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RAPID COMMUNICATION

An analysis of the transcriptional landscape in hypoxia-treated primary nucleus pulposus cells

Intervertebral disc degeneration (IDD) is the main cause of lower back pain. Lower back pain places a huge burden on society, and all current treatments for IDD cannot restore the original function of the intervertebral disc. Proposing new treatments for IDD requires clarifying the mechanisms of IDD. Physiological hypoxia is an important feature of the nucleus pulposus because of its special anatomical structure. Using RNA sequencing (RNA-seq), we obtained the whole transcriptome of nucleus pulposus cells (NPCs) under hypoxia and hypoxia-inducible factor (HIF1A) deletion. Results demonstrated possible effects of oxygen concentration and HIF1A on NPCs. In addition, our results showed that hypoxia can affect lipid metabolism in NPCs.

The degree of disc degeneration in the patients (Table S1) was evaluated by MRI scans, and we obtained the patients' surgically resected discs after obtaining the consent of the patients. We knocked down the expression of HIF1A in NPCs using small RNA interference technology (RNA-si), and cultured NPCs in a normoxia chamber (NXNC group) or hypoxia chamber (HXNC group) for subsequent RNA-seq. Before RNA-seq, we assessed the expression of HIF1A in different groups of NPCs by q-PCR, and the results showed that our treatment effectively reduced the expression of HIF1A in NPCs (Fig. 1A).

Our results show that the treatment conditions described above significantly affect the transcriptomics of NPCs. Compared with the NXNC group, the HXNC group had 77 up-regulated differential genes (DEGs) and 30 down-regulated DEGs (Fig. 1B and Table S2). We also explored the biological processes and pathways in which these DEGs might be involved. Results show that, DEGs enriched in various Gene Ontology (GO) enrichment analysis terms including regulation of cell cycle, cell division, DNA replication initiation, and response to hypoxia (Fig. 1C and Table S3). Gene Set Enrichment Analysis (GSEA) of DEGs showed 63 significantly enriched pathways (Table S4), including the lipoprotein metabolic process and positive regulation of the reactive oxygen species (ROS) metabolic process (Fig. 1D).

Hypoxia-inducible factor (HIF1A), a key transcription factor of cells in a hypoxic environment, regulates a series of genes for cell survival under hypoxic conditions. HIF1A has been identified as involved in all mechanisms underlying IDD.[1] Interestingly, the deletion of HIF1A under hypoxic conditions resulted in 28 DEGs (Table S5), while the deletion of HIF1A under normoxic conditions resulted in 68 DEGs (Table S6) that included the former 28 DEGs (Fig. 1B). This result suggests that HIF1A may play significant roles in NPCs under normoxic conditions. In order to fully understand the effect of HIF1A on NPCs, we compared and analyzed the DEGs of the NXSI and NXNC groups. After analyzing DEGs under HIF1A loss and normoxic conditions, we found that DEGs enriched in multiple GO terms including negative regulation of growth, response to hypoxia, negative regulation of oxidative stress-induced neuron intrinsic apoptotic signaling pathway, and hypoxia-inducible factor-1 alpha signaling pathway (Fig. 1E and Table S7). GSEA of these DEGs showed 14 significantly enriched pathways, including canonical glycolysis, response to hypoxia, negative regulation of ROS metabolic process, and response to vitamin D (Fig. 1F and Table S8).

In order to confirm our sequencing results, we selected 4 genes and verified whether their expression trends were consistent with Western blotting results (Fig. 1G). Comparing Western blotting results with RNA-seq results (Fig. 1H), the change trends of gene expression are roughly the same. To further explore the contribution of HIF1A to the production of ROS in NPCs under different oxygen concentrations, we examined the production of ROS by NPCs (Fig. 1I). The results are consistent with the sequencing results that hypoxia can inhibit the production

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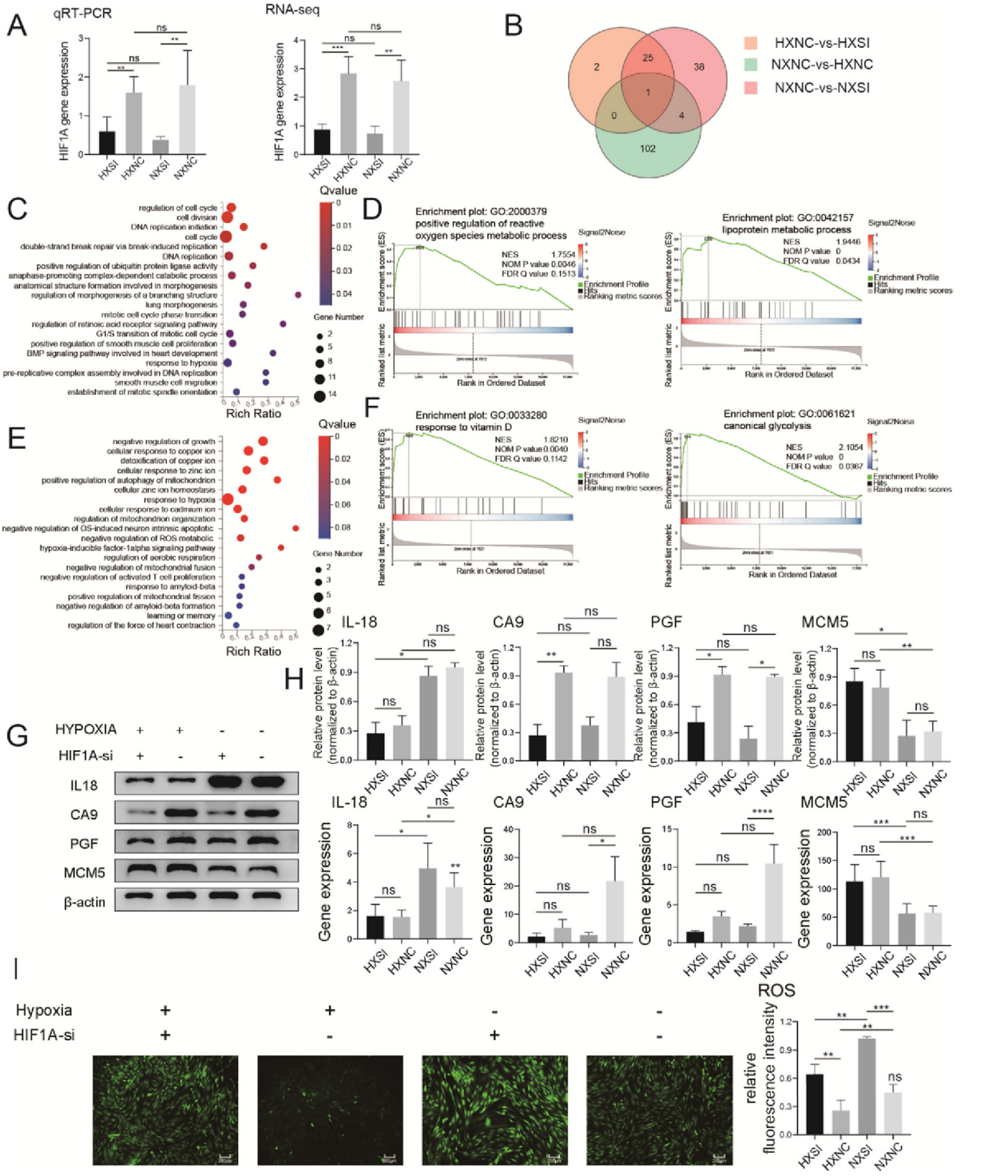

Figure 1 Transcriptomic analysis and validation of NPCs. **(A)** The qPCR and RNA-seq results of treatment of NPCs with HIF1A-si under hypoxia (HXSI) or negative control (HXNC) and treatment of NPCs with HIF1A-si under normoxia (NXSI) or negative control (NXNC). **(B)** Venn diagram of DEG volcano plots for HXNC *vs*. NXNC, HXSI *vs*. HXNC, and HXSI *vs*. HXNC. **(C)** GO enrichment analysis of DEGs in HXNC *vs*. NXNC. **(D)** GSEA analysis of GO, including lipoprotein metabolic process and positive regulation of reactive oxygen species metabolic process. **(E)** GO enrichment analysis of DEGs in HXNC *vs*. NXSI. **(F)** GSEA analysis of GO, including canonical glycolysis and response to vitamin D. **(G)** Western blot experiments and densitometric analysis of NPCs treated with HIF1A-si and negative control at different oxygen concentrations (β-actin as internal control). **(H)** The sequencing results of the expression levels of each gene are roughly consistent with the Western blotting results. **(I)** The detection of reactive oxygen species and fluorescence intensity analysis of nucleus pulposus treated with HIF-1A and negative control at different oxygen concentrations. ns, no significant difference. $^{*}P < 0.1$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Scale bar = 200 μm.

of ROS in NPCs and, when HIF1A is depleted, the production of ROS increases regardless of the oxygen concentration.

Physiological hypoxia is a characteristic feature of the nucleus pulposus,[2] and the oxygen concentration of the environment in which NPCs live may change with the progression of IDD.[3] Our results suggest that when the oxygen concentration increases, the expression levels of genes involved in lipid metabolism (ABAC1, PCSK9, LDLR, and LRP1) increase. In addition, when HIF1A is disrupted, the gene expression of LRP4 is down-regulated, which requires further study. Previous studies have reported that lipid metabolism disorders may promote IDD.[4] Our results reveal that oxygen concentration can affect lipid metabolism in NPCs. On the other hand, previous studies successfully constructed IDD models using APOE knockout rabbits.[5] Taken together with our results, studies on lipid metabolism in NPCs should take hypoxia into consideration. In conclusion, our results explain the possible effects of oxygen concentration and HIF1A on NPCs, including the effect of oxygen concentration on NPC lipid metabolism. Further study is required to apply these findings to the subsequent treatment of IDD.

Author contributions

Zhicai Peng and Zhuo Wang: manuscript preparation; Shuaichi Guo, Bing Tan and Ruichao Cao: extraction and processing of NPCs and validation of RNA-seq results; Shengqiang Cheng, Jun Chen and Chunwang Xie: data acquisition and analysis; Dehong Mao and Zhenming Hu: concept and design of the study and revision of the manuscript. We ensure that all authors have agreed to the manuscript's content and its submission to *Genes & Diseases*.

Conflict of interests

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.04.009.

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