Acta Histochemica 118 (2016) 505–512

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

The expression of KRT2 and its effect on melanogenesis in alpaca skins

Yucong Cui [a], Yajun Song [b], Qingling Geng [a], Zengfeng Ding [a], Yilong Qin [a], Ruiwen Fan [a], Changsheng Dong [a], Jianjun Geng [a,*]

[a] College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu, 030801, PR China
[b] Tianshui Entry-Exit Inspection and Quarantine Bureau, Gansu, 741020, PR China

ARTICLE INFO

Article history:
Received 21 February 2016
Received in revised form 17 May 2016
Accepted 19 May 2016

Keywords:
KRT2
Hair color
Alpaca
Melanocyte
Skin

ABSTRACT

In order to investigate the effects of the keratin 2 (KRT2) on alpaca melanocyte in vivo and vitro, the immunohistochemistry (IHC), quantitative real-time PCR (qPCR), Western blot, and alpaca melanocytes transfection methods were used. The results showed that mRNA and protein expression of KRT2 was highly expressed in brown skin in comparison with that in white skin. Moreover, we found that KRT2 was expressed in alpaca melanocytes in vitro by immunocytochemistry. After transfection with KRT2 in alpaca melanocytes, the relative mRNA and protein expression of KRT2, microphthalmia-associated transcription factor (MITF), tyrosinase (TYR) and tyrosinase-related protein 1 (TYRP1) in alpaca skin melanocytes was increased with significant differences; a further result was the increase of melanin production. The results suggested that KRT2 functions in alpaca hair color formation, which offered an essential theoretical basis for further exploration of the role of melanogenesis.

1. Introduction

In adult animals, both hair and skin colors depend on pigment-producing melanocytes that remain stationary at the base of the epithelium and transfer melanin-containing organelles to adjacent keratinocytes that are pushed upward as they proliferate (Barsh and Cotsarelis, 2007). Melanocytes in mammals and birds produce two chemically distinct types of melanin, black to brown eumelanin and yellow to reddish-brown pheomelanin within membrane-bound organelles termed melanosomes; subsequently, those melanosomes are transferred to surrounding epidermal cells, which are known as keratinocytes (Ito, 2003; Ito and Wakamatsu, 2007, 2008). The combination and distribution of melanin in keratinocytes and hair determines color (OYEHAUG et al., 2002). Current research indicates that many genes regulate hair and skin colors in human and other vertebrate species (Lamason et al., 2005; Passeron et al., 2007; Park et al., 2014). However, the molecular and cellular mechanisms that regulate coat colors in fiber-producing species have not been completely elucidated.

Alpaca coat color is an important feature of breeds; color is a quality trait and controlled by many genes. Although the role of numerous genes in regulating the coat color of mice, dog, cat, and sheep has been verified (Sturm, 2009; Fan et al., 2013; Kaelin and Barsh, 2013), KRT2 gene in the forming of alpaca coat color is available. Keratin can maintain hair follicle structure and contains the expression of the most abundant protein in the hair follicles. The major proteins synthesized in the hair shaft are the keratin intermediate filament (IF or KRT) and keratin-associated proteins (KAP) (Dunn et al., 1998). More than 30 different keratins are known, all of which fall into either of two groups, type I (K9-20) or type II (K1-8) (Virtanen et al., 2001). Keratin IFs are composed of type I and type II proteins that are co-expressed in specific pairs to form 8–10 nm diameter heteropolymeric filaments (Steinert and Roop, 1988). In hair growth, as the follicle bulb cells rapidly differentiates into either cortical or cuticle hair keratinocytes, approximately 50–100 keratin genes are transcriptionally activated (Powell et al., 1991, 1992; Powell and Rogers, 1996). One prominent motif, originally termed HK-1 (Powell et al., 1991), is now recognized as a binding site for lymphoid enhancer factor 1(LEF-1) (Zhou et al., 1995). During follicle morphogenesis and hair growth, LEF-1 is expressed in several locations, i.e., the embryonic ectoderm, the ectodermal cells at the leading edge of the follicle placode, the follicle bulb and lower shaft of adult follicles, and the dermal papilla (Zhou et al., 1995).

KRT2 is among the differentially expressed genes identified in the white vs black sheep skin transcriptome profiles (Fan et al., 2013). Thus far, the expression pattern and localization of this gene regulation have never been elucidated in alpaca skin melanocyte development and melanogenesis. We therefore conducted this

* Corresponding author at: Science and Veterinary Medicine, Shanxi Agricultural University. PR China
E-mail address: sxndgjj@126.com (J. Geng).

http://dx.doi.org/10.1016/j.acthis.2016.05.004
0065-1281/

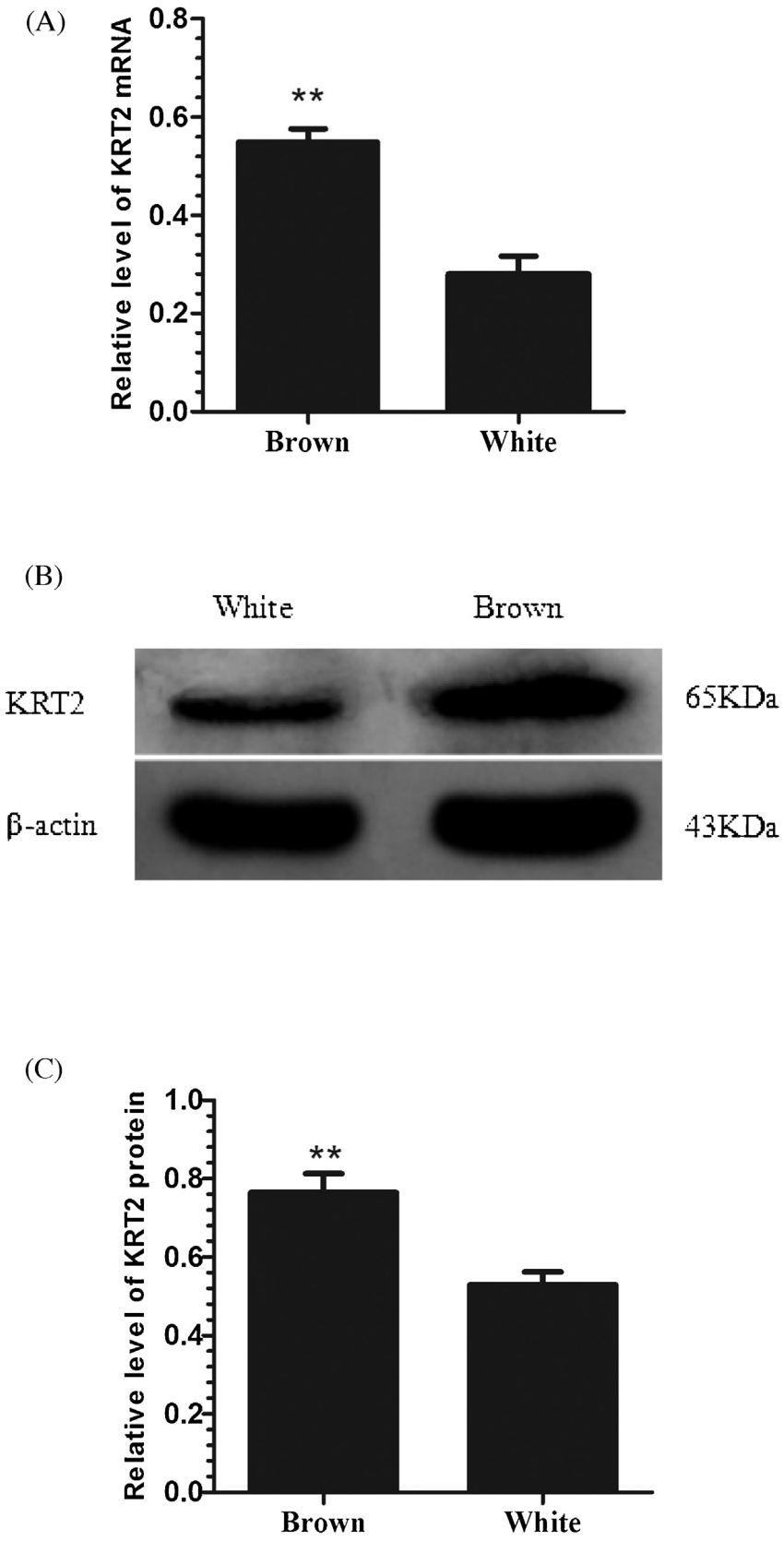

Fig. 1. Real-time PCR and Western blot analysis of KRT2 in the same areas of skin samples collected from different coat colors of alpaca. (A). Relative KRT2 mRNA expression in alpaca brown skin was 5.668 times higher than that of white skin. (B). Representative Western blot of KRT2 protein abundance in protein extracts from the same areas of skin samples collected from different coat colors alpacas. (C). Quantitative analysis of KRT2 protein abundance in same areas of skin samples collected from different coat colors alpacas as determined by densitometry. Abundance of KRT2 was normalized relative to abundance of β-actin. $^{**}P < 0.01$. The bars in each panel represent the mean ± SD from three replicates.