Dihydroartemisinin-induced mitochondrial mRNA degradation and apoptosis in keloid fibroblasts

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To the Editor: Keloid is a type of proliferative fibroma that may form after trauma. It is characterized by an exacerbated proliferation and inhibited apoptosis of fibroblasts coupled with an imbalance between synthesis and degradation of collagen in the extracellular matrix.[1] Although surgical resection and combined radiotherapy are effective treatment methods, keloids commonly recur after resection, resulting in decreased patient satisfaction and damage to their physical and mental health. Therefore, search for more effective treatments needs to continue. Dihydroartemisinin (DHA), a derivative of artemisinin isolated from the traditional Chinese herb by the same name, was initially employed as an effective anti-malaria agent with minimal side effects. Studies have shown that DHA prevents the progression of lung,[2] liver,[3] and kidney[4] fibrosis by inhibiting the proliferation of fibroblasts. However, DHA inhibition of keloid fibroblasts or clinical treatment of keloid has not been reported yet. This study explored the possible mechanisms involved in DHA-induced differential expression of multiple genes, mitochondrial mRNA (mt-mRNAs) degradation, cell viability inhibition, and apoptosis in keloid fibroblasts.

Keloid samples were obtained from enrolled patients with keloids who underwent surgical treatment between February 2016 and March 2018 at the Department of Plastic and Burn Surgery of West China Hospital, Sichuan University (Chengdu, China). The study protocol was reviewed and approved by the Ethics Committee of West China Hospital of Sichuan University (No. 2014-65) and informed consent was obtained from all the participants. These samples were then used for the primary culture of keloid fibroblasts and treated with either solvent or 40 μmol/L DHA for 4 hours to evaluate their gene expression profile. Database construction and sequencing analysis of the transcriptome were performed by Anoroad Gene Technology Co., Ltd. (Beijing, China). The DEGSeq software was used to compare and analyze the differentially expressed genes in keloid fibroblasts between the solvent- and DHA-treated groups. Differentially expressed genes were defined as those that met the following criteria: $|\log2 \text{ (Fold Change:)}| \geq 1$ and $q < 0.5$. Compared with the solvent group, there were 1606 upregulated and 642 downregulated genes in keloid fibroblasts treated with DHA [Figure 1A]. Furthermore, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) database for pathway analysis. The related signaling pathways of differentially expressed genes were sorted in descending order according to their adjusted *P* value. These genes were mainly distributed in 50 signaling pathways (data not shown), including the Phosphoinositide 3-kinase (PI3K)/Protein Kinase B (Akt) pathway, which was consistent with the previous studies showing that DHA alleviated fibrosis by inhibiting this pathway.[4] The top 10 signaling pathways were as follows [Figure 1B]: Tumor necrosis factor (TNF) signaling pathway, pathways in cancer, breast cancer, MAP kinase (MAPK) signaling pathway, interleukin (IL)-17 signaling pathway, transcriptional misregulation in cancer, measles, Janus kinase/signal transducer and activator of tran-ions (Jak-STAT) signaling pathway, signaling pathways regulating pluripotency of stem cells, and osteoclast differentiation. Thus, DHA induced the differential expression of multiple genes, involved in multiple signaling pathways, in

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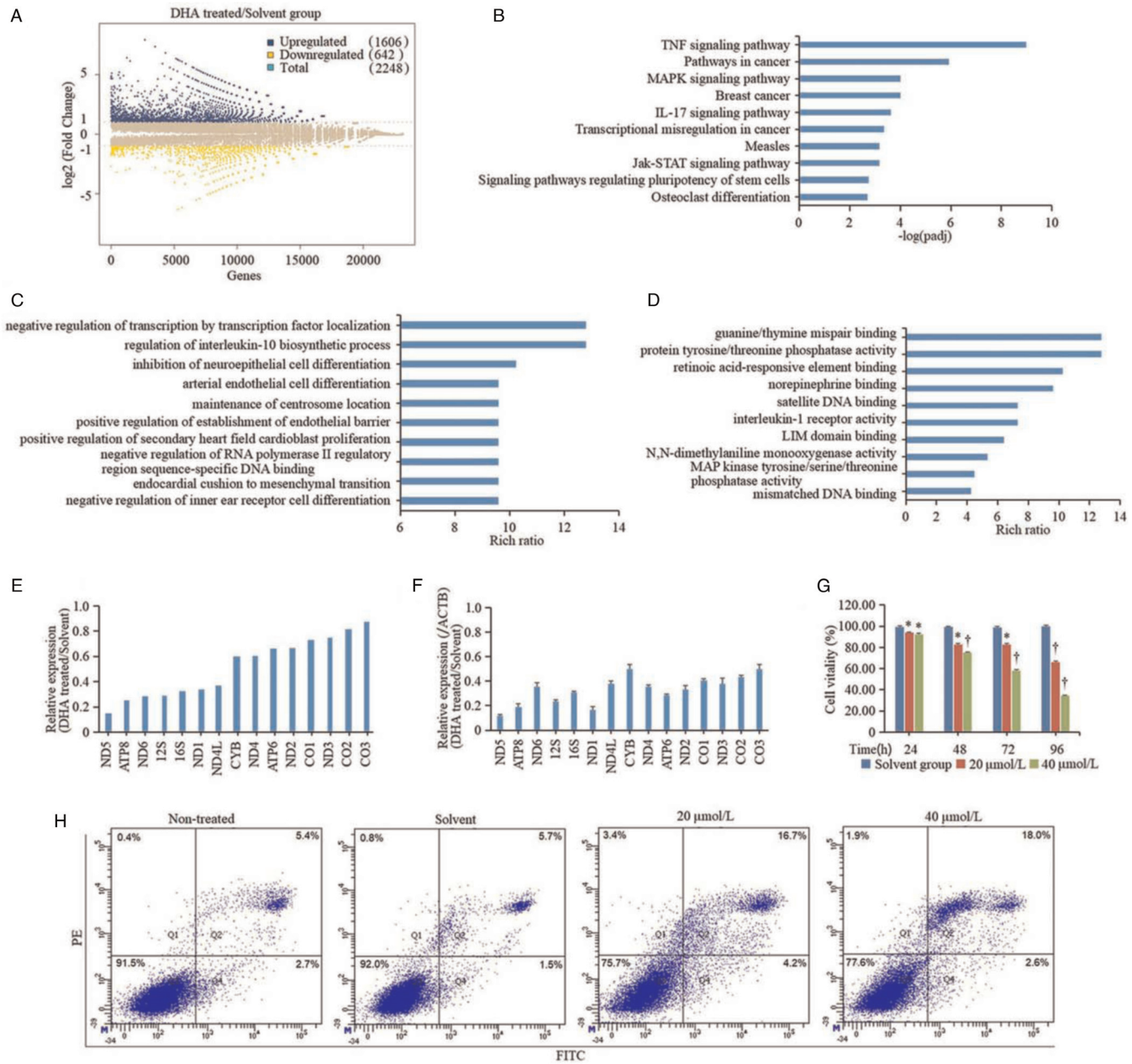

Figure 1: Keloid fibroblasts were treated with solvent or 40 μmol/L DHA for 4 hours. Based on the results from database construction and sequencing analysis of the transcriptome, we analyzed the differentially expressed genes between treatment groups. (A) Scatter diagram of Log2 (fold change) value distribution of differentially expressed genes in keloid fibroblasts. Yellow represents genes with Log2 (fold change) >1, blue represents those genes with Log2 (fold change) <−1, and gray represents gene in between. Log2 (fold change) represents the log2 value of the difference multiple. (B) The bar chart represents the top 10 related signaling pathways according to the degree of significant difference of differentially expressed genes in keloid fibroblasts between solvent- and DHA-treated groups. The bar chart represents the top 10 BPs (C) and MFs (D) of differentially expressed genes according to the rich ratio. (E) The relative expression from the read count ratio of gene expression profile between DHA- and solvent-treated keloid fibroblasts. (F) Keloid fibroblasts were treated with DHA or solvent and the levels of mt-mRNA were detected by real-time qPCR, where ACTB served as the reference gene ($n = 6$). (G) Keloid fibroblasts were treated with solvent or with 20 μmol/L or 40 μmol/L DHA ($n = 6$), respectively, for 24 hours, 48 hours, 72 hours, and 96 hours, using the non-treated group as control, after which cell viability was quantified using the CCK-8 method. Compared with the solvent group, $^*P < 0.05$, $\dagger P < 0.01$. Error bars represent SD. (H) Keloid fibroblasts were treated with solvent or with 20 μmol/L or 40 μmol/L DHA for 24 hours, using the non-treated group as control, after which apoptosis was detected by flow cytometry. BPs: Biological pathways; DHA: Dihydroartemisinin; MFs: Molecular functions; mt-mRNAs: Mitochondrial mRNAs.

keloid fibroblasts, which provided reliable basic data for the study of its mechanism.

Additionally, we reported the biological processes (BPs) and molecular functions (MFs) of differentially expressed genes in keloid fibroblasts treated with DHA compared with those in the solvent group, among which the top 10 according to the rich ratio are shown. In this case, the calculation formula was as follows: (differential genes of the pathway/all differential genes)/(genes annotated to this pathway/all genes that can be annotated). The top 10 BPs were [Figure 1C]: regulation of the interleukin-10 biosynthetic process, negative regulation of transcription by transcription factor localization, inhibition of neuro-

epithelial cell differentiation, negative regulation of inner ear receptor cell differentiation, endocardial cushion to mesenchymal transition, negative regulation of RNA polymerase II regulatory region sequence-specific DNA binding, positive regulation of secondary heart field cardioblast proliferation, positive regulation of establishment of endothelial barrier, maintenance of centrosome location, and arterial endothelial cell differentiation. In addition, the top 10 MFs [Figure 1D] were: protein tyrosine/threonine phosphatase activity, guanine/thymine mispair binding, retinoic acid-responsive element binding, norepinephrine binding, interleukin-1 receptor activity, satellite DNA binding, LIM domain binding, N, N-dimethylaniline monooxygenase activity, MAPK tyrosine/serine/threonine phosphatase activity, and mismatched DNA binding. Previous studies examining the effect of DHA on fibrotic diseases have shown that DHA decreases the level of inflammatory cytokines, oxidative stress, and collagen synthesis in lung tissue, leading to the inhibition of fibroblast proliferation and alveolar inflammation as well as attenuation of lung injury and fibrosis.[2] Moreover, DHA-induced senescence, proautophagic and anti-inflammatory effects in liver fibrosis.[3] Therefore, these studies combined with ours indicated that the effects of DHA treatment on keloid fibroblasts involved the interaction with multiple genes and may regulate multiple systems.

As mentioned above, DHA action on keloid fibroblasts is a complex process. In this study, we surprisingly found that DHA induced the degradation of mt-mRNAs in keloid fibroblasts. In the expression profile analysis, the expression levels of mt-mRNAs (NADH Dehydrogenase (ND) 5, Adenosine triphosphate (ATP) 8, ND6, 12S, 16S, ND1, ND4L, Cytochrome B (CYB), ND4, ATP6, ND2, Cytochrome C Oxidase (CO)1, ND3, CO2, and CO3) were all downregulated in keloid fibroblasts treated with DHA, compared with those in the solvent group [Figure 1E]. Additionally, we corroborated this phenomenon using polymerase chain reaction [Figure 1F]. Mitochondrial DNA mainly encodes proteins associated with the respiratory chain. Thus, mt-mRNAs are essential to the function of mitochondria and the maintenance of cell viability. Previous reports showed that the degradation of mitochondrial 16S ribosomal RNA is a new marker of mammalian cell apoptosis.[5] However, no correlation between the degradation of other mt-mRNAs and apoptosis has been reported. Furthermore, the inhibitory effect of DHA on keloid fibroblasts was confirmed using a Cell Counting Kit-8 (CCK-8) assay, in which cell viability after treatment with 20 μmol/L or 40 μmol/L DHA for 24 hours, 48 hours, 72 hours, or 96 hours was significantly inhibited compared with that in the solvent group [Figure 1G]. Lastly, apoptosis was significantly induced in keloid fibroblasts treated with 20 μmol/L or 40 μmol/L DHA for 24 hours compared with non-treated or solvent groups, as detected by flow cytometry [Figure 1H]. In summary, decreased cell viability, increased apoptosis, and degradation of mitochondrial RNA simultaneously occur in the keloid fibroblasts treated with DHA. Therefore, we speculated that there may be a close relationship between these phenomena.

In conclusion, the findings of the present study suggested that DHA treatment in keloid fibroblasts alters the expression of numerous genes, which are involved in multiple BPs, MFs, and signaling pathways. Remarkably, treatment with DHA induced both degradation of mt-mRNAs and apoptosis in keloid fibroblasts, along with inhibiting their cell viability, which may be one of the mechanisms by which DHA regulates keloid fibroblasts. This study will provide new directions and useful insight for the elucidation of the mechanism by which DHA inhibits keloid fibroblasts.

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Conflicts of interests

None.

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