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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REFERENCES

Altieri DC (2003) Survivin in apoptosis control and cell cycle regulation in cancer. *Prog Cell Cycle Res* 5:447–52

Ambrosini G, Adida C, Sirugo G, Altieri DC (1998) Induction of apoptosis and inhibition of cell proliferation by survivin gene targeting. *J Biol Chem* 273:11177–82

Banks DP, Plescia J, Altieri DC, Chen J, Rosenberg SH, Zhang H *et al.* (2000) Survivin does not inhibit caspase-3 activity. *Blood* 96:4002–3

Botchkarev VA, Kishimoto J (2003) Molecular control of epithelial–mesenchymal interactions during hair follicle cycling. *J Invest Dermatol Symp Proc* 8:46–55

Botchkareva NV, Ahluwalia G, Shander D (2006) Apoptosis in the hair follicle. *J Invest Dermatol* 126:258–64

Botchkareva NV, Khlgatian M, Longley BJ, Botchkarev VA, Gilchrest BA (2003) SCF/c-kit signaling is required for cyclic regeneration of the hair pigmentation unit. *FASEB J* 15:645–58

Cotsarelis G, Millar SE (2001) Towards a molecular understanding of hair loss and its treatment. *Trend Mol Med* 7:293–301

DasGupta R, Fuchs E (1999) Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. *Development* 126:4557–68

Emami KH, Nguyen C, Ma H, Kim DH, Jeong KW, Eguchi M *et al.* (2004) A small molecule inhibitor of beta-catenin/CREB-binding protein transcription. *Proc Natl Acad Sci USA* 101:12682–7

Grossman D, Kim PJ, Blanc-Brude OP, Brash DE, Tognin S, Marchisio PC *et al.* (2001) Transgenic expression of survivin in keratinocytes counteracts UVB-induced apoptosis and cooperates with loss of p53. *J Clin Invest* 108:991–9

Kappler M, Bache M, Bartel F, Kotzsch M, Panian M, Wurl P *et al.* (2004) Knockdown of survivin expression by small interfering RNA reduces the clonogenic survival of human sarcoma cell lines independently of p53. *Cancer Gene Ther* 11:186–93

Kim PJ, Plescia J, Clevers H, Fearon ER, Altieri DC (2003) Survivin and molecular pathogenesis of colorectal cancer. *Lancet* 362:205–9

Li F, Ackermann EJ, Bennett CF, Rothermel AL, Plescia J, Tognin S *et al.* (1999) Pleiotropic cell-division defects and apoptosis induced by interference with survivin function. *Nat Cell Biol* 1:461–6

Ma H, Nguyen C, Lee KS, Kahn M (2005) Differential roles for the coactivators CBP and p300 on TCF/beta-catenin-mediated survivin gene expression. *Oncogene* 24:3619–31

Paus R, Foitzik K (2004) In search of the "hair cycle clock": a guided tour. *Differentiation* 72:489–511

Philpott MP, Sanders D, Westgate GE, Kealey T (1994) Human hair follicle growth *in vitro*: a model for the study of hair follicle biology. *J Dermatol Sci* 7:S55–S72

Rodel F, Hoffmann J, Distel L, Herrmann M, Noisternig T, Papadopoulos T *et al.* (2005) Survivin as a radioresistance factor, and prognostic and therapeutic target for radiotherapy in rectal cancer. *Cancer Res* 65:4881–7

Stenn KS, Paus R (2001) Controls of hair follicle cycling. *Physiol Rev* 81:449–94

Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T *et al.* (1998) IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. *Cancer Res* 58:5315–20

Temme A, Rieger M, Reber F, Lindemann D, Weigle B, Diestelkoetter-Bachert P *et al.* (2003) Localization, dynamics, and function of survivin revealed by expression of functional survivinDsRed fusion proteins in the living cell. *Mol Biol Cell* 14:78–92

Van Mater D, Kolligs FT, Dlugosz AA, Fearon ER (2003) Transient activation of beta-catenin signaling in cutaneous keratinocytes is sufficient to trigger the active growth phase of the hair cycle in mice. *Genes Dev* 17:1219–24

Wheatley SP, McNeish IA (2005) Survivin: a protein with dual roles in mitosis and apoptosis. *Int Rev Cytol* 247:35–88

Xia C, Xu Z, Yuan X, Uematsu K, You L, Li K *et al.* (2002) Induction of apoptosis in mesothelioma cells by antisurvivin oligonucleotides. *Mol Cancer Ther* 1:687–94

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

Journal of Investigative Dermatology (2007) **127,** 482–485. doi:10.1038/sj.jid.5700525; published online 31 August 2006

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

ter region of *ARPC3* (Zhang *et al.*, 2005), were identified in Family 2. A frameshift mutation, p.Ser19CysfsX24 in an alternative variant (isoform f) of *SSH1*, was identified in Family 3 (Zhang *et al.*, 2004). Do lesions develop based on haploinsufficiency of *SSH1* or does *SSH1* function as a tumor suppressor gene requiring inactivation of the second, otherwise normal allele in a given cell? Sequence analyses of genomic DNA derived from lesional tissues were performed to determine if a second, acquired *SSH1* mutation was present, or whether there was drop out of the wild-type sequence.

Deletion of the wild-type sequence was observed at *SSH1* in two of the six lesional tissues, which were IV:3 and V:8 in Family 2 (Table 1). In addition, loss of heterozygosity (LOH) was found at *ARPC3* in these two lesional tissues. However, no allelic loss was found in this region in other matched samples. Figure 1 shows the LOH sequences, for example, in c.188G>A (p.Ser63Asn) at *SSH1* and

Table 1. Clinical and molecular findings, including results of LOH analysis, in six patients with DSAP

No.	ID number in the family we described	Sex	Age (years)	Location	Known *SSH1* mutations[1]	LOH on chromosome 12*q*
1	IV:3 in Family 2	F	53	Left forearm	p.Ser63Asn	+
2	IV:4 in Family 2	M	50	Left forearm	p.Ser63Asn	–
3	IV:8 in Family 2	M	49	Left forearm	p.Ser63Asn	–
4	IV:10 in Family 2	M	40	Chest	p.Ser63Asn	–
5	V:8 in Family 2	F	25	Left hand	p.Ser63Asn	+
6	III:2 in Family 3	F	23	Left ankle	p.Ser19CysfsX24 in an alternative variant (isoform f)	–

DSAP, disseminated superficial actinic porokeratosis; F, female; LOH, loss of heterozygosity; M, male; +, loss of heterozygosity; –, no loss of heterozygosity.
[1]The amino-acid change within the *SSH1* gene (Zhang *et al.*, 2004).

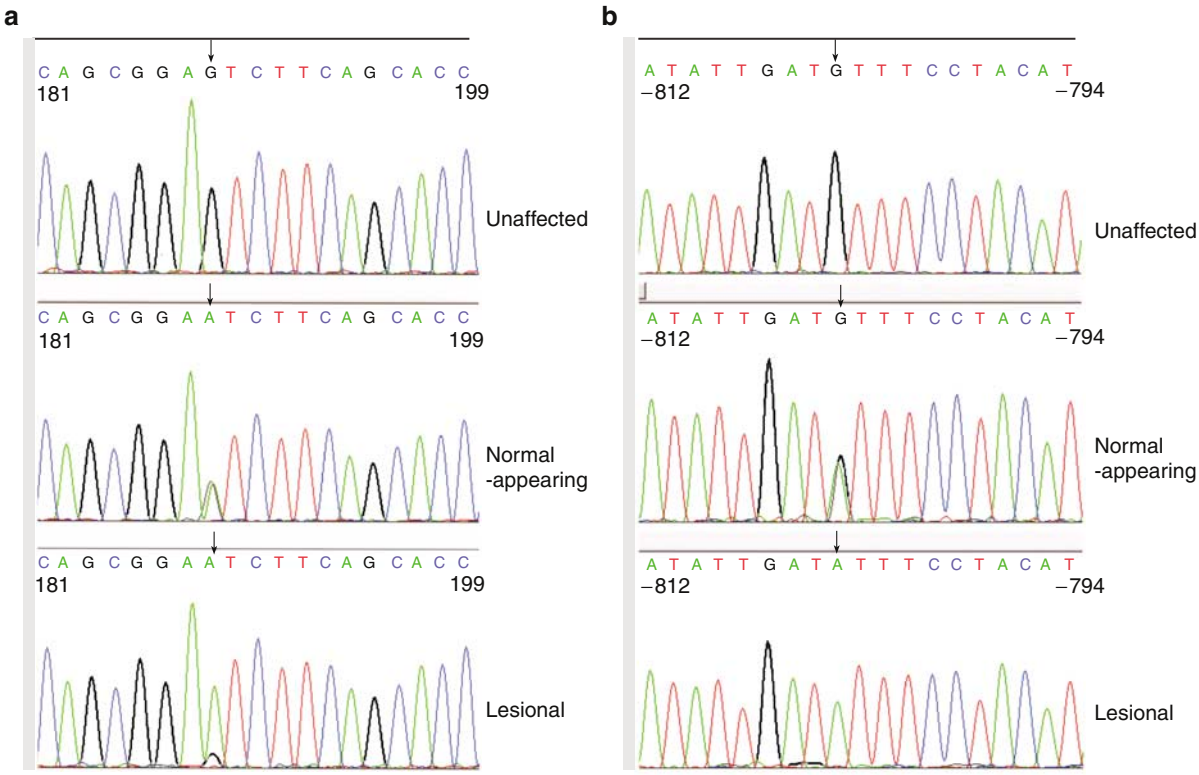

Figure 1. Chromatograms of the normal and mutated sequences and LOH in two affected members in Family 2. (**a**) NM_018984.2: c.188G>A (p.Ser63Asn) at *SSH1*. (**b**) NM_005719.2: c. –804G>A (dbSNP3759383: G>A) at *ARPC3* (The DNA numbering is based on cDNA sequence with a "c." symbol before the number.).

c.−804G>A (dbSNP3759383: G>A) at *ARPC3*. Both patients were from the same family, with close linked variation in *ARPC3* as reported previously (Zhang *et al.*, 2005). No difference was found between DNA samples from the normal-appearing skin and peripheral blood in the two patients with LOH at *SSH1* and *ARPC3* by sequence analysis. The loss of an allele from lesional tissue in Figure 1a was not very pure, because admixture with normal tissue may constitute a major problem in LOH studies (Tomlinson *et al.*, 2002). The genomic distance between *SSH1* and *ARPC3* is approximately 1.58 Mb, which is located between D12S1605 and D12S354 (Table 2).

Therefore, 10 highly polymorphic microsatellite markers on spanning chromosome 12*q*23–24.3 were selected from Genethon Linkage Maps to define the regions of LOH. The results of PCR-based microsatellite analysis confirmed the LOH we found in two of the six lesional tissues, and the deleted regions were also determined. According to Sequence Map on chromosome 12, the LOH region of IV:3 in Family 2 was 27.07 Mb, located from D12S1588 to D12S1679. The LOH region of V:8 in Family 2 was 27.90 Mb, defined by D12S78 and D12S1628 (Table 2). D12S86 showed homozygosity in all DNA samples from V:8 in Family 2, and did not provide reliable information of LOH to this patient. Moreover, no microsatellite instability was observed in any of the paired DSAP samples on chromosome 12*q*, and no LOH was found in the samples obtained from normal-appearing skin of six patients.

So far, there are few studies on the potential genetic mechanism leading to the development of DSAP lesions. LOH was considered to be a predictor to map tumor suppressor genes in cancer, and a powerful tool to gain a better understanding of various skin diseases (Happle, 1999; Thiagalingam *et al.*, 2002). As a rule, LOH is a genetic mechanism by which a heterozygous somatic cell becomes either homozygous or hemizygous, originating from a deletion, gene conversion, translocation, mitotic recombination, or nondisjunction. The percentage of patients with porokeratosis and associated skin malignancies was estimated at between 6.9 and 11.6% (Maubec *et al.*, 2005). Malignant transformation has been reported to occur in 19% of linear porokeratosis (Sasson and Krain, 1996). LOH or allelic loss was proposed to explain the oncogenic potential of linear porokeratosis (Happle, 1997). Moreover, it was taken as segmental type 2 manifestation of autosome-dominant skin diseases, used to explain the coexistence of linear porokeratosis and DSAP (Freyschmidt-Paul *et al.*, 1999). Because there were no linear porokeratotic lesions superimposed on DSAP and no malignant transformation in the pedigrees we studied, our results could not show a molecular proof for these genetic concepts in DSAP. Neither microsatellite instability nor allelic loss was found in two lesional tissues of disseminated superficial porokeratosis, which was reported previously associated with hereditary non-polyposis colorectal cancer (Takata *et al.*, 2000; Hussein and Wood, 2002). To our knowledge, our study is the first report of LOH in DSAP lesions. The course of disease and regions exposed to light might affect the frequency of LOH, but the haploinsufficiency of *SSH1* in lesions was not essential to develop DSAP. It is speculated that normal-appearing skin of some dermatoses could be genetically altered by UV exposure. In the literature, LOH in histologically normal-appearing skin was demonstrated in two of the 37 lesions of actinic keratosis (Kushida *et al.*, 1999); however, we did not find LOH in adjacent normal-appearing skin of DSAP in this study. The limited number of samples and microsatellite markers may account for this negative result.

Table 2. LOH at microsatellite markers on chromosome12*q* in DSAP

		IV:3 in Family 2			V:8 in Family 2		
Marker	**Location (Mb)**	**B**	**N**	**L**	**B**	**N**	**L**
D12S1588	100.26	HE	HE	HE	HO	HO	HO
D12S78	102.79	HE	HE	**LOH**	HE	HE	HE
D12S1605	107.23	HE	HE	**LOH**	HE	HE	**LOH**
D12S354	113.61	HE	HE	**LOH**	HE	HE	**LOH**
D12S86	117.65	HE	HE	**LOH**	HO	HO	**HO**
D12S1612	123.47	HE	HE	**LOH**	HE	HE	**LOH**
D12S1658	126.14	HE	HE	**LOH**	HE	HE	**LOH**
D12S1679	127.33	HE	HE	HE	HE	HE	**LOH**
D12S367	128.53	HE	HE	HE	HE	HE	**LOH**
D12S1628	130.69	HE	HE	HE	HE	HE	HE

B, blood; DSAP, disseminated superficial actinic porokeratosis; HE, heterozygous; HO, homozygous; L, lesional tissue; N, normal-appearing skin.
Note: Genetic coordinates in million base pairs (Mb) according to Sequence Map on Chromosome 12 are in column ''Location''.
Bold indicates the LOH region could be obvious.

In summary, LOH was observed in only two of the six lesions of DSAP, and our results showed that the development of DSAP lesions may sometimes occur independently from LOH. Whereas, LOH at the candidate region might be a random event caused by environment factors, such as sun exposure.

Six adult patients were selected at random from two DSAP families with known *SSH1* germline mutations, which we reported before (Zhang *et al.*, 2004). These patients were IV:3, 4, 8, 10 and V:8 in Family 2 (S2) and III:2 in Family 3 (S3), respectively. Clinical data of Family 2 were described in detail elsewhere (Zhang *et al.*, 2005). An overview of clinical and molecular data of six patients from two families is presented in Table 1. The lesions with clear borderlines and from mostly sun-exposed areas were selected. With patients' written informed consent, lesional tissue and adjacent normal-appearing skin were dissected with a disposable scalpel from each patient. All procedures were approved by the ethical code of Fudan University in accordance with the Declaration of Helsinki Principles. The paired samples were stored in RNAlater (Qiagen, Germany) at −80°C. After the tissues were ground into powder in liquid nitrogen, genomic DNA was isolated by proteinase K-SDS lysis, followed by phenol–chloroform extraction and ethanol precipitation. DNA isolated from each patient's peripheral blood was used for control. DNA sequence analysis of each lesional tissue was compared with its matched normal-appearing skin and blood. The coding and the promoter regions of *SSH1* and *ARPC3* were sequenced, as described previously (Zhang *et al.*, 2004).

All of these markers were amplified by multiplex PCR, as described previously (Zhang *et al.*, 2004). The PCR products were separated on a 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA), and GeneMapper software (v3.5) was used for gel analysis and genotype assignment. Both PCR and electrophoresis steps were performed twice. LOH was visually scored, and defined as a significant reduction in peak intensity (>84%) or loss of one allele in lesional tissues (Dieffenbach and Dveksler, 2003). Microsatellite instability was defined as a difference in length of amplified microsatellite markers between the blood and lesional tissue or adjacent normal-appearing skin of the same individual (Boland *et al.*, 1998).

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work is supported by National Nature Science Foundation of China (No. 30500439). We thank all the members of the families who have so willingly participated in this study. We are most grateful to the members and associates from Chinese National Human Genome Center at Shanghai for their enthusiastic support and great contribution to this project. Special thanks should be given to Dr Meredith Crosby for helpful discussion.

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REFERENCES

Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW *et al.* (1998) A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 58:5248–57

Dieffenbach CW, Dveksler GS (eds) (2003) *PCR primer: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press: New York, 223–35

Freyschmidt-Paul P, Hoffmann R, Konig A, Happle R (1999) Linear porokeratosis superimposed on disseminated superficial actinic porokeratosis: report of two cases exemplifying the concept of type 2 segmental manifestation of autosomal dominant skin disorders. *J Am Acad Dermatol* 41:644–7

Happle R (1997) Cancer proneness of linear porokeratosis may be explained by allelic loss. *Dermatology* 195:20–5

Happle R (1999) Loss of heterozygosity in human skin. *J Am Acad Dermatol* 41:143–64

Hussein MR, Wood GS (2002) Microsatellite instability and its relevance to cutaneous tumorigenesis. *J Cutan Pathol* 29:257–67

Kushida Y, Miki H, Ohmori M (1999) Loss of heterozygosity in actinic keratosis, squamous cell carcinoma and sun-exposed normal-appearing skin in Japanese: difference between Japanese and Caucasians. *Cancer Lett* 140:169–75

Maubec E, Duvillard P, Margulis A, Bachollet B, Degois G, Avril MF (2005) Common skin cancers in porokeratosis. *Br J Dermatol* 152:1389–91

Sasson M, Krain AD (1996) Porokeratosis and cutaneous malignancy. A review. *Dermatol Surg* 22:339–42

Takata M, Shirasaki F, Nakatani T, Takehara K (2000) Hereditary non-polyposis colorectal cancer associated with disseminated superficial porokeratosis. Microsatellite instability in skin tumours. *Br J Dermatol* 143:851–5

Thiagalingam S, Foy RL, Cheng KH, Lee HJ, Thiagalingam A, Ponte JF (2002) Loss of heterozygosity as a predictor to map tumor suppressor genes in cancer: molecular basis of its occurrence. *Curr Opin Oncol* 14:65–72

Tomlinson IPM, Lambros MBK, Roylance RR (2002) Loss of heterozygosity analysis: practically and conceptually flawed? *Gene Chromosome Cancer* 34:349–53

Zhang ZH, Niu ZM, Yuan WT, Liu WD, Xiang LH, Zhang J *et al.* (2004) Fine mapping and identification of a candidate gene, SSH1, in disseminated superficial actinic porokeratosis. *Hum Mutat* 24:438

Zhang ZH, Huang W, Niu ZM, Liu WD, Xiang LH, Yuan WT *et al.* (2005) Two closely linked variations in actin cytoskeleton pathway in a Chinese pedigree with disseminated superficial actinic porokeratosis. *J Am Acad Dermatol* 52:972–6