Secreted Frizzled-Related Protein 2 (sFRP2) Functions as a Melanogenic Stimulator; the Role of sFRP2 in UV-Induced Hyperpigmentary Disorders

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In this study, we found that secreted frizzled-related protein 2 (sFRP2) is overexpressed in the hyperpigmentary skin of melasma and solar lentigo and in acutely UV-irradiated skin. To investigate the effect of sFRP2 on melanogenesis, normal human melanocytes were infected with sFRP2-lentivirus or sh-sFRP2. It was found that sFRP2 stimulates melanogenesis through microphthalmia-associated transcription factor and/or tyrosinase upregulation via β-catenin signaling. The stimulatory action of sFRP2 in pigmentation was further confirmed in melanocytes cocultured with fibroblasts and in ex vivo cultured skin. The findings suggest that sFRP2 functions as a melanogenic stimulator and that it plays a role in the development of UV-induced hyperpigmentary disorders.

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INTRODUCTION

Wnt signaling is known to play a role in the development of neural crest melanoblasts. Recently, increasing evidence has highlighted the contribution of Wnt signaling in the regulation of melanogenesis in adult melanocytes (Bellei et al., 2011; Kim et al., 2013; Park et al., 2014; Yamaguchi et al., 2004). Wnt/β-catenin signaling induces the formation of a microphthalmia-associated transcription factor (MITF)/β-catenin/lymphoid enhancer-binding factor (LEF)-1 complex that in turn elevates the transcription of the tyrosinase gene (Schepsky et al., 2006). Wnt3a activates the MITF promoter and increases melanin synthesis, and Wnt5a inhibits the proliferation and melanogenesis of mouse melanocytes (Guo et al., 2012; Takeda et al., 2000; Zhang et al., 2013). In addition, it has been suggested that β-catenin also plays a key role in the physiological regulation of cutaneous pigmentation, as α-melanocyte-stimulating hormone reportedly stimulates Wnt/β-catenin signaling (Bellei et al., 2011). It was also shown that UV irradiation elevates the expression of Wnt7a in normal human keratinocytes, resulting in the elevation of melanogenic proteins in normal melanocytes (Yamada et al., 2013), thus suggesting that abnormal Wnt/β-catenin signaling is responsible for various UV-induced pigmentary disorders.

Wnt signaling is modulated by a number of secreted and transmembrane Wnt inhibitors and activators (Cruciat and Niehrs, 2013). Extracellular secreted proteins include the Wnt inhibitory factor Dickkopf and secreted frizzled-related proteins (sFRPs). sFRP proteins (sFRP1—sFRP5) appear to represent the largest family of Wnt modulators. The sFRP protein family can be separated into two subgroups, sFRP1/2/5 and sFRP3/4, based on sequence homology studies (Kawano and Kypta, 2003). Early studies found that sFRP binding to Wnt prevented the activation of Wnt receptors, leading to the initial classification of sFRPs as Wnt signaling inhibitors. However, subsequent reports have indicated that sFRP2 is an agonist rather than an antagonist of β-catenin that synergizes or mimics Wnt activities (Kress et al., 2009; Lee et al., 2004; Mirotsou et al., 2007; von Marschall and Fisher, 2010; Yamamura et al., 2010).

Our interest in sFRP2 involves its upregulation in the melasma, a common UV-induced hyperpigmentary disorder. In our previous large-scale gene expression profiling using lesional and perilesional normal skin of melasma, the *sFRP2* gene was found to be one of the 20 most upregulated genes in melasma hyperpigmented skin. The *sFRP2* expression level increased by 1.76-fold compared with perilesional normal skin (Kang et al., 2011). A molecular network analysis of 279 differentially expressed genes identified the Wnt pathway and the MITF-associated pathway as significantly modified pathways. A microarray analysis of solar lentigo also identified the upregulation of the *sFRP1* gene (Goyarts et al., 2007). We therefore investigated the role of sFRP2 in the regulation of skin pigmentation.

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Abbreviations: LEF, lymphoid enhancer-binding factor; MITF, microphthalmia-associated transcription factor; sFRP2, secreted frizzled-related protein 2; TCF, T cell factor

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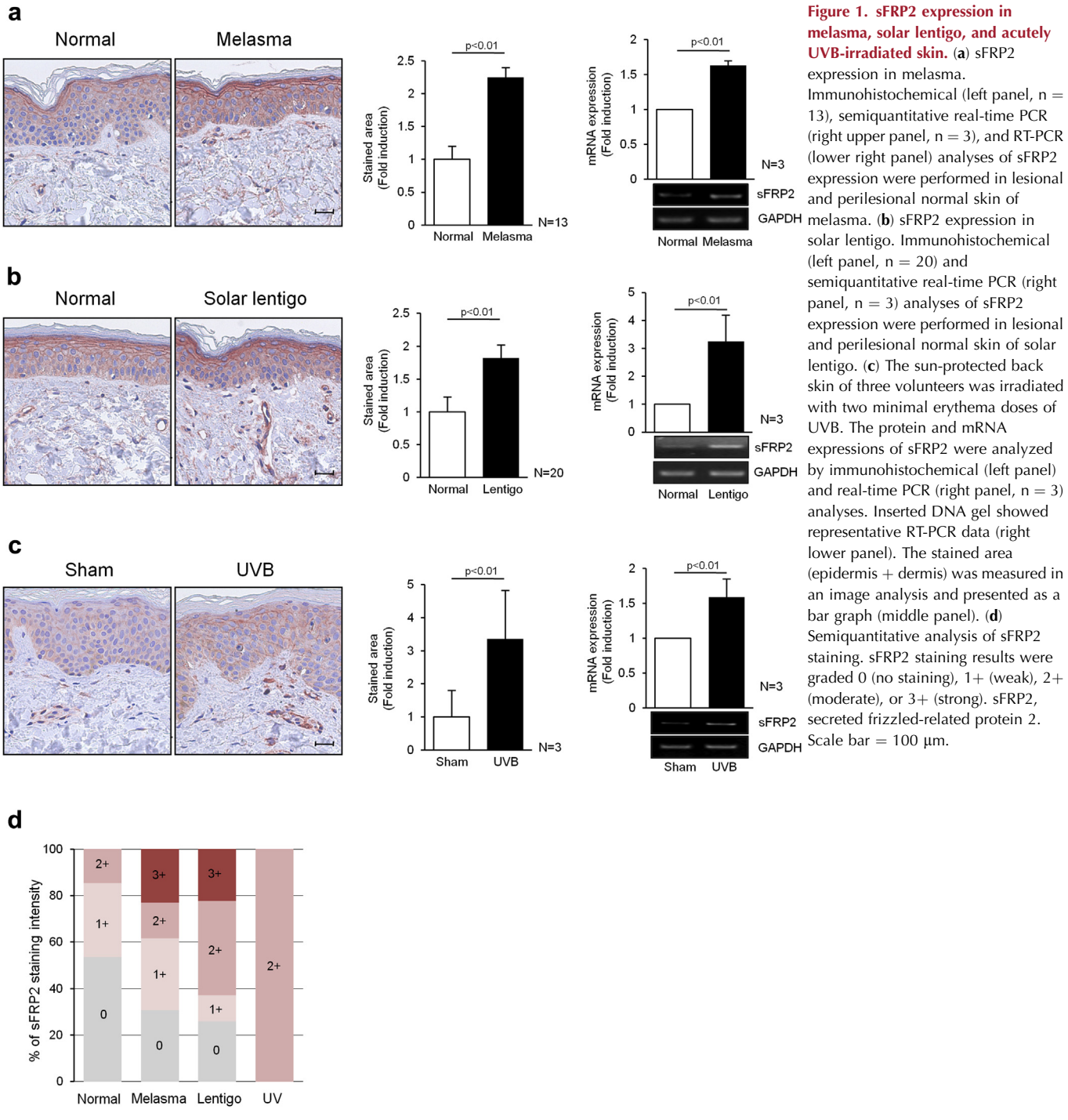

Figure 1. sFRP2 expression in melasma, solar lentigo, and acutely UVB-irradiated skin. (**a**) sFRP2 expression in melasma. Immunohistochemical (left panel, n = 13), semiquantitative real-time PCR (right upper panel, n = 3), and RT-PCR (lower right panel) analyses of sFRP2 expression were performed in lesional and perilesional normal skin of melasma. (**b**) sFRP2 expression in solar lentigo. Immunohistochemical (left panel, n = 20) and semiquantitative real-time PCR (right panel, n = 3) analyses of sFRP2 expression were performed in lesional and perilesional normal skin of solar lentigo. (**c**) The sun-protected back skin of three volunteers was irradiated with two minimal erythema doses of UVB. The protein and mRNA expressions of sFRP2 were analyzed by immunohistochemical (left panel) and real-time PCR (right panel, n = 3) analyses. Inserted DNA gel showed representative RT-PCR data (right lower panel). The stained area (epidermis + dermis) was measured in an image analysis and presented as a bar graph (middle panel). (**d**) Semiquantitative analysis of sFRP2 staining. sFRP2 staining results were graded 0 (no staining), 1+ (weak), 2+ (moderate), or 3+ (strong). sFRP2, secreted frizzled-related protein 2. Scale bar = 100 μm.

RESULTS

sFRP2 expression is increased in UV-induced hyperpigmented skin

In our previous microarray study, we found that *sFRP2* expression was significantly increased at the transcriptional level in melasma lesional skin (Kang et al., 2011; NCBI GEO accession number GSE72140). The levels of sFRP2 expression in melasma were analyzed by immunohistochemical staining (left and middle panel), semiquantitative real-time PCR (right upper panel), and RT-PCR (right lower panel). The findings revealed that in 9 of the 13 patients, sFRP2 immunoreactivity was higher in the basal layer and around fibroblasts in lesional skin (Figure 1a). An image analysis demonstrated an increase in the stained area in hyperpigmented skins compared with perilesional skin in melasma (n = 13, epidermis, 0.567 ± 0.062 vs. 0.256 ± 0.086; dermis, 0.052 ± 0.017 vs. 0.019 ± 0.012, $P < 0.01$). The sFRP2 expression level was also examined with skin samples showing solar lentigo, which is the most common photoaged hyperpigmentary disorder. It was found that sFRP2 expression was increased in 18 of 20 solar lentigo legions (Figure 1b, n = 20, epidermis, 0.483 ± 0.089 vs. 0.262 ± 0.081; dermis,