Survivin in the Human Hair Follicle

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TO THE EDITOR

The hair follicle (HF) is a skin appendage that shows cyclic activity with periods of growth and hair fiber production (anagen), apoptosis-driven involution (catagen), and relative resting/hair shedding (telogen/exogen) (Stenn and Paus, 2001). Proliferation, differentiation, and apoptosis in HF keratinocytes are controlled by a number of signaling molecules that belong to the bone morphogenic protein/transforming growth factor-β, epidermal growth factor, fibroblast growth factor, Hedgehog, IGF, Notch, neurotrophin, tumor necrosis factor, and Wnt families (Stenn and Paus, 2001; Botchkarev and Kishimoto, 2003; Paus and Foitzik, 2004). The decrease in proliferative activity or activation of apoptosis in the matrix region leads to hair growth retardation and/or alterations in HF cyclic activity. Therefore, molecules involved in the control of cell proliferation and/or apoptosis are of interest for hair growth modulation (Cotsarelis and Millar, 2001).

Survivin is a member of the inhibitor of apoptosis protein family, and has been implicated in the control of cell proliferation, as well as in the inhibition of apoptosis (Ambrosini *et al.*, 1998; Wheatley and McNeish, 2005). Survivin functions as a component of the chromosomal passenger complex, which is essential for cell division (Li *et al.*, 1999; Temme *et al.*, 2003). In addition, survivin inhibits apoptosis by either directly or indirectly interfering with the functions of caspases (Tamm *et al.*, 1998; Banks *et al.*, 2000). Transgenic mice expressing survivin under control of the keratin 14 promoter show significantly reduced number of apoptotic cells in the epidermis after UV exposure (Grossman *et al.*, 2001). In contrast, molecular survivin antagonists increase the susceptibility of numerous cell lines to apoptosis (Xia *et al.*, 2002; Kappler *et al.*, 2004). Survivin expression is elevated in majority of human cancers. It has potential as a marker for diagnostics, as well as a prognostic and therapeutic target for radio- and chemotherapy (Altieri, 2003; Rodel *et al*, 2005). The dual functions of survivin in promoting cell proliferation and preventing apoptosis raises a question about its involvement in regulating anagen and catagen phases of the hair cycle, during which hair matrix keratinocytes extensively proliferate and undergo apoptosis (Stenn and Paus, 2001; Botchkareva *et al.*, 2006).

To explore survivin expression in human skin, survivin gene transcription and survivin protein expression in the epidermis and HFs was characterized by real-time PCR analysis and immunohistochemistry, respectively (human scalp skin was obtained from face-lifts remains with written patient consent from five different individuals; the study was approved by an independent Institutional Review Board to ensure subject protection and adherence to the Declaration of Helsinki Principles). By real-time PCR analysis, survivin mRNA expression was observed in anagen HFs, as well as in the epidermis (PCR primer set was obtained from SuperArray Bioscience Corporation, Frederick, MD). Relative quantification revealed that levels of survivin mRNA are substantially higher in the HF than in the epidermis (Figure 1a).To determine localization of survivin in skin, rabbit polyclonal antibody against human survivin protein (1:1,000; Chemicon International Inc., Temecula, CA) was applied, using 8-μm frozen tissue sections fixed in 10% formalin (10 min) and post-fixed in ethanol-acetic acid (5 min, −20°C) and the tyramide-amplification method, as described before (Botchkareva *et al.*, 2003). In the epidermis, survivin was expressed only in few basal cells (Figure 1b). In the anagen HF, survivin was prominently expressed in the hair matrix and outer root sheath, and survivin colocalized with proliferative marker Ki-67, as determined by using rabbit monoclonal antibody against Ki-67 (Dako, Carpinteria, CA) (Figure 1c and d). Double immunostaining with antibody against survivin and the marker of melanocytes pMel-17 (using chicken anti-gp100/pMel-17; Zymed Laboratories, San Francisco, CA) revealed that none of the cells expressing survivin either in the outer root sheath or hair matrix showed pMel-17 immunoreactivity in anagen and early-catagen HFs, suggesting that survivin expression is restricted to hair matrix and outer root sheath keratinocytes (Figure 1e and f).

In early-catagen HFs, survivin expression decreased in the hair matrix and disappeared from the outer root sheath (Figure 1g). During mid-catagen, the number of survivin-positive cells further decreased in the regressing hair matrix (Figure 1h). Finally, in late catagen, only a few surviving-positive cells were detected in the regressing epithelial portion of the HF (Figure 1i). Because of the involvement of survivin in inhibiting apoptosis, co-visualization of survivin and apoptotic cells was also performed using commercially available Apo-Direct TUNEL Assay kit (Chemicon International Inc.). Lack of colocalization of survivin and TUNEL was detected in catagen HFs (Figure 1j). Collectively, these data suggested that survivin is involved in the maintenance of HF growth by supporting proliferation and protecting cells against apoptosis.

It was recently shown that transcription of *survivin* is regulated by T-cell factor (TCF)/β-catenin signaling: TCF-binding sites were identified in the promoter region of *survivin*, and the

Abbreviation: HF, hair follicle

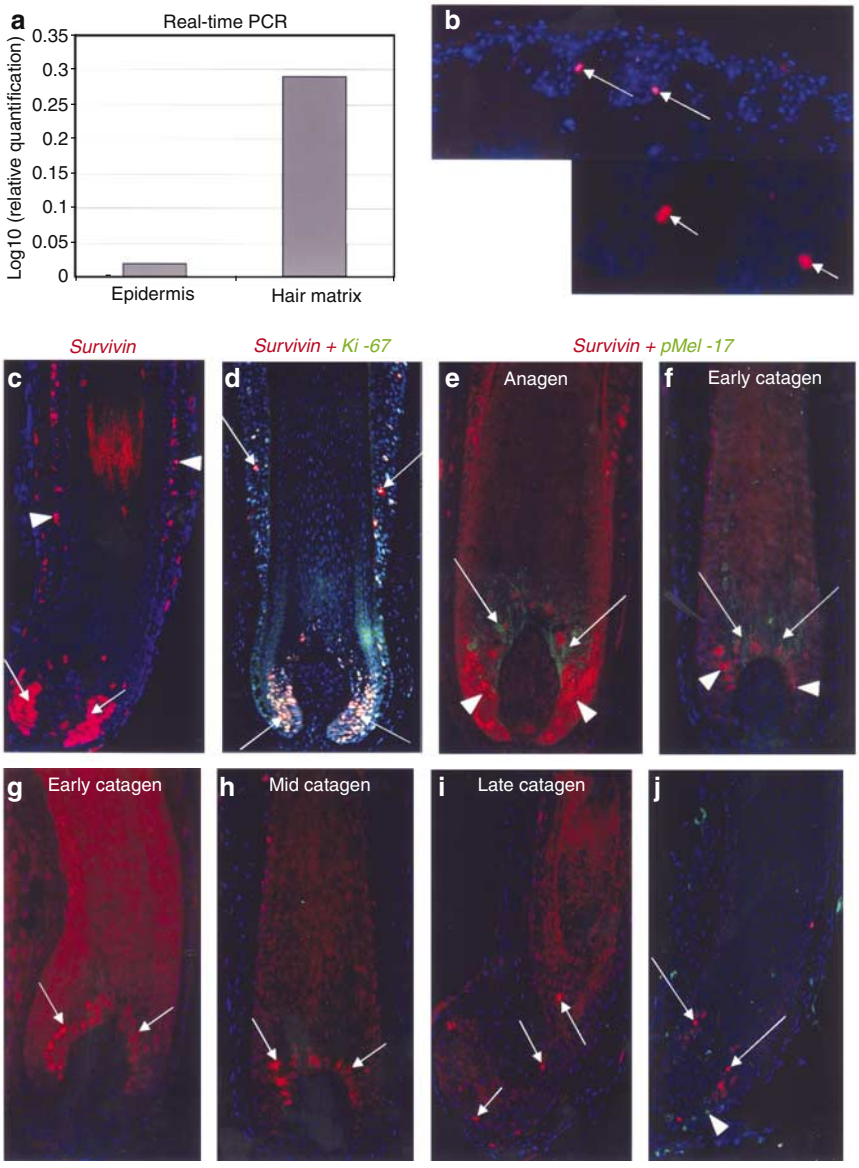

Figure 1. Expression of survivin in human skin and HF. (**a**) Real-time relative PCR quantification revealed that survivin mRNA is expressed at higher levels in anagen HFs than in the epidermis. (**b**) In the full thickness skin, only few cells in the basal layer of the epidermis are survivin+ (arrows). (**c**) In anagen HF, survivin is detected in the hair matrix and outer root sheath (arrows and arrowheads, respectively). (**d**) Expression of survivin (Rhodamine) in Ki-67+ cells (FITC) of the HF epithelium (arrows). (**e**, **f**) Double immunostaining of survivin (Rhodamine, arrowheads) and pMel-17 (FITC, arrows) revealed that the melanocytes do not express survivin in anagen and early-catagen HFs. (**g, h**) Survivin expression disappears from the outer root sheath and progressively decreases in the hair matrix during catagen development (arrows). (**i**) A few survivin+ cells are detected in the regressing epithelial strand during late catagen (arrows). (**j**) Lack of survivin expression on TUNEL+ cells (survivin shown in red, arrows; TUNEL shown in green, arrowhead).

dominant-negative TCF isoform was able to block *survivin* expression (Kim *et al.*, 2003). The Wnt/β-catenin pathway plays key roles in HF growth and differentiation (DasGupta and Fuchs, 1999; Van Mater *et al.*, 2003), and we wished to clarify whether interactions between survivin and β-catenin are functional in the HF. By double immunostaining with antibodies against survivin and β-catenin (1:50; R&D Systems Inc., Minneapolis, MN), we observed that some cells expressing survivin in the hair matrix are also β-catenin-positive (Figure 2a–c). These data suggested that the expression of survivin in hair matrix keratinocytes may be under the control of and/or coordinated with Wnt/β-catenin signaling.

To provide an experimental evidence for crosstalk between survivin and Wnt/β-catenin signaling in the HF, ICG-001 (Institute for Chemical Genomics, Seattle, WA), a small molecule that inhibits *survivin* transcription via blocking TCF/β-catenin binding to its co-activator CREB-binding protein (Emami *et al.*, 2004; Ma *et al.*, 2005), was tested in HF organ culture model,

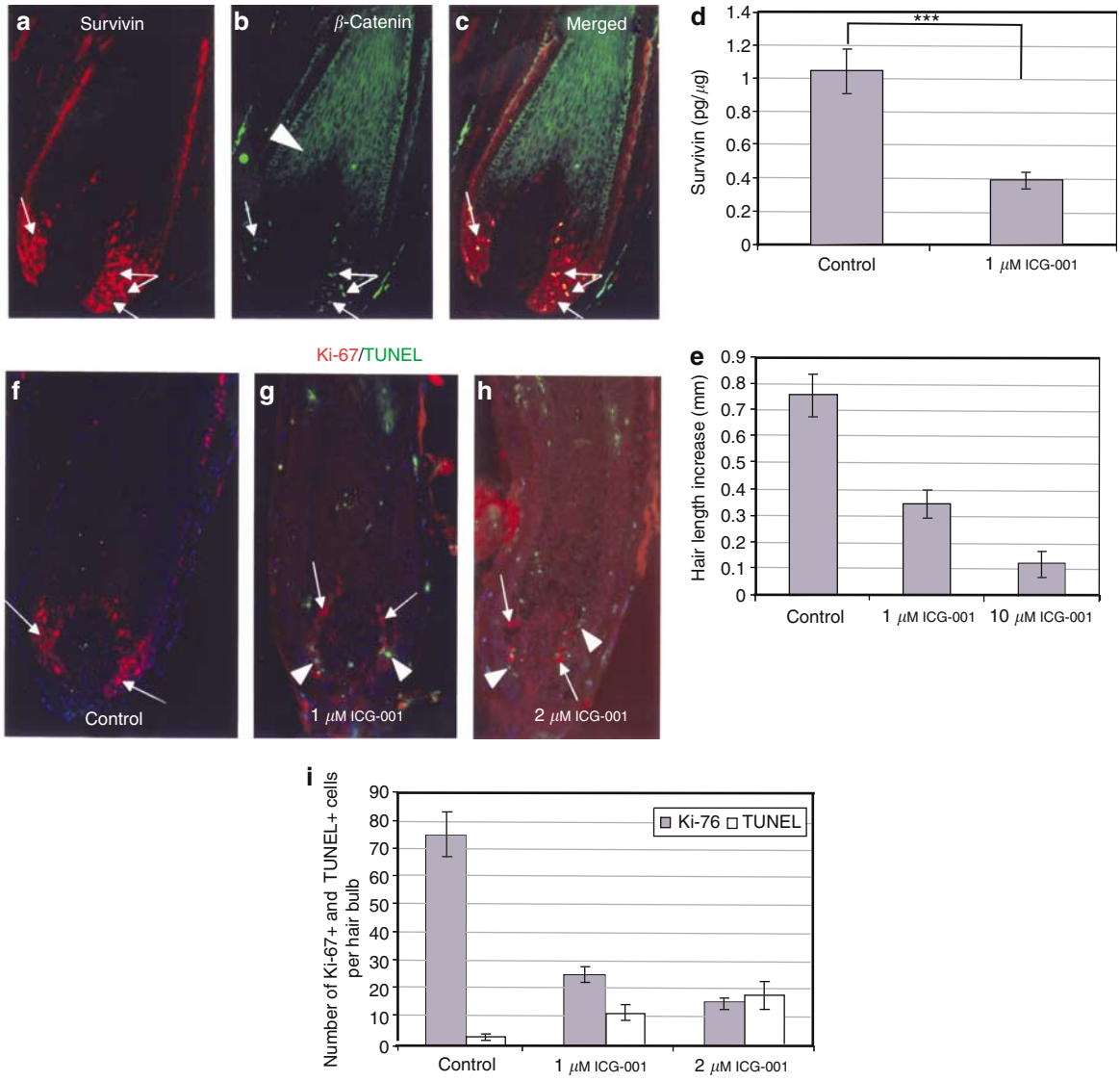

Figure 2. *β*-Catenin–survivin interactions in human HF. (**a**) In anagen HF, survivin immunofluorescence (Rhodamine) was detected in the hair matrix (arrows); (**b**) cell membrane staining of *β*-catenin (FITC) was seen in the upper part of the hair matrix (arrowhead), (**c**) and nuclear localization of *β*-catenin in the hair matrix cells (arrow); colocalization of survivin and *β*-catenin was detected in hair matrix cells (yellow immunofluorescence, arrows). (**d**) By ELISA, survivin protein levels are significantly reduced in the HFs treated with ICG-001, compared to the control. (**e**) Hair shaft elongation in ICG-001-treated HFs compared to vehicle control after 4 days in culture. (**f–h**) Ki-67/TUNEL double staining of HFs after 4 days in culture: (**f**) numerous Ki-67+ cells are seen in the hair matrix of the control (arrows);(**g** and **h**) treatment with ICG-001 (1–2 μM) decreases the number of Ki-67+ cells (TRITC, arrows), as well as increased number of TUNEL+ cells in the hair bulb and the outer root sheath (arrowheads); (**i**) total number of Ki-67- and TUNEL-positive cells in the hair matrix of ICG-001- and vehicle-treated follicles after 4 days in culture.

as described previously (Philpott *et al.*, 1994). ICG-001 was added to the microdissected anagen HFs from occipital human scalp skin derived from six different female donors (aged 45–55 years). To determine survivin protein content in the HFs treated with ICG-001, a commercially available ELISA kit was used (Assay Design Inc., Ann Arbor, MI). By ELISA, a significant decrease in the survivin protein levels were observed in the HFs after 1 μM ICG-001 treatment for 4 days, compared to vehicle control ($P<0.001$; Figure 2d). Concurrently, ICG-001 significantly reduced hair fiber elongation rate in a dose-dependent manner ($P<0.0001$; Figure 2e). However, ICG-001 did not cause premature catagen development in cultured HFs. Hair growth inhibition caused by ICG-001 treatment was accompanied by decrease in the number of proliferating (Ki-67+) cells and increase in the number of apoptotic (TUNEL+) cells in the hair bulb and the outer root sheath, compared to the controls (Figure 2f–i). These data are consistent with previous report on similar effects of ICG-001 (inhibition of proliferation and stimulation of apoptosis) associated with downregulation of survivin and increase of caspase-3 activity in several cell lines (Ma *et al.*, 2005). However, very limited number of reports is available on the interactions between

survivin and known regulators of apoptosis in the HF. Therefore, it would be very interesting to further explore and/or identify survivin upstream and downstream components in the control of proliferation and apoptosis in the HF.

In summary, we provide the first evidence that (1) survivin is expressed in the proliferating keratinocytes of the hair matrix and outer root sheath of human anagen HF and its expression is decreased with the progression of catagen phase; (2) expression of survivin in anagen HF may be controlled by Wnt/β-catenin signaling. The dual functions of survivin may be involved in the control of the delicate proliferation–apoptosis balance controlling HF cyclic behavior.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Loss of Heterozygosity Analysis on Chromosome 12*q* in Disseminated Superficial Actinic Porokeratosis

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TO THE EDITOR

Genome-wide scanning and linkage analysis were performed in three Chinese families with disseminated superficial actinic porokeratosis (DSAP) and the gene was localized to an 8.0-cM interval defined by D12S330 and D12S354 on chromosome 12. Meanwhile, both missense mutations, p.Ser63Asn in slingshot 1 (*SSH1*) (Zhang *et al.*, 2004) and a variation (dbSNP3759383: G>A) in the promo-

Abbreviation: DSAP, disseminated superficial actinic porokeratosis; LOH, loss of heterozygosity; SSH1, slingshot 1