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Tetranectin gene deletion induces Parkinson's disease by enhancing neuronal apoptosis

Zhifeng Chen [a, 1], Ersong Wang [b, 1], Rong Hu [a], Yu Sun [a], Lei Zhang [a], Jue Jiang [a], Ying Zhang [a, **], Hong Jiang [a, *]

[a] Department of Anesthesiology, Shanghai Ninth People's Hospital Affiliated to Shanghai Jiao Tong University, School of Medicine, 639 Zhizaoju Road, Shanghai 200011, China
[b] Department of Neurosurgery, Jinshan Hospital, Fudan University, Shanghai 201508, China

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ABSTRACT

Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). We previously identified tetranectin (TET) as a potential biomarker for PD whose expression is downregulated in the cerebrospinal fluid of PD patients. In the present study, we investigate the role of TET in neurodegeneration *in vitro* and *in vivo*. Our results showed that siRNA knockdown of TET decreased cell viability and the number of tyrosine hydroxylase (TH) positive cells, whereas it increased caspase-3 activity and the Bax/Bcl-2 ratio in cultured primary dopaminergic neurons. Overexpression of TET protected dopaminergic neurons against neuronal apoptosis in 1-methyl-4-phenylpyridinium cell culture model *in vitro*. In TET knockdown mouse model of PD, TET gene deletion decreased the number of TH positive cells in the SNpc, induced apoptosis via the p53/Bax pathway, and significantly impaired the motor behavior of transgenic mice. The findings suggest that TET plays a neuroprotective role via reducing neuron apoptosis and could be a valuable biomarker or potential therapeutic target for the treatment of patients with PD.

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1. Introduction

Parkinson's disease (PD) is the second most common age-dependent neurodegenerative disorder after Alzheimer's disease, affecting approximately 1% of the population over 50 [1]. The disease presents with bradykinesia, resting tremor, cogwheel rigidity and postural instability [2] and is characterized by the progressive degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) leading to a reduction of striatal dopamine levels [3]. In sporadic PD, which accounts for approximately 90% of PD cases [4], alterations associated with the pathogenesis of PD include oxidative stress, mitochondrial dysfunction, inflammatory responses, and the activation of pro-apoptotic and autophagic pathways [5–8]. Cell apoptosis, also known as programmed cell death (PCD), maintains internal environment homeostasis mainly through endogenous DNA enzyme activation to induce natural cell death process and clear redundant cells. In many diseases including PD, aberrant regulation of apoptosis is the central abnormality [9]. Many studies indicate that activation of PCD pathways especially apoptosis may contribute to SNpc dopaminergic neurodegeneration in PD [10].

Tetranectin (TET) is a plasminogen-binding, homotrimeric protein belonging to the C-type lectin family of proteins that can be detected in serum, the cerebrospinal fluid (CSF) and the extracellular matrix [11,12], and was shown to regulate proteolytic processes by enhancing plasminogen activation [13,14]. Our previous study suggested that TET and apoA-I may serve as potential biomarkers for PD [15], and TET knockdown ($TET^{-/-}$) mice exhibit several key features of PD and so may be a valuable model for testing candidate neuroprotective therapies for PD [16], although its exact involvement remains unclear. In this study, we studied the role of TET *in vitro* and *in vivo* and found that TET plays a neuroprotective role via reducing neuron apoptosis in PD and could serve as a potential biomarker or therapeutic target for the treatment of

* Corresponding author.
** Corresponding author.
E-mail addresses: zhangyingyxy@126.com (Y. Zhang), hongjiangyxy@126.com (H. Jiang).
[1] These authors contribute equally to the study.

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Table 1
Primers and siRNA sequences used in the study of TET RNA.

TET	sense	5′-GCAGTATGGGATTTTGGG-3′
	antisense	5′-GGCACTTCAAGTTCACCTTGGTG-3′
α-Tubulin	sense	5′-GATCTTGATCTTCATGGTGCTAG-3′
	antisense	5′-TTGTAACCACCTGGGACGATATGG-3′
TET siRNA	sense	5′-GAUUUGGUGAGCUCAAAGAUU-3′
	antisense	5′-UCUUUGAGCUCACCAAAUCUU-3′
siRNA control	sense	5′-GAUUUCCUGACGUGAAAGAUU-3′
	antisense	5′-UCUUUGAGCUCACCAAAU CUU-3′

patients with PD.

2. Materials and methods

2.1. Cell culture and preparation

All animal studies were approved by the Institutional Animals Ethics Committee. Primary midbrain DA neurons were prepared from embryonic day 14 mice as described previously [17,18]. Briefly, ventral mesencephalon tissues were isolated and incubated in 0.1% trypsin/DMEM at 37 °C for 20 min and then treated with 0.05% DNase/DMEM. The tissue was mechanically dissociated by trituration followed by centrifugation at 600 rpm for 5 min. The pellet was resuspended in DMEM: F12 and cells were plated onto poly-L-lysine coated 24-well plates. Within 12 h after initial plating, the medium was replaced with Neurobasal with B27, 0.5 mM L-glutamine but without glutamate (Invitrogen, Carlsbad, CA) and 3 days after with fresh medium supplemented with 2 μM cytosine-beta-D-arabinofuranoside (Ara-C) to inhibit the replication of non-neuronal cells. 93% of the cells were immunoreactive for the neuronal marker microtubule-associated protein 2, and more than 50% of neurons were TH-positive DA neurons.

2.2. Cell transfection

Isolated DA neurons were transfected with Cy5-labeled small interfering RNA (siRNA) sequence against TET of siRNA control using Lipofectamine 2000 according to the manufacturer's instructions. Briefly, cells are incubated in fresh Neurobasal medium with B27, 0.5 mM L-glutamine but without glutamate 24 h prior to transfection. Replace the culture medium in each well with fresh Neurobasal/B27/L-glutamine medium and then add the OptiMEM I containing DNA/Lipofectamine 2000 mixture 100 μl per well (all transfection materials from Invitrogen). Transgene expression was evaluated 24 h from the start of transfection. The transfection procedure was repeated several times for enrichment of successfully transfected neurons by flow cytometry. The siRNA sequences were shown in Table 1. For TET overexpression, mouse TET cDNA was cloned into pCMS-eGFP, encoding the eGFP (Clontech, Palo Alto, CA), and the transfection procedure was performed as described above.

2.3. MTT assay

Cells were seeded in 96-well culture plates. Cell viability was assessed using the colorimetric MTT ([3−4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium) metabolic activity assay following

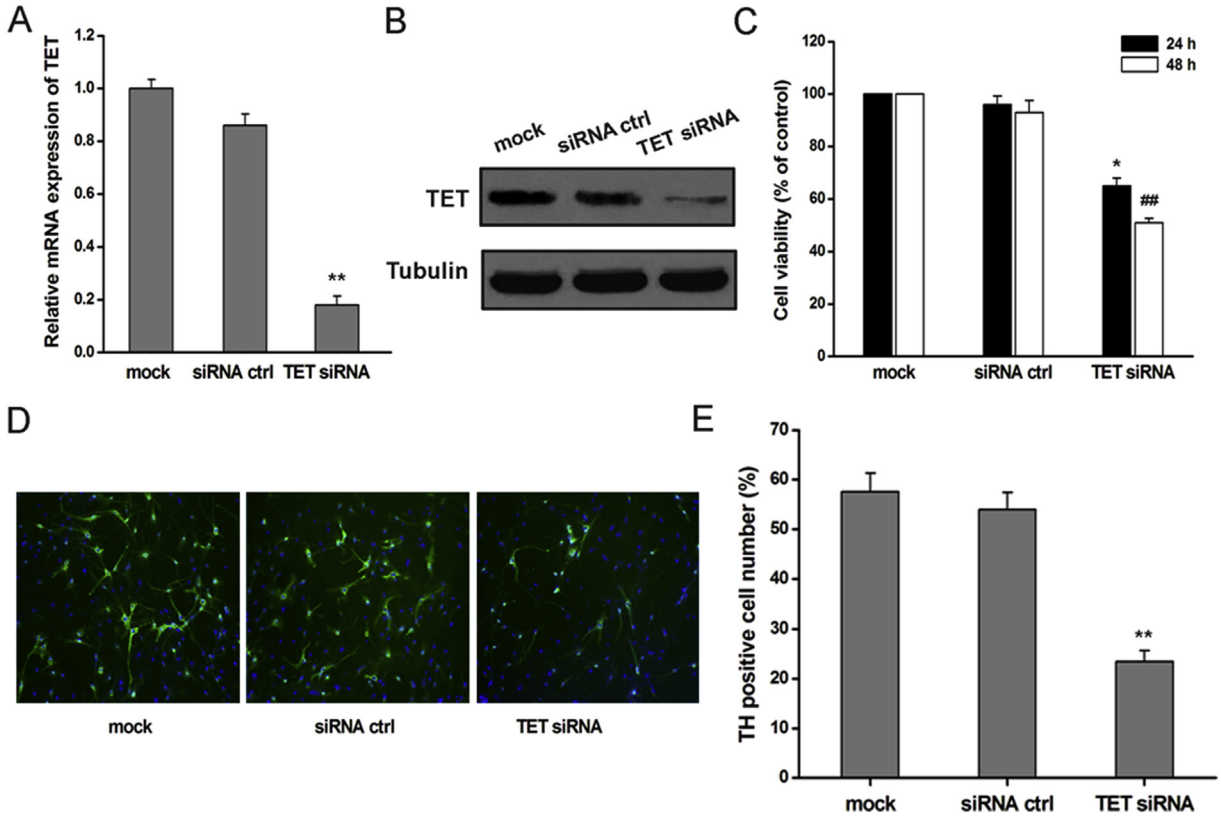

Fig. 1. Gene knockdown of TET in neurons. (A) TET mRNA expression in neurons transfected with TET siRNA or siRNA control by qRT-PCR with untreated neurons as mock controls. $^{**}p < 0.01$. (B) TET protein expression in neurons was determined by western blotting with Tubulin as the loading control. (C) Neuronal cell viability was evaluated by MTT assay after transfection. $^{*}p < 0.05$ vs mock (24 h); $^{\#\#}p < 0.01$ vs mock (48 h). Data are expressed as means ± SD of three independent experiments. (D) Representative micrographs of TH-immunopositive neurons (100×). (E) Quantification of TH-immunopositive neurons. Bars represent means ± SD of four random fields from each of three independent experiments. $^{**}p < 0.01$.

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