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MicroRNA-130b transcriptionally regulated by histone H3 deacetylation renders Akt ubiquitination and apoptosis resistance to 6-OHDA

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ABSTRACT

Apoptosis of DA neurons is a contributing cause of disability and death for Parkinson's disease (PD). Akt may become a potential therapeutic target for PD since Akt has been deactivated during DA neuron apoptosis. We previously demonstrated that Akt confers apoptosis resistance against 6-OHDA in DA neuron-like PC12 cells, yet the underlying mechanisms accounted for this are not fully understood. Here we report that microRNA-130b (miR-130b)-dependent and cylindromatosis (CYLD) repression-mediated Akt ubiquitination renders apoptosis resistance of PC12 cells to 6-OHDA, which elicits histone H3 deacetylation-induced transcriptional down-regulation of miR-130b vice versa. CYLD deficiency ubiquitinates Akt at Lys63, thereby phosphorylating Akt and antagonizing 6-OHDA-initiated apoptosis. MiR-130b targetedly represses CYLD and increases apoptosis resistance to 6-OHDA. CYLD repression by miR-130b restores Akt ubiquitination and activation, GSK3β and FoxO3a phosphorylation, FoxO3a removal from Bim promoter as well as Bim downregulation during 6-OHDA administration. CYLD deficiency-mediated Akt activation is instrumental for the apoptosis-resistant phenotypes of miR-130b. In addition, 6-OHDA transcriptionally downregulates miR-130b through recruitment of HDAC3 at the promoter. Furthermore, EPO potentiates the ability of miR-130b to activate Akt and augment apoptosis resistance. Our findings identify the apoptosis-resistant function of miR-130b and suggest that histone H3 deacetylation plays a pivotal role in regulating miR-130b transcription in response to 6-OHDA.

1. Introduction

Akt, also known as protein kinase B, is a key signaling molecule that can trigger adaptive responses to stress microenvironment and has crucial roles in most, if not all, cellular biological functions. The strength and duration of phosphoinositide (the polyphosphorylated derivatives of phosphatidylinositol (PI)) signaling from phosphatidylinositol-3-kinase (PI3K) to Akt are tightly balanced by subcellularly localized PI kinases and phosphatases under physiological conditions [1]. Phosphorylation of Akt kinase at Thr308 and Ser473 residues evoked by lysine-63 (K-63) chain ubiquitination is sufficient to activate Akt, which further activates multiple downstream intermediates, including glycogen synthase kinase-3 (GSK3β) and FoxO-family transcription factors and ultimately promotes cell survival, growth and proliferation [2]. Emerging evidences have shown that phosphatase and tensin homologue (PTEN) is a negative regulator of phosphoinositide signaling to turn off PI3K/Akt signaling cascade. For instance, loss of PTEN heterozygosity or reduced expression of PTEN is frequently correlated with constitutive activation of PI3K/Akt signaling [3]. Meanwhile, Akt activity is controlled through negative feedback mechanisms mediated by cylindromatosis (CYLD), the K63-specific deubiquitinating (DUB) enzyme that dismantles Lys63-linked poly-Ub chains from multiple signaling molecules, such as TRAF-2, TAK1 and NEMO [4,5]. Hence, a better understanding of how these negative regulators drive Akt signaling activity may provide novel clues for apoptosis-related diseases.

Parkinson's disease (PD) is a distinctive type of neurogenerative disease worldwide. The main pathological hallmarks of PD are severe degeneration and apoptosis of the dopamine (DA) neurons in substantia nigra pars compacta (SNpc) [6]. 6-hydroxydopamine (6-OHDA), which has been identified as a chemical manner to analyze the effect of DA neuronal loss as a relevant feature of PD, is thought to enter DA neurons

Abbreviations: DA, dopamine; 6-OHDA, 6-hydroxydopamine; HDAC3, histone deacetylase 3; EPO, erythropoietin; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; PI3K, phosphatidylinositol-3-kinase; TRAF2, TNF receptor associated factor-2; TAK1, TGF-β-activated kinase-1

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and induce DA neurons apoptosis via the DA reuptake transporters [7]. Clinical patients with PD often suffer from motor dysfunctions (e.g., bradykinesia, tremor and postural instability) and have a much higher disability rate than those without [8]. Recovery therapy of neurological function based on regeneration medicine holds great promise for PD treatment and has achieved remarkable outcomes in recent years, despite the molecular mechanisms involved remain elusive. In agreement with the consensus notion that preventing degeneration and apoptosis of DA neurons is helpful to restrain PD development and progression, the aspect of PD biology offers a unique therapeutic opportunity.

MicroRNAs (miRNAs) are the small, non-coding, single-stranded RNAs that sequence-specifically interact with the 3′ untranslated region (3′-UTR) of mRNA for degradation and block the expression of downstream targets [9]. miRNAs play pivotal roles in a wide range of physiological and pathological processes including differentiation, metabolism and apoptosis. Functioning as a upstream inhibitor of PTEN to dephosphorylate Akt, miR-17-92 contributes to insulin secretion and pancreatic β-cells survival [10]. Aside from conferring drug resistance, miR-130b leads to downregulation of PTEN, which inhibits apoptosis of breast cancer MCF-7 cells [11]. MiR-130b upregulation in children acute promyelocytic leukemia (APL) is essential for maintenance of the apoptosis-resistant phenotypes [12]. Dysregulation of miR-130b is found to be associated with the proliferation and differentiation of adult neural progenitor cells (aNPCs) [13]. However, the biological roles and mechanisms of miR-130b in 6-OHDA-initiated apoptosis are hitherto poorly understood.

In the current study, we investigated the biological roles of miR-130b in PC12 cells apoptosis in response to 6-OHDA and uncovered the possible mechanism: restoration of CYLD repression-dependent Akt ubiquitination and activation. We also unearthed the histone H3 deacetylation-mediated transcriptional regulation of miR-130b during 6-OHDA stimulation. Our data thus provide a novel theoretical basis for PD biology.

2. Materials and Methods

2.1. Cell culture and transfection

PC12 cells were grown in Dulbecco's modified Eagle medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 10% heat-inactivated horse serum (Gibco, Carlsbad, USA), 100 U/mL penicillin and 100 mg/mL streptomycin under a humidified atmosphere at 37°C and 5% CO_2 as previously reported [14,15]. Transfection of plasmids were performed on cells seeded at 2×10^5 cells/well in six-well culture plates as described previously [14,16,17]. Briefly, cells were transfected with miR-130b mimic (GenePharma, Shanghai, China), negative controls (NC) of lin4 miRNA mimic (GenePharma, China) and miR-130b inhibitor (GenePharma) at a final concentration of 100 nM according to the manufacturer's instructions. The siRNA and plasmid DNA transfection was carried out with specific small interfering RNA (siRNA) duplex oligonucleotides targeting CYLD (GenePharma, Shanghai, China) or PTEN (Santa Cruz, CA) and human pReceiver-M11 vector expressing CYLD (GeneCopoeia, Rockville, USA) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) in OPTI-MEM medium (Invitrogen). The medium was then replaced with fresh medium and the cells were harvested for the subsequent experiments 48 hours later.

2.2. Cellular ubiquitination assay

Cellular ubiquitination assays were performed essentially as described in our previous studies [14]. Briefly, cells underwent variable treatments were lysed by radio-immunoprecipitation assay (RIPA) buffer with protease inhibitors (KeyGene, China) and phosphatase inhibitor cocktail (KeyGene, China). After pulling down Akt using protein A/G–sepharose beads (Cwbiotech, China) overnight at 4°C, lysates were subjected to SDS-PAGE and immunoblotted with an anti-K63 ubiquitin antibody.

2.3. Western blotting

Western blotting analyses were performed as described previously using the primary antibodies as below [14–16,18]: CYLD (1:500), anti-Akt pSer473 (1:1000), anti-Akt pThr308 (1:1000), anti-Akt (1:2000), anti-FoxO3a pSer253 (1:1000), anti-FoxO3a (1:2000), anti-Bim (1:500), anti-acetyl histone H3 (1:1000), anti-histone H3 (1:2000; Cell Signaling Technology, USA), anti-GSK3β pTyr216 (1:1000), anti-GSK3β (1:2000), anti-HDAC3 (1:1000; Abcam, USA) and anti-Flag (1:1000; ProteinTech group, USA). In brief, cell lysates were fractionated on 10% sodium dodecyl sulfate (SDS)-polyacrylimide gel (PAGE) electrophoresis and then transferred to the Immobilon™ PVDF Transfer Membranes (Millipore Corporation, Billerica, MA). The membranes were then blocked in 5% bovine serum albumin (BSA) and separately incubated with the indicated primary antibodies, followed by incubation with the horseradish peroxidase (HRP) conjugated secondary antibody. After the initial western blot assay, the membranes were stripped and re-probed with anti-GAPDH (1:3000, Biosynthesis, China) and proliferating cell nuclear antigen (PCNA) (1:3000, Biosynthesis, China) as a cytoplasmic and nuclear proteins loading control.

2.4. Cell viability and apoptosis detection

For cell viability, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide reduction (MTT) assay was performed and the absorbance was measured at 490 nm according to the protocols described previously [15,17]. For apoptosis detection, Hoechst 33342/PI staining and Caspase-3 activity assay were carried out as previously described [14,15]. The percentage of PI-positive apoptotic cells in Hoechst 33342/PI staining was observed and quantified using fluorescent microscope (IX71; Olympus, Japan) and the caspase-3 activities in Caspase-3 activity assay were measured at 405 nm by spectrometer (Wellscan MK3; Labsystems Dragon).

2.5. Luciferase assay

Luciferase assays were carried out as described previously using the Dual Luciferase Reporter Assay Kit (Promega, Madison, WI) [14]. Briefly, one hundred nanograms of pGL3 luciferase reporter plasmids (Promega, USA) containing fragments of gene promoters with pRL-TK Renilla plasmid (Promega, USA) as an internal control were cotransfected into cells seeded in 96-well plates by Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's recommendation. Forty-eight hours later, luciferase activities were measured and the data were normalized by the ratio of Firefly and Renilla. The primers used for luciferase reporter plasmid construction were as follow: CYLD 3′ UTR-Luc: 5′-CGAGCTCCCATGCCAAGACCGAGCA-3′ (forward) and 5′-CCCGGGGAGAGTGCAAAATGCTTCTT-3′ (reverse); Bim-Luc: 5′-CGAGCTCTCAGGTGGCCTGGCCACT-3′ (forward) and 5′-CCCGGGGTCTCCAGAGACACAACTTCG-3′ (reverse); miR-130b-Luc: 5′-CGAGCTCGTCTTCAAATCCTGGGCTCAA-3′ (forward) and 5′-CCCGGGGCGCTGCGCCCGGTGATGTT-3′.

2.6. Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed with Pierce Agarose ChIP Kit (26156, Thermo) as described previously [14]. In brief, the indicated cells (2×10^6) were subjected to 1% formaldehyde to cross-link proteins to DNA. The cell lysates were sonicated to shear the DNA into 300-1000 bp lengths. Aliquots containing equal amounts of chromatin supernatants were separately incubated on a rocking bed at 4°C overnight with 2 μg anti-FoxO3a antibody, 2 μg anti-HDAC3 antibody, 2 μg anti-