The Long Noncoding RNA UCA1 Negatively Regulates Melanogenesis in Melanocytes

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The long noncoding RNA UCA1 was first discovered in bladder cancer and is known to regulate the proliferation and migration of melanoma. However, its role in melanogenesis is unclear. In this study, we aimed to explore the role and mechanism of UCA1 in melanogenesis. Our findings showed that the expression of UCA1 was negatively correlated with melanin content in melanocytes and pigmented nevus. Overexpression of UCA1 in melanocytes decreased melanin content and the expression of melanogenesis-related genes, whereas knockdown of UCA1 in melanocytes had the opposite effect. High-throughput sequencing revealed that microphthalmia-associated transcription factor (MITF), an important transcription factor affecting melanogenesis, was also negatively correlated with the expression of UCA1. Furthermore, the transcription factor CRE-binding protein (CREB), which promotes MITF expression, was negatively regulated by UCA1. The cAMP/protein kinase A (PKA), extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) signaling pathways, which are upstream of the CREB/MITF/melanogenesis axis, were activated or inhibited in response to silencing or enhancing UCA1 expression, respectively. In addition, enhanced UCA1 expression downregulates the expression of melanogenesis-related genes induced by UVB in melanocytes. In conclusion, UCA1 may negatively regulate the CREB/MITF/melanogenesis axis through inhibiting the cAMP/PKA, ERK, and JNK signaling pathways in melanocytes. UCA1 may be a potential therapeutic target for the treatment of pigmented skin diseases.

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INTRODUCTION

Long noncoding RNA (lncRNA) is a type of noncoding RNA that encodes a transcript longer than 200 nucleotides and does not code for proteins (Bergmann and Spector, 2014; Kaikkonen and Adelman, 2018; Krause, 2018). A small number of studies have confirmed that lncRNAs play important roles in pigmented skin diseases (Pei et al., 2018; Zeng et al., 2016). For example, Nan-Hyung Kim and others found that microRNA (miRNA)-67, which was derived from the lncRNA H19 in keratinocytes, could enter melanocytes (MCs) through exosomes to affect the expression of melanogenesis-related genes (Kim et al., 2014). Our previous study also found that H19 could affect melanogenesis by a paracrine effect (Pei et al., 2018). LncRNA UCA1 was first discovered in bladder cancer (Li et al., 2015; Li et al., 2017b) and is also involved in the development of various tumors, such as breast cancer (Liu et al., 2016; Wu and Luo, 2016), non–small cell lung cancer (Wu and Zhou, 2018), gastric cancer (Gu et al., 2018), and liver cancer (Hu et al., 2018; Li et al., 2017c). Studies have demonstrated that UCA1 can regulate downstream target molecules by competitively binding to miRNAs (Li et al., 2018a; Liang et al., 2018). At the same time, UCA1 also promotes tumor development, invasion, and metastasis by regulating the PI3K/protein kinase B (Akt) (Yang et al., 2012), Wnt/β-catenin (Liu et al., 2016; Lu and Liu, 2018), and Akt/mTOR (Li et al., 2017a; Wu and Luo, 2016) pathways, as well as other signaling pathways. In melanoma, the expression of UCA1 is significantly increased, and UCA1 promotes the invasion and metastasis of melanoma (Chen et al., 2018c; Wei et al., 2016). However, no literature has reported if UCA1 plays a regulatory role in the melanogenesis of MCs.

The synthesis and transportation of melanin in MCs play an important role in skin pigmentation (Correia et al., 2018; Zhou et al., 2016). The key enzyme that regulates the melanin synthesis pathway is tyrosinase (TYR), and its activity and expression directly reflect the melanin synthesis levels (Mann et al., 2018). TYRP1 and TYRP2 are also key enzymes...
that promote melanin production (Camacho-Hubner and Beermann, 2000; Sarangarajan et al., 2000). Studies have demonstrated that TYR, TYRP1, and TYRP2 can exist as a high-molecular-weight complex that is not easily attacked and cleaved by melanin intermediates in melanosomes (Kobayashi and Hearing, 2007; Kobayashi et al., 1998). Therefore, TYR, TYRP1, and TYRP2 can be used as molecular markers for melanin synthesis. When melanin is deposited in melanosomes, the melanin will be transported from the nucleus to the dendrites (Fujita et al., 2009; Hasse et al., 2018). Various transport-related proteins, such as RAB27A, FSCN1, and MYOSA, are involved in the transportation of melanosomes and thus can be regarded as molecular markers for melanin transportation (Chiaverini et al., 2008; Lopes et al., 2007).

Melanogenesis is regulated by a series of transcription factors, among which microphthalmia-associated transcription factor (MITF) plays an important role (Young et al., 2018b; Ishii et al., 2017). MITF promotes melanogenesis by inducing melanogenesis-related genes, such as TYR, TYRP1, TYRP2, and RAB27A (Alves et al., 2017; Sultana et al., 2018). Studies have shown that the phosphorylation state of the transcription factor CRE-binding protein (CREB) promotes the expression of MITF by binding to and activating the promoter of MITF, thereby promoting the activation of MC function (Alam et al., 2018; Shin et al., 2018). The transcription factor CREB is regulated by a variety of signaling pathways, among which are confirmed pathways such as the cAMP/protein kinase A (PKA) (Park et al., 2018; Xie et al., 2018), mitogen-activated protein kinase (MAPK) family (Fujimura and Usuki, 2018; Yu et al., 2019), Ca²⁺/CaMK (Chen et al., 2018a; Liu et al., 2018), and PI3K/Akt signaling pathways (Dong et al., 2018; Nishina et al., 2018). The cAMP/PKA signaling pathway is one of the key regulatory pathways that promotes the activation of MITF (Choi et al., 2018; Sun et al., 2017). Under UVB, α-melanocyte-stimulating hormone (α-MSH), and other types of stimulation, MCs will exhibit increased intracellular cAMP levels, and PKA will be activated; this further enhances the expression of MITF by activating CREB phosphorylation (Shin et al., 2018). Meanwhile, the MAPK family members extracellular signal–regulated kinase (ERK) and P38 also have been shown to regulate the phosphorylation levels of CREB, which in turn regulates the expression of MITF (Byun et al., 2017; Castro-Vega et al., 2016).

In this study, we explored the role of UCA1 in the process of melanogenesis. Moreover, the mechanism underlying the regulation of melanogenesis by UCA1 was also investigated.

RESULTS
The expression of UCA1 is negatively correlated with the melanin content in MCs and pigmented nevus
The basal expression levels of UCA1 in keratinocytes, HaCaT cells, fibroblasts, human immortalized fibroblast cells, MCs, and human immortalized MC (PIG1) cells were examined. The results show that the UCA1 levels were the lowest in MCs and PIG1 cells and were relatively high in keratinocytes and HaCaT cells (Figure 1a). Meanwhile, it was observed that when the same numbers of MCs and PIG1, SK-MEL-28, and A375 cells, which are all melanin-producing but differ in color, were centrifuged, the quantitative detection by NaOH assay demonstrated that melanin content was the highest in MCs; this was followed by PIG1, SK-MEL-28, and A375 cells (Figure 1b). In contrast, UCA1 levels were the highest in SK-MEL-28 cells, followed by A375 cells, PIG1 cells, and MCs (Figure 1c). During the subsequent MC subculture, it was found that the melanin content of cells decreased (Figure 1d) but the expression of UCA1 increased (Figure 1e). Furthermore, we collected eight different melanin-containing nevus tissues. Hematoxylin and eosin staining results showed that the content of melanin granules increased with the darkening of the pigmented nevus (Figure 1f). The UCA1 levels in tissues were detected by quantitative real-time reverse transcriptase–PCR (qRT-PCR) assay, and the expression of UCA1 decreased in nevus tissues with high melanin content (Figure 1g).

UCA1 negatively regulates melanogenesis
Because the expression level of UCA1 is moderate in PIG1 cells, PIG1 cells were selected for follow-up experiments. We overexpressed UCA1 in PIG1 cells using an overexpression plasmid (Figure 2a). qRT-PCR and western blot assays demonstrated that the expression of TYR, TYRP1, TYRP2, RAB27A, FSCN1, and MYOSA was significantly downregulated (Figure 2b and c). We also knocked down UCA1 in PIG1 cells using three short hairpin RNAs. The results showed that sh-UCA1-1 and sh-UCA1-2 significantly inhibited the expression of UCA1 (Figure 2d) but increased the expression of TYR, TYRP1, TYRP2, RAB27A, FSCN1, and MYOSA (Figure 2e and f). In MCs, in which the expression of UCA1 was low, we overexpressed UCA1 (Figure 2g) and found that, with overexpressed UCA1, the color of the cells was significantly lighter, and the melanin content and the expression of melanogenesis-related genes were reduced (Figure 2h and i). The distribution of melanosomes in the cells was observed by immunofluorescence, and the number of melanosomes decreased after overexpression of UCA1 (Figure 2j). A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide experiment showed that UCA1 had no significant effect on cell proliferation activity (Supplementary Figure S1).

UCA1 negatively regulates the expression of MITF
High-throughput sequencing was used to detect differentially expressed genes at the transcriptomic level after UCA1 was overexpressed in PIG1 cells. The results showed that there were 805 differentially expressed genes exhibiting changes of more than two-fold, of which 144 genes were upregulated and 661 genes were downregulated (Figure 3a). The details of the differentially expressed genes are shown in the Supplementary Material (Supplementary Figure 2a–d and Supplementary Table S1). Gene Ontology and pathway enrichment analysis demonstrated that the melanogenesis-related genes were highly enriched (Figure 3b and c). The distribution of these pigment-related genes in the Kyoto Encyclopedia of Genes and Genomes pathway is shown in Supplementary Figure S2e. A total of 16 melanogenesis-related genes were identified; 15 genes were downregulated, with the exception being PLCB1. Among those genes, the key transcription factor MITF, which regulates melanogenesis, was significantly downregulated (Figure 3d). qRT-PCR and western blot assays further revealed that MITF expression decreased after UCA1 overexpression in PIG1.
Figure 1. The expression of UCA1 is negatively correlated with the melanin content in melanocytes and pigmented nevus. (a) The UCA1 expression levels in different skin cells are shown. (b) The color and melanin contents of MCs and PIG1, SK-MEL-28, and A375 cells were determined by NaOH assay. (c) qRT-PCR shows the UCA1 levels in A375, SK-MEL-28, and PIG1 cells and MCs. (d) The melanin contents of different generations of MCs are shown. (e) qRT-PCR shows the expression of UCA1 in different cell generations. (f) H&E staining was performed with eight different melanin-containing nevus tissues. (g) The UCA1 levels of the eight tissues were detected by qRT-PCR assay. (b, d, g) Mean ± SEM; n = 3. Two-tailed t-test, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. BJ, human immortalized fibroblasts; FB, fibroblast; H&E, hematoxylin and eosin; KC, keratinocyte; MC, melanocyte; PIG1, human immortalized melanocytes; qRT-PCR, quantitative real-time reverse transcriptase-PCR; SEM, standard error of the mean. Bar = 50 μm.
UCA1 negatively regulates the CREB/MITF/melanogenesis axis by inhibiting the cAMP/PKA, ERK, and c-Jun N-terminal kinase (JNK) signaling pathways.

We further found that the phosphorylation of CREB was significantly reduced after overexpression of UCA1 in PIG1 cells but increased after inhibition of UCA1 expression (Figure 4a). We further explored the changes in key proteins in the cAMP/PKA, MAPK, Ca\(^{2+}\)/CaMK, and PI3K/Akt signaling pathways. Both the expression levels and the phosphorylation levels of PKA, ERK, and JNK were significantly downregulated with UCA1 overexpression in PIG1 cells, whereas they were significantly upregulated after inhibition of UCA1 expression in PIG1 cells. However, there were no significant changes in the key protein levels in the other pathways (Figure 4a). The statistical charts are shown in the Supplementary Material (Supplementary Figure S3). We further examined the intracellular cAMP levels using the cAMP-Glo kit (Promega, Madison, WI) and found a negative correlation between UCA1 expression and cAMP content (Figure 4b and c). qRT-PCR and western blot assays further revealed that the expression of the adenylyl cyclase subtypes ADCY2 and ADCY5 decreased after overexpression of UCA1 but increased after knockdown of UCA1 in PIG1 cells (Figure 4d–f).

UCA1 may antagonize UVB-induced melanogenesis.

In our study, it was found that both the melanin content and the expression of melanogenesis-related genes in MCs were significantly promoted after three days of continuous UVB irradiation, which is one of the important external causes of skin pigmentation (Figure 5a and b). In addition,
melanosomes increased after UVB irradiation and were transported from the nucleus to the dendrites (Figure 5c).

Interestingly, the expression of UCA1 was upregulated after repeated irradiation of MCs with low-dose UVB (Figure 5d). Therefore, we irradiated cells with low doses of UVB for three consecutive days after overexpression of UCA1 in MCs; we found that UCA1 downregulated the expression of melanogenesis-related genes induced by UVB (Figure 5e). Meanwhile, we performed the same experiments in Pig1 cells, and the same results were obtained (Supplementary Figure S4). We further explored the effects of stimuli, such as oxidative stress, nutritional deficiencies, α-MSH, and FGF2 on UCA1 expression. The results show that under nonlethal dose stimulation, α-MSH can inhibit the expression of UCA1 but that others cannot (Supplementary Figure S5).

**DISCUSSION**

The expression level of UCA1 in skin cells is not well defined. This study reveals that UCA1 is differentially expressed in different skin cells, and that UCA1 is expressed at low levels in MCs. Furthermore, we found that the expression of UCA1 is negatively correlated with melanin content, and we showed that UCA1 can inhibit melanogenesis by negatively regulating the expression of melanogenesis-related genes.

MITF is a key transcription factor that regulates the expression of many melanogenesis-related genes (Nguyen et al., 2018). Binding to the promoters of genes such as TYR, TYPR1, TYPR2, and MITF promotes their expression; this, in turn, induces melanin synthesis and increased transport (Lee et al., 2018; Sultana et al., 2018). In this study, high-throughput sequencing revealed a decrease in MITF expression after overexpression of UCA1 in Pig1 cells. Our experiments further confirmed that UCA1 could negatively regulate MITF expression. Previous studies have found that transcription factors, such as CREB and PAX3; miRNAs, such as miRNA-26a (Qian et al., 2017), miRNA-340 (Zhao et al., 2017) and miRNA-143 (Ji et al., 2018); and proteins, such as SC7A11 (Yang et al., 2018) and BRN-2 (Fane et al., 2017), can regulate the expression or transcriptional activity of MITF at the levels of transcription, posttranscription, and translation. To our knowledge previously unreported, our study found that UCA1 negatively regulated CREB and its
phosphorylation levels. Additionally, it is known that the transcriptional activity of CREB increases 10–20 times after phosphorylation (Kim et al., 2019). Therefore, at the transcriptional level, UCA1 inhibits MITF-induced melanogenesis by regulating the transcriptional activity of CREB. Whether UCA1 plays a role in inhibiting melanogenesis through post-transcriptional and translational regulation mechanisms remains to be confirmed by subsequent experiments.

The transcription factor CREB is regulated by a variety of signaling pathways. It has been confirmed that the signaling pathways that regulate CREB include cAMP/PKA, ERK, and JNK signaling pathways.
Figure 5. UCA1 may antagonize UVB-induced melanogenesis. (a, b) The expression levels of melanogenesis-related genes in MCs were examined after three days of continuous UVB irradiation. (c) The transportation of melanosomes was observed by immunofluorescence. (d) The expression of UCA1 in MCs was detected after repeated UVB irradiation. (e) The expression of melanogenesis-related genes was examined in UCA1-overexpressing MCs treated with low doses of UVB. (b, d, e) Mean ± SEM; n = 3. Two-tailed t-test, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. MC, melanocyte; NC, negative control; SEM, standard error of the mean. Bar = 20 μm.
(Konar et al., 2018; Park et al., 2018), MAPK (Daniel et al., 2018; Fujimura and Usuki, 2018; Kim et al., 2019), Ca$^{2+}$/CaMK (Chen et al., 2018b; Liu et al., 2018), STAT (Deng et al., 2018), and PI3K/Akt (Daniel et al., 2018; Nishina et al., 2018). In this study, we investigated the possible mechanism of UCA1 regulation of CREB through signaling pathways. The experimental results showed that the expression levels of PKA, ERK, and JNK and their phosphorylation levels were negatively regulated by UCA1. The amount of the second messenger cAMP, which activates PKA, was also negatively correlated with UCA1 levels. Therefore, UCA1 may negatively regulate the CREB/MITF/melanogenesis axis through the cAMP/PKA, ERK, and JNK signaling pathways. Our study also found that UCA1 negatively regulates the expression of ADCY2 and ADCY5, the subtypes of adenylyl cyclase (Halls and Cooper, 2017) that promote the synthesis of cAMP. These results indicate that UCA1 may specifically and negatively regulate cAMP/PKA signaling by inhibiting the expression of ADCY2 and ADCY5. It is worth noting that in tumor cells, UCA1 can promote tumor proliferation and metastasis by activating signaling pathways such as PI3K/Akt and MAPK (C Li et al., 2017; Wang et al., 2017). Factors such as tissue specificity and heterogeneity between tumor cells and normal cells may be the reason for the different regulatory effects of UCA1 on signaling pathways. Notably, the regulation of signaling pathways for melanogenesis is a complex and interactional network. Signaling pathways such as MAPK may also regulate the expression of melanogenesis-related genes, such as MITF and TYR, in a non−CREB-dependent manner (Figure 6).

UV radiation is the most important external cause of melanogenesis (Glady et al., 2018; Mishima and Tanay, 1968). Current studies have found that UV light can promote melanogenesis through epigenetic modifications (Zhang et al., 2019), DNA damage repair (Kemp et al., 2019; Oonincx et al., 2018), signal transduction (Hu et al., 2019), and the alteration of the MC microenvironment (Glady et al., 2018; Pei et al., 2018). It is well known that any biological behavior exerts positive and negative feedback to maintain homeostasis. Unfortunately, there are currently few reports of negative feedback mechanisms that antagonize UV-induced melanogenesis. Our results show that the expression of UCA1 is increased after UV irradiation. We also found that with upregulation of UCA1, UVB-induced melanogenesis is significantly downregulated, suggesting that UCA1 plays a negative feedback role in UVB-induced melanogenesis. We also found that UCA1 was affected by α-MSH stimulation (Supplementary Figure S5). However, further experiments are needed to demonstrate the mechanism of UCA1 expression changes caused by those stimuli.

We found that the UCA1 expression levels in MCs are very low. However, the reason for the low expression of UCA1 in MCs is not fully understood. The regulation of lncRNA expression is mainly determined by transcriptional and post-transcriptional mechanisms. UPF1 is an RNA-binding protein that specifically binds to RNAs and causes the degradation of the target RNA (Chicois et al., 2018; Kim and Maquat, 2019). StarBase v2.0 software was used to predict that there are binding sites between UPF1 and UCA1. Additionally, in our consecutive experiments, UPF1 was found to be highly
expressed in MCs compared with the expression in melanoma cells (Supplementary Figure S6a). We wondered whether the low expression of UCA1 in MCs is associated with the degradation of UCA1 by UPF1. Unfortunately, there was no significant change in UCA1 expression after inhibition of UPF1 expression in MCs (Supplementary Figure S6b and c). Studies have shown that the promoter of the UCA1 gene has no CpG island (Xue et al., 2013), so the low expression of UCA1 is unlikely to be regulated by DNA methylation–related enzymes. Transcription factors are involved in the transcriptional regulation of genes. It was reported in the literature that the transcription factors HIF-1α and c-Myb can bind to the UCA1 gene promoter and promote the expression of UCA1 (Li et al., 2018b; Wang et al., 2018). Studies have revealed that HIF-1α and c-Myb have low expression levels in MCs relative to the levels in melanoma cells (Slominski et al., 2014; Walker et al., 1998). Therefore, we hypothesize that the low expression of UCA1 in melanocytes may be associated with the low expression of HIF-1α and c-Myb. However, more experiments are needed to further confirm this hypothesis.

In our study, we confirmed that UCA1 plays an important role in melanogenesis. Therefore, upregulating or downregulating the expression of UCA1 in local skin could be a potential treatment for hyperpigmentation and hypopigmentation skin disorders. The abnormal expression of UCA1 is related to a variety of tumor types, and whether its upregulation or downregulation will affect tumors remains unclear. Therefore, a large number of preclinical trials are needed before UCA1 can be used as a therapeutic target.

In conclusion, UCA1 is expressed at low levels in MCs and negatively regulates melanogenesis. UCA1 may reduce the CREB/MITF/melanogenesis axis by inhibiting the cAMP/PKA, ERK, and JNK signaling pathways. In addition, UCA1 plays a negative feedback role in UVB-induced melanogenesis. Our results suggest that UCA1 may be a potential therapeutic target for the treatment of pigment skin diseases.

MATERIALS AND METHODS

Cell culture

PIG1 cells (a gift from Dr. Caroline Le Poole at Loyola University, Chicago, IL) were cultured in M254 cell culture medium (#M-254-500, Gibco, Waltham, MA) containing 5% fetal bovine serum (Biological Industries, Beit-Haemek, Israel). HaCaT cells, human immortalized fibroblasts, and the human cutaneous malignant melanoma cell lines SK-MEL-28 and A375 were cultured in DMEM cell culture medium with 10% fetal bovine serum. Human skin keratinocytes, human dermal fibroblasts, and human cutaneous MCs were extracted from fresh foreskin tissue donated after circumcision in adolescents. Prior patient consent and approval from the Institutional Research Ethics Committee were obtained.

Sample collection

Completely excised pigmented nevus tissue was collected from the Dermatology Department of the Third Xiangya Hospital of Central South University. The criteria for sample collection were as follows: (i) written informed consent was obtained from each patient; (ii) patients had no history of systemic diseases or malignancies; (iii) the samples were pathologically diagnosed as pigmented nevus; (iv) the weight of each sample was greater than 200 mg; and (v) no degradation was found in the RNA extracted from the samples. In total, eight pigmented nevus samples with different shades of color were selected. The experiment was approved by the Institutional Research Ethics Committee.

Determination of melanin content

The melanin content in the cells was determined with a sodium hydroxide assay. The same number of cells was seeded into a 96-well plate, and after the cells were attached, they were mixed with 100 μl of 1 mol/L sodium hydroxide and allowed to stand in a water bath at 70°C for 2 hours. Then, the absorbance of the cells at 490 nm was measured with a microplate reader.

Cell infection

Virus particle–based plasmids for overexpression of UCA1 and the negative control were provided by Shanghai GeneChem Company (Shanghai, China). Virus particle–based short hairpin RNAs targeting UCA1 and nonsilencing short hairpin RNAs were purchased from GenePharma Biotechnology Company (Shanghai, China). The viral infection was performed according to the manufacturer’s instructions. According to the sequence of UPF1, three target UPF1 small interfering RNAs with different sites were designed to avoid an off-target effect. The target small interfering RNAs and the control small interfering RNA were synthesized by GenePharma Company. Small interfering RNAs were transfected into PIG1 cells using Lipofectamine 3000 (#L3000015, Invitrogen, San Diego, CA) according to the corresponding instructions.

UV irradiation and other stimuli of MCs

Human primary MCs or PIG1 cells (normal cells or cells infected with lentivirus) were inoculated into the culture dishes at an appropriate density. After the cells attached, they were irradiated with different doses and repeated low doses of UVB by UV-inducing instruments (SS-03B, Sigma, Shanghai, China). Detailed procedures are described in our published articles (Zeng et al., 2016). We used 150 μM α-MSH, 100 ng/ml FGF2, a nonlethal dose (1 mM) of H2O2, and serum-free DMEM to treat the melanocytes for 24 hours, and we detected the level of UCA1.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

The cells were seeded at a suitable density into 96-well plates and were allowed to attach. The cell proliferation activity was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Beyotime, Shanghai, China), and a multifunction microplate reader was utilized to measure the absorbance value of each well at a wavelength of 490 nm.

Immunofluorescence

The cells were fixed with 4% paraformaldehyde and were incubated with a PMEL17 antibody (Novus Biologicals, Centennial, CO) overnight. Then, the cells were incubated in the dark with a fluorescent secondary antibody and DAPI staining solution. Finally, the cells were observed under a fluorescence confocal microscope (LSM800, Zeiss, Oberkochen, Germany). Green fluorescence indicated melanin, and blue fluorescence revealed the nuclei.

RNA extraction and qRT-PCR

The total cellular RNA was extracted with the E.Z.N.A. Total RNA Kit II (#R6934-02, Omega Bio-Tek, Norcross, GA) and was then reverse-transcribed into cDNA using a reverse transcription kit (#FS1-301, ToyoBo, Osaka, Japan). The PCR system was prepared according to the SYBR qPCR Mix (#QPS-201T, Toyobo) kit. Variance in the
expression of UCA1, TYR, TYRP1, TYRP2, RAB27A, FSCN1, MYO5A, ADCY1, ADCY2, UPIF1, and GAPDH (Supplementary Table S2) was detected using a real-time PCR instrument (Roche LightCycler480II, Basel, Switzerland) with GAPDH as the internal reference; ΔCT = 2^ΔΔCT.

High-throughput sequencing
Transcriptomic changes after the overexpression of UCA1 in PIG1 cells (three replicates for each group) were analyzed using the BGISEQ-50 high-throughput sequencing platform (BGI, Shenzhen, China). The processes of library construction and RNA sequencing were performed according to a procedure described previously (Zhu et al., 2018).

Western blotting
The total cellular protein was extracted from RIPA lystate (Beyotime). The protein concentration was determined by a bicinchoninic acid protein concentration quantification kit (#KGBPCA, Nanjing Kaki Biotechnology, Nanjing, China). Primary antibodies against MITF (#MABE78, Millipore, San Diego, CA), TYR (#ab180753, Abcam, Cambridge, United Kingdom), TYRP1 (#MABC592, Millipore), TYRP2 (NBPI-56058, Novus Biologicals), RAB27A (#69295, Cell Signaling Technology, Danvers, MA), FSCN1 (#9269, Cell Signaling Technology), MYO5A (#3402, Cell Signaling Technology), CREB (#9197, Cell Signaling Technology), p-CREB (#9198, Cell Signaling Technology), ERK (#4695, Cell Signaling Technology), p-ERK (#4370, Cell Signaling Technology), JNK (#9252, Cell Signaling Technology), p-JNK (#4668, Cell Signaling Technology), p38 (#8690, Cell Signaling Technology), p-p38 (#4311, Cell Signaling Technology), Akt (#6491, Cell Signaling Technology), p-Akt (#4060, Cell Signaling Technology), STAT (#4904, Cell Signaling Technology), p-STAT (#9145, Cell Signaling Technology), PKA (#3927, Cell Signaling Technology), CaMKIV (13263-1-AP, Proteintech, Rosemont, IL), ADCY2 (A9805, ABclonal, Woburn, MA), ADCY5 (bs-3922R, Bioss, Woburn, MA), and GAPDH (#2118, Cell Signaling Technology) were incubated with the membranes overnight at 4 °C in 5% milk. After washing three times with tris-buffered saline–Tween20, the membranes were incubated with a goat anti-mouse secondary antibody or a goat anti-rabbit secondary antibody. Fluorescence development analysis was performed to detect protein changes using a two-color infrared laser imaging system (Odyssey CLx, Nanjing, China).

cAMP-Glo
Normal PIG1 cells and PIG1 cells overexpressing or downregulating UCA1 were seeded at the same cell density into black 96-well plates. cAMP content in the cells was detected according to the instructions of the cAMP-Glo content detection kit.

Statistical analysis
Statistical analysis was performed with SPSS 22.0 (SPSS Inc., Chicago, IL). Data are presented as the mean ± standard deviation. Significance tests were conducted on the data groups using analysis of variance followed by a comparison between the specific groups using Student’s t-test. P-values < 0.05 were considered statistically significant (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). Each experiment was repeated at least three times.

Data availability statement
The high-throughput sequencing datasets related to this article can be found at http://cdts-wh.genomics.cn:90, hosted at the BGI Data Delivery System, which is available after making an account.


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Supplementary Figure S2. Details of the DEGs identified after UCA1 was overexpressed in PIG1 cells. (a) Heatmap for NC and UCA1. The X-axis represents the sample, the Y-axis represents the DEGs. The color represents the log10-transformed gene expression level. (b) GO analysis of DEGs. The X-axis represents the number of DEGs, the Y-axis represents the GO terms. (c) GO classification of upregulated and downregulated genes. The X-axis represents the GO terms, the Y-axis represents the amount of up- or downregulated genes. (d) Pathway classification of DEGs. The X-axis represents the number of DEGs, the Y-axis represents KEGG functional classifications. There are seven branches for KEGG pathways. (e) The melanogenesis pathway. The green boxes indicate downregulation, and the red box indicates upregulation. DEG, differentially expressed gene; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; NC, negative control; OD, optical density; PIG1, human immortalized melanocytes.

Supplementary Figure S1. The effect of UCA1 on PIG1 proliferation activity was detected by MTT assay. H, hours; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NC, negative control; OD, optical density; PIG1, human immortalized melanocytes.
Supplementary Figure S3. Relative quantitative results of Figure 4a. The changes in PKA, ERK, JNK and their phosphorylation levels are statistically significant. ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; PKA, protein kinase A.
Supplementary Figure S4. UCA1 may antagonize UVB-induced melanogenesis in PIG1 cells. (a) Cell density and cell status were observed 24 hours after a single exposure to different doses of UVB irradiation. (b, c) The cell viability was measured by MTT assay after a single exposure and after consecutive three days of UVB irradiation. (d, e) The expression of UCA1 in PIG1 cells was detected after single and repeated UVB irradiation. (f, g) qRT-PCR and western blot results show the expression of melanogenesis-related genes in PIG1 cells after three days of continuous UVB irradiation. (h) The expression of melanogenesis-related genes was examined in overexpressed UCA1 PIG1 cells treated with low doses of UVB. (d–f, h) Mean ± SEM; n = 3. Two-tailed t-test, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PIG1, human immortalized melanocytes; qRT-PCR, quantitative real-time reverse transcriptase–PCR; SEM, standard error of the mean. Bar = 100 μm.
Supplementary Figure S6. The change of UCA1 expression after inhibiting UPF1 expression in MCs.

(a) The expression of UPF1 was detected by qRT-PCR in MCs, PIG1, SK-MEL-28, and A375. (b) Three siRNA including si-UPF1-1, si-UPF1-2, and si-UPF1-3 were used to infect in PIG1 cells, and the first two siRNAs could downregulate the expression of UPF1. (c) The expression of UCA1 was detected by qRT-PCR assay. Mean ± SEM; n = 3. Two-tailed t-test, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. MC, melanocyte; PIG1, human immortalized melanocytes; qRT-PCR, quantitative real-time reverse transcriptase–PCR; SEM, standard error of the mean; siRNA, small interfering RNA.

Supplementary Figure S5. Stimuli of oxidative stress, nutritional deficiencies, α-MSH, and FGF2 on UCA1 expression. (a–d) The expression of UCA1 in melanocytes was detected after respective treatment of 150 μM α-MSH, 100 ng/ml FGF2, nonlethal dose (1 mM) of H2O2, and serum-free DMEM for 24 hours. Mean ± SEM; n = 3. Two-tailed t-test, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. α-MSH, α-melanocyte-stimulating hormone.
### Supplementary Table S2. PCR Primer Sequences

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### Supplementary Table S1. The Top 29 DEGs and Their Variation Multiples

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Abbreviations: DEG, differentially expressed gene; NC, negative control.