

Research Article

Down-Regulation of Insulin Like Growth Factor 1 Involved in Alzheimer's Disease via MAPK, Ras, and FoxO Signaling Pathways

Kexin Kang ¹, Jun Bai,² Shanshan Zhong,³ Rongwei Zhang,¹ Xiaoqian Zhang,³ Ying Xu,^{2,4} Mei Zhao ⁵, Chuansheng Zhao ³, and Zhike Zhou ¹

¹Department of Geriatrics, The First Affiliated Hospital, China Medical University, Shenyang, 110001 Liaoning, China

²Cancer Systems Biology Center, The China-Japan Union Hospital, Jilin University, Changchun, 130033 Jilin, China

³Department of Neurology, The First Affiliated Hospital, China Medical University, Shenyang, 110001 Liaoning, China

⁴Computational Systems Biology Lab, Department of Biochemistry and Molecular Biology and Institute of Bioinformatics, The University of Georgia, USA

⁵Department of Cardiology, The Shengjing Affiliated Hospital, China Medical University, Shenyang, 110004 Liaoning, China

Correspondence should be addressed to Zhike Zhou; zkHzou@cmu.edu.cn

Received 19 February 2022; Revised 7 April 2022; Accepted 19 April 2022; Published 4 May 2022

Academic Editor: Giulia Sita

Copyright © 2022 Kexin Kang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The inability to halt or even delay the course of Alzheimer's disease (AD) forces the development of new molecular signatures and therapeutic strategies. Insulin like growth factor 1 (IGF1) is a promising target for AD treatment, yet exact mechanisms of AD ascribed to IGF1 remain elusive. Herein, gene expression profiles of 195 samples were analyzed and 19,245 background genes were generated, among which 4,424 differentially expressed genes (DEGs) were overlapped between AD/control and IGF1-low/high groups. Based on such DEGs, seven co-expression modules were established by weight gene correlation network analysis (WGCNA). The turquoise module had the strongest correlation with AD and IGF1-low expression, the DEGs of which were enriched in GABAergic synapse, long-term potentiation, mitogen-activated protein kinase (MAPK), Ras, and forkhead box O (FoxO) signaling pathways. Furthermore, cross-talking pathways of IGF1, including MAPK, Ras, and FoxO signaling pathways were identified in the protein-protein interaction network. According to the area under the curve (AUC) analysis, down-regulation of IGF1 exhibited good diagnostic performance in AD prediction. Collectively, our findings highlight the involvement of low IGF1 in AD pathogenesis via MAPK, Ras, and FoxO signaling pathways, which might advance strategies for the prevention and therapy of AD based on IGF1 target.

1. Introduction

Alzheimer's disease (AD), an age-related and progressive neurodegenerative disorder in the elderly, is characterized by extracellular deposits of amyloid-beta ($A\beta$) peptides and intracellular accumulation of hyperphosphorylated tau proteins [1]. In addition to these aggregates, it shares additional pathological alterations with diabetes mellitus, including abnormal glucose metabolism, insulin resistance, oxidative stress, inflammation, and neuron atrophy [2–4]. Such common hallmarks possibly represent the identical etiology for both clinical entities, a viewpoint supported by an extensive

array of epidemiological observations that patients with diabetes mellitus remarkably and independently increase the risk of AD onset [5–7]. Among multiple metabolic hormones in diabetes mellitus, insulin like growth factor 1 (IGF1) is found to be closely related to $A\beta$ clearance and tau phosphorylation, suggesting IGF1 as an important mediator with a wide spectrum of effects on the pathophysiology of diabetes mellitus and AD [7, 8].

IGF1, similar in function and structure to insulin, encodes a regulatory protein involving a series of intracellular biological actions, ranging from DNA synthesis to cell metabolism, cycle, and death [9]. It has been shown that

systemic IGF1 enhances insulin activity and neuronal excitability, thus to play a neuroprotective role in brain insults with different etiologies and anatomies [10]. In neurodegenerative conditions, particularly AD, subnormal levels of IGF1 are detected in the brain and serum of affected individuals, a large proportion of whom are accompanied with insulin resistance [11, 12]. Indeed, increased accumulation of soluble A β rather than overproduction is thought to be responsible for AD senile-plaque pathology, due at least in part to impaired A β clearance mediated by low IGF1 levels [13]. One plausible interpretation is that IGF1 binding to tyrosine kinases of insulin receptors and auto-receptors not only facilitates A β clearance but also protects cells against apoptosis in a IGF1-binding protein-sensitive manner [14, 15]. Moreover, a substantial amount of evidence [16–18] has confirmed that IGF1 elicits the bond of tau protein with microtubules to reduce tau phosphorylation, pointing to a direct role of IGF1 in neurofibrillary-tangle pathology of AD. Despite the causal relationship between IGF1 and AD, precise mechanisms of IGF1 underlying AD occurrence have not been well defined. There is convincing evidence that bioinformatics analysis is a promising method to identify potential biomarkers of AD (e.g., NPIPA1) and validated them through functional enrichment annotations [19]. Herein, we performed an integrative genomic analysis to investigate the role played by IGF1 during AD development on basis of gene expression data and functional annotations, aiming to elucidate the mechanistic pathways of IGF1 in AD.

2. Materials and Methods

2.1. Data Collection. Based on our previous study [20], microarray chips of temporal cortex tissues between AD and non-dementia controls were obtained from the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). We selected the GSE132903 dataset as the test set for genomic analysis, while GSE5281 and GSE37264 are served as the training sets (Table 1). Clinical phenotypic information (e.g., age, gender, AD, and IGF1 expression) for each sample were collected in Supplementary Table 1. In *limma* package of R software, the *normalizeBetweenArrays* function was used to normalize ribonucleic acid sequencing (RNA-seq) data from all datasets.

2.2. Differential Expression Analysis. Using *lmFit* and *eBayes* functions in R package *limma*, RNA-seq data were processed to screen for differentially expressed genes (DEGs) between AD and non-dementia controls [21]. Based on DEGs values, the samples were grouped by two-dimensional hierarchical clustering method, and the results were displayed in the forms of volcano plot and heatmap. The threshold value for DEGs identification was $|\log_{2}(\text{fold change})| > 0.15$ with a false discovery rate (FDR)-adjusted p value of < 0.05 .

2.3. Co-Expression Network Analysis. The co-expression network on DEGs overlapped from AD/control and IGF1-low/high groups was established by weight gene correlation network analysis (WGCNA). The procedure for co-expression network construction using WGCNA package consisted of

TABLE 1: Sample data in test and training sets. AD: Alzheimer’s disease; GEO: gene expression omnibus.

GEO	Test/training set	Platform	AD	Controls
GSE132903	Test	GPL10558	97	98
GSE5281	Training	GPL570	16	11
GSE37264	Training	GPL5188	8	8

the following steps: (1) remove outliers from sample clustering diagram; (2) select the soft threshold power of 8 to construct an adjacency matrix; (3) transform the adjacency matrix into a topological overlap matrix (TOM); (4) form a hierarchical clustering tree by hierarchical clustering on basis of the TOM dissimilarity; (5) obtain the modules from the branches of the hierarchical clustering tree with the *minModuleSize* = 30; and (6) cluster the modules to build a co-expression network by the average-linkage hierarchical clustering method according to the value of module eigengene (ME), an index indicating the overall expression of a module [22]. Gene functional annotation on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was conducted by employing the *clusterProfiler*, *org.Hs.eg.db*, and *topGo* packages with default parameters.

2.4. Protein-Protein Interaction (PPI) Network and Cross-Talking Pathways of IGF1. Module-trait relationships were assessed by the correlations between intramodular connectivity and genetic phenotype, as measured by module membership (MM) and gene significance (GS), respectively [23]. Scatterplots of the association between MM for module genes and GS for IGF1 expression were drawn using the *verboseScatterplot* function. Through the STRING (Search Tool for the Retrieval of Interacting Genes, <http://www.stringdb.org/>) online database, protein-protein interaction (PPI) network was established to investigate the physical or functional interaction between proteins that were encoded by the DEGs under consideration [24, 25]. The *cytoscape* software (Version 3.8.0, <http://www.cytoscape.org/>) was utilized to analyze and visualize the PPI network and the cross-talking pathways of IGF1 [26].

2.5. Diagnostic Performance of the Area Under the Curve (AUC). AUC analysis is performed by comparing the receiver operating curve (ROC) of each subgroup in the cohort, providing the value of AUC, sensitivity, and specificity, as well as the best accuracy value at an optimal cut-off to estimate the positivity of a diagnosis or biomarker for AD [27]. The *pROC* function was used to detect the differentiation of IGF1 between AD cases and non-dementia controls. An AUC value of 100% represented a complete prediction, relative to a random selection with an AUC value of 50%.

2.6. Pathway Signature Genes and Gene Set Enrichment Analysis (GSEA). Pearson’s correlation coefficient (PCC) analysis was used to quantitatively evaluate the association between genes [28]. In each cross-talking pathway, the six genes with the top-rank PCC values were defined as the pathway signature genes, the expression of which were most

strongly correlated with other genes of the pathway [29]. If IGF1 was significantly associated with a set of all pathway signature genes ($p < 0.05$), this pathway could be served to be mediated or regulated by IGF1. The entire predefined differential expression gene lists were subjected to GSEA analysis to gain a complete biological process (BP) of gene ontology (GO) terms using the *GSEABase*, *enrichplot*, and *ClusterProfiler* packages [30]. The number and type for permutations were set to 1000 and phenotype, respectively. The *ggplot2* function was adopted to visualize the top 10 representative BPs enriched by GSEA analysis. All data were expressed as mean \pm standard deviation (SD). Unpaired Student's *t*-test, one-way analysis of variance (ANOVA), or two-way ANOVA with Bonferroni's correction were used to assess the differences between groups. GraphPad Prism software (La Jolla, CA, USA) was utilized to perform statistical analysis. A two-tailed value of *p* less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. Differentially Expressed Gene Identification. The procedure for this study was followed by steps outlined in the flowchart (Figure 1). In test set of GSE132903, the expression level of IGF1 was significantly down-regulated in temporal lobes of 97 AD patients (5.38 ± 0.54) compared with 98 non-dementia controls (5.67 ± 0.45 ; $p < 0.0001$) (Figure 2(a)). Differential expression analysis on 19,245 background genes was conducted after eliminating invalid and repeated genes. As shown in volcano plots, 5,591 DEGs were selected in AD versus non-dementia controls, of which 2,805 were up-regulated and 2,786 were down-regulated (Figure 2(b)); meanwhile, 6,742 DEGs were identified in IGF1-low versus high group, of which 3,366 were up-regulated and 3,376 were down-regulated (Figure 2(c)). Ultimately, 4,424 DEGs (2,076 up-regulated and 2,348 down-regulated) were overlapped between AD/control and IGF1-low/high cohorts, which were subsequently extracted for two-dimensional hierarchical clustering analysis. The clustering results of the top 50 up- and down-regulated DEGs were presented in the heatmap (Figure 2(d)).

3.2. Gene Co-Expression Modules and Functional Enrichment Analysis. After removing outliers above the cut-off line (height = 60), 185 out of 195 samples were included for hierarchical clustering (Figure 3(a)). The DEGs with highly relevant expression were integrated into seven co-expression modules, while the remaining non-co-expressed DEGs were grouped into the gray module (Figure 3(b)). Heatmap of module-trait relationships (Figure 3(c)) exhibited that the turquoise module was most negatively associated with AD (correlation coefficient = -0.45 , $p = 2e-10$) and most positively related to IGF1 expression (correlation coefficient = 0.74 , $p = 1e-33$). Co-expression modules were functionally annotated by KEGG pathway enrichment analysis (Figure 3(d)). In the turquoise module, DEGs were enriched in GABAergic synapse, long-term potentiation, mitogen-activated protein kinase (MAPK), Ras, and forkhead box O (FoxO) signaling pathways; in the black module, DEGs were involved in hippo

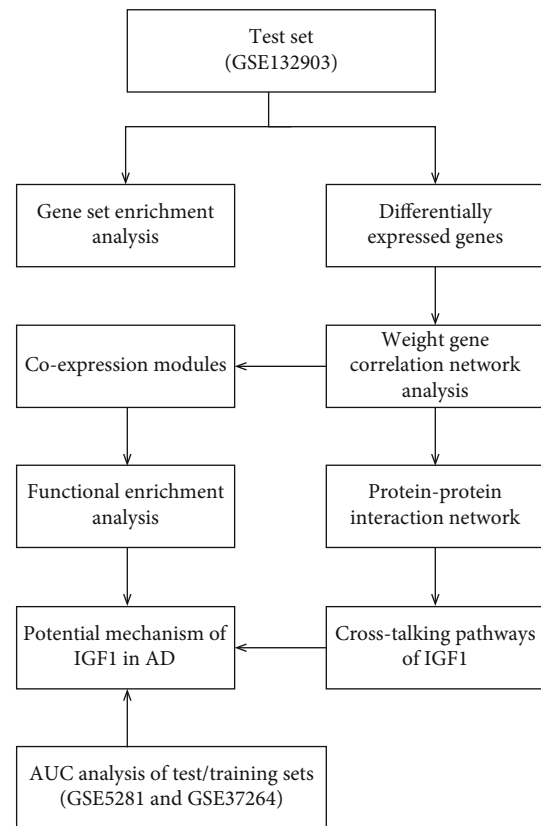


FIGURE 1: The flowchart for study design. AD: Alzheimer's disease; AUC: area under the curve.

and phosphoinositol-3-Kinase (PI3K)-Akt signaling pathways; in the blue module, genes participated in apoptosis and hippo signaling pathways; in the brown module, DEGs were implicated in cytokine-cytokine receptor interaction and axon guidance; and in the yellow, green, and red modules, DEGs were responsible for synaptic vesicle cycle, axon guidance, cAMP, and PI3K-Akt signaling pathways.

3.3. PPI Network and the AUC Analysis. Correlation analysis between MM for genes in each module and GS for IGF1 expression was conducted to detect the correlation coefficient of intramodular connectivity with genetic phenotypes. As shown in Figure 4(a), the turquoise module had the highest correlation coefficients between MM and GS (correlation coefficient = 0.73 , $p < 1e-200$), which was consistent with the results of module-trait (IGF1) relationships. The MM and GS of each DEG in the co-expressed module are detailed in Supplementary Table 2. PPI network was constructed to display the interaction of DEGs in the turquoise module that were most closely correlated with IGF1 (Figure 4(b)). Further cross-talking pathways of IGF1 including MAPK, Ras, and FoxO signaling pathways were identified by functional enrichment analysis of KEGG (Figure 4(c)). According to the results of AUC analysis, low expression of IGF1 had a good diagnostic performance in AD prediction in test set (GSE132903: AUC = 69.4%) and training sets (GSE5281: AUC = 90.9%; GSE37264: AUC = 84.4%) (Figure 4(d)). In comparison

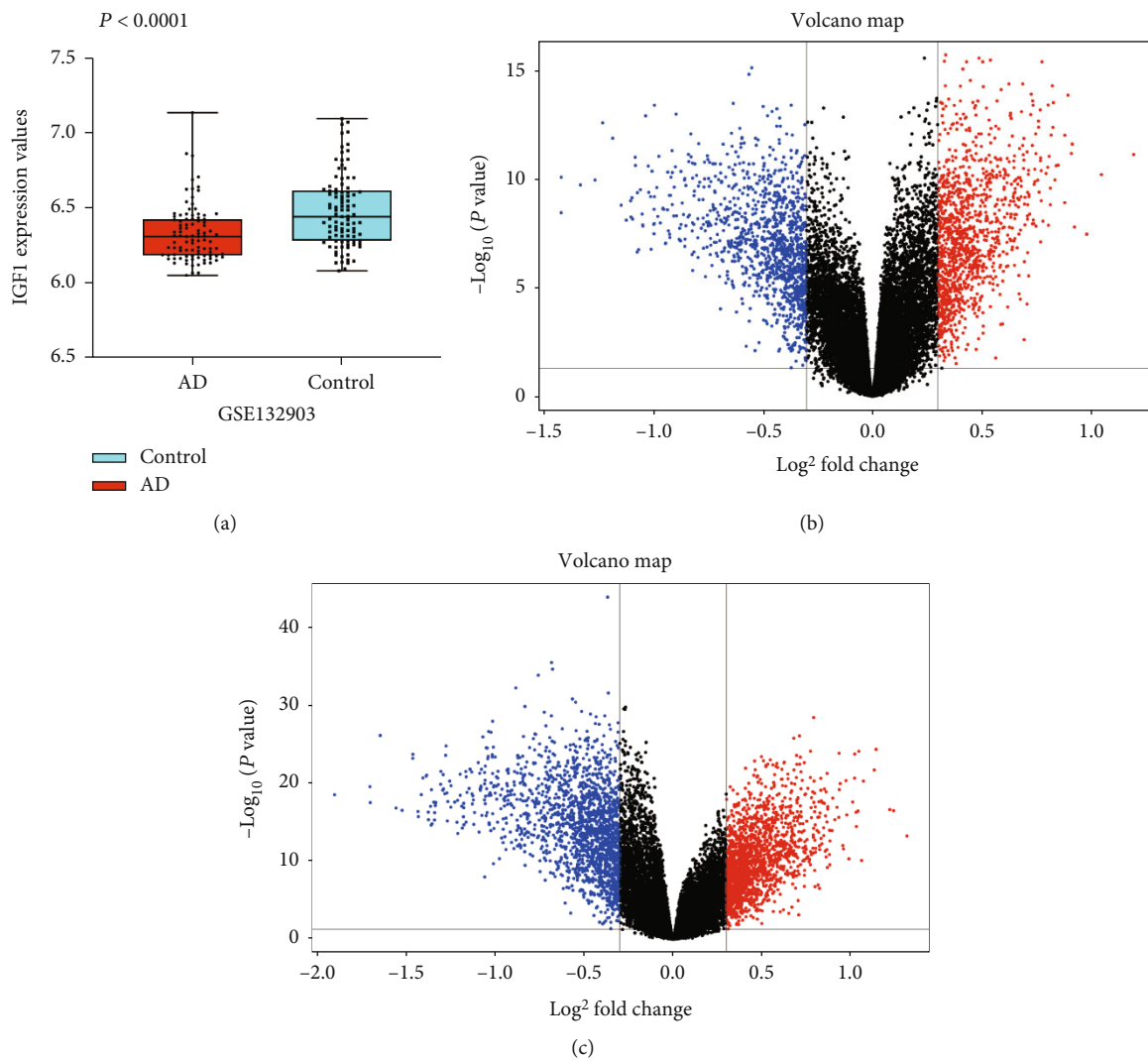


FIGURE 2: Continued.

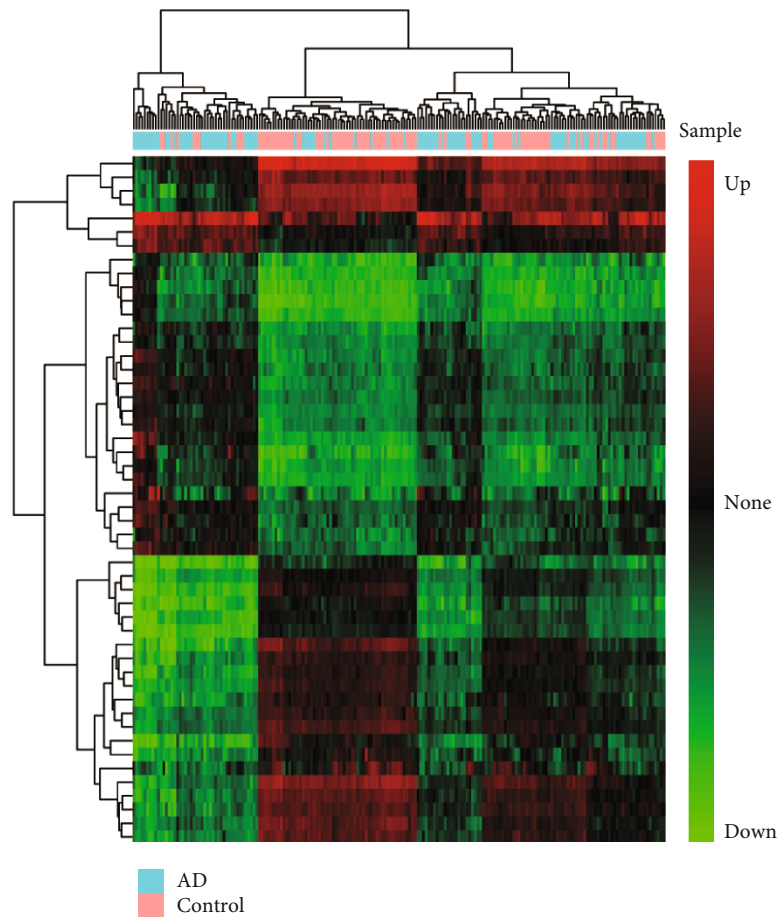


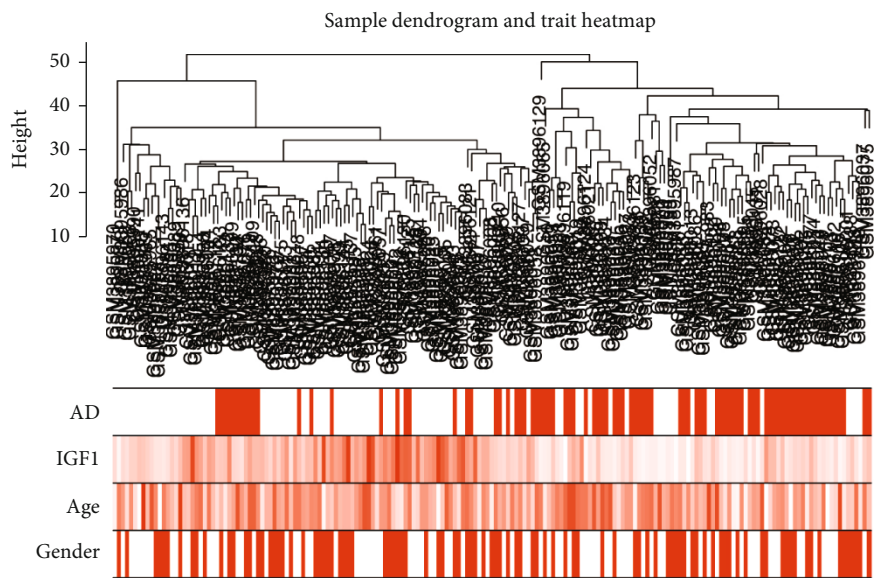
FIGURE 2: Differential expression analysis. (a) IGF1 expression between AD and non-dementia controls in GSE132903. Volcano plots of DEGs in AD/control (b) and IGF1-low/high (c) groups: blue and red represent down-regulated and up-regulated, respectively. (d) Clustering heatmap of the top 50 down- and up-regulated DEGs between AD and non-dementia controls: the gradual change of color from green to red indicates that the gene expression changes from down-regulation to up-regulation. AD: Alzheimer's disease; DEGs: differentially expressed genes.

with non-dementia controls, down-regulation of IGF1 in AD was also observed in training sets (Figure 4(e)), including GSE5281 (AD: 4.76 ± 1.27 vs. controls: 6.63 ± 0.90 ; $p < 0.001$) and GSE37264 (AD: 5.04 ± 1.07 vs. controls: 5.99 ± 0.54 ; $p = 0.04$).

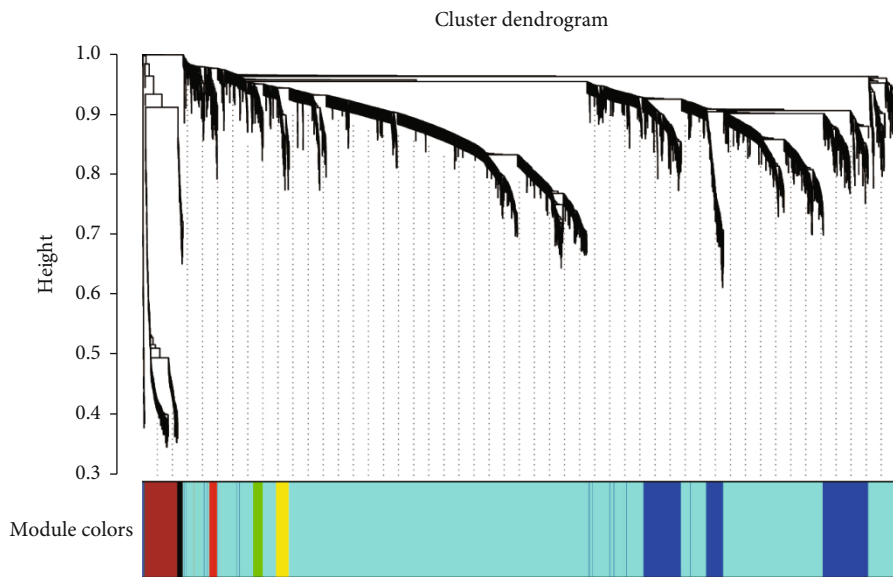
3.4. Validation of IGF1-Mediated Pathways and Biological Processes by GSEA. The signature genes in each cross-talking pathway are listed in Supplementary Table 3. As shown in Supplementary Figure 1, all pathway signature genes exhibited significantly positive or negative correlation with IGF1 expression ($p < 0.05$). Gene functional annotations of BPs in AD were related to neurotransmitter secretion, regulation of synaptic vesicle cycle, signal release from synapse, synaptic vesicle exocytosis, and synaptic vesicle transport (Figure 5(a)). Furthermore, the enriched BPs in IGF1-low cohort referred to neurotransmitter secretion, regulation of neurotransmitter transport, regulation of postsynaptic membrane potential, synaptic vesicle exocytosis, and synaptic vesicle transport (Figure 5(b)).

4. Discussion

In this study, we used publicly available RNA-seq data to screen for differentially expressed genes between AD and non-dementia cohorts. In line with the proteomic study [31], significant down-regulation of IGF1 expression in AD brains was observed in both test and training datasets. The GSEA analysis involving 19,245 background genes demonstrated that BPs of neurotransmitter secretion, synaptic vesicle exocytosis, and synaptic vesicle transport were associated with AD and low IGF1 expression. This was supported by *in vitro* evidence from PC12 cell lines that brief treatment with IGF1 activated intimal PI3K to enhance neurotransmitter secretion in a concentration-dependent manner [32], underlining the regulatory role of IGF1 in synaptic transmission of neurotransmitters. To decipher the signaling pathway of IGF1 in AD, we constructed a PPI network and co-expression modules of DEGs interacting with low IGF1 expression, thus to illuminate the genome-level pathogenesis of IGF1 underlying AD onset.



(a)



(b)

FIGURE 3: Continued.

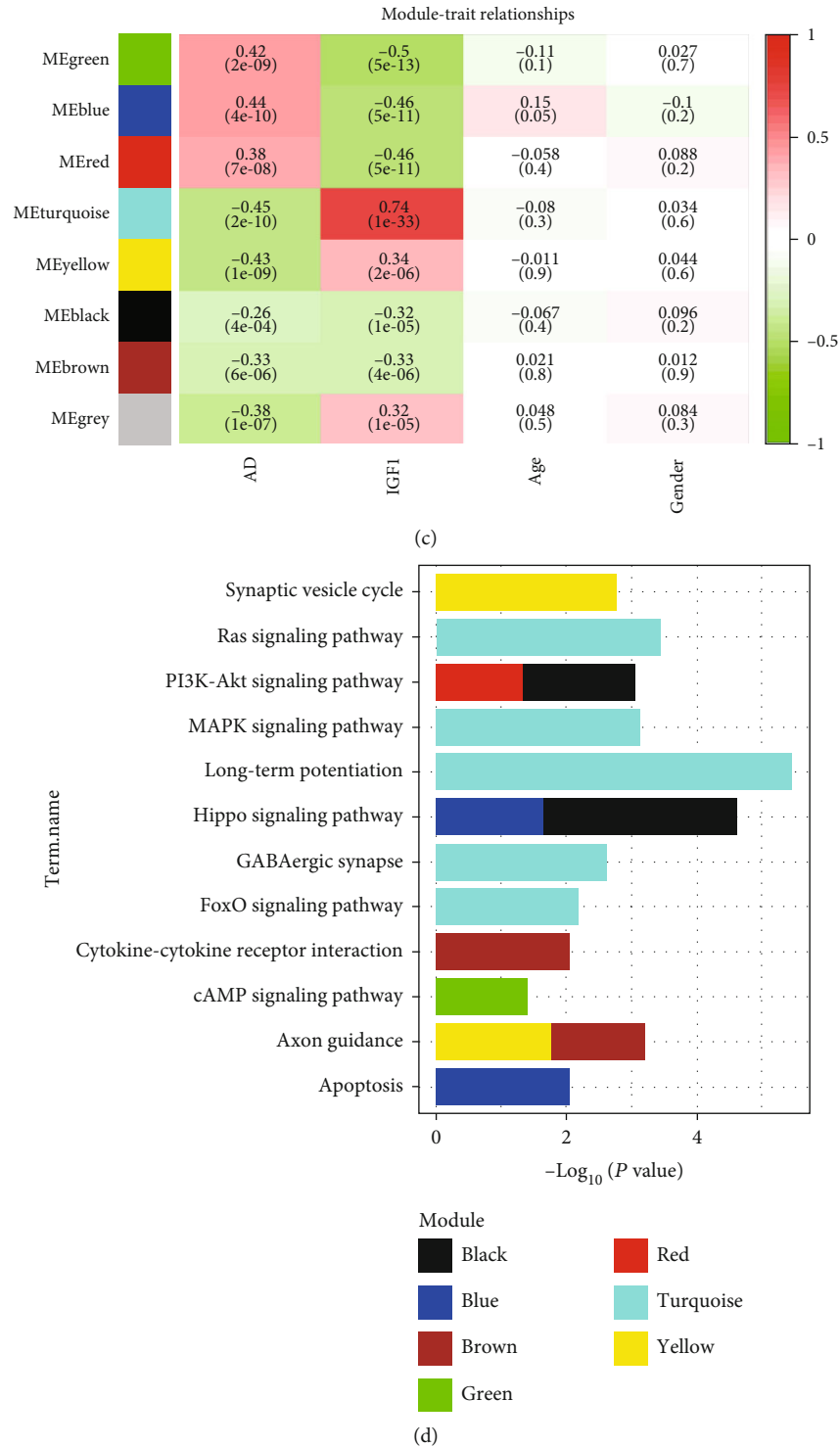
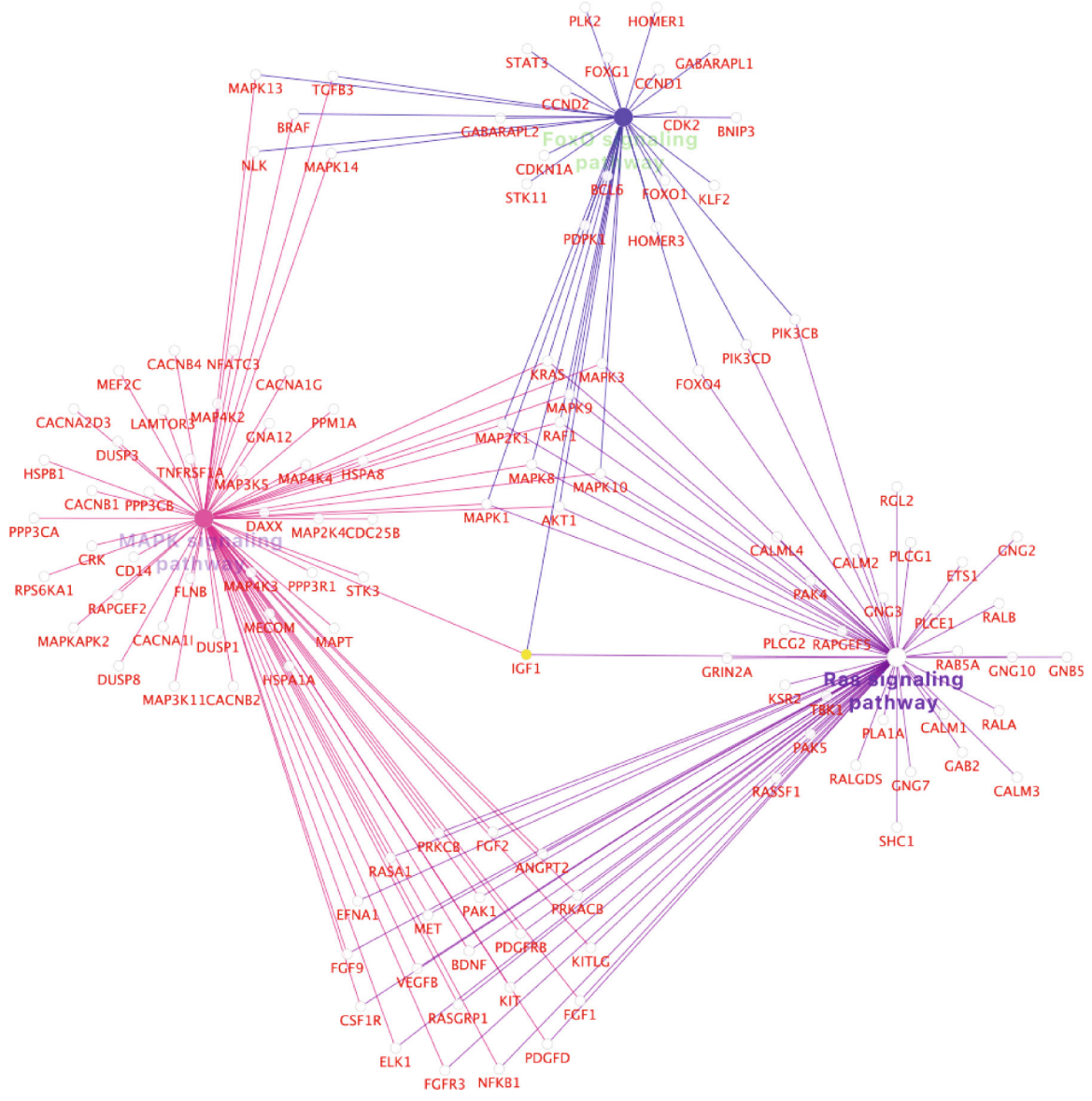


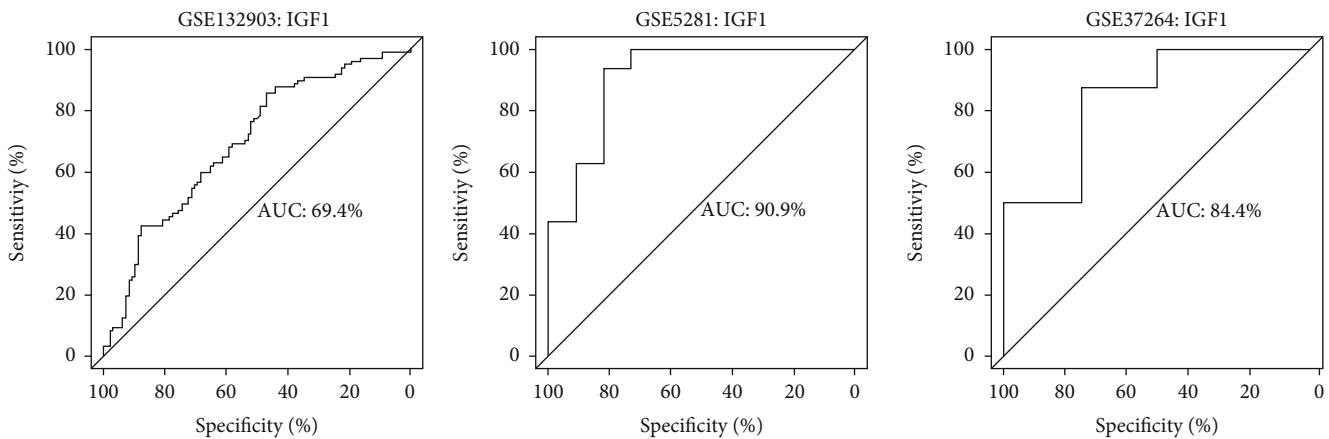
FIGURE 3: Weighted gene correlation network analysis. (a) Sample dendrogram and trait heatmap. (b) Cluster dendrogram of all DEGs with assigned module colors: non-clustering genes are displayed in gray. (c) Module-trait associations: the gradual change of color from green to red represents the changing correlation between ME and trait from negative to positive. (d) KEGG pathways enriched by module genes. AD: Alzheimer’s disease; KEGG: Kyoto Encyclopedia of Genes and Genomes; ME: module eigengene.

The results emerging from WGCNA showed that the turquoise module was overwhelmingly correlated with AD and IGF1 expression, in which DEGs were enriched in GABAergic synapse, long-term potentiation, MAPK, Ras,

and FoxO signaling pathways. Under pathological stimulation, MAPK signaling is involved in a variety of intracellular events, such as proliferation, differentiation, survival, apoptosis, and transformation [33, 34]. As confirmed by



(c)



(d)

FIGURE 4: Continued.

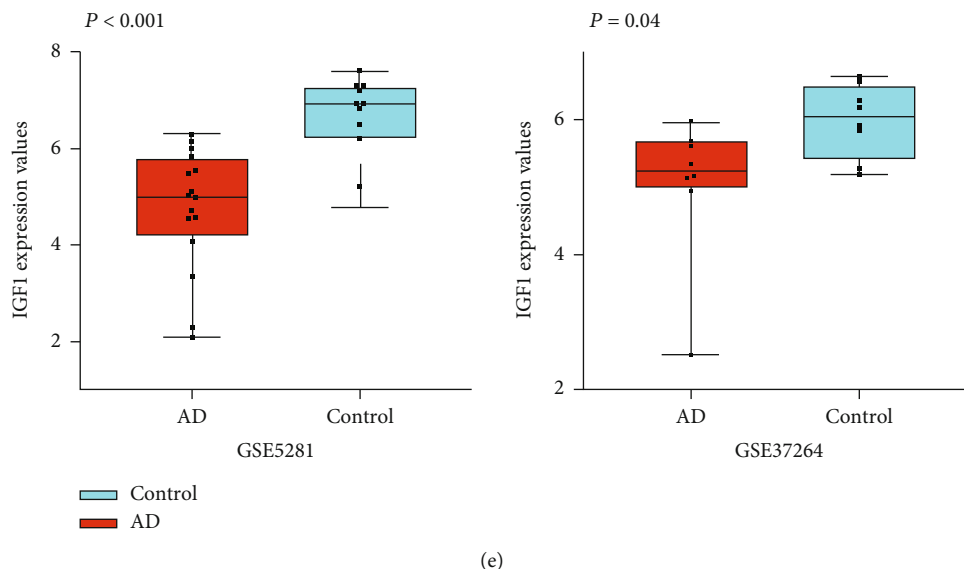


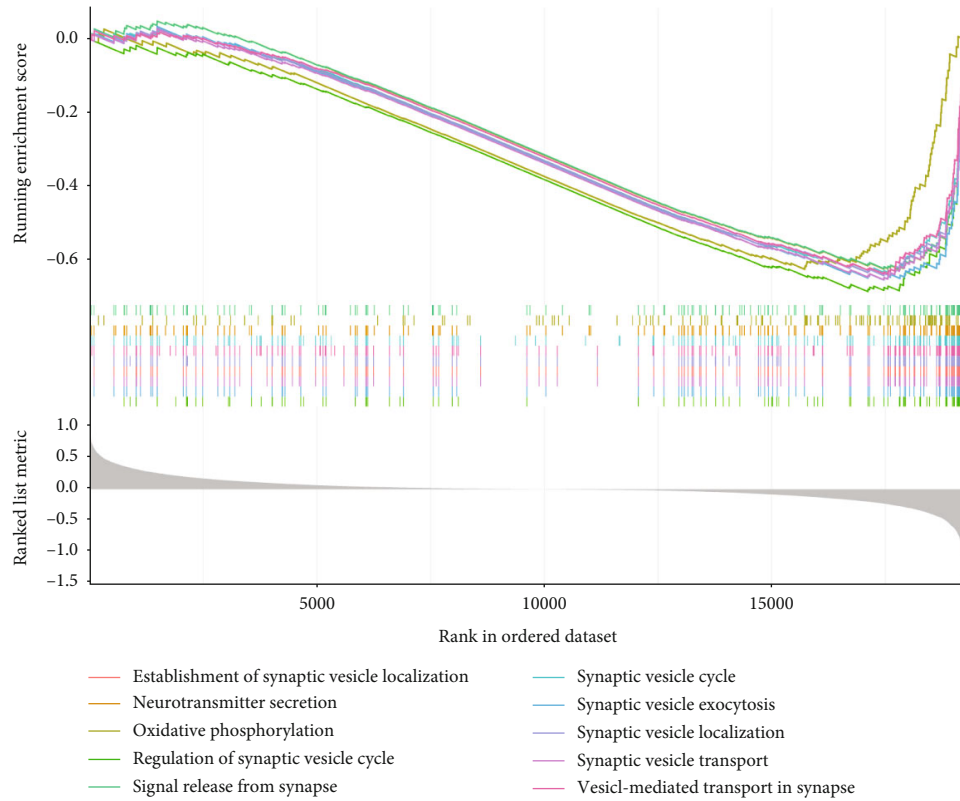
FIGURE 4: PPI network and AUC analysis. (a) Scatterplots of the relationship between MM (x -axis) and GS (y -axis). (b) PPI network based on turquoise module genes: node size reflects the degree of gene connectivity; down-regulated IGF1 is marked in yellow; blue and red represent down-regulated and up-regulated, respectively. (c) Cross-talking pathways of IGF1: down-regulated IGF1 is marked in yellow. (d) The predictive performance of IGF1 in AD measured by AUC analysis. (e) IGF1 expression between AD and non-dementia controls in training sets. AD: Alzheimer's disease; AUC: area under the curve; GS: gene significance; MM: module membership; PPI: protein-protein interaction.

astrocyte activation during the development of AD, multiple key stress-related responses are modulated by the MAPK cascades [35]. Additional evidence in support of this argument originates from *in vivo* and *in vitro* experiments with AD, wherein MAPKs subtypes, including p38 and c-Jun NH2 terminal kinase (JNK), are activated by $A\beta$ -treated cultured astrocytes and cerebral cortex of rats [34]. Conversely, blockade of p38 signaling by the mitochondrial antioxidant SkQ1 alleviates phosphorylation of intermediate kinases and α B-crystallin, both of which have implications in senile plaques and tau protein inclusions [36]. Moreover, several lines of evidence have linked IGF1 to MAPKs by influencing cell proliferation and differentiation through extracellular signal related kinase 1 and 2 (ERK1/2) and p38 signaling pathways [37, 38]. For instance, IGF1 elicits the expression and translocation of intracellular androgen receptors, thereby enhancing p38 and ERK1/2 activities to increase the proliferation of C2C12 myoblasts [39]. Similar outcomes are also replicated in muscle satellite cells and skeletal muscle cells, i.e., ERK1/2 signaling mediates the cell proliferation of IGF1 [40, 41], a role that can be completely suppressed by the ERK1/2 specific inhibitor PD98059 [37].

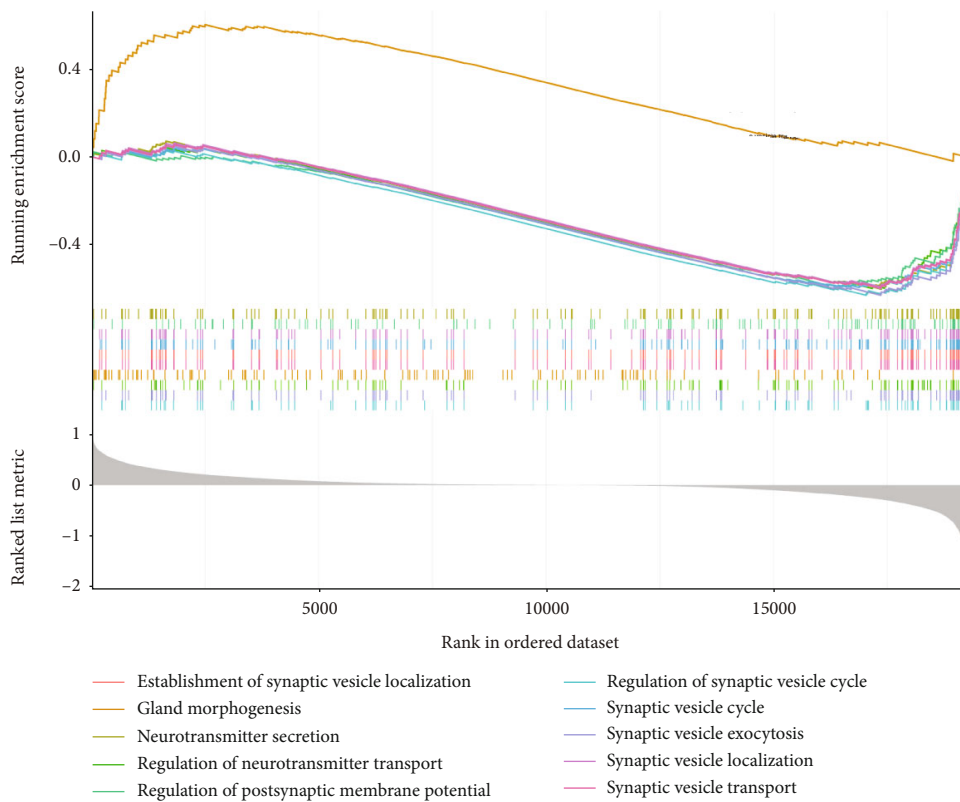
With except of the MAPK signaling pathway, Ras signaling has been linked to AD by regulating induction and post-translational modification of tau and amyloid-beta precursor protein (APP) [42, 43]. In frontal cortex of early AD, up-regulation of Ras expression acts on lysosomes and glycohydrolases, contributing to $A\beta$ deposition and formation of paired helical filaments, which is suggestive of the progression of AD neurodegeneration [44, 45]. In this process, it is noteworthy that the Ras must be routed and anchored to the inner leaflet of plasma membrane for signal reception and transmission [46]. Dislodge of Ras from anchorage domains by the

synthetic Ras inhibitor (e.g., FTS) enhances N-methyl-D-aspartate receptor (NMDAR)-dependent long-term potentiation (LTP) and blocks ERK-dependent LTP, hence improving synaptic plasticity and spatial memory [47, 48]. There is compelling evidence to support a relationship between IGF1 and Ras signaling pathway. Previous study has shown that IGF1 enhances Ras signaling through PI3K activation and thus to promote Lef/Tcf-dependent transcription, a process inhibited by dominant-negative mutants of Ha-Ras [49]. In addition, mice deficient in *Rasgrf1*, a guanine nucleotide-releasing factor of Ras, exhibits lower IGF1 levels responsible for learning and memory dysfunctions [50]. Consistently, our results provide evidence for the involvement of low IGF1 expression in Ras signaling pathway and that the up-regulation of IGF1 might be neuroprotective in AD.

In terms of FoxO signaling, transcription factors of the pathway are crucial regulators of cellular response to oxidative stress, a pathophysiological hallmark shared by insulin resistance, diabetic complications, and AD [51]. Mechanistically, short-term FoxO activation induced by oxidative stress involving Wingless proteins and beta-catenin pathway, which exerts antioxidant effects via regulating the transcription of antioxidant enzymes, manganese-superoxide dismutase, and catalase [52, 53]. Alternatively, long-term activation of FoxO due to an inability to cope with oxidative stress may predispose to hyperglycaemia and hyperinsulinaemia, which in turn fosters secondary oxidative stress related to the initiation of apoptosis [54, 55]. In the brain of established AD, oxidative stress-mediated FoxO activation is one of the important mechanisms underlying $A\beta$ -induced cell death. Specifically, $A\beta$ triggers FoxO phosphorylation in a p66Shc-dependent manner, promoting the generation of reactive oxygen species (ROS), lipid peroxides, and oxidized proteins,



(a)



(b)

FIGURE 5: Gene set enrichment analysis. (a) Top 10 representative BPs enriched by GSEA in AD. (b) Top 10 representative BPs enriched by GSEA in IGF1-low cohort. AD: Alzheimer's disease; BP: biological process; GSEA: gene set enrichment analysis.

thus to the release of neurotoxic H_2O_2 ; intriguingly, ectopic expression of p66ShcS36A reduces FoxO phosphorylation, thereby preventing oxidative cell death against $A\beta$ toxicity [56]. On the other hand, IGF1 encoding a paracrine or autocrine hormone is associated with FoxO transcription factors. In *Caenorhabditis elegans*, mutations in the IGF1 receptor homolog DAF-2 lead to metabolic, longevity, and developmental defects, which are alleviated by deletion of the FoxO homolog DAF-16 [57]. Additionally, FoxOs are transcriptional targets for fast induction of IGF1-mediated gluconeogenesis, which is reversed by insulin-stimulated nuclear rejection, implying a regulatory role of IGF1 in FoxO signaling pathway [58]. Likewise, these data are consistent with our findings that low expression of IGF1 is involved in the pathogenesis of AD through FoxO signaling pathway.

The scatterplot results between MM and GS confirmed the strongest correlation of DEGs with low IGF1 expression in the turquoise module. Based on these DEGs, a PPI network was constructed to identify the cross-talking pathways of IGF1, which supports the pluripotency of IGF1 in AD pathophysiology via MAPK, Ras, and FoxO signaling pathways. Of note, low expression of IGF1 is an important contributor to increased susceptibility of these relevant pathways during the development of AD, giving rise to subsequent impaired $A\beta$ clearance and neuronal apoptosis [59–61]. According to AUC analysis of test set and validation of training sets, cohort subgroup comparisons revealed that low IGF1 could actually distinguish AD from non-dementia controls, indicating IGF1 to be a molecular target in predicting the onset of AD. As demonstrated by quantitative real-time polymerase chain reaction (qRT-PCR) experiment in advanced AD brains, IGF1 mRNA levels and overall expression of its receptors were apparently diminished with the increase of Braak stage [62]. Thenceforth, Fredue et al. found that insufficient expression or impaired function of IGF1 was dose-responsive to the severity of AD progression [63]. In view of this, IGF1 is envisioned as a potential candidate for the perspective of AD therapy that can be administered safely and systemically [31]. Additionally, PCC analysis demonstrated the significant correlation of IGF1 with all pathway signature genes, indicating that signature genes of each cross-talking pathway changed with the expression of IGF1. These data provided computational statistical evidence for the regulatory or mediated role of low IGF1 expression in MAPK, Ras, and FoxO signaling pathways. Future work should focus on the biological verification of the cross-talking pathways involved in the pathogenesis of low IGF1-mediated AD, thus facilitating the transition from a single target of IGF1 to multiline understanding of the holistic AD spectrum.

5. Conclusions

In summary, integrative genomic analysis is an attractive and effective approach to decipher complex mechanisms of the molecular signature that contribute to AD onset. Our findings reveal a pleiotropic role of low IGF1 expression in the pathogenesis of AD via MAPK, Ras, and FoxO signaling

pathways, which may lead to a promising strategy in the prevention and treatment of AD by targeting IGF1.

Data Availability

The datasets referenced during the study are available in public repositories from the Gene Expression Omnibus database (<https://www.ncbi.nlm.nih.gov/geo/>) under accession numbers GSE132903, GSE5281, and GSE37264.

Conflicts of Interest

The authors confirm that there are no conflicts of interest.

Authors' Contributions

KXK and ZKZ were the principal researchers who conceived and designed this study. JB, RWZ, SSZ, and XQZ carried out the experiments and analyzed the data. KXK and MZ wrote the original draft. CSZ, YX, and ZKZ reviewed and edited the paper. All authors have read, revised, and approved the final manuscript.

Acknowledgments

This work is supported by the National Natural Science Foundation of China (No. 82071467); the National Natural Science Foundation of China (No. 81801053); the National Science Foundation of Liaoning Province (No. 20180540150); Innovation Team Support Plan of Universities in Liaoning Province (No. LT2019015); Guidance plan for key research and development plans of Liaoning Province (No. 2019JH8/10300002); Discipline Cultivation Support Plan-Stroke Center of China Medical University; Scientific Research Fund of Education Department of Liaoning Province (No. ZF2019012); Revitalizing Talents Plan of Liaoning Province (Chuansheng Zhao).

Supplementary Materials

Supplementary 1. Supplementary Table 1: Sample phenotypic information. AD; Alzheimer's disease.

Supplementary 2. Supplementary Table 2: MM and GS for each differentially expressed gene in co-expression modules. MM: Module membership; GS: gene significance.

Supplementary 3. Supplementary Table 3: Identification of pathway representative genes by PCC analysis: pathway representative genes are highlighted in yellow. PCC: Pearson's correlation coefficient.

Supplementary 4. Supplementary Figure 1: Correlation between IGF1 and pathway signature genes. * $p < 0.05$.

References

- [1] K. D. Ryneerson, M. Ponnusamy, O. Prikhodko et al., "Preclinical validation of a potent γ -secretase modulator for Alzheimer's disease prevention," *The Journal of Experimental Medicine*, vol. 218, no. 4, 2021.

- [2] T. Wakabayashi, K. Yamaguchi, K. Matsui et al., “Differential effects of diet- and genetically-induced brain insulin resistance on amyloid pathology in a mouse model of Alzheimer’s disease,” *Molecular Neurodegeneration*, vol. 14, no. 1, p. 15, 2019.
- [3] S. E. Arnold, Z. Arvanitakis, S. L. Macauley-Rambach et al., “Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums,” *Nature Reviews. Neurology*, vol. 14, no. 3, pp. 168–181, 2018.
- [4] K. Talbot, H.-Y. Wang, H. Kazi et al., “Demonstrated brain insulin resistance in Alzheimer’s disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline,” *The Journal of Clinical Investigation*, vol. 122, no. 4, pp. 1316–1338, 2012.
- [5] T. Ohara, Y. Doi, T. Ninomiya et al., “Glucose tolerance status and risk of dementia in the community: the Hisayama study,” *Neurology*, vol. 77, no. 12, pp. 1126–1134, 2011.
- [6] L. A. Profenno, A. P. Porsteinsson, and S. V. Faraone, “Meta-analysis of Alzheimer’s disease risk with obesity, diabetes, and related disorders,” *Biological Psychiatry*, vol. 67, no. 6, pp. 505–512, 2010.
- [7] A. Jones, P. Kulozik, A. Ostertag, and S. Herzig, “Common pathological processes and transcriptional pathways in Alzheimer’s disease and type 2 diabetes,” *Journal of Alzheimer’s Disease*, vol. 16, no. 4, pp. 787–808, 2009.
- [8] E. Carro, J. L. Trejo, T. Gomez-Isla, D. LeRoith, and I. Torres-Aleman, “Serum insulin-like growth factor I regulates brain amyloid- β levels,” *Nature Medicine*, vol. 8, no. 12, pp. 1390–1397, 2002.
- [9] F. Hakuno and S.-I. Takahashi, “IGF1 receptor signaling pathways,” *Journal of Molecular Endocrinology*, vol. 61, no. 1, pp. T69–T86, 2018.
- [10] E. Carro, J. L. Trejo, S. Busiguina, and I. Torres-Aleman, “Circulating insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy,” *The Journal of Neuroscience*, vol. 21, no. 15, pp. 5678–5684, 2001.
- [11] S. Craft, S. Asthana, G. Schellenberg et al., “Insulin effects on glucose metabolism, memory, and plasma amyloid precursor protein in Alzheimer’s disease differ according to apolipoprotein-E genotype,” *Annals of the New York Academy of Sciences*, vol. 903, no. 1 VASCULAR FACT, pp. 222–228, 2000.
- [12] L. Xie, E. Helmerhorst, K. Taddei, B. Plewright, W. Van Bronswijk, and R. Martins, “Alzheimer’s beta-amyloid peptides compete for insulin binding to the insulin receptor,” *The Journal of Neuroscience*, vol. 22, no. 10, p. RC221, 2002.
- [13] D. J. Selkoe, “Clearing the brain’s amyloid cobwebs,” *Neuron*, vol. 32, no. 2, pp. 177–180, 2001.
- [14] T. Niikura, Y. Hashimoto, T. Okamoto et al., “Insulin-like growth factor I (IGF-I) protects cells from apoptosis by Alzheimer’s V642I mutant amyloid precursor protein through IGF-I receptor in an IGF-binding protein-sensitive manner,” *The Journal of Neuroscience*, vol. 21, no. 6, pp. 1902–1910, 2001.
- [15] L. Gasparini, G. K. Gouras, R. Wang et al., “Stimulation of beta-amyloid precursor protein trafficking by insulin reduces intraneuronal beta-amyloid and requires mitogen-activated protein kinase signaling,” *The Journal of Neuroscience*, vol. 21, no. 8, pp. 2561–2570, 2001.
- [16] M. Lesort and G. V. Johnson, “Insulin-like growth factor-1 and insulin mediate transient site-selective increases in tau phosphorylation in primary cortical neurons,” *Neuroscience*, vol. 99, no. 2, pp. 305–316, 2000.
- [17] M. Lesort, R. S. Jope, and G. V. Johnson, “Insulin transiently increases tau phosphorylation: involvement of glycogen synthase kinase-3 β and Fyn tyrosine kinase,” *Journal of Neurochemistry*, vol. 72, no. 2, pp. 576–584, 1999.
- [18] M. Schubert, D. P. Brazil, D. J. Burks et al., “Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation,” *The Journal of Neuroscience*, vol. 23, no. 18, pp. 7084–7092, 2003.
- [19] L. Wang and Z.-P. Liu, “Detecting diagnostic biomarkers of Alzheimer’s disease by integrating gene expression data in six brain regions,” *Frontiers in Genetics*, vol. 10, p. 157, 2019.
- [20] Z. Zhou, J. Bai, S. Zhong et al., “Downregulation of ATP6V1A involved in Alzheimer’s disease via synaptic vesicle cycle,” *Oxidative Medicine and Cellular Longevity*, vol. 2021, Article ID 5555634, 15 pages, 2021.
- [21] A. Reiner, D. Yekutieli, and Y. Benjamini, “Identifying differentially expressed genes using false discovery rate controlling procedures,” *Bioinformatics*, vol. 19, no. 3, pp. 368–375, 2003.
- [22] P. Langfelder and S. Horvath, “WGCNA: an R package for weighted correlation network analysis,” *BMC Bioinformatics*, vol. 9, no. 1, p. 559, 2008.
- [23] E. Fredlund, J. Staaf, J. K. Rantala, O. Kallioniemi, A. Borg, and M. Ringnér, “The gene expression landscape of breast cancer is shaped by tumor protein p53 status and epithelial-mesenchymal transition,” *Breast Cancer Research*, vol. 14, no. 4, article R113, 2012.
- [24] D. Szklarczyk, J. H. Morris, H. Cook et al., “The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible,” *Nucleic Acids Research*, vol. 45, no. D1, pp. D362–D368, 2017.
- [25] R. Sriroopreddy, R. Sajeed, P. Raghuraman, and C. Sudandiradoss, “Differentially expressed gene (DEG) based protein-protein interaction (PPI) network identifies a spectrum of gene interactome, transcriptome and correlated miRNA in nondisjunction Down syndrome,” *International Journal of Biological Macromolecules*, vol. 122, pp. 1080–1089, 2019.
- [26] D. Otasek, J. H. Morris, J. Bouças, A. R. Pico, and B. Demchak, “Cytoscape automation: empowering workflow-based network analysis,” *Genome Biology*, vol. 20, no. 1, p. 185, 2019.
- [27] T. K. Karikari, T. A. Pascoal, N. J. Ashton et al., “Blood phosphorylated tau 181 as a biomarker for Alzheimer’s disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts,” *Lancet Neurology*, vol. 19, no. 5, pp. 422–433, 2020.
- [28] D.-M. Ma, Z. Wang, L. Wang, F. Alejos-Gonzales, M.-A. Sun, and D.-Y. Xie, “A genome-wide scenario of terpene pathways in self-pollinated *Artemisia annua*,” *Molecular Plant*, vol. 8, no. 11, pp. 1580–1598, 2015.
- [29] H. Sun, Y. Zhou, M. F. Skaro et al., “Metabolic reprogramming in cancer is induced to increase proton production,” *Cancer Research*, vol. 80, no. 5, pp. 1143–1155, 2020.
- [30] A. Subramanian, P. Tamayo, V. K. Mootha 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*, vol. 102, no. 43, pp. 15545–15550, 2005.

- [31] L. Gasparini and H. Xu, "Potential roles of insulin and IGF-1 in Alzheimer's disease," *Trends in Neurosciences*, vol. 26, no. 8, pp. 404–406, 2003.
- [32] M. Itakura, S. Yamamori, R. Kuwahara, M. Sekiguchi, and M. Takahashi, "Two distinct regulatory mechanisms of neurotransmitter release by phosphatidylinositol 3-kinase," *Journal of Neurochemistry*, vol. 94, no. 2, pp. 502–509, 2005.
- [33] J. M. Morganti, D. S. Goulding, and L. J. Van Eldik, "Deletion of p38 α MAPK in microglia blunts trauma-induced inflammatory responses in mice," *Journal of Neuroinflammation*, vol. 16, no. 1, p. 98, 2019.
- [34] P. Saha, S. Guha, and S. C. Biswas, "P38K and JNK pathways are induced by amyloid- β in astrocyte: implication of MAPK pathways in astrogliosis in Alzheimer's disease," *Molecular and Cellular Neurosciences*, vol. 108, article 103551, 2020.
- [35] J. K. Lee and N.-J. Kim, "Recent advances in the inhibition of p38 MAPK as a potential strategy for the treatment of Alzheimer's disease," *Molecules*, vol. 22, no. 8, p. 1287, 2017.
- [36] N. A. Muraleva, N. A. Stefanova, and N. G. Kolosova, "SkQ1 suppresses the p38 MAPK signaling pathway involved in Alzheimer's disease-like pathology in OXYS rats," *Antioxidants*, vol. 9, no. 8, p. 676, 2020.
- [37] S. Fu, L. Yin, X. Lin, J. Lu, and X. Wang, "Effects of cyclic mechanical stretch on the proliferation of L6 myoblasts and its mechanisms: PI3K/Akt and MAPK signal pathways regulated by IGF-1 receptor," *International Journal of Molecular Sciences*, vol. 19, no. 6, p. 1649, 2018.
- [38] P. Liu, F. Kong, J. Wang et al., "Involvement of IGF-1 and MEOX2 in PI3K/Akt1/2 and ERK1/2 pathways mediated proliferation and differentiation of perivascular adipocytes," *Experimental Cell Research*, vol. 331, no. 1, pp. 82–96, 2015.
- [39] Y. Ma, S. Fu, L. Lu, and X. Wang, "Role of androgen receptor on cyclic mechanical stretch-regulated proliferation of C2C12 myoblasts and its upstream signals: IGF-1-mediated PI3K/Akt and MAPKs pathways," *Molecular and Cellular Endocrinology*, vol. 450, pp. 83–93, 2017.
- [40] X. Ge, Y. Zhang, and H. Jiang, "Signaling pathways mediating the effects of insulin-like growth factor-I in bovine muscle satellite cells," *Molecular and Cellular Endocrinology*, vol. 372, no. 1–2, pp. 23–29, 2013.
- [41] M. Yu, H. Wang, Y. Xu et al., "Insulin-like growth factor-1 (IGF-1) promotes myoblast proliferation and skeletal muscle growth of embryonic chickens via the PI3K/Akt signalling pathway," *Cell Biology International*, vol. 39, no. 8, pp. 910–922, 2015.
- [42] J. M. Cosgaya, M. J. Latasa, and A. Pascual, "Nerve growth factor and ras regulate beta-amyloid precursor protein gene expression in PC12 cells," *Journal of Neurochemistry*, vol. 67, no. 1, pp. 98–104, 1996.
- [43] E. Sadot, H. Jaaro, R. Seger, and I. Ginzburg, "Ras-signaling pathways: positive and negative regulation of tau expression in PC12 cells," *Journal of Neurochemistry*, vol. 70, no. 1, pp. 428–431, 1998.
- [44] C. Emiliani, L. Urbanelli, L. Racanicchi et al., "Up-regulation of glycohydrolases in Alzheimer's disease fibroblasts correlates with Ras activation," *The Journal of Biological Chemistry*, vol. 278, no. 40, pp. 38453–38460, 2003.
- [45] U. Gärtner, M. Holzer, and T. Arendt, "Elevated expression of p21^{ras} is an early event in Alzheimer's disease and precedes neurofibrillary degeneration," *Neuroscience*, vol. 91, no. 1, pp. 1–5, 1999.
- [46] J. M. Shields, K. Pruitt, A. McFall, A. Shaub, and C. J. Der, "Understanding Ras: 'it ain't over 'til it's over'," *Trends in Cell Biology*, vol. 10, no. 4, pp. 147–154, 2000.
- [47] M. A. Patterson, E. M. Szatmari, and R. Yasuda, "AMPA receptors are exocytosed in stimulated spines and adjacent dendrites in a Ras-ERK-dependent manner during long-term potentiation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 36, pp. 15951–15956, 2010.
- [48] Y. Wang, T. Chen, Z. Yuan et al., "Ras inhibitor S-trans, trans-farnesylthiosalicylic acid enhances spatial memory and hippocampal long-term potentiation via up-regulation of NMDA receptor," *Neuropharmacology*, vol. 139, pp. 257–267, 2018.
- [49] C. Desbois-Mouthon, A. Cadoret, M. J. Blivet-Van Eggelpoël et al., "Insulin and IGF-1 stimulate the β -catenin pathway through two signalling cascades involving GSK-3 β inhibition and Ras activation," *Oncogene*, vol. 20, no. 2, pp. 252–259, 2001.
- [50] N. M. Drake, Y. J. Park, A. S. Shirali, T. A. Cleland, and P. D. Soloway, "Imprint switch mutations at Rasgrf1 support conflict hypothesis of imprinting and define a growth control mechanism upstream of IGF1," *Mammalian Genome*, vol. 20, no. 9–10, pp. 654–663, 2009.
- [51] K. N. Manolopoulos, L. O. Klotz, P. Korsten, S. R. Bornstein, and A. Barthel, "Linking Alzheimer's disease to insulin resistance: the FoxO response to oxidative stress," *Molecular Psychiatry*, vol. 15, no. 11, pp. 1046–1052, 2010.
- [52] G. J. P. L. Kops, T. B. Dansen, P. E. Polderman et al., "Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress," *Nature*, vol. 419, no. 6904, pp. 316–321, 2002.
- [53] S. Nemoto and T. Finkel, "Redox regulation of forkhead proteins through ap66shc-dependent signaling pathway," *Science*, vol. 295, no. 5564, pp. 2450–2452, 2002.
- [54] D. Cao, H. Lu, T. L. Lewis, and L. Li, "Intake of sucrose-sweetened water induces insulin resistance and exacerbates memory deficits and amyloidosis in a transgenic mouse model of Alzheimer disease," *The Journal of Biological Chemistry*, vol. 282, no. 50, pp. 36275–36282, 2007.
- [55] S. C. Martinez, K. Tanabe, C. Cras-Méneur, N. A. Abumrad, E. Bernal-Mizrachi, and M. A. Permutt, "Inhibition of Foxo1 protects pancreatic islet beta-cells against fatty acid and endoplasmic reticulum stress-induced apoptosis," *Diabetes*, vol. 57, no. 4, pp. 846–859, 2008.
- [56] W. W. Smith, D. D. Norton, M. Gorospe et al., "Phosphorylation of p66Shc and forkhead proteins mediates Abeta toxicity," *The Journal of Cell Biology*, vol. 169, no. 2, pp. 331–339, 2005.
- [57] S. Ogg, S. Paradis, S. Gottlieb et al., "The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*," *Nature*, vol. 389, no. 6654, pp. 994–999, 1997.
- [58] B. T. O'Neill, K. Y. Lee, K. Klaus et al., "Insulin and IGF-1 receptors regulate FoxO-mediated signaling in muscle proteostasis," *The Journal of Clinical Investigation*, vol. 126, no. 9, pp. 3433–3446, 2016.
- [59] V. E. Bianchi, V. Locatelli, and L. Rizzi, "Neurotrophic and neuroregenerative effects of GH/IGF1," *International Journal of Molecular Sciences*, vol. 18, no. 11, p. 2441, 2017.
- [60] H. Zhang, Y. Gao, Z. Dai, T. Meng, S. Tu, and Y. Yan, "IGF-1 reduces BACE-1 expression in PC12 cells via activation of PI3K/Akt and MAPK/ERK1/2 signaling pathways," *Neurochemical Research*, vol. 36, no. 1, pp. 49–57, 2011.

- [61] P. Zheng and W. Tong, "IGF-1: an endogenous link between traumatic brain injury and Alzheimer disease?," *Journal of Neurosurgical Sciences*, vol. 61, no. 4, pp. 416–421, 2017.
- [62] E. J. Rivera, A. Goldin, N. Fulmer, R. Tavares, J. R. Wands, and S. M. de la Monte, "Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: link to brain reductions in acetylcholine," *Journal of Alzheimer's Disease*, vol. 8, no. 3, pp. 247–268, 2005.
- [63] S. Freude, M. M. Hettich, C. Schumann et al., "Neuronal IGF-1 resistance reduces A β accumulation and protects against premature death in a model of Alzheimer's disease," *The FASEB Journal*, vol. 23, no. 10, pp. 3315–3324, 2009.