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Impaired neurite development associated with mitochondrial dysfunction in dopaminergic neurons differentiated from exfoliated deciduous tooth-derived pulp stem cells of children with autism spectrum disorder

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ABSTRACT

Autism spectrum disorder (ASD) is a highly heterogeneous neurodevelopmental disorder characterized by impaired social interactions, restrictive interests, and repetitive stereotypic behaviors. Among the various mechanisms underlying the pathogenesis of ASD, dysfunctions of dopaminergic signaling and mitochondria have been hypothesized to explain the core symptoms of children with ASD. However, only a few studies focusing on the pathological association between dopaminergic neurons (DN) and mitochondria in ASD have been performed using patient-derived stem cells and *in vitro* differentiated neurons. Stem cells from human exfoliated deciduous teeth (SHED) are neural crest-derived mesenchymal stem cells present in the dental pulp of exfoliated deciduous teeth; these cells can differentiate into dopaminergic neurons (DN) *in vitro*. This study aimed to investigate the pathological association between development of DN and mitochondria in ASD by using SHED as a disease- or patient-specific cellular model. The SHED obtained from three children with ASD and three typically developing children were differentiated into DN, and the neurobiology of these cells was examined. The DN derived from children with ASD showed impaired neurite outgrowth and branching, associated with decreased mitochondrial membrane potential, ATP production, number of mitochondria within the neurites, amount of mitochondria per cell area and intracellular calcium level. In addition, impaired neurite outgrowth and branching of ASD-derived DN were not improved by brain-derived neurotrophic factor (BDNF), suggesting impairment of the BDNF signaling pathway in ASD. These results imply that intracerebral dopamine production may have decreased in these children. The earliest age at which deciduous teeth spontaneously exfoliate in humans, and SHED can be noninvasively collected, is approximately 6 years. Our results suggest that *in vitro* analysis of SHED-derived DN obtained from children with ASD provides neurobiological information that may be useful in determining treatment strategies in the early stages of ASD.

1. Introduction

Autism spectrum disorder (ASD) is a highly heterogeneous neurodevelopmental disorder [1]. The core symptoms of this disorder include impaired social interaction, restrictive interests, and repetitive stereotypic behaviors. Previous studies have reported abnormalities in a wide variety of genetic, environmental, and neurobiological factors related to the differentiation, growth, and function of the central nervous system of patients with ASD [2–4]. However, a common model for the pathogenesis of ASD has not yet been established, possibly because

Abbreviations: ASD, Autism spectrum disorder; ASD-DN, DN differentiated from a child with ASD; Ctrl-DN, DN differentiated from a typically developing child; DN, Dopaminergic neurons; MMP, Mitochondrial membrane potential; SHED, Stem cells from human exfoliated deciduous teeth

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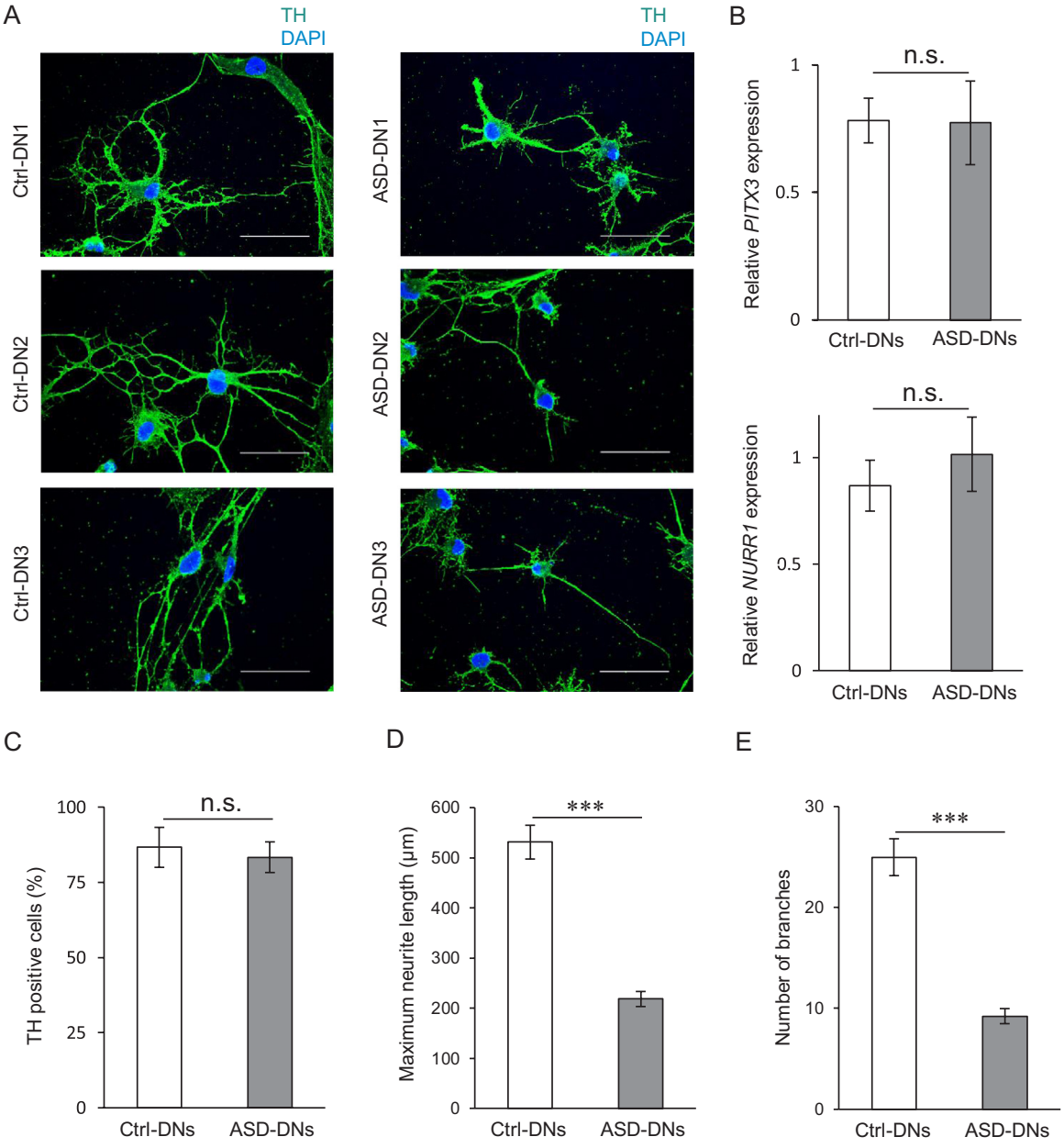

Fig. 1. Neurite development of DN derived from children with ASD. (A) TH expressing cells are shown for each of the three Ctrl-DNs (1–3) and three ASD-DNs (1–3). Cells were immunostained with anti-TH antibody and counterstained with DAPI. Scale bar = 50 μm. (B) The mRNA expression of PITX3 and NURR1 were measured using quantitative reverse transcription polymerase chain reaction. The relative expression of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method. Graphs show the mean ± SEM from three experiments. (C) The percentage of TH-positive cells were measured. The mean ± SEM from 30 cells from each of the three Ctrl-DNs and ASD-DNs are shown. (D, E) Maximum neurite length (D) and total number of branches per cell (E) of DN were measured. The mean ± SEM from 30 cells from each of the three Ctrl-DNs and ASD-DNs are shown. ***P < 0.001. DN, Dopaminergic neurons; ASD, Autism spectrum disorder; TH, tyrosine hydroxylase; Ctrl-DNs, DN differentiated from stem cells derived from exfoliated deciduous teeth of typically developing children; ASD-DNs, DN differentiated from stem cells derived from exfoliated deciduous teeth of children with ASD; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride; SEM, standard error of the mean; n.s., not significant.

ASD exists as a spectrum, and has highly heterogeneous variants.

In silico analysis suggests that various genes associated with the dopaminergic pathway contribute to the pathogenesis of ASD, supporting experimental evidences and the dopamine hypothesis [5,6]. On the other hand, mitochondrial dysfunction has been reported in the analysis of various tissues and postmortem brains of patients with ASD [7,8]. However, only a few studies focusing on the neuropathological association between dopaminergic neurons (DN) and mitochondria in ASD have been conducted, since the direct examination of neurons in vivo is invasive and restricted.

Stem cells from human exfoliated deciduous teeth (SHED) are neural crest-derived mesenchymal multipotent stem cells obtained from the dental pulp of exfoliated deciduous teeth [9]. Some groups have succeeded in differentiating SHED into DN *in vitro* [10,11]. The earliest age for spontaneous exfoliation of deciduous teeth in humans is approximately 6 years [12]. This age corresponds to the early stage of ASD, when it is important to determine the optimal treatment strategies for children suspected to have, or those diagnosed with, ASD [13–15].

The purpose of this study was to clarify the pathological association between development of DN and mitochondria in ASD by using SHED as a disease- or patient-specific cellular model for neurobiological analysis. For this purpose, we obtained SHED from three children with ASD, and examined the neurobiological features of DN differentiated from the SHED, comparing them with those differentiated from SHED