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Basic Study

KAT7/HMGN1 signaling epigenetically induces tyrosine phosphorylation-regulated kinase 1A (DYRK1A) expression to ameliorate insulin resistance in Alzheimer's disease

KAT7 on insulin resistance in AD

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Abstract

BACKGROUND

The correlation between Alzheimer's disease (AD) and type 2 diabetes mellitus (T2D) has been disclosed by epidemiological studies and basic research. Insulin resistance in brain is a common feature that shared by T2D and AD. KAT7 is a histone acetyltransferase that participates in modulation of various genes.

AIM

This work aimed to determine the effects of KAT7 on insulin resistance in AD.

METHODS

APPswe/PS1-dE9 (APP/PS1) double-transgenic mice and db/db mice were used to mimic AD and diabetes, respectively. *In vitro* model of AD was established by Aβ stimulation. Insulin resistance was obtained by chronic stimulation of high-level insulin. The expression of MAP2 was assessed by immunofluorescence assay. The protein levels of MAP2, Aβ, DYRK1A, IRS-1, p-AKT, total AKT, p-GSK3β, total GSK3β,

DYRK1A, and KAT7 were measured by western blotting. Accumulation of ROS, MDA level, and SOD activity was measured to determine cell oxidative stress. Flowcytometry andCCK-8 assay were performed to evaluate neuron cell death and proliferation. Relative RNA levels of KAT7 and DYRK1A was checked by quantitative PCR. ChIP assay was performed to determine H3K14ac on DYRK1A gene.

RESULTS

KAT7 expression was suppressed in AD model. Overexpression of KAT7 decreased Aβ accumulation and MAP2 expression in AD brains. KAT7 overexpression decreased ROS level and MDA level, elevated SOD activity in brain tissues and neurons, simultaneously suppressed neuron apoptosis. KAT7 upregulated level of p-AKT and p-GSK3β to alleviate insulin resistance, along with elevated expression of DYRK1A. Depletion of KAT7 suppressed expression of DYRK1A and impaired H3K14ac of DYRK1A. Overexpression of HMGN1 recovered the DYRK1A level and reversed insulin resistance that caused by KAT7 depletion.

CONCLUSION

We determined that overexpression of KAT7 recovered insulin sensitivity in AD *via* recruiting the HMGN1 to enhance acetylation of DYRK1A gene. Our findings suggested KAT7 as a novel and promising therapeutic approach for insulin resistance in AD.

Key Words: Alzheimer's disease, diabetes, insulin resistance, KAT7, DYRK1A, HMGN1

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Core Tip: Type 2 diabetes mellitus is closely associated with neurodegenerative diseases such as Alzheimer's disease, during which the dysfunction of insulin resistance

plays a critical role. However, the pathological mechanisms underlying the diabetes mellitus-correlated Alzheimer's disease remain unclear. Our study demonstrated that Histone acetyltransferase KAT7 ameliorated neuron death and oxidative stress in AD, as well as recovered insulin sensitivity in insulin-resistant neurons through recruiting the HMGN1 to enhance acetylation of DYRK1A gene, suggesting the promising therapeutic potential of KAT7 in diabetes mellitus -associated Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is a complicated and prevalent neurodegenerative disease that commonly occurs among older adults globally [1, 2]. AD is featured by progressively declined cognition ability and memory loss [3]. The deposition of Aβ-comprised extracellular plaques and neurofibrillary tangles are the main pathological hallmarks of AD [3]. Moreover, most patients with AD are accompanied with cerebrovascular diseases, including impaired integrity of the blood-brain barrier (BBB) [4]. Increasing number of epidemiological studies have shown a strong association between AD and type 2 diabetes mellitus (T2D), in which insulin resistance is a common and critical pathological feature [5, 6]. However, the pathological mechanisms underlying the correlation between insulin resistance and AD remains unclear.

Histone acetyltransferases (HATs) are divided into different families according to homology of structure and sequence, including the P300 /CBP, MYST and GCN5 family [7]. The HATs play central role in transcriptional regulation by catalyzing the transfer of acetyl from acetyl CoA to ε-amino of histone lysine residues [8]. The abnormal function of HATs is closely correlated with various diseases, including developmental disorders and cancers [9-11]. HATs of the MYST family are characterized by conserved MYST catalytic domain, which include the KAT5 (TIP60), KAT6A (MOZ and MYST3), KAT6B (MORF and MYST4), KAT7 (HBO1 and MYST), and KAT8 (MOF) [12]. KAT7 acetylates the K14 and K23 on histone H3 *via* interacting with scaffolding protein BRPF, and acetylates K5, K8 and K12 on histone H4 *via* scaffolding protein JADE [13, 14]. During

tissue development, depletion of KAT7 Led to significantly decreased H3K14ac in erythrocytes of fetal liver and embryo of mice [15].

Dual-specificity tyrosine phosphorylation-regulated kinase-1A (DYRK1A) is a highly conserved protein kinase that phosphorylates its own tyrosine and the silk/threonine residues of exogenous substrates [16]. Studies have determined that DYRK1A catalyzed multiple critical proteins, such as NOTCH, CREB, STAT3, eIF2B, and caspase-9, and so on [17]. The transgenic mice with high level of DYRK1A exhibited impaired motor skill and spatial learning ability [18]. DYRK1A knockout mice died in embryonic stage, and the heterozygous mice exhibited low survival rate and abnormal neurological behavior [19]. DYRK1A is also reported to participate in the development of Alzheimer's disease, Down syndrome, diabetes, and tumors [20, 21].

In this work, we explored the mechanisms underlying insulin resistance in AD, determined that KAT7 epigenetically upregulated the acetylation and expression of DYRK1A to ameliorate the insulin resistance during AD. Our work provided novel therapeutic targets for AD.

MATERIALS AND METHODS

AD mouse model

APPswe/PS1-dE9 (APP/PS1) double-transgenic mice aged 8-months were brought from Vital River Laboratory (China). The mice were randomly divided into experimental groups, the KAT7 overexpressing lentivirus (1×10^9 IU/mL) were stereotactic injected (3 μl/min) to the CA1 area of the hippocampus. All experiments were approved by the Animal Ethic Committee of Qilu Hospital of Shandong University.

Diabetic mouse model

The db/db mice and control mice that aged 12 wk-old were purchased from Vital River Laboratory (China). The brain tissues were collected from these mice, and protein expression was checked using western blotting assay.

Cell lines

Primary neurons were isolated from mice and maintained in specific culture medium at 37°C humidified atmosphere with 5% CO_2 [22]. To mimic insulin resistance, cells were stimulated with culture medium that contains insulin (3 μM), no FBS and no B27 for 24 h, followed by deprivation of insulin for 30 minutes. After that, cells were stimulated with or without insulin (10 nM) for 15 minutes and were collected for following experiments.

Cell transfection

A lentivirus system for KAT7 and HMGN1 overexpression, siRNAs that target KAT7 (siKAT7) and HMGN1 (siHMGN1) were synthesized by GenePharma (China). The oligonucleotides were transfected into cells by using Lipofectamine 2000 (Invitrogen, USA) following manufacturer's description.

Cell viability and apoptosis

The viability of cells was checked using cell counting kit-8 (CCK-8) (Beyotime, China). In brief, at 5,000 cells were placed in each well of 96-well plates and incubated for 24 h. Then, 20 μl CCK-8 reagent was added and hatched for another two hours at 37°C Absorbance values were checked at 450 nm by a microplate detector (Thermo, USA). Cell apoptosis was assessed by flow cytometry using an Annexin V/PI apoptosis detection kit (Beyotime, China)

Immunofluorescence (IF) staining

For immunofluorescence staining, brain tissues were fixed and coated with OCT, made into 5 μm slices, then probed with primary antibodies against MAP2 overnight at 4 °C. Next day, the samples were hatched with Alexa Fluor 633-conjugated secondary antibodies (Thermo, USA) for 1 h at room temperature. DAPI (Thermo) reagent was

adopted to label the nucleus. Five random images were taken under microscope (Leica, Germany).

Quantitative Real-time PCR (qRT-PCR) assay

The brain tissues and cells were homogenized by Trizol reagent (Thermo, USA) to extract total RNA, followed by reverse-transcription to cDNA using First Strand cDNA Synthesis Kit (Thermo, USA). Gene expression levels were quantified by using SYBR Green system (Thermo, USA). The relative gene expression was normalized to GAPDH.

Western blotting

Total proteins were obtained from brain tissues and cells using ice-cold RIPA lysis buffer (Thermo, USA) that contains protease inhibitors (Sigma, USA). Equal amounts of proteins were separated by SDS-PAGE gel, blotted onto the PVDF membranes (Millipore, USA), blocked with 5% non-fat milk, then hatched with anti-Aβ, anti-MAP2, anti-KAT7, anti-DYRK1A, anti-AKT, anti-pAKT, anti-GSK3β, and ani-β-actin for one night at 4°C The blots were visualized after incubation with secondary antibodies and ECL reagent (Millipore, USA). All antibodies were purchased from Abcam and used following the protocols.

Evaluation of ROS level

The reactive oxygen species (ROS) level was evaluated by staining with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) (Sigma, USA) in line with manufacturer's protocol. Samples were hatched with DCF-DA (25 μM) at 37° C incubator in dark for 30 minutes. The relative values of fluorescence at 485 nm were checked by a microplate detector (Thermo, USA).

Evaluation of oxidative stress

The levels of *malondialdehyde (MDA)* and *superoxide dismutase* [23] *activity* was assessed by MDA kit and SOD kit (Beyotime, China) in line with manufacturers' description.

Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was performed following the instruction of EZ-ChIP kit (Millipore, USA). In short, neurons were treated with formaldehyde for 10 minutes to obtain crosslink between DNA and protein. The chromatin fragments were obtained after sonication of cell lysates, then incubated with antibody that targets H3K27me3. The precipitated DNA was evaluated by qPCR.

Statistical analysis

All data were exhibited as mean ± standard deviation, and analyzed by using SPSS software (SPSS, USA). Data comparisons between two groups or among multiple groups were conducted using student's t test or one-way analysis of variance [24]. The value of $p<0.05$ was regarded as statistically significant.

RESULTS

KAT7 expression is correlated with AD and insulin resistance

To determine the role of KAT7 in insulin resistance of AD, we established an *in vivo* AD model. We observed notable accumulation of Aβ, and decreased expression of microtubule-associated protein 2 (MAP2), the biomarker of neuron generation (Fig S1A and B) in brain tissues from AD mice, comparing with control mice, which suggested the successful establishment of AD model. In contrast, we observed decreased expression of KAT7 in AD group (Fig S1A and B). Besides, the KAT7 is coordinately overexpressed with IRS-1 and DYPK1A in the diabetic mice (db/db), comparing with normal mice (m/m) (Fig S1C). The insulin receptor substrate-1 (IRS1) is an important regulator of insulin homeostasis, and its downregulation promotes insulin resistance [25, 26]. Recent work has indicated that the DYPK1A/IRS-1 signaling represses insulin resistance [27]. Hence, we speculated that KAT7 may modulate insulin resistance in AD.

KAT7 alleviated AD-caused neurological damages in vivo

We next determined how KAT7 overexpression affect damage and oxidative stress brain. As shown in Fig 1A, treatment with KAT7 overexpression vectors led to significant elevation of KAT7 in brain tissues, along with decreased Aβ accumulation, which revered the phenotype of AD brains. KAT7 treatment also enhanced portion of MAP2-positive neurons (Fig 1B), comparing with the AD brains. Moreover, AD brains exhibited elevated accumulation of ROS, enhanced MDA level, and decreased activity of SOD, whereas KAT7 overexpression reversed these phenomena (Fig 1C-E).

KAT7 alleviated AD-caused neurological damages in vitro

We also adopted an *in vitro* model to assess the effects of KAT7 overexpression on Aβ-induced neuron cell damages. Stimulation with Aβ repressed the expression of KAT7, and transfection with KAT7 vectors enhanced its protein levels in brains (Fig 2A). Results from flow cytometry and CCK-8 demonstrated suppressed cell viability and increased apoptosis of neurons in Aβ-stimulated cell model, whereas KAT7 overexpression recovered cell viability and alleviated cell apoptosis (Fig 2B-D). In contrast with the *in vivo* model, KAT7 also alleviated oxidative stress induced by Aβ (Fig 2E-G). These data indicated that KAT7 alleviated AD-caused neuron cell death and oxidative stress.

KAT7 ameliorates chronic high insulin-induced insulin resistance

Insulin resistance could be caused by sustained stimulation of high level-insulin. Here, we firstly treated neurons with insulin (3μM) for 24 h to achieve insulin resistance, and treatment with serum-free medium reached basal status, followed by acute stimulation with 10 nM insulin for 15 minutes. As shown inpanel1 and panel 2 in Fig 4A to C, acute stimulation by insulin caused elevated ration of p-AKT and p-GSK3β, indicating sensitivity to insulin. On the other hand, neurons pre-treated with insulin (3μM) for 24 h presented no significant alteration of p-AKT and p-GSK3β ratio (Fig 3A-C, panel 3 and 4), indicating the acquired insulin resistance. We also found that IRS-1 expression was decreased by pre-stimulation of insulin and increased by acute stimulation (Fig

3D), which is consistent with reported findings. Noteworthy, chronic stimulation with insulin caused increased expression of DYRK1A with or without pre-stimulation of insulin (Fig 3E). Overexpression of KAT7 upregulated the sensitivity to insulin in both stimulated and basal neurons, manifested by elevated levels of p-AKT and p-GSK3β ratio. These data suggested that KAT7 ameliorates chronic high insulin-induced insulin resistance.

KAT7 epigenetically induce DYRK1A expression and ameliorates insulin resistance via HMGN1

We next explored the regulatory downstream of KAT7 during insulin resistance in AD. We administrated KAT7 depletion in neurons and checked the expression of DYRK1A. Transfection with siKAT7-3 could effectively downregulate the level of KAT7 and DYRK1A (Fig S2A and B). Results from ChIP revealed that depletion of KAT7 alleviated the acetylation of K14 on histone 3 of DYRK1A gene (Fig S2C). Moreover, HMGN1 is a protein that has been reported to bind to nucleosome and facilitate the H4K14 acetylation [28]. We observed that siHMGN1-3 effectively suppressed HMGN1 and DYRK1A expression in neurons (Fig S2D and E). Overexpression of HMGN1 reversed both the RNA and protein level of DYRK1A (Fig S2F and G). We next used the insulin-resistant neurons to evaluate the function of KAT7/HMGN1/DYRK1A. We observed that p-AKT, p-GSK3β, and IRS-1 expression were decreased by knockdown of KAT7 (Fig 4A-D) or HMGN1 (Fig 4E-H), whereas overexpression of DYRK1A reversed this phenomenon. These findings indicated that KAT7 modulate DYRK1A expression *via* recruiting HMGN1 and ameliorates neuron insulin resistance *via* DYRK1A/HMGN1 signaling.

DISCUSSION

The correlation between AD and T2D has been disclosed by epidemiological studies and basic research [4, 29]. Diabetes is even considered as a novel risk factor of AD [5]. Nevertheless, the mechanisms underlying the correlation between AD and T2D remains

unclear. Insulin resistance in brain is a common feature that shared by T2D and AD [30]. Studies have reported that diabetic mouse with cognitive disorders presented notable insulin resistance in brain [31]. Accumulating evidence demonstrated that insulin resistance promotes Tau phosphorylation and Aβ plaques accumulation in AD brains [31]. Here we established an *in vivo* AD model and determined notable decrease of KAT7 in AD brains, comparing with control mice. Overexpression of KAT7 alleviated the accumulation of Aβ and increased MAP2 positive neurons, simultaneously suppressed oxidative stress and apoptosis of neurons, suggesting the protective function of KAT7 against AD.

DYRK1A is a protein kinase that phosphorylates the serine and tyrosine on targeted proteins [18]. It has been reported that the dosage of DYRK1A is critical in the central nervous system (CNS) during development and aging, and abnormal DYRK1A level occurs in neurodegenerative diseases such as AD and Parkinson's diseases [18]. Previous study has reported that DYRK1A interacts with IRS-1 through serine phosphorylation [27]. Besides, DYRK1A inhibitors have been frequently proposed as potential therapeutic approach for diabetes [32-34]. Consistently, we proved that DYRK1A and IRS-1 were both elevated in brain tissues of diabetic mice, along with elevated KAT7 expression. IRS-1 is the critical factor that mediates insulin signaling transduction, and decreased IRS-1 Level is a feature of insulin resistance [35]. Studies revealed that drugs that upregulating IRS-1 expression caused alleviated insulin resistance [36]. Here we established an insulin resistance neuron model by chronic stimulation of high-level insulin. The levels of p-AKT and pGSK3β in established insulin-resistant neurons did not change under stimulation of insulin, indicating the successful establishment of the model. Subsequently, we found that overexpression of KAT7 Led to elevated p-AKT and p-GSK3β levels.

KAT7 is a histone acetyltransferase that acetylates the K14 and K23 on histone H3 *via* interacting with scaffolding protein [13, 14]. Here, we evaluated the acetylation of DYRK1A in neurons, and determined decreased enrichment of H3K14ac on DYRK1A gene upon depletion of KAT7. HMGN1 is a DNA binding protein [37, 38]. Recent study

reported that HMGN1 could increase the acetylation of H3K14 *via* enhancing the function of histone acetyltransferase [28]. Hence, we wonder if KAT7 modulate DYRK1A expression through recruiting HMGN1. As was expected, depletion of HMGN1 downregulated DYRK1A and H3K14ac enrichment on DYRK1A gene. The knockdown of HMGN1 also recovered the phosphorylation of AKT and GSK3βin insulin-resistant neurons. However, the direct interaction between KAT7 with HMGN1 and DYRK1A gene is not identified in current work. And verification of KAT7/ HMGN1/ DYRK1A axis in *in vivo* model requires further experiments.

CONCLUSION

To summarize, we determined decreased level of KAT7 in AD. Overexpression of KAT7 ameliorated neuron death and oxidative stress in AD, as well as recovered insulin sensitivity in insulin-resistant neurons through recruiting the HMGN1 to enhance acetylation of DYRK1A gene. Our findings suggested KAT7 as a potential therapeutic approach for insulin resistance in AD.

ARTICLE HIGHLIGHTS

Research background

Epidemiological studies increasingly suggest a significant connection between Alzheimer's disease and type 2 diabetes mellitus, primarily attributed to insulin resistance, a prominent and pivotal pathological characteristic.

Research motivation

The precise pathological mechanisms that underlie the correlation between insulin resistance and Alzheimer's disease remain elusive.

Research objectives

This study aims to investigate the impact of KAT7, a histone acetyltransferase involved in regulating multiple genes, on insulin resistance in Alzheimer's disease (AD).

Research methods

APPswe/PS1-dE9 (APP/PS1) transgenic mice were employed to study AD, while db/db mice were utilized as a model for diabetes. An *in vitro* AD model was established through Aβ stimulation.

Research results

Overexpression of KAT7 decreased Aβ accumulation, alleviated ferroptosis and apoptosis in brain tissues and neurons. KAT7 epigenetically regulated the expression of DYRK1A *via* recruiting the HMGN1 and activated AKT and GSK3β to alleviate insulin resistance.

Research conclusions

Our study revealed that upregulation of KAT7 restored insulin sensitivity in AD by recruiting HMGN1 to augment acetylation of the DYRK1A gene.

Research perspectives

Our findings highlight KAT7 as a novel and promising therapeutic target for addressing insulin resistance in AD.

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