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RAPID COMMUNICATION

The tRNA-derived fragment tRF-5022a positively regulates melanogenesis

The tRNA-derived fragments (tRFs) are small non-coding RNAs containing fewer than 50 nucleotides that are abundant in humans and have various biological functions. However, their roles and mechanisms in melanogenesis are unclear. In this study, we firstly investigated the change in the expression profile of tRFs in melanocytes after UVB irradiation through tsRNA sequencing. UVB-induced melanogenesis led to the upregulation of 119 tRFs and the downregulation of 103 tRFs. Among the tRFs with greater than 1.5-fold change in expression level, tRF-Ser-AGA-002 (tRF-5022a) was significantly upregulated after UVB irradiation. Overexpression of tRF-5022a in melanocytes significantly increased melanin production and upregulated melanogenesis-related genes. On the other hand, inhibition of tRF-5022a expression had the opposite effects. CCDC88A, which regulates the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway, was significantly downregulated in the tRF-5022a-overexpressing melanocytes. Collectively, our data indicates that the expression profile of tRFs was significantly altered in melanocytes after UVB irradiation, and tRF-5022a is involved in regulating melanogenesis.

UV radiation is the main external cause of chloasma and freckles, and UVB is the main band promoting melanogenesis in melanocytes. To fully simulate UVB-induced skin pigmentation, we constructed a cellular model of UVB-induced melanogenesis as described in our previous study.[1] Melanocytes irradiated with 60 mJ/cm^2 UVB showed a significant increase in melanin content (Fig.1 A–C), and in the expression levels of key melanogenic genes including *MITF*, *TYR*, *TYRP1*, *DCT*, *RAB27A*, and *MYO5A* (Fig. 1D). These results suggested that the UVB-induced melanogenesis model was successfully established. Furthermore, 726 differentially expressed tRFs were detected in the irradiated cells, of which 119 tRFs were upregulated and 103 tRFs were downregulated (Fig. S1A). There are currently 152 tRFs in the tRFtb database, of which 86 tRFs overlapped with our sequencing results (Fig. S1B). We further screened for the tRFs that showed more than a 1.5-fold change (log2) in expression, and detected that 47 upregulated and 16 downregulated tRFs (Fig. 1E, F, and Table S1). Overall, the sequencing results showed that the expression levels of tRFs in melanocytes were significantly altered after UVB irradiation. Given that different tRFs may have different biological roles, we performed cluster analysis of the detected tRFs. As shown in Figure 1G, tRF-3 was the predominant tRFs in both the control and UVB groups, followed by tRF-5. However, among the tRFs with more than 1.5-fold change after UVB irradiation, tRF-5 showed the maximum change in expression level, followed by tRF-3b (Fig. 1H). These findings suggested that tRF-5 and tRF-3b may play important roles in UVB-induced melanogenesis.

Among tRFs differentially expressed with a more than 1.5-fold change, tRF-Ser-AGA-002 (tRF-5022a) included in tRFdb database with CPM value $>$ 50 was selected among the up-regulated tRFs for further research. tRF-5022a belongs to tRF-5a, and its sequence and location on tRNA were shown in Figure S1F. We measured the expression level of tRF-5022a in the UVB-irradiated melanocytes and MNT1 cells (a well-established cellular model of melanogenesis with high melanin content) through qRT-PCR. The results showed that UVB irradiation upregulated tRF-5022a expression (Fig. 1I), which was consistent with the sequencing results. The MNT1 cells were transfected with tRF-5022a mimics or tRF-5022a inhibitor to ascertain its function during melanogenesis. Transfection efficiency was verified using qRT-PCR (Fig. 1J). The results of Masson-Fontana staining showed that tRF-5022a mimics increased melanin content, while the tRF-5022a inhibitor led to lower melanin production in the MNT1 cells (Fig. 1K, M). Furthermore, *MITF*, *TYR*, *TYRP1*, and *DCT* were upregulated in cells transfected with tRF-5022a mimics (Fig. 1L) and downregulated upon tRF-5022a inhibition (Fig. 1N). These results suggested that tRF-5022a promotes melanogenesis.

To further explore the mechanisms underlying the pro-melanogenic function of tRF-5022a, we preformed high-throughput RNA sequencing of MNT1 cells transfected with tRF-5022a mimics. A total of 771 differentially expressed

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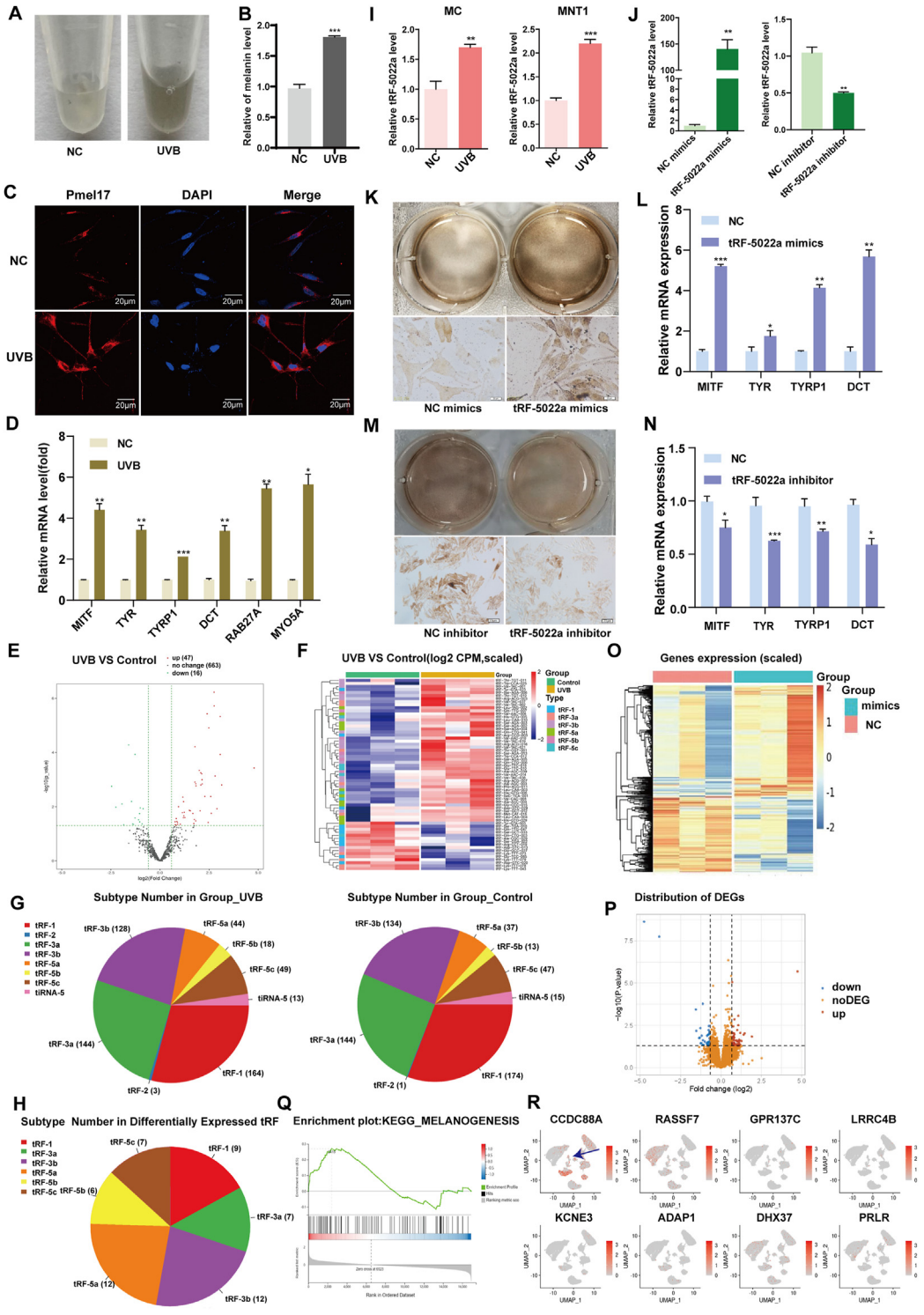

Figure 1 tRF-5022a promotes melanogenesis. **(A)** Melanocytes (MC) were irradiated with UVB and the cell colors were shown. **(B)** The content of melanin was detected by NaOH method. **(C)** The distribution of melanosomes in the cells was observed by immunofluorescence. **(D)** The expression levels of melanogenesis-related genes were detected by qRT-PCR. **(E)** Volcanic map of differentially expressed tsRNAs. **(F)** Heat map of differentially expressed tRFs. **(G)** Classification of tRFs detected in control and UVB-stimulated melanocytes. **(H)** Classification of tRFs differentially expressed in the UVB-induced melanogenesis model of melanocytes. **(I)** The expression level of tRF-5022a in UVB- irradiated melanocytes and MNT1 cells. **(J)** The transfection efficiency of tRF-5022a was verified by qRT-PCR. **(K, M)** The melanin content was detected by Masson-Fontana staining kit. **(L, N)** *MITF*, *TYR*, *TYRP1* and *DCT* mRNA levels were detected by qRT-PCR. **(O)** Heat map of DEGs. **(P)** Volcano map of DEGs. **(Q)** GSEA enrichment analysis of DEGs. **(R)** CCDC88A was highly expressed in melanocytes.

genes (DEGs) with more than a 1.5-fold change were identified in the cells overexpressing tRF-5022a. A heat map of the DEGs is shown in Figure 1O, and a volcano map is shown in Figure 1P. Gene set enrichment analysis (GSEA) of DEGs showed a significant positive correlation between tRF-5022a and the melanogenesis pathway (Fig. 1Q). Since tRFs can be similar to miRNAs and inhibit target gene expression through direct binding with mRNA,[2,3] we next predicted the target genes of tRF-5022a using Targetscan and miRanda databases, and identified 193 putative targets. A Venn diagram of these target genes and 771 DEGs revealed eight overlapping genes - CCDC88A, RASSF7, GPR137C, LRRC4B, KCNE3, ADAP1, DHX37, and PRLR (Fig. S1C). The expression of these genes was analyzed in different skin cells using the GSE150672 single-cell data. We found that only CCDC88A was highly expressed in melanocytes (Fig. 1R), while the others were extremely low. Furthermore, tRF-5022a mimics significantly inhibited CCDC88A expression (Fig. S1D). The binding target of CCDC88A mRNA to tRF-5022a was shown in Figure S1E. Downregulation of CCDC88A is associated with the inhibition of the PI3K/Akt signaling pathway,[4] which can reduce Akt phosphorylation and activate glycogen synthase kinase 3 (GSKβ). Following activation, GSKβ phosphorylates *MITF* and increases the transcriptional activity of the downstream melanogenesis-related genes such as *TYR*, *TYRP1*, and *TYRP2*. GSKβ also promotes the nuclear translocation of β-catenin, where in the latter binds to transcription factors such as LEF and CREB, and promotes the transcription of *MITF*.[5] Therefore, inhibition of the PI3K/Akt pathway can upregulate *MITF* expression and phosphorylation by activating GSKβ and inducing melanogenesis. Based on the results of previous studies, we hypothesize that tRF-5022a promotes melanogenesis by inhibiting CCDC88A expression, thus inhibiting the PI3K/Akt signaling pathway.

In summary, UVB irradiation significantly altered the expression profile of tRFs in melanocytes, and tRF-5022a promoted melanogenesis by inhibiting CCDC88A. Our study provides novel insights into the mechanisms of melanogenesis, and identifies new possible therapeutic targets for pigmented dermatosis. Nevertheless, the targeting relationship between tRF-5022a and CCDC88A needs to be experimentally confirmed. Second, the function of CCDC88A in regulating melanogenesis via the PI3K/Akt signaling pathway also needs to be explored. Third, the effect of tRF-5022a on melanogenesis has not been verified in clinical trials or in animal experiments. In addition, whether tRF-5022a regulates melanogenesis through other mechanisms remains to be investigated.

Author contributions

K Cao and QH Zeng designed the study. S Li and L Zhang did the experiments and collated the data. A Huang, XX Lei, J Chen and SY Yang carried out data analyses and produced the initial draft of the manuscript. L Jiang and CH Fu contributed to revising the manuscript. All authors have read and approved the final submitted manuscript. S Li and L Zhang contributed equally to this work. Corresponding authors: K Cao and QH Zeng.

Conflict of interests

We declare no competing 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.2022.07.020.

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