Cellular and Molecular Biology

Wang *et al. Cell. Mol. Biol.*2016, 62 (12): 37-43
ISSN: 1165-158X
doi: 10.14715/cmb/2016.62.12.7

Original Research

miR-488 determines coat pigmentation by down-regulating the pigment-producing gene pro-opiomelanocortin

H. Wang[1], S. Ma[2], L. Xue[3], Y. Li[1], J. Wang[4], X. He[1], Z. Zhu[1], C. Dong[1*]

[1] College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi 030801, China
[2] College of Animal Science and Veterinary Medicine, China Agricultural University, Beijing 264670, China
[3] College of Information, Shanxi Agricultural University, Taigu, Shanxi 030800, China
[4] Department of Geriatric, Tongji Hospital of Shanghai affiliated to Tongji university, Shanghai 20065, China

Abstract: Coat color is a key economic trait in wool- and fur-producing animals. Coat color is controlled by complex mechanisms. Pro-opiomelanocortin (POMC) is a gene involved in pigment formation. Previous studies suggested that miR-488 might target the POMC mRNA. This study aimed to determine whether miR-488 could affect coat color by regulating POMC and to explore the regulatory roles of miR-488 on coat color in mammals. A dual fluorescence report vector containing the 3'-UTR of POMC was built to determine whether miR-488 could post-transcriptionally regulate POMC expression. Then, a eukaryotic vector expressing miR-488 was built and transfected into mouse keratinocytes to confirm the regulatory mechanism in vitro. Compared with gray mice, the expression of POMC mRNA was 3.36-fold higher in black mice and 1.29-fold higher in brown mice. The results showed that miR-488 could control mice coat color by combining with the 3'-UTR seed sequence of POMC mRNA to achieve the degradation of POMC mRNA, therefore playing a role in POMC expression. This study revealed the roles of miR-488 in animal coat color and enriches our knowledge about the determination of coat color in mammals.

Key words: miR-488; pigment, POMC, cell transfection, keratinocytes, bioinformatics, coat color.

Introduction

Coat color is one of the most important economic characteristics of animals and plays a decisive role in the economic value of wool and fur. Traditionally, white coats held the highest value in the wool and fur industry because white coats can be dyed, but chemical dyes damage the environment. Therefore, finding novel ways to control coat color in the living animal could be of economic and environmental values. Coat color is determined by the synthesis and distribution of melanin, which is controlled by complex regulatory mechanisms (1). More than 300 genes are involved in the regulation of coat color in mammals (2,3).

Pro-opiomelanocortin (POMC) is a gene involved in pigment formation (4). α-melanocyte-stimulating hormone (α-MSH) is the most complex and multifunctional of all POMC-derived peptides (5,6). POMC generates the melanocortin peptides adrenocorticotrophin (ACTH), the melanocyte-stimulating hormones (MSH) α, β, and γ, and the opioid-receptor ligand β-endorphin-1; these derived peptides play roles in coat color through combining with MC1R to control intracellular cAMP levels (2,3). POMC-produced α-MSH activates the cAMP-coupled MC1 receptor to activate the tyrosinase to stimulate melanogenesis (6). Melanocortin receptor ligation by α-MSH leads to the activation of adenylate cyclase and the subsequent increase of intracellular cAMP levels, enhancing the expression of eumelanin (5). The functions of POMC and POMC-related receptors become impaired with aging in humans, suggesting their involvement in skin ageing and white hairs (7).

MicroRNAs (miRNAs) are small endogenous non-coding RNAs of about 22 nucleotides and play key roles in the regulation of gene expression by binding to the 3'-UTRs of their target mRNAs, leading to mRNA degradation or inhibition of mRNA translation (8,9). miRNAs expressed by the skin cells may play key roles in skin development, coat color, and melanogenesis. A previous study identified 105 miRNAs involved in the characteristics of hair follicles of goats and sheep (10). Zhu et al. (11) investigated the expression of nine miRNAs in the skin of brown vs. white alpacas and identified potential mRNA and miRNAs responsible for coat color. The murine ortholog of miR-203 has been shown to have important functions in the regulation of epidermal differentiation (12). Previous studies suggested that miR-488 might target the POMC mRNA (13,14), suggesting a possible role of miR-488 in coat color.

Therefore, the aim of this study was to validate that miR-488 targets POMC using bioinformatics, to determine whether miR-488 could affect coat color by regulating POMC, and to explore the regulatory roles of this miRNA on coat color in mammals.

Materials and Methods

Animal and sample collection

All experiments were conducted in accordance with the International Guiding Principles for Biomedical Research Involving Animals (http://www.cioms.ch/frame

Received May 16, 2016; **Accepted** October 14, 2016; **Published** October 31, 2016

*** Corresponding author:** Changsheng Dong, College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi 030801, China. Email: 13623541993@163.com

1985 texts of guidelines.htm). All experiments were approved by the ethics committee of the Shanxi Agricultural University. All efforts were taken to minimize the suffering and the number of animals used.

Biopsies were obtained from the dorsal skin of nine mice (2-weeks old C57BL/6 mice; Shanxi Agricultural University Animal Centre) after general anesthesia, with three mice of each color (black, gray, and brown). Three biopsies were taken from each mouse: one was used of protein extracted, one for RNA isolation, and the last was fixed in 4% formalin overnight, processed, embedded in paraffin, and cut into 5-µm-thick sections.

Bioinformatics analysis of miR-488 targets

The homology of miR-488 among different species was determined using NCBI blast. The target binding relationship between miR-488 and POMC was predicted using TargetScan (http://www.targetscan.org/) to locate the seed sequence (Supplementary Figure S1 A).

RNA preparation and real-time PCR analysis

Total RNA was isolated from skin samples using the Trizol reagent (Invitrogen Inc., Carlsbad, CA, USA). RNA concentration was determined using the NanoDrop 1000 spectrophotometer (NonoDrop products, Wilmington, DE, USA), and RNA integrity was confirmed by 1% agarose gel electrophoresis. cDNA synthesis was performed using a RT-PCR kit (Beijing Comwin Pharm-culture Co., Ltd, Beijing, China). The primers for POMC and miR-488 were designed and synthesized by the Beijing Genomics Institute (Beijing, China) (Table 1). All samples were assayed in triplicate using an ABI fast 7500 Real-Time QPCR system (Applied Biosystems, Foster City, CA, USA). Quantification of miR-488 was performed using the $2^{-\Delta\Delta Ct}$ method. The abundance of miR-488 was normalized to that of U6 snRNA. The expression of POMC mRNA was normalized relative to that of β-actin.

Western blot

Total proteins were extracted from the skin samples using a protein extraction kit (RIPA Lysis Buffer, Beyotime Institute of Biotechnology, Haimen, China) according to the manufacturer's instructions. Protein concentrations were measured by the BCA method using bovine serum albumin as the standard. Extracts were denatured at 95°C for 5 min. Equal amounts (100 mg/lane) of proteins from each sample were separated by 10% SDS-PAGE and electroblotted on nitrocellulose membranes. Anti-POMC antibodies (SC-20148, 1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used, diluted in fresh 5% skim milk blocking buffer, and incubated overnight at 4°C. After washing in TBS 0.1% with Tween 20, the appropriate horseradish peroxidase conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was incubated 1 h at room temperature (1:10,000 in 3% blocking buffer). After washing, the blot was exposed using SupersignalWest Pico (Tiangen Biotech (Beijing) Co., Ltd., Beijing, China). The signals were quantified using Image-Pro Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA) and normalized to β-actin. All experiments were performed in triplicate.

Table 1. Primer sequences for PCR.

Gene		Primers (5'→3')
POMC	F	CACTTCCGCTGGGGCAAGCCG
	R	TAGCGCTTGTCCTTGGGCGGG
β-actin	F	TTGCTGACAGGATGCAGAAG
	R	ACATCTGCTGGAAGGTGGAC
miR-488	RT	CTCAACTGGTGTCGTGGAGTC GGCAATTCAGTTGAGGACCAAGA
	F	ACACTCCAGCTGGGTTGAAAGGCTGTTTC
	R	TGGTGTCGTGGAGTCG
U6	F	CTCGCTTCGGCAGCACA
	R	AACGCTTCACGAATTTGCGT

Immunohistochemistry

Sections of mice skin were washed three times (3 min each time) in 0.1 M PBS and were incubated at room temperature in 3% hydrogen peroxide for 15 min to block endogenous peroxidase. After washing with 0.1 M PBS three times (15 min each time), sections were boiled 10 min in 0.01 M citric acid, followed by a 20-min incubation in PBS containing 5% BSA (bovine serum albumin) at 37°C. Sections were incubated for 15-24 h at 4°C with anti-POMC antibodies (SC-20148, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 30 min at room temperature. After three washes (5 min each time) in 0.1 M PBS, the sections were incubated with HRP-conjugated goat anti-rabbit IgG (cwBiotech, Beijing, China) for 20-30 min at 37°C. After washing with 0.1 PBS three times for 15 min, DAB was added for 2-3 min and washed three times in 0.1 M PBS. Sections were stained with hematoxylin, dehydrated, and cleared using different concentrations of ethanol and xylene. Staining was observed under a microscope (Leica Microsystems, Wetzlar, Germany). In negative controls, PBS was substituted for the primary antibody.

Double fluorescence report carrier and luciferase reporter assay

To determine the binding relation between POMC and miR-488, two expression vectors were built (PmiGLO-POMC 3'-UTR and pMSCV-Puro-miR-488) (Supplementary Figure S1D). After PCR amplification and SacI and XbaI digestion, the POMC 3'-UTR fragment was connected to a dual fluorescence reporter vector that has two reporter genes connected to the SV40 and HPK promoters.

The pre-miR-488 fragment (Table 2) was digested by XhoI and EcoRI, and connected to the pMSCV-Puro-miR eukaryotic expression vector, which contains the Green Fluorescent Protein (GFP) reporter gene.

The mouse POMC 3'-UTR containing the predicted binding sites for miR-488 and the mutation (Supplementary Figure S1 B C) were cloned into the pmiR-GLO dual luciferase reporter plasmid (Promega, Madison, WI, USA) downstream of the firefly luciferase-coding region. The pre-miR-488 construct was generated through the amplification of DNA sequences following PCR based on mouse cDNA template, and then cloning into the pMSCV puro vector (Clontech Laboratories Inc., Mountain View, CA, USA). These two constructs were transformed into *Escherichia coli* (DH5α), and the positive colonies were verified by sequencing.

Table 2. miR-488 sequence in mice.

Oligo name	oligo DNA sequence (5'-3')
mmu-miR-488-3p mature sequence: UUGAAAGGCUGUUUCUUGGUC	
Pre-miR-488-F	TGCTGTTGAAAGGCTGTTTCTTGGTCGTTTTGGCCACTGACTGACGACCAAGA CAGCCTTTCAA
Pre-miR-488-R	CCTGTTGAAAGGCTG TCTTGGTCGTCAGTCAGTGGCCAAAACGACCAAGAAACAGCCTTTCAAC
Negative control -F	TGCTGAAATGTACTGCGCGTGGAGACGTTTTGGCCACTGACTGACGTCTCCACGCAGTACATTT
Negative control -R	CCTGAAATGTACTGCGTGGAGACGTCAGTCAGTGGCCAAAACGTCTCCACGCGCAGTACATTTC

Table 3. Experimental design of the transfection group and control group using the dual fluorescence detection reporter vector.

Group	*Pmir-GLO reporter*	*pMSCVpuro Vector-GFP-miR488*
Transfection	Pmir-GLO-POMC 3'-UTR	pMSCVpuro Vector-GFP-miR488
Control 1	Pmir-GLO-POMC 3'-UTR	pMSCVpuro Vector-GFP-Negative
Control 2	Pmir-GLO-mismatch POMC 3'-UTR	pMSCVpuro Vector-GFP-miR488
Control 3	Pmir-GLO-mismatch POMC 3'-UTR	pMSCVpuro Vector-GFP-Negative
Blank	**Blank 293T without any transfection**	

HEK293T cells and keratinocytes were cultured before co-transfection with pmiR-GLO-POMC 3'-UTR reporter and MSCV-Pre-miR-488 using the Attractene Transfection Reagent kit (Qiagen, Venlo, Netherlands). Cells were lysed 48 h after transfection and analyzed for firefly and Renilla luciferase activities using the Dual-Luciferase Assays (Promega, Madison, WI, USA) on a GloMax 96 Microplate Luminometer (Promega, Madison, WI, USA). Table 3 presents the experimental design.

Construction of the expression vector for miR-488

An oligonucleotide sequence corresponding to the sequence of the pri-miR-488 was synthesized by Cyagen Biosciences Inc. (Santa Clara, CA, USA) and ligated into the mammalian expression vector pLV.ExBi.P/Puro-K14-eGFP-MCS-miR (Supplementary Figure S2), which contains a K14 promoter driving the expression of GFP and miR-488 (Supplementary Figure S1B). The pri-miR-488 sequence was the same as the endogenous sequence and it produced miR-488. This plasmid is capable of expressing mature miRNA and GFP simultaneously. The GFP expression unit was used to monitor the expression of miR-488. The pri-miR-488 construct was transformed into *Escherichia coli* (DH5α), and the positive colonies were confirmed by sequencing.

Mouse keratinocytes and transfection of miR-488

The skin of 1-2-day-old newborn mice (black mice) was removed using sterile methods and digested by 0.25% dispase II for 12-14 h. The epidermis was separated from the dermis with scalpel and forceps, cut into pieces, and trypsinized with 0.05% trypsin-0.01% EDTA for 10 min at 37°C. The keratinocytes were seeded in a keratinocyte-SFM medium supplemented with bovine pituitary extract (20-30 μg/mL) and EGF (human recombinant; 0.1-0.2 ng/mL). The cell culture plate was coated with 0.01% of rat tail collagen. The transfection of miR-488 was carried out when the cells reached 60-80% confluence, according to the manufacturer's instructions (Qiagen, Venlo, Netherlands). After 12 h, the keratinocyte-SFM medium was changed. Total RNA and proteins were extracted for qRT-PCR and western blot.

MTT assay

Keratinocytes were seeded on 96-well plates at a density of 5×10^3 cells per well, incubated for 12 h, and then treated with vector-GFP (NC) or the miR-488 construct. After 48 h, MTT (Sigma, St Louis, MO, USA) was added to each well and the cells were incubated at 37°C for 4 h. The medium was removed and dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The absorbance was measured at 490 nm with an ELISA reader. The experiments were performed in triplicate.

Statistical analysis

Continuous data are expressed as means ± standard deviation and were analyzed using analysis of variance followed by the LSD post hoc test. SPSS 16.0 (IBM, Armonk, NY, USA) was used for analysis. Two-sided P-values <0.05 were considered significant.

Results

Bioinformatics analysis of POMC and candidate miR-488

Bioinformatics revealed that the degree of homology of miR-488 is high (94-100%) among humans, mice, cattle, sheep, and other mammals (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Bioinformatics using TargetScan (http://www.targetscan.org/) indicated that POMC from numerous species might be targeted by miR-488. The 7-base sequence of the binding site is located at "CCUUUCA" in POMC and at "GGAAAGU" in miR-488 (3'-5') (Supplementary Figure S1 A).

Expression of POMC and miR-488 in mice of different colors

Compared with gray mice, the expression of POMC mRNA was 3.36-fold higher in black mice and 1.29-fold higher in brown mice (Figure 1A). On the other hand, miR-488 expression in black mice was 0.28-fold that of grey mice and 0.46-fold that of brown mice (Figure 1B). The protein levels of POMC in black mice was 1.44-fold that of grey mice and 1.20-fold that of brown mice (Figure 1C-D). Immunohistochemistry showed that mice of different colors expressed POMC in the hair follicles, located in the hair bulb and hair root sheath keratinocytes (Figure 1E).

POMC targeting by miR-488

HEK293T cells were transiently co-transfected with

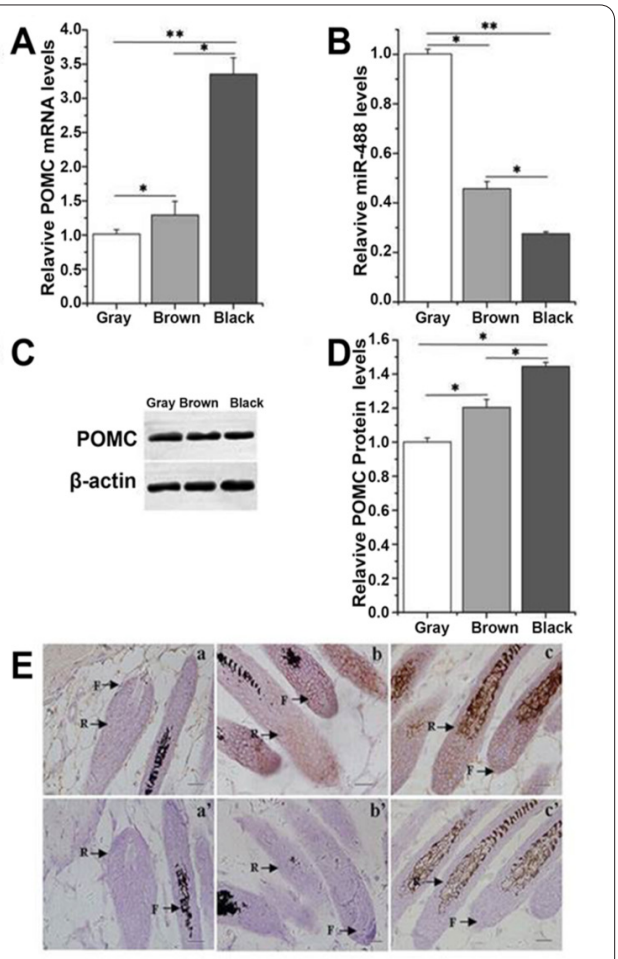

Figure 1. Analysis of POMC mRNA and miR-488 expression in mice of different colors. (A) Real-time PCR analysis of POMC mRNA expression in mice of different colors. (B) miR-488 levels in mice of different colors (n = 3). Data are shown as mean±SD, n=3. *P<0.05, **P<0.01. (C, D) Western blot analysis of POMC protein expression in mice of different colors. (E) Immunohistochemistry showing the distribution and expression of the POMC protein. Scale bar, 100μm. Top panel is the positive result. The bottom panel is the negative control. a a': grey mice; b b': brown mice; c c': black mice. F: follicle; R: root sheath.

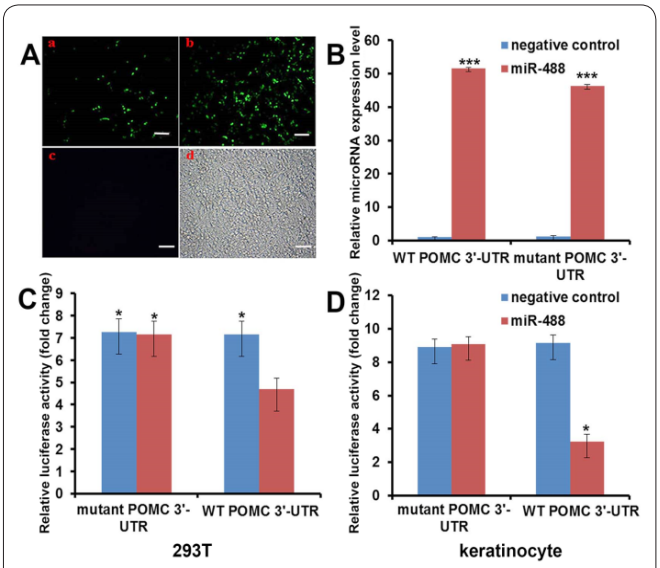

Figure 2. MiR-488 directly targets POMC. (A) Efficiency of cotransfected 293T cells by pmirGLO-POMC 3'-UTR and pms-cv-miR-488. (a) 293T with Pmir-GLO-POMC 3'-UTR and pMS-CVpuro Vector-GFP-miR488 transfection. (b) 293T with Pmir-GLO-POMC 3'-UTR transfection. (c and d) Blank 293T without any transfection. Scale bar, 100μm. (B) qRT-PCR validation of the effect of miR-488 overexpression in 293T cell. Data are shown as mean±SD, n=3. ***P<0.001. (C) 293T cells were transfected with luciferase reporter plasmid containing WT or mutant (Mut) POMC -UTR or empty vector (Vector) together with miR-488 or NC control. Data are shown as mean±SD, n=3, normalized to the blank group (blank 293T without any transfection). *P<0.05 compared to the WT POMC 3'UTR+miR-488 group. (D) Keratinocytes were transfected with luciferase reporter plasmid containing WT or mutant (Mut) POMC -UTR or empty vector (Vector) together with miR-488 or NC control. Data are shown as mean±SD, n=3. *P<0.05 compared to the WT POMC 3'UTR +NC group, **P<0.01, ***P<0.001, normalized to the blank group (blank keratinocyte without any transfection).

pri-miR-488/negative control reporter plasmids. After 12 h, GFP expression was observed and cell growth was good (Figure 2A). The reporters were significantly suppressed by pri-miR-488 in the co-transfected cells, while there was no significant change in the control groups (Figure 2C). In order to demonstrate the interaction between POMC and miR-488, the reporter assays were repeated in keratinocytes (Figure 2D) and the results were consistent with Figure 2C. Therefore, the results indicated that miR-488 might affect the expression of the dual fluorescence reporter vector sequence upstream of the firefly luciferase by combining with the 3'-UTR of POMC.

Detection of POMC expression after transfection of mouse keratinocytes by miR-488

To determine the effects of mir-488, miR-488 was over-expressed by transfecting logarithmic-phase primary keratinocytes with the miR-488 plasmid. The keratinocytes were growing well and their morphology showed polygon and dendritic protrusions in the cytoplasm. After transfection, the keratinocytes still had a normal growing status (Figure 3A-B). Cells transfected with the pLV.ExBi.P/Puro-K14-MCS construct had a 15-fold higher expression of miR-488 compared with cells transfected with the vector control or the non-transfected cells (Figure 3C).

The qRT-PCR results showed that the POMC mRNA levels of the transfected group were 0.87-fold that of the negative control group (Figure 3D). Interestingly, POMC showed no significant changes in transcriptional expression compared with the control group, suggesting that miR-488 does not trigger the degradation of POMC mRNAs and may trigger the translational repression of its mRNAs. Indeed, western blot analyses showed that the POMC protein levels were significantly reduced to 0.65-fold in cells over-expressing miR-488 compared with the negative control group (Figure 3E-F).

Discussion

Coat color is a key economic trait in wool- and fur-producing animals. Coat color is controlled by complex mechanisms. This study aimed to determine whether miR-488 could affect coat color by regulating POMC and to explore the regulatory roles of this miRNA on coat color in mammals. The results showed that miR-488 could control the coat color of mice by combining with the 3'-UTR seed sequence of POMC mRNA to

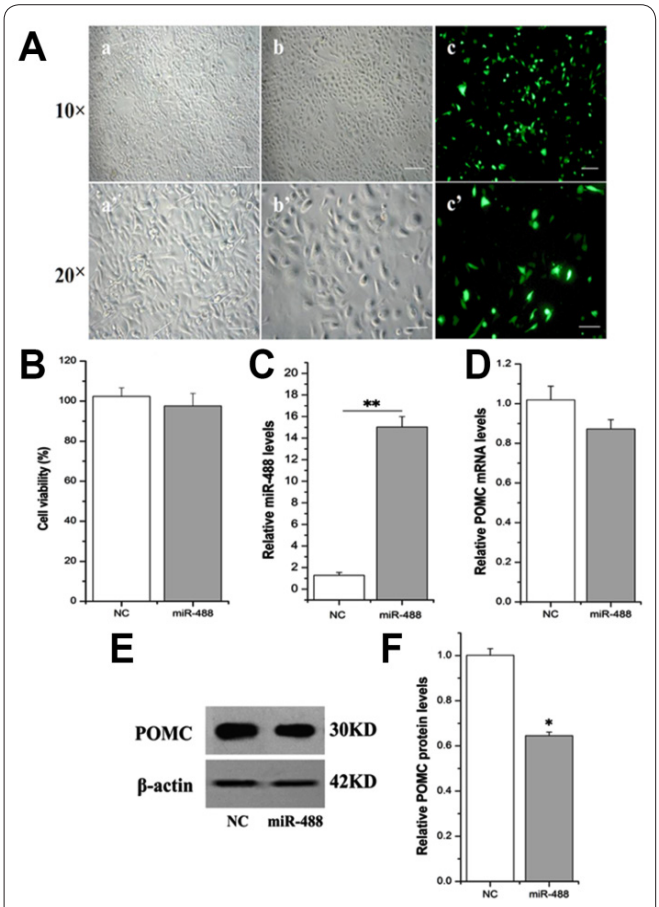

Figure 3. miR-488-overexpressing keratinocytes displayed an altered expression of POMC. (A) Mouse keratinocytes morphology. Scale bar, 100 μm in a, b, and c. Scale bar, 50 μm in a', b', and c'. a a', keratinocyte morphology before transfection; b b', keratinocyte morphology after transfection; c c', GFP of transfected keratinocytes. (B) Real-time PCR analysis of miR-488 expression in keratinocytes transfected with the miR-488 construct and vector-GFP negative control (NC). (C) Real-time PCR analysis of POMC expression in keratinocytes transfected with the miR-488 construct and NC. (D and E) Western blot analysis of POMC expression in keratinocytes overexpressing miR-488 versus NC. β-actin was used as a protein loading control. Data are shown as mean±SD, n=3. *P<0.05, **P<0.01.

achieve the degradation of POMC mRNA or to inhibit post-translational regulation of POMC transcription.

In the skin, melanocytes residing at the dermal-epidermal border propel their melanosomes into their dendrites as they mature. The movement of these keratinocytes towards the surface of the skin eventually yields visible skin color (15). The follicular melanin synthesis units are constituted of active melanocytes surrounding keratinocyte precursors (16,17). POMC is one of the genes affecting the formation of melanin (1,16), which have been identified in the keratinocytes of the skin (15,18). Ultraviolet B (UVB) radiation stimulates the expression of the POMC gene, which is accompanied by production and release of α-MSH, ACTH, and other derived peptides (19).

miRNA-mediated regulation have a widespread impact on the transcriptome and evolution of gene regulatory networks (20,21). Recently, the identification of skin miRNAs has added a new dimension to the regulatory network and attracted significant interest on this novel layer of gene regulation (22). miR-203 was the first miRNA found in the skin, but more miRNAs were subsequently found to influence the skin and coat color (e.g., miR-25, miR-137, miR-337, miR-340, miR-182, etc.) (11,23,24). Although several signaling pathways are involved in the regulation of skin and hair growth in mammals, the regulation networks of skin miRNAs are still poorly known.

In this study, bioinformatics showed that POMC and miR-488 are highly homologous among different species. Bioinformatics predictions from publicly available databases (http://www.targetscan.org/; miRBase, microrna, sanger.ac.uk) indicated that POMC may be targeted by multiple miRNAs (including miR-488) and that the 7-base binding site is present. A study predicted that miR-488 might be associated with panic disorders by regulating POMC (13). Therefore, miR-488 was a probable candidate for POMC modulation in keratinocytes. Luciferase reporter assays were performed to validate the predicted miR-488 target site; miR-488 significantly suppressed the activity of a luciferase reporter containing the 3'-UTR of POMC.

This study showed that different coat colors were associated with different levels of POMC and miR-488 expression. In addition, the expression of POMC and miR-488 were inversely correlated, supporting a probable role in coat color. Immunohistochemistry showed that POMC was expressed in the hair follicle keratinocytes (Figure 4).

POMC could affect the pigmentation by generating its different derived peptides including ACTH, β-endorphin, and α-MSH (25,26). Yu et al. (27) found that α-MSH could induce dendritic elongation, proliferation, melanin synthesis, and expression of MC1R, MITF, and TYR of alpaca skin melanocytes. Excessive skin pigmentation resulting from high levels of circulating ACTH is occasionally reported as one of the primary complaints in many diseases (e.g., Addison's disease) (28). A study showed that the levels of β-endorphin in the tissue fluids from vitiligo skin lesions were significantly higher than those from uninvolved skin (29). Another study showed that β-endorphin has potent

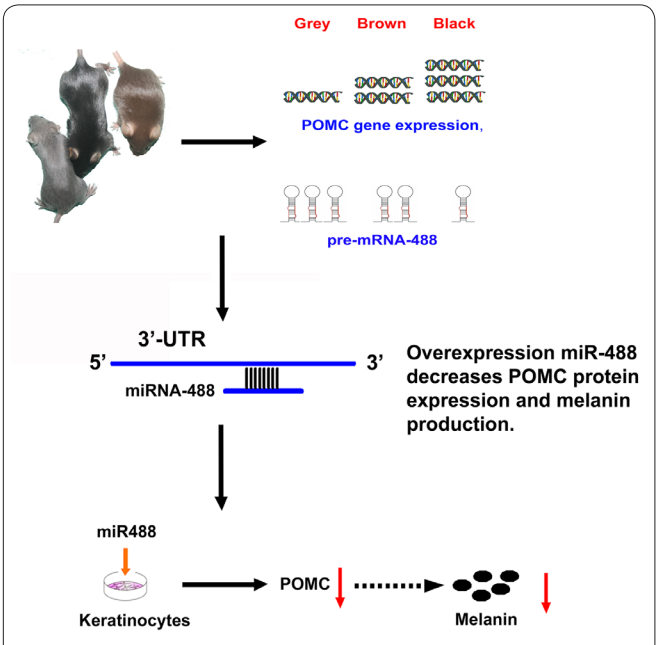

Figure 4. miR-488 decrease the POMC protein expression via binding its 3'-UTR.

melanogenic, mitogenic, and dendritogenic effects in cultured epidermal melanocytes deprived of any exogenous supply of pro-opiomelanocortin peptides, and that the β-endorphin/μ-opiate receptor system participates in the regulation of skin pigmentation (30). It was found that β-MSH was also associated with increases in skin tyrosinase, which is associated with dark skin and hair (31).

This study is not without limitations. Indeed, only one miRNA was analyzed, and only one of the genes involved in hair pigmentation was explored. Additional comprehensive studies are necessary before developing strategies resulting in controlled coat color in mammals.

In conclusion, this study revealed the roles of miR-488 and POMC in the formation of animal coat pigmentation and enriches our knowledge of coat color in mammals.

Acknowledgments

This work was supported by grants from National Natural Science Foundation of China (31302049, 31172283), Doctoral Program of Higher Education (20131403120002), and Agro-scientific Research in the Public Interest (201303119). We thank Cyagen Biosciences for assistance in the vectors and Haixia Han for editing the manuscript.

References

1. Hearing VJ. Biochemical control of melanogenesis and melanosomal organization. J Investig Dermatol Symp Proc 1999; 4:24-8.
2. Corre S, Primot A, Sviderskaya E, Bennett DC, Vaulont S, Goding CR *et al.* UV-induced expression of key component of the tanning process, the POMC and MC1R genes, is dependent on the p-38-activated upstream stimulating factor-1 (USF-1). J Biol Chem 2004; 279:51226-33.
3. Beaumont KA, Wong SS, Ainger SA, Liu YY, Patel MP, Millhauser GL *et al.* Melanocortin MC(1) receptor in human genetics and model systems. Eur J Pharmacol 2011; 660:103-10.
4. Krude H, Gruters A. Implications of proopiomelanocortin (POMC) mutations in humans: the POMC deficiency syndrome. Trends Endocrinol Metab 2000; 11:15-22.
5. Elliott RJ, Szabo M, Wagner MJ, Kemp EH, MacNeil S, Haycock JW. alpha-Melanocyte-stimulating hormone, MSH 11-13 KPV and adrenocorticotropic hormone signalling in human keratinocyte cells. J Invest Dermatol 2004; 122:1010-9.
6. Thody AJ. alpha-MSH and the regulation of melanocyte function. Ann N Y Acad Sci 1999; 885:217-29.
7. Pain S, Dezutter C, Reymermier C, Vogelgesang B, Delay E, Andre V. Age-related changes in pro-opiomelanocortin (POMC) and related receptors in human epidermis. Int J Cosmet Sci 2010; 32:266-75.
8. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75:843-54.
9. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE *et al.* The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature 2000; 403:901-6.
10. Wenguang Z, Jianghong W, Jinquan L, Yashizawa M. A subset of skin-expressed microRNAs with possible roles in goat and sheep hair growth based on expression profiling of mammalian microRNAs. OMICS 2007; 11:385-96.
11. Zhu Z, He J, Jia X, Jiang J, Bai R, Yu X *et al.* MicroRNA-25 functions in regulation of pigmentation by targeting the transcription factor MITF in Alpaca (Lama pacos) skin melanocytes. Domest Anim Endocrinol 2010; 38:200-9.
12. Yi R, Poy MN, Stoffel M, Fuchs E. A skin microRNA promotes differentiation by repressing 'stemness'. Nature 2008; 452:225-9.
13. Muinos-Gimeno M, Espinosa-Parrilla Y, Guidi M, Kagerbauer B, Sipila T, Maron E *et al.* Human microRNAs miR-22, miR-138-2, miR-148a, and miR-488 are associated with panic disorder and regulate several anxiety candidate genes and related pathways. Biol Psychiatry 2011; 69:526-33.
14. Derghal A, Djelloul M, Airault C, Pierre C, Dallaporta M, Troadec JD *et al.* Leptin is required for hypothalamic regulation of miRNAs targeting POMC 3'UTR. Front Cell Neurosci 2015; 9:172.
15. Weiner L, Han R, Scicchitano BM, Li J, Hasegawa K, Grossi M *et al.* Dedicated epithelial recipient cells determine pigmentation patterns. Cell 2007; 130:932-42.
16. Van Den Bossche K, Naeyaert JM, Lambert J. The quest for the mechanism of melanin transfer. Traffic 2006; 7:769-78.
17. Joshi PG, Nair N, Begum G, Joshi NB, Sinkar VP, Vora S. Melanocyte-keratinocyte interaction induces calcium signalling and melanin transfer to keratinocytes. Pigment Cell Res 2007; 20:380-4.
18. Schauer E, Trautinger F, Kock A, Schwarz A, Bhardwaj R, Simon M *et al.* Proopiomelanocortin-derived peptides are synthesized and released by human keratinocytes. J Clin Invest 1994; 93:2258-62.
19. Chakraborty AK, Funasaka Y, Slominski A, Ermak G, Hwang J, Pawelek JM *et al.* Production and release of proopiomelanocortin (POMC) derived peptides by human melanocytes and keratinocytes in culture: regulation by ultraviolet B. Biochim Biophys Acta 1996; 1313:130-8.
20. Selbach M, Schwanhausser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. Nature 2008; 455:58-63.
21. Farh KK, Grimson A, Jan C, Lewis BP, Johnston WK, Lim LP *et al.* The widespread impact of mammalian MicroRNAs on mRNA repression and evolution. Science 2005; 310:1817-21.
22. Yi R, Fuchs E. MicroRNA-mediated control in the skin. Cell Death Differ 2010; 17:229-35.
23. Changsheng D, Haidong W, Linli X, Yanjun D, Lei Y, Ruiwe F *et al.* Coat color determination by miR-137 mediated down-regulation of microphthalmia-associated transcription factor in a mouse model. RNA 2012; 18:1679-86.
24. Segura MF, Hanniford D, Menendez S, Reavie L, Zou X, Alvarez-Diaz S *et al.* Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. Proc Natl Acad Sci U S A 2009; 106:1814-9.
25. Krude H, Biebermann H, Luck W, Horn R, Brabant G, Gruters A. Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nat Genet 1998; 19:155-7.
26. Gantz I, Fong TM. The melanocortin system. Am J Physiol Endocrinol Metab 2003; 284:E468-74.
27. Zhihui Y, Rui B, Ruiwen F, Gang Y, Yanjun D, Junping H *et al.* Effect of α-MSH on Proliferation and Melanin Synthesis of Alpaca Skin Melanocytes in vitro. Acta Veterinaria Et Zootechnica Sinica 2010; 41:1095-101.
28. Clark AJ, Weber A. Adrenocorticotropin insensitivity syndromes. Endocr Rev 1998; 19:828-43.
29. Caixia T, Daming Z, Xiran L. Levels of beta-endorphin in the plasma and skin tissue fluids of patients with vitiligo. J Dermatol Sci 2001; 26:62-6.
30. Kauser S, Schallreuter KU, Thody AJ, Gummer C, Tobin DJ. Regulation of human epidermal melanocyte biology by beta-endorphin. J Invest Dermatol 2003; 120:1073-80.
31. Pomerantz SH, Chuang L. Effects of beta-MSH, cortisol and ACTH on tyrosinase in the skin of newborn hamsters and mice.

Endocrinology 1970; 87:302-10.