has its ligand as Nlgn1 was also expressed in cluster8. And we known above, Nrxn1 also has interacted with PRG-1 that might be involved in synaptic plasticity related signaling pathway. As we known, BDNF/TrkB axis is widely expressed in the brains of developing and adult mammals. BDNF/TrkB stimulated intracellular signaling is essential for neuronal survival, morphogenesis, and plasticity [44]. Binding of BDNF to TrkB is known to trigger a variety of intracellular signaling pathways, including mitogen activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK), phospholipase Cg (PLCg), and phosphoinositol 3-kinase (PI3K) pathways, and BDNF has biological effects on neurons by activating similar mechanisms [45]. Actually, our current work showed that down-regulated expression of p-Akt, p-ERK were found in DCD groups, compared to WT. In fact, PI3K-Akt signaling pathway plays key roles in a variety of cellular processes, such as glucose metabolism, apoptosis, cell proliferation, transcription, and cell migration [46]; Dysregulation of the PI3K/AKT signaling pathway has been found in a variety of human diseases, including cancer, diabetes, cardiovascular disease, and neurological diseases [47]. Moreover, MAPK-ERK signaling pathway is a key signaling pathway for cell proliferation, differentiation, apoptosis, and stress response under normal and pathological conditions [48]. In summary, our research work identified the cellular and heterogeneity of type I/ IC spiral ganglion neurons, the potential crucial ganglion neuron types correlated with DCD. Meanwhile, the functional role of PRG-1 in synaptic plasticity was also illustrated; which might find new view on diversity of ganglion neurons and the potential therapeutic targets for DCD.

Conclusion

In conclusion, through integrated analysis and experimental validation, we obtained in-depth understanding of the synaptic plasticity and heterogeneity of spiral ganglion neurons in hippocampus of normal and DCD animals. We investigated changes of neuroplasticity in hippocampal spiral ganglion neurons, which confirm the prominent view that neurons play a crucial role in plasticity regulation of DCD and that PRG-1 could be involved in this process (Fig. 9). Our findings in this paper should be validated in other DCD- associated models and their relevance in patients. In addition, differential expression pathways need to be further evaluated in functional experiments to assess their harmful or protective role in DCD.

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Approval date of registry and the registration no. of the study/trial
N/A.

Animal studies
All the animal experiments were studied under the requirements of the National Laboratory Animal Use Act of China and approved by the Laboratory Animal Use Committee of the Medical Research Institute of Jishou University (NO:JSDX-2021-0004).

Authors' contributions
QX, and XL designed the experiments; wrote and revised the draft; HL, JT, CF, LL and JD performed the experiments; Data collection, analysis and interpretation by QX, HL, JT , CF and XL. All of the authors approved the final version of the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
N/A.

Consent for publication
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Competing interests
The authors declare no competing interests.

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