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LETTER TO THE EDITOR

Reactive oxygen species-mediated mitochondrial dysfunction plays a critical role in high glucose-induced nucleus pulposus cell injury

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Dear Editor,

We read with interest and appreciation the article by Park et al. [1] attempting to illustrate the role of reactive oxygen species (ROS) in notochordal cells under high glucose concentrations. The study indicated that high glucose-induced oxidative stress promotes autophagy through mitochondrial damage of rat notochordal cells in a dose- and time-dependent manner. In humans, most notochordal cells are larger than nucleus pulposus (NP) cells, containing vacuoles, and disappear within the first decade of life [2]. A previous study showed that notochordal cells will be first replaced by smaller chondrocyte-like NP cells and then replaced by cells with a fibrocartilagenous phenotype with age [3]. The chondrocyte-like NP cells are the major cells in the adult nucleus pulposus and excessive apoptosis of NP cells plays an important role in the development of intervertebral disc degeneration[4]. As we know, there are two main types of diabetes: type1 diabetes and type 2 diabetes. Type 2 diabetes accounts for 90–95 % percent of all diabetes cases, and it usually occurs in people aged over 40. Moreover, intervertebral disk disease occurs primarily in middle aged and older persons. Therefore, NP cells may be the main cells which are affected by hyperglycaemia in diabetic patients with degenerative disc diseases. However, it is still unknown whether ROS-mediated mitochondrial dysfunction also play a critical role in high glucose-induced NP cell injury. Therefore, we performed a study to investigate the question.

In our study, rat NP cells showed chondrocyte-like morphology under phase-contrast microscope (Fig. 1A1). By immunohistochemical staining (Fig. 1A2) and immunofluorescence staining (Fig. 1A3), cells were demonstrated to exhibit collagen II protein expression. To determine the effects of high glucose concentrations on cell viability, cells were treated with two concentrations (0.1M and 0.2M) of high glucose for 24 hours. Hoechst 33258 nuclear staining showed that the majority of NP cells exhibited significant morphological features of apoptosis such as bright nuclear condensation or fragments after treatment with high glucose concentrations (Fig. 1B2 and 1B3). To further explore whether ROS-mediated mitochondrial dysfunction plays a causal role in high glucose-induced NP cell injury, the level of ROS and mitochondrial membrane potential in cells was detected by DCFH-DA assay and JC-1 assay, respectively. As anticipated, the intracellular ROS levels which exhibit a green fluorescence signal were obviously increased in both high glucose groups compared with the control group (Fig. 1c). Mitochondrial membrane potential ($\Delta\psi m$) is an important indicator of mitochondrial function. We observed that in both high glucose groups (Fig. 1D2 and 1D3), there was an obvious decrease in the $\Delta\psi m$ with a decrease in red fluorescence (JC-1 polymers) which indicates the intact $\Delta\psi m$, and an increase in green fluorescence (JC-1 monomers), which indicates the disruption of $\Delta\psi m$.

Combined with the previous research by Park et al. [1], we can draw a conclusion that ROS-mediated mitochondrial dysfunction not only plays a critical role in notochordal cells but also in NP cells under high glucose concentration. Further, it

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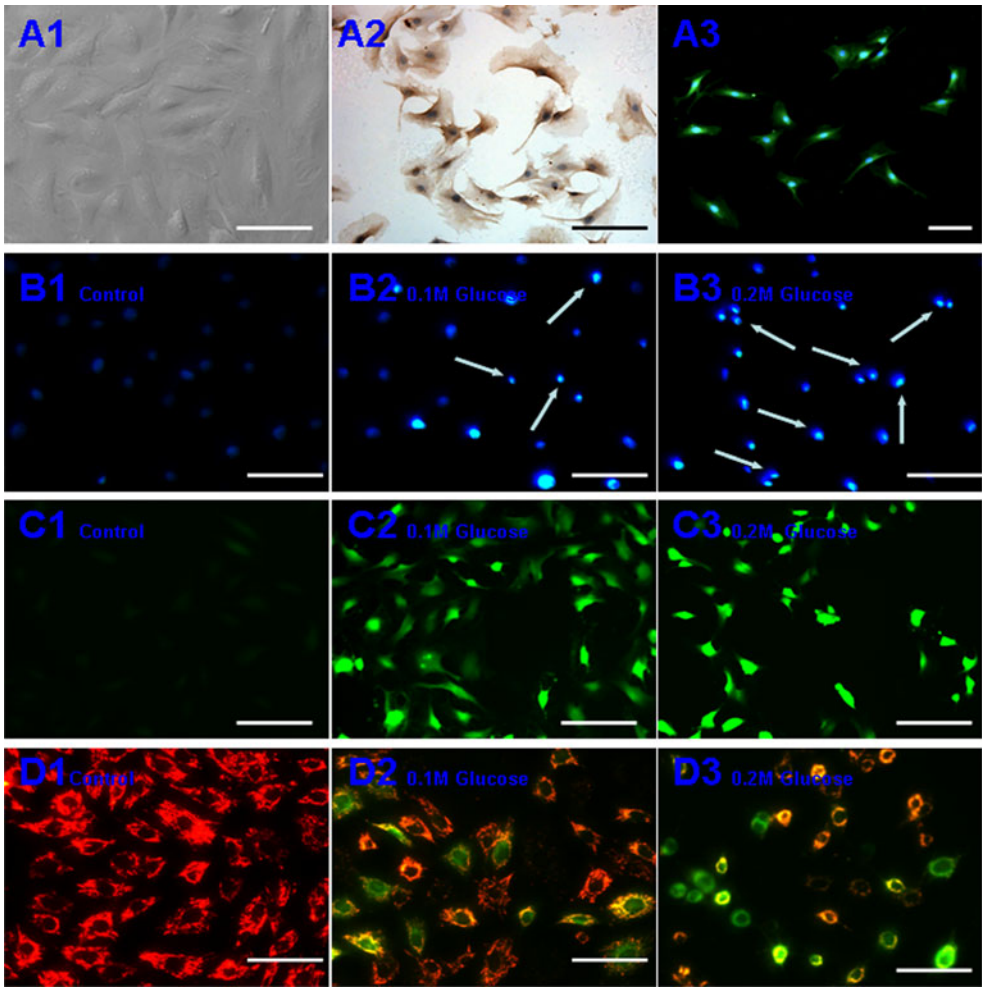

Fig. 1 The effect of high glucose concentrations on NP intracellular ROS production and mitochondrial membrane potential (ΔΨm). (**a** 1) Morphology of NP cells observed by phase-contrast microscopy. (**a** 2) NP cells identified by collagen II immunohistochemical staining and collagen II immunofluorescence staining (**a** 3). (**b**) Hoechst33258 staining. (**c**) Typical fluorescence photomicrograph of ROS imaged by fluorescence microscopy. (**d**) Typical fluorescence photomicrograph of in situ JC-1 staining imaged by fluorescence microscopy

confirmed our previous hypothesis that antioxidants may be a novel treatment option for diabetic patients with degenerative disc diseases [5].

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