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Interplay of MicroRNA-21 and SATB1 in Epidermal Keratinocytes during Skin Aging

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TO THE EDITOR

Aging is a complex process characterized by progressive decline in physiological and biochemical performance of individual tissues and organs. In aged skin, reduced cell proliferation and functional decline of epithelial and mesenchymal cells underlie age-related changes, such as dry skin (xerosis), loss of elasticity, and functional senescence, leading to increased susceptibility to aging-associated conditions such as skin cancer and poor wound healing (Engelke et al., 1997;Zhang et al., 2009). MicroRNAs (miRNAs) are small noncoding RNAs involved in the post-transcriptional regulation of coding-gene expression. They provide an additional level of control for important cellular processes such as growth, differentiation, and remodeling of skin (Botchkareva, 2017). In addition, miRNAs can regulate the expression of important epigenetic regulators, including DNA methyltransferases, histone deacetylases, and polycomb group genes. Disruption of the miRNA-epigenetic regulatory network was shown to interfere with normal physiological cellular functions, leading to activation of disease processes (reviewed in [Sato et al., 2011]).

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AUTHOR CONTRIBUTIONS

Conceptualization: MIA, NVB, VAB; Data Curation: MIA, AGR, KP, MAA, MEP, ANM, NVB, VAB; Formal Analysis: MIA, AGR, KP, MAA, MEP, ANM, NVB, VAB; Funding Acquisition: MIA, VAB, NVB; Investigation: MIA, AGR, KP, MAA, MEP, ANM, NVB, VAB; Supervision: MIA, NVB, VAB; Writing - Original Draft Preparation: MIA, NVB, VAB.

CONFLICT OF INTEREST

The authors state no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.04.022.

By fine-tuning biological systems, miRNAs can contribute to healthy aging or development of age-related diseases, and may serve as useful diagnostic or prognostic biomarkers for age-related diseases (Olivieri et al., 2012).

MicroRNA-21 (miR-21) plays an important role in the development of a number of pathological skin conditions including psoriasis, tumorigenesis, and poor wound-healing (Ahmed et al., 2011;Meisgen et al., 2012;Yang et al., 2011). However, the role of miR-21 in skin aging has not been investigated yet. Here, we identify miR-21 as a contributor to skin aging, at least in part, by negative regulation of the chromatin remodeler SATB1 in keratinocytes.

The expression of miR-21 was examined in skin of young and aged mice (8-week-old vs 2-year-old) and human female donors (48, 60–62, and 78 years old; Supplementary Materials and Methods). Quantitative reverse transcriptase–PCR (RT-qPCR) analysis revealed a prominent increase in miR-21 expression in both mouse and human aged skin (Figure 1a and b). In contrast to miR-21, the level of miRNA-199a, which is not detectable in the epidermis of either mouse or human skin, was used as a control for this study (Sonkoly et al., 2007;Yi et al., 2006) and was not altered during the aging process in human skin (Figure 1b). Using in situ hybridization, we confirmed increased miR-21 expression in the epidermis and dermis of aged mouse and human skin (Figure 1c and d). Our data are consistent with published reports showing the elevation of miR-21 in age-associated cardiovascular diseases in human patients (Olivieri et al., 2012) and in kidneys of aged mice (Sataranatarajan et al., 2012).

Human skin aging is associated with a decrease in the expression of keratinocyte differentiation-associated markers (Engelke et al., 1997). Indeed, a significant reduction in the expression of keratinocyte differentiation-related genes was observed in aged mouse ($P < 0.05$) and human skin (Figure 1e and f). Forced expression of miR-21 in both primary mouse and human keratinocytes transfected with pro-miR-21 mimic resulted in significant reduction in *Krt1* and *Ivl* ($P < 0.05$, mouse) and *KRT1* ($P < 0.01$), *KRT10, KRT14*, and *IVL* expressions ($P < 0.05$, human) (Figure 1g and h). This suggests that miR-21 potentially can contribute to skin aging by downregulating keratinocyte differentiation-related genes, possibly leading to cellular senescence (Dellago et al., 2013) and contributing to increased susceptibility to age-related pathological conditions.

To identify potential putative miR-21 targets, we performed bioinformatics analysis as done previously (Ahmed et al., 2014). By interrogating predicted miR-21 targets from three different databases, we identified 35 potential genes whose expression may be regulated by miR-21. Ten of these genes, including *Satb1*, have highly conserved miR-21 target sequences between human and mouse genomes (Figure 1i). SATB1 is a nuclear protein operating as a genome organizer, which originally was identified as an essential mediator of normal T-cell development regulating the large-scale chromatin remodeling and enhancer–promoter interactions in several lineage-specific gene loci (Cai et al., 2003). In the skin, SATB1 is essential for higher-order chromatin folding and transcriptional regulation of the epidermal differentiation complex locus in keratinocytes (Fessing et al., 2011). Interestingly, genetic ablation of *Satb1* in mouse skin causes thinning of the epidermis accompanied by

downregulation in the expression of terminal differentiation-associated genes (Fessing et al., 2011).

We confirmed the direct regulation of *Satb1* by miR-21 using a luciferase reporter assay. Cotransfection of HaCaT cells with pro-miR-21 mimic and the *Satb1* 3′ untranslated region reporter construct caused a significant reduction in luciferase activity ($P < 0.001$) compared with their corresponding controls, whereas this effect was not detected when miR-21 binding sites in the *Satb1* 3′ untranslated region were mutated (Figure 1j). This is consistent with published data showing miR-21 targeting of SATB1 in rectal cancer cells (Lopes-Ramos et al., 2014). The functional interactions of miR-21 and SATB1 in keratinocytes were evaluated by transfecting primary human and mouse epidermal keratinocytes with pro-miR-21 mimic and anti-miR-21, which resulted in the decreased and increased expression of SATB1 mRNA and protein, respectively, as determined by RT-qPCR and western blot (Figure 1k–n, Supplementary Materials and Methods). Additionally, reduced *Satb1* expression was confirmed by RT-qPCR and immunofluorescent analysis in both mouse and human aged epidermis (Figure 2a–d).

To further explore the plausible functional link between miR-21 and SATB1 in skin aging, we overexpressed SATB1 and miR-21 in keratinocytes using SATB1-expressing lentiviral particles or pro-miR-21 mimic. We confirmed the increased expression of *Satb1* (SATB1 Leti + miR-Control) or miR-21 (Control Leti + pro-miR-21) in primary mouse epidermal keratinocytes as determined by RT-qPCR (Figure 2e and f, Supplementary Materials and Methods). However, coexpression of both SATB1 and miR-21 (SATB1 Leti + pro-miR-21) significantly reduced SATB1 expression (Figure 2e). RT-qPCR analysis also revealed that *Satb1* induces expression of differentiation-associated genes, supporting its role as a promoter of terminal keratinocyte differentiation (Fessing et al., 2011). Forced expression of miR-21 abolished SATB1-induced upregulation of *Krt1, Krt10*, and *Krt17* (Figure 2g). Therefore, our data suggest that miR-21 contributes to the age-associated alterations in gene expression, at least in part, by targeting *Satb1*.

The downregulation of SATB1 in human keratinocytes by miRNA-191 has been shown to establish epigenetic modifications leading to senescence (Lena et al., 2012). SATB1 has also been associated with increased lifespan, whereas a reduction in its expression was seen with age and in age-related pathologies, such as diabetes in mice, demonstrating the general involvement of SATB1 in counteracting the senescence and/or aging pathways (Zhang et al., 2009). An increasing number of studies have identified miR-21 as a senescence, inflammation, and cancer-associated miRNA (Olivieri et al., 2013). Therefore, our data suggest that the negative regulation of SATB1 by miR-21 in keratinocytes may be an important age-phase–specific regulation leading to senescence and promoting disease states in skin.

Taken together, we demonstrate that (i) miR-21 expression is increased in human and mouse aging skin; (ii) SATB1 expression is inversely correlated with miR-21 in young and aged skin;and (iii) *Satb1* serves as a genuine direct target of miR-21 in keratinocytes. Thus, by regulating SATB1 in epidermal keratinocytes, miR-21 may contribute to the higher-order chromatin remodeling and establishment of enhancer–promoter networks involved in

epidermal differentiation, as well as increase susceptibility to age-related pathological conditions, such as tumorigenesis. These data provide a platform for the establishment of novel approaches for pharmacological manipulation of skin aging via modulation of the miR-21 activity in keratinocytes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

miR-21	microRNA-21
miRNA	microRNA
RT-qPCR	quantitative reverse transcriptase–PCR

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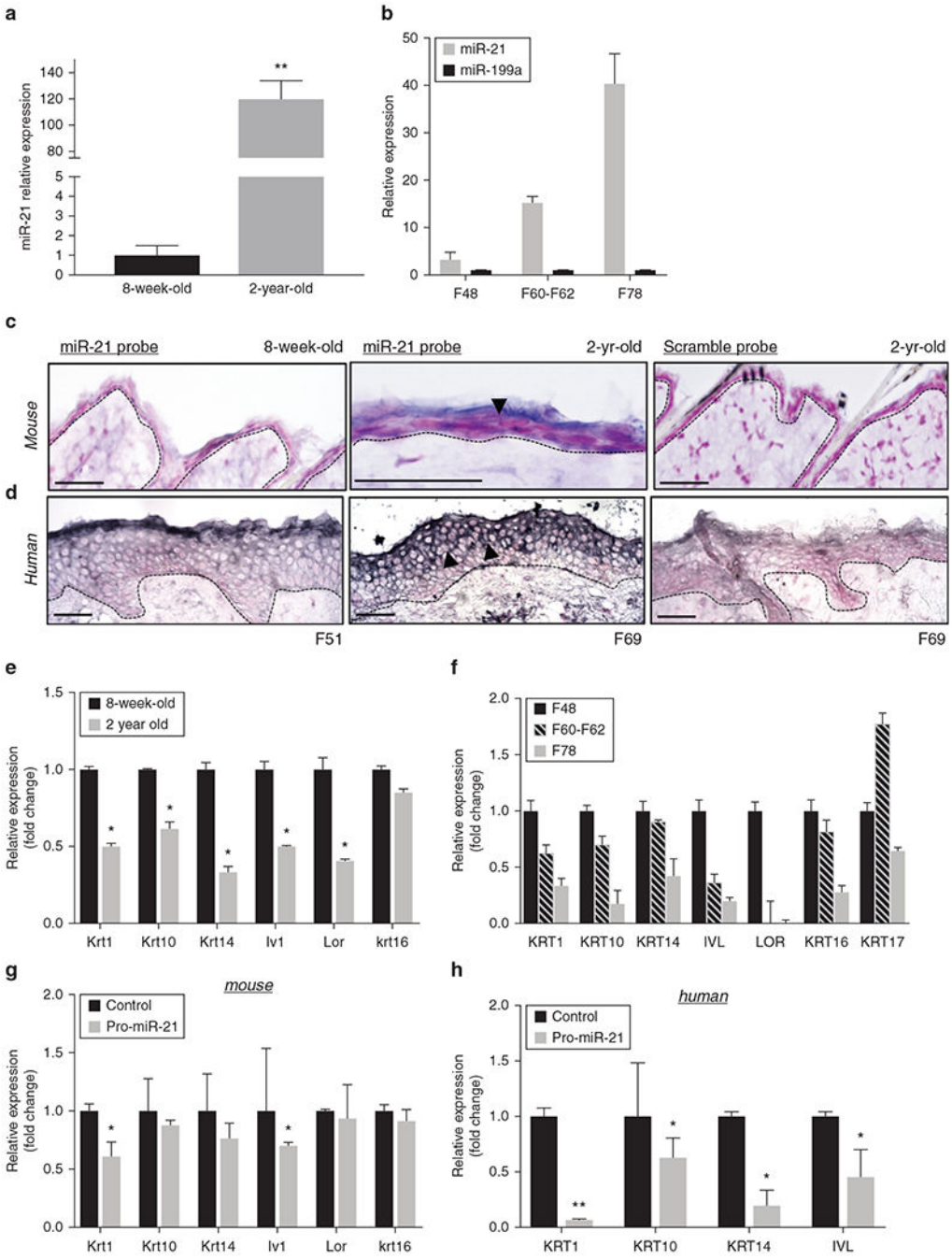

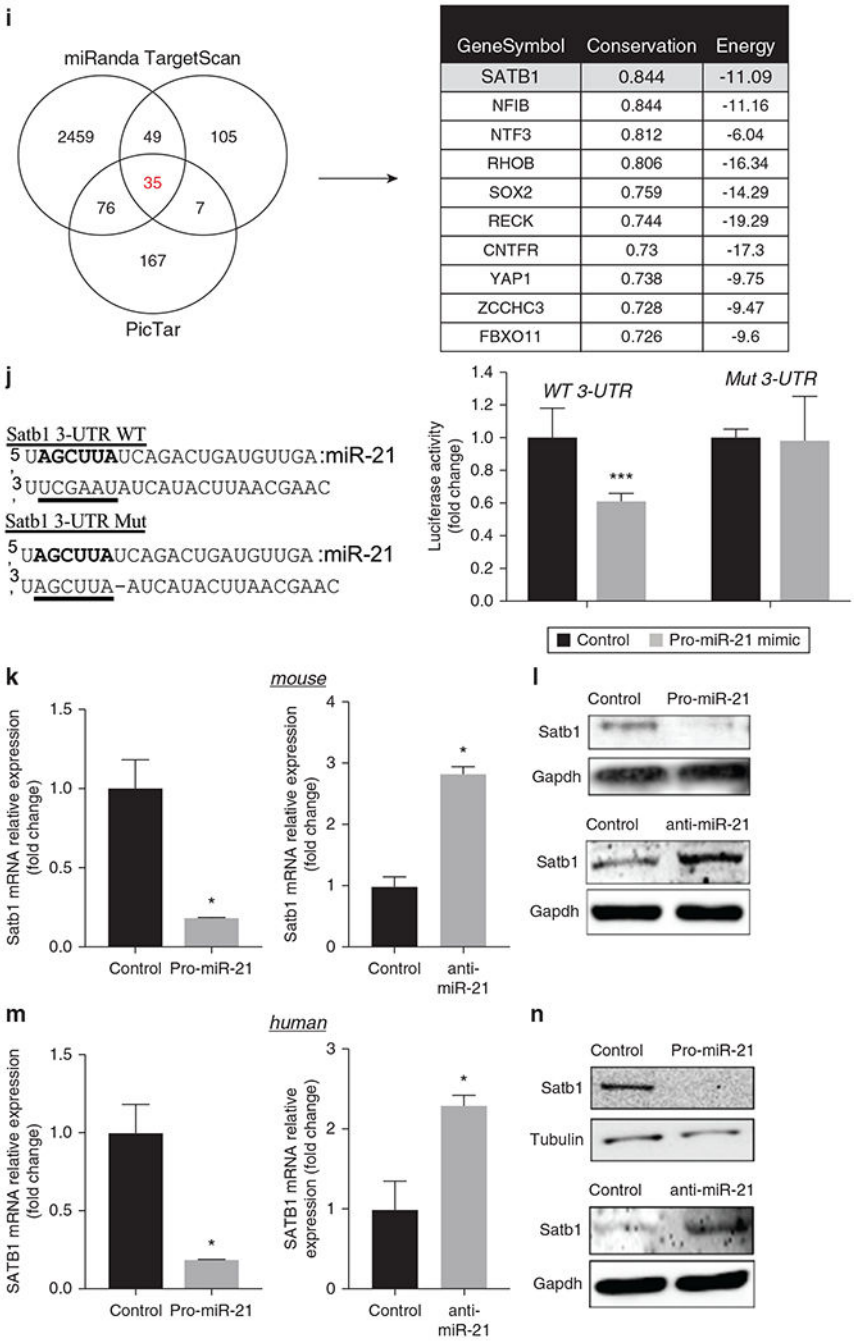

Figure 1. MiR-21 is elevated in aging skin and targets SATB1 in keratinocytes.

(**a, b**) RT-qPCR analysis of miR-21 expression in young vs aged mice (8-week-old vs. 2-year-old) and human female donor skins (F48, F60–62, F78); miR-21 expression is upregulated in aged mouse and human epidermis. Data are presented as mean ± SEM values from three (mouse) or two (human) independent samples and three independent experiments each. (**c, d**) Representative photomicrographs of in situ hybridization for miR-21; miR-21 expression is elevated in the epidermis (arrowheads) and dermis of aged mouse and human skins. Data are presented from three (mouse) and two (human) independent samples. (**e**) RT-

qPCR analysis of differentiation-related genes in young versus aged mice; a decrease in expression is observed for all genes analyzed. Data are presented as mean ± SEM values from three independent samples and experiments. (**f**) RT-qPCR analysis in young versus aged human skin; a decrease in expression is observed for all differentiation-associated genes analyzed. Data are presented as mean ± SEM values from two independent samples and three independent experiments. (**g, h**) Transfection with pro-miR-21 mimic in primary mouse and human keratinocytes causes a significant decrease in the expression of *Krt1* and *Ivl* (mouse) and *KRT1, KRT10, KRT14*, and *IVL* (human). Data is presented as mean ± SEM values from three independent experiments. (**i**) Venn diagram of predicted miR-21 gene targets. A table showing the top ten miR-21 target genes listed as the most conserved between human and mouse genomes, including *SATB1*. (**j**) Significant reduction in luciferase activity in HaCaT cells cotransfected with pro-miR-21 mimic and the *SATB1*-3′ UTR (wt-3′UTR) construct encompassing the putative miR-21 target site. No changes in luciferase activity were detected when the miRNA binding site was mutated (mut-3′UTR). Bold letters represent miR-21 seed region. Underlined letters represent predicted binding sites within *SATB1*-3′UTR. Each sample was normalized to Renilla luciferase activity. Data is presented as mean ± SEM values from three independent experiments. (**k**–**n**) RT-qPCR and western blot analysis; SATB1 mRNA and protein levels are significantly decreased and increased after transfection with pro-miR-21 or anti-miR-21, respectively, in both primary mouse and human keratinocytes. Data are presented as mean ± SEM values from three independent experiments. Western blot data shown are from a single representative experiment out of three repeats. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; Student's *t*-test. The broken lines demarcate the epidermal–dermal border. miR-199a, microRNA-199a; miR-21, microRNA-21; mut, mutated; RT-qPCR, quantitative reverse transcriptase–PCR; SEM, standard error of the mean; UTR, untranslated region; wt, wild type. Bar = 50μm.

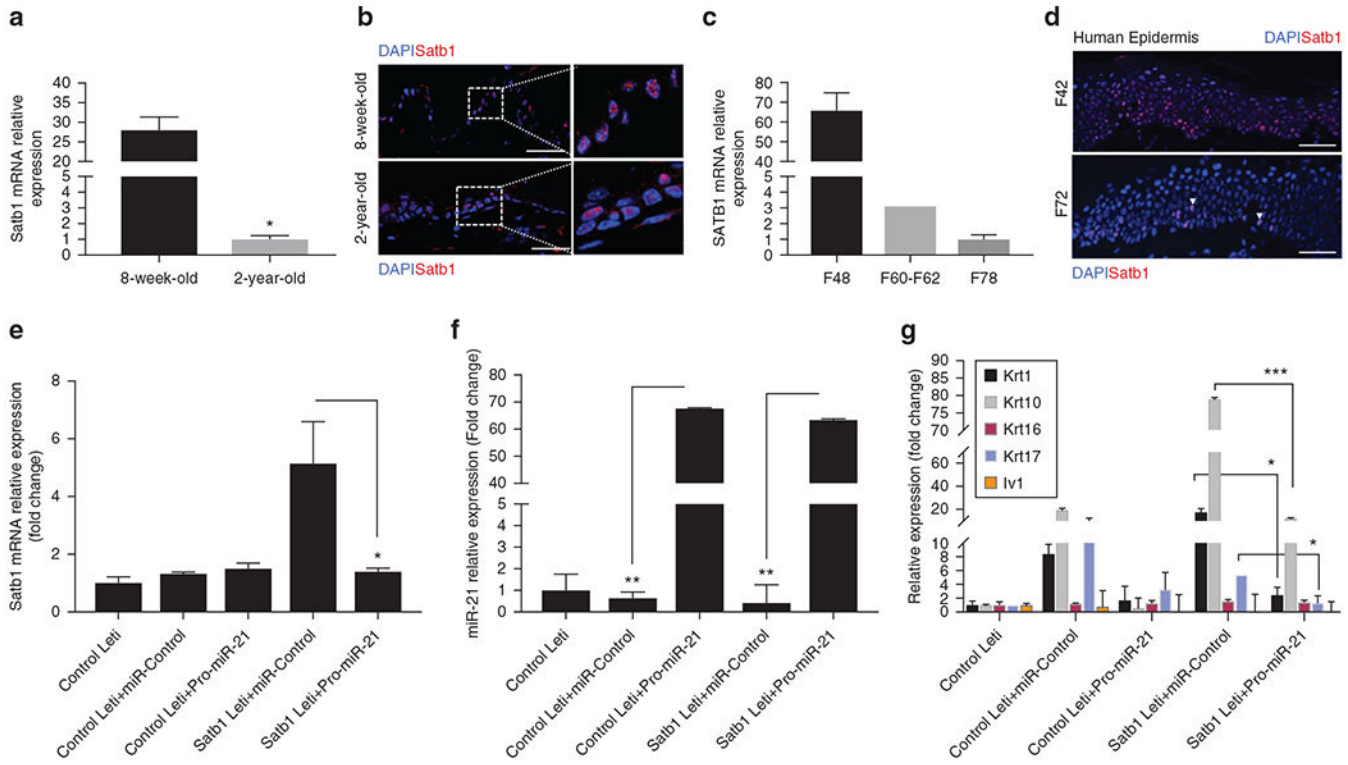

Figure 2. miR-21 modulates expressions of terminal differentiation-associated markers by regulating SATB1 in keratinocytes.

SATB1 mRNA and protein expression in (**a, b**) young versus aged mice (8-week-old vs. 2-year-old) and (**c, d**) human female donor skins (F48, F60-62, F78). (**a, b**) SATB1 mRNA and protein expression are downregulated in aged mouse skins. RT-qPCR and immunostaining data are presented as mean ± SEM values and representative images from three independent samples and experiments. (c, d) SATB1 mRNA and protein expression are downregulated in aged human skin (arrowheads). RT-qPCR and immunostaining are presented as mean ± SEM values and representative images from two independent samples and three independent experiments. (**e, f**) *Satb1* and miR-21 expression were upregulated after either Satb1-lentivral overexpression or pro-miR-21 mimic transfection in primary mouse keratinocytes as determined by RT-qPCR analysis. Data are presented as mean ± SEM values from three independent experiments. (**g**) RT-qPCR analysis of differentiation-associated genes in primary mouse keratinocytes; overexpression of SATB1 induces expression of *Krt1, Krt10*, and *Krt17*, whereas cotransfection with pro-miR-21 mimic diminishes these effects. Data are presented as mean ± SEM values from three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Student's *t*-test. miR, microRNA; miR-21, microRNA-21; RT-qPCR, quantitative reverse transcriptase–PCR; SEM, standard error of the mean. Bar = 50μm.