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Enhanced StefinA and Sprr2 expression during papilloma formation in HPV8 transgenic mice

Daliborka Lazić^a, Farnoosh Alborzi^a, Gian Paolo Marcuzzi^a, Peter Angel^b, Jochen Hess^{b,c}, Herbert Pfister^a, Baki Akgül^{a,*}

^a Institute of Virology, University of Cologne, Fürst-Pückler-Str. 56, 50935 Cologne, Germany

^b Division of Signal Transduction and Growth Control, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

^c Research Group Experimental Head and Neck Oncology, Department of Otolaryngology, Head and Neck Surgery, University Hospital Heidelberg, Im Neuenheimer Feld 400, 69120 Heidelberg, Germany

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ABSTRACT

Background: The human papillomavirus type 8 (HPV8) is associated with the development of non-melanoma skin cancer. Transgenic mice expressing the complete early gene region of HPV8 (E6/E7/E1/E2/E4 = CER) or E6 separately under the control of the keratin14 promoter spontaneously developed papillomas characterized by varying degrees of epidermal dysplasia. Papilloma growth could be synchronized by a single UVA/B irradiation of the skin, which led to the development of papillomas within three weeks.

Objective: The objective of this study was to identify alterations in cellular gene expression correlated with HPV8 oncogene expression in transgenic mice.

Methods: We applied global gene expression profiling by microarray analysis and confirmed deregulation of cellular genes by qRT-PCR and immunohistochemical analysis.

Results: By comparison of non-lesional HPV8-CER skin with skin of the parental mouse strain FVB/n, two cellular genes, namely StefinA and Sprr2, coding for precursor proteins of the cornified envelope, were predicted to be strongly upregulated in transgenic skin, which could be confirmed in subsequent qRT-PCR experiments. StefinA and Sprr2 mRNA expression was enhanced until day 7 after UV treatment with higher levels in HPV8 positive skin. While the expression of both genes returned to a normal level in the course of epidermis regeneration in wt mice, the expression persisted elevated in hyperplastic transgenic skin. Staining of an UV induced papilloma of FVB/n wt mouse revealed also strong expression of StefinA and Sprr2 indicating that upregulation in later stages of papilloma formation is independent of HPV8.

Conclusion: In non-lesional HPV8-CER transgenic skin StefinA and Sprr2 were found to be indirect/direct transcriptional targets of HPV8.

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1. Introduction

Non-melanoma skin cancer (NMSC), which includes basal cell carcinoma (BCC), Bowen's disease and squamous cell carcinoma (SCC), is the most frequent malignancy in fair-skinned populations. The incidence of NMSC has dramatically increased during the last decade and at present it accounts for 30% of all cancers [1,2]. The established main risk factor for cutaneous SCC is long-term exposure to UV-radiation particularly during childhood [3]. In recent years epidemiological and experimental studies also support a role of human papillomaviruses (HPV) of the genus beta in the development of cutaneous SCCs [4]. The association

between beta-HPV and skin cancer was first identified in patients with the rare inherited disorder epidermodysplasia verruciformis (EV). These individuals have a predisposition to the early development of disseminated, persistent flat warts and macular lesions following infection with beta-HPV genotypes. About half of EV patients develop premalignant skin lesions and SCC by the age of 40, primarily in sun-exposed areas. DNA from these lesions was found to harbour HPV genomes, suggesting a positive correlation between beta-HPV and UV irradiation in the development of EV cancers. HPV-DNA sequences have also been detected in NMSC from immunosuppressed and immunocompetent individuals [5,6]. The detection of a high prevalence of beta-HPV, including the high-risk cutaneous types HPV5 and HPV8, in low- and high-grade, premalignant actinic keratoses and the fact that HPV-DNA loads in actinic keratoses were found to exceed those of NMSC suggests an involvement of these viruses in the early stages of

* Corresponding author. Tel.: +49 221 4783911; fax: +49 221 4783904.

E-mail address: baki.akguel@web.de (B. Akgül).

cutaneous oncogenesis [7,8,9]. The presence of antibodies against HPV8 virus-like-particles also turned out to be associated with the development of large numbers of both actinic keratoses [10] and cutaneous SCC [11].

In order to analyze the carcinogenic potential of HPV8 *in vivo*, different transgenic mouse lines were recently established, in which the HPV8 oncogenes E2 or E6 or all early genes (CER = complete early region = E1/E2/E4/E6/E7) were expressed under the control of the human keratin14 promoter [12–14]. Nearly all HPV8 transgenic mice spontaneously developed papillomas, characterized by varying degrees of epidermal dysplasia. In all transgenic mouse lines UVA/B irradiation led to an increase of early gene expression, which was paralleled by papilloma development in about three weeks. Furthermore, a direct link between elevated viral gene expression and papilloma development could be demonstrated by siRNA-mediated HPV8-E6 knock-down during the early phase of papilloma initiation, which clearly reduced papilloma formation in transgenic mice [15]. In order to identify alterations in the gene regulatory network correlated with the presence of the HPV8 oncogenes in the skin of transgenic mice, we applied global gene expression profiling using RNA samples from non-lesional skin of HPV8-CER mice and the parental mouse strain FVB/n (FVB/n wt). Interestingly, the microarray result predicted altered transcript levels for only few cellular genes. The two genes coding for StefinA (also named Cystatin A) and Sprr2, which mainly participate in the development and maintenance of the epidermal cornified envelope, were by far the most strongly upregulated genes.

2. Material and methods

2.1. UV irradiation of transgenic mice

FVB/n mice used in this study include transgenic hemizygous mouse lineages either expressing the complete early region of HPV8 (E1/E2/E4/E6/E7, referred to as HPV8-CER) or -E6 or -E2 separately under the control of the hK14 promoter [12–14]. Mice at an age of 5 weeks were anesthetized prior to UV irradiation with ketamine hydrochloride and xylazine hydrochloride and shaved on their back with an electric shaver (Wella, Karlsruhe, Germany). UV-irradiation was performed on a 4 cm² mouse skin area with 10 J/cm² UVA and 1 J/cm² UVB using an UV lamp 801 as reported before [13,14]. The remaining skin was protected with an UV radiation-impermeable aluminum foil. For certain time points after UV irradiation in either case two siblings transgenic and two FVB/n wt mice of three different litter were irradiated to generate biological replicates. Skin samples were collected as followed: each irradiated 4 cm² skin area was split into three parts, one was fixed in formalin over night and embedded in paraffin for immunohistochemistry, one was snap frozen for protein analysis and one was stored at –20 °C in RNALater (Qiagen, Hilden, Germany).

2.2. Global gene expression profiling and data analysis

Global gene expression profiling was accomplished on self-printed microarrays employing a set of 35,852 oligomers representing approximately 25,000 genes (MouseOligo Set Version 4.0, Operon, Cologne, Germany). Sample preparation, hybridization and data analysis were performed as described previously [16].

2.3. RNA isolation and reverse transcription

Total RNA was isolated from tissues using the miRNAeasy Kit and DNase digestion was performed on column using RNase-free DNase according to the manufacturer's instructions (Qiagen, Hilden, Germany). 1 µg of total RNA was reverse transcribed

using the Omniscript RT Kit (Qiagen, Hilden, Germany) with 10 µM Random nonamers (TIB MOLBIOL, Berlin, Germany) and 1 µM oligo-dT₂₃-primer (Sigma, Deisenhofen, Germany) as well as 10 units RNase Inhibitor (Fermentas, St. Leon-Rot, Germany).

2.4. Real time PCR

StefinA and Sprr2 mRNA levels were measured by quantitative real time PCR (qRT-PCR) using the Light-Cycler System (Roche, Mannheim, Germany). Total copy numbers of the target gene were normalized to the total copy number of the house-keeping gene hypoxanthine phosphoribosyltransferase 1 (HPRT1). One PCR reaction contained 2 µl of 1:10 dilution cDNA in a total volume of 20 µl, 1.25 units Platinum Taq Polymerase and the provided buffer (Invitrogen, Karlsruhe, Germany), 4 mM MgCl₂, 1.6 µl of a 1:1000 dilution of SYBGreen (Sigma, Deisenhofen, Germany), 5% DMSO, 0.5 µM of forward and backward primer, 500 ng/µl non-acetylated bovine serum albumin (Fermentas, St. Leon-Rot, Germany) and 0.2 mM deoxynucleotide triphosphates each. Amplified PCR fragments of StefinA, Sprr2 and HPRT1 were cloned before into pJET1.2 (Qiagen, Hilden, Deutschland) to generate absolute standards with primers also used for subsequent qRT-PCR analysis. Samples were analyzed in duplicate together with a 10-fold dilution series of standard plasmid. The cycling protocol conditions were 60 s at 95 °C, followed by 40 cycles of 1 s at 95 °C (20 °C/s), 5 s at T_{an} (20 °C/s) and 15 s at 72 °C (20 °C/s). The primers used in this study had the following 5' to 3' sequences,

StefinA-fw: CAAGATGAGTCTTGGAGGTGTT,
StefinA-bw: ATCTTTTCCAGTAGGTCCATT,
Sppr2-fw: CTGCTCCGGAGAACCTGAT,
Sppr2-bw: ACAAGGCTCAGGGCACTTC,
HPRT1-fw: CCTAAGATGAGCGCAAGTTGAA,
HPRT1-bw: CCACAGGACTAGAACACCTGCTAA.

2.5. Immunohistochemical staining

Deparaffinization of 4 µm tissue sections was done by incubation in xylene for 10 min. Afterwards sections were hydrated through a descending alcohol series (100%, 90%, 70%; 5 min each). Tissue sections were then incubated 10 min in 3% hydrogen peroxidase in methanol to quench endogenous peroxidases followed by 30 min incubation with normal horse serum to block non-specific binding. Primary antibodies for StefinA (1:8000, Abcam, Cambridge, UK) and Sprr2 (1:1000, Enzo, Lörrach, Germany) were incubated at 4 °C over night. At the following day sections were washed three times in PBS and treated with secondary biotinylated antibody using the Vectastain Universal ABC Kit (Linaris, Wertheim – Bettingen, Germany) according to the manufacturer's instructions. Following three washes with PBS DAB staining was performed with the liquid DAB Concentrated Substrate Pack (Biogenex, San Ramon, USA) according to the manufacturer's instructions. Finally, sections were counterstained with hematoxylin, dehydrate with an ascending alcohol series and embedded with mounting medium. The specificity of the staining was proved by omitting the primary antibody. E2 staining was performed as described previously in [15].

2.6. Western blotting on protein extracts from PM1 keratinocytes coding for HPV8 early genes

The PM1 cell line was cultivated at 37 °C and 6% CO₂ in DMEM/F12 with 10% FCS and supplements as described in Akgül et al. [17]. The PM1 cells are spontaneously immortalized human cutaneous keratinocytes retaining many features of normal keratinocytes

including keratin phenotype, differentiation capacity and non-tumorigenicity in athymic mice [18]. Retroviral infection of PM1 cells was performed with supernatants from PT67 cells stably expressing pLXSN based HPV8-E2, -E6, -E7, and -CER [19]. The HPV8 E1 ORF (nt. 933–2787) was amplified by PCR using the sense primer 8-E1-fw: GGAATTCGCGCGGTAAGTCAAAC and anti-sense primer 8-E1-bw: CGGGATCCCGTGCCTCAAGTGTGTTCTG, cleaved with EcoRI and BamHI and subcloned into pLXSN, giving rise to pLXSN-8-E1. Total protein extract were isolated by homogenizing and incubating tissue in lysis buffer (with 150 mM NaCl, 50 mM Tris pH 7.4, 1% NP-40, and 1% SDS) containing 1 mM PMSF (Boehringer, Mannheim, Germany) and protease inhibitors over night at 4 °C. Protein lysates were separated by SDS gel electrophoresis and transferred to a nitrocellulose membrane (GE Healthcare, Munich, Germany). The membranes were blocked with 5% dry milk in TBS-T (10 mM Tris/HCL, pH 8.0; 150 mM NaCl; 0.05% Tween 20) for Sprr2 and 5% BSA in case of GAPDH detection. Following primary antibodies were used: Sprr2 (Enzo, Lörrach, Germany, dilution 1:2000) and GAPDH (Abcam, Cambridge, UK, dilution 1:1000). The membranes were then washed three times 5 min in TBS-T and incubated with peroxidase-coupled secondary antibody for 1 h. Detection of the reactive proteins was performed with ECL+ Kit (Amersham, Bioscience, Little Chalfont, UK). The nitrocellulose blot was finally exposed to autoradiographic film. To quantify the Western blot bands, we applied Quantity One software based analysis (Biorad, München, Germany). The SPPr2 specific signal was corrected for the GAPDH loading control. The value for the pLXSN condition was set as 1 and other conditions were recalculated correspondingly to allow ratio comparisons.

3. Results

3.1. StefinA and Sprr2 expression is enhanced in HPV8-CER transgenic mouse skin

Total RNA was isolated from tissue samples of FVB/n wt skin as well as non-lesional HPV8-CER skin and global gene expression profiling was done by hybridization on a 70-mer oligonucleotide-microarray platform (MouseGenom V.4.0, Operon). After data processing and normalization only 15 cellular genes were found to be deregulated. However, only the predicted strong upregulation of StefinA and Sprr2 could be confirmed by qRT-PCR. Generally, we observed a 8-fold and 2.4-fold overexpression of StefinA and Sprr2 ($p < 0.01$, student *t*-test) in untreated HPV8-CER compared to FVB/n wt skin (Fig. 1). To address the question which HPV8 oncogene might be involved in deregulation of StefinA and SPPr2, we further measured the mRNA levels in untreated HPV8-E2 and -E6 mouse

Fig. 2. Sprr2 protein levels are reduced in human cutaneous keratinocytes expressing HPV8-E6. Total cell extracts from PM1 cells expressing HPV8-E1, -E2, -E6, -E7 or -CER or the empty retroviral vector pLXSN were analyzed for Sprr2. Beneath the western blot, the relative intensity of the Sprr2 specific bands is indicated adjusted for sample loading. The intensity for pLXSN was set as 1.

skin. Expression levels of StefinA were unchanged in HPV8-E2 and slightly upregulated (2.4-fold, $p < 0.01$, student *t*-test) in HPV8-E6 transgenic skin indicating that E6 alone is not able to enhance StefinA expression up to the level detected in HPV8-CER mice and that an interplay of additional HPV8 oncogenes is required. Sprr2 expression levels were unchanged in HPV8-E2 mice and interestingly strongly reduced in HPV8-E6 animals (Fig. 1).

3.2. Reduced levels of Sprr2 in human cutaneous keratinocytes expressing HPV8-E6

To examine the E6 mediated downregulation measured in the mouse skin, Sprr2 protein levels were also analyzed in total cell extracts from the human cutaneous keratinocytes PM1 expressing the CER or single oncogenes of HPV8 (Fig. 2). In line with the qRT-PCR data on transgenic mouse skin, we also detected a strong downregulation of Sprr2 protein levels in PM1-8E6 cells. An about 2-fold and 2.4-fold increase of Sprr2 protein level was found in PM1-8E2 and -E7 cells compared to control cells (PM1-pLXSN). When all oncogenes are expressed simultaneously within the CER, a 2.5-fold increase of Sprr2 could be detected.

3.3. StefinA and Sprr2 mRNA levels are increased after UVA/B irradiation

In order to analyze the expression kinetics of StefinA and Sprr2 during the early steps of papillomatosis in HPV8-CER mice, animals were irradiated once with a UVA/B dose known to stimulate synchronized papilloma formation. Until day 7 StefinA and Sprr2 mRNA levels were continuously higher in transgenic mice compared to FVB/n animals (Fig. 3). Beginning at around day 13 after UV treatment the expression of StefinA and Sprr2 started to

Fig. 1. StefinA and Sprr2 mRNA levels are enhanced in skin biopsies of non-lesional HPV8-CER mice. StefinA and Sprr2 expression was measured via qRT-PCR and normalized to the expression level of HPRT1 in untreated FVB/n, HPV8-CER, -E6 and -E2 mice. Expression ratios were related to the median of untreated FVB/n wt skin. The results are an average of six independent experiments with standard errors of mean and fold changes are displayed above the bars.

Fig. 3. StefinA and Sprr2 expression is enhanced in HPV8-CER transgenic after UV irradiation. FVB/n wt and HPV8-CER mice were treated with UV at an age of 5 weeks and skin biopsies were collected at indicated time points. StefinA and Sprr2 mRNA expression was quantified in qRT-PCR and normalized to the expression level of HPRT1. Expression ratios were related to the median of untreated FVB/n wt skin. The results are an average of six independent experiments with standard errors of mean and fold changes for early time points (0–7 days) are displayed above the bars. Statistical analysis was generated with student *t*-test and *p < 0.05 and **p < 0.01.

decrease in wt mice until they reached the base line level of the untreated skin at around 24 days. In contrast, in transgenic mice strong StefinA and Sprr2 mRNA expression persisted over the whole observation period of 24 days.

3.4. Strong StefinA and Sprr2 protein expression in HPV8-CER skin after UV treatment

To study whether the increase of StefinA and Sprr2 mRNA expression in the skin of HPV8-CER mice is mirrored at the protein level and to characterize the expression pattern of both proteins, immunohistological staining experiments were performed on sections of UVA/B irradiated in comparison to untreated mouse skin. In case of StefinA, untreated skin revealed an endogenous expression in hair follicles and enhanced expression in the interfollicular epidermis (Fig. 4A, a and b). Five and 10 days post treatment, StefinA protein was detected in all layers of the epidermis (Fig. 4A, c–f). Starting at around day 10 post treatment, the skin of wt mice became less hyperproliferative and changed morphologically to a normal two layer epidermis within 24 days. Simultaneously StefinA expression decreased to the base line level of untreated skin (Fig. 4A, g). In contrast, the skin of transgenic mice still stayed hyperproliferative and led to papilloma formation, which was accompanied by a strong StefinA expression (Fig. 4A, h).

Staining for Sprr2 revealed moderately increased protein level in untreated HPV8-CER transgenic skin (Fig. 4B, a and b). Five days post treatment the whole epidermis displayed faint Sprr2 expression increasing in differentiating epidermal layers (Fig. 4B, c and d). 10 days after UV irradiation the basal and

suprabasal epidermal layers were negative for Sprr2 staining and expression was restricted to the upper differentiated layers of the epidermis (Fig. 4B, e and f). As described for StefinA, expression of Sprr2 decreased within the investigation period in wt mice, while papilloma formation in HPV8-CER transgenic mice exhibit strong Sprr2 expression (Fig. 4B, g and h). In comparison to the StefinA staining pattern, which was found in all epidermal layers during the whole investigation period, Sprr2 expression persisted only in the differentiating layers.

3.5. StefinA and Sprr2 protein expression is independent of the presence of HPV8 oncogenes in UV induced papilloma

To test, if the overexpression of StefinA and Sprr2 detected at late time points (day 13–24 after UV irradiation) is still linked to the presence of HPV8, an UV induced papilloma of a FVB/n wt animal was also stained for StefinA and Sprr2. As shown in Fig. 5a staining of HPV8-CER papilloma displayed conspicuous E2 expression. In contrast, an UV induced FVB/n wt papilloma fixed on day 20 after UV showed no E2 staining (Fig. 5b). As shown in Fig. 5c and d strong expression of both proteins could also be detected in the HPV8 negative papilloma suggesting that overexpression in fully developed papillomas is independent of the presence of HPV8.

4. Discussion

In this report we confirmed an upregulation of StefinA and Sprr2 expression in non-lesional skin of HPV8-CER transgenic

A FVB/n wt

untreated

5 days after UV

10 days after UV

24 days after UV

HPV8-CER

B FVB/n wt

untreated

5 days after UV

10 days after UV

24 days after UV

HPV8-CER

Fig. 4. StefinA (A) and Sprr2 (B) expression pattern in FVB/n wt and HPV8-CER skin sections. For each point in time skin samples were taken, formalin fixed and paraffin embedded. 4 μ m sections were stained for StefinA and Sprr2 and counterstained with hematoxylin. Magnification: 200 $\times$.

mice. Interestingly, we observed a strong downregulation of Sprr2 in HPV8-E6 transgenic skin although it was upregulated in HPV8-CER skin. Using cell extracts from PM1 cells expressing HPV8-E6, -E7 or -CER, respectively, an inhibitory effect of HPV8-E6 on Sprr2 expression could also be confirmed in human cutaneous keratinocytes. The observed downregulation of Sprr2 by HPV-E6 is in line with previous observations, demonstrating an inhibitory effect of HPV16-E6 on Sprr genes in human keratinocytes [20].

Early after UV irradiation (time points 0–10 days) the expression of StefinA and Sprr2 was continuously higher in the transgenic mice compared to FVB/n wt animals indicating that the UV induced increase of HPV8 early proteins is directly/indirectly triggering StefinA and Sprr2 expression. Beginning around 10 days after irradiation the wt skin started to heal, which was accompanied by decreasing levels of StefinA and Sprr2. In the transgenics higher levels of StefinA and Sprr2 were still present during the early

phases of wound healing. By staining the UV induced papilloma in FVB/n wt and HPV8-CER mice we could show that in papilloma (time points 13–24 days) strong expression of StefinA and Sprr2 is associated with hyperproliferation, indicating that expression is independent of HPV8.

In the human skin, StefinA expression is known to be upregulated in psoriatic lesions, characterized by epidermal hyperproliferation, abnormal keratinocyte differentiation and premature desquamation of the stratum corneum [21]. Recently, Takahashi et al. [22] could also demonstrate an antiapoptotic effect of StefinA in cultured normal human keratinocytes. StefinA expression protects normal human keratinocytes from UVB-induced apoptosis by inhibiting caspases-3. The epidermis of established transgenic mice expressing higher levels of StefinA was more resistant to UVB-induced apoptosis.

Fig. 5. StefinA and Sprr2 protein expression is independent of the presence of HPV8 oncogenes in UV induced papilloma. Staining of a HPV8-CER induced papilloma revealed strong E2 protein expression (a) while an UV induced FVB/n wt papilloma, fixed 20 days after UV, was negative for E2 (b). UV-induced FVB/n wt papilloma was stained for StefinA (c) and Sprr2 (d) and showed strong expression of both proteins. Magnification: 200 $\times$.

The Sprr proteins are differentially regulated in various types of epithelia and represent cornified envelop precursor proteins. Sprr2 is known to be expressed in the spinous and granular layers of the normal interfollicular epidermis [23] and is also associated with hyperproliferative skin diseases [23,24,25]. Abnormalities were identified in the CE isolated from genital epithelium infected with HPV11 (low-risk type) and HPV59 (high-risk type). These abnormalities included reduced thickness and increased fragility compared to the cornified envelope in healthy skin. In line with our observations, increased expression of Sprr2 could also be detected in HPV11 and 59 positive genital epithelia [26].

The StefinA and Sprr2 genes are both located within the epidermal differentiation complex (EDC) on mouse chromosome 3 [27]. The EDC plays a role in the generation and maintenance of the epidermis [28] and deregulation of EDC genes is known to be associated with hyperproliferative skin diseases [29]. As shown for HPV11 and 59, HPV8 induced expression of Sprr2 in non-lesional transgenic skin and during the early steps in papillomatosis may have contributed to the cornified envelope fragility, which was recently characterized by O'Shaughnessy et al. [30] for non-lesional HPV8-CER transgenic mice. The weakening of the epidermal barrier function is known to be accompanied by increased DNA synthesis in the basal cells resulting in epidermal hyperplasia [31]. This suggests that Sprr2 may contribute to keratinocyte proliferation in the CER mice. Although HPV8-E6 mice are phenocopies of the HPV8-CER transgenics in terms of papilloma development, the E6 only mice showed reduced levels of Sprr2, suggesting that Sprr2 induction does not appear to be necessary for HPV8-induced papillomatosis. In contrast, StefinA was induced in CER and E6 mice so that it is tempting to speculate that its known anti-apoptotic activities are generally relevant to HPV8 mediated oncogenesis.

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References

- [1] Nindl I, Gottschling M, Stockfleth E. Human papillomaviruses and non-melanoma skin cancer: basic virology and clinical manifestations. *Dis Markers* 2006;23(4):247–59.
- [2] Madan V, Lear JT, Szeimies RM. Non-melanoma skin cancer. *Lancet* 2010;375(9715):673–85.
- [3] Staples MP, Elwood M, Burton RC, Williams JL, Marks R, Giles GG. Non-melanoma skin cancer in Australia: the 2002 national survey and trends since 1985. *Med J Aust* 2006;184:6–10.
- [4] Akgül B, Cooke JC, Storey A. HPV-associated skin disease. *J Pathol* 2006;208(2):165–75.
- [5] Bouwes Bavinck JN, Feltkamp M, Struijk L, ter Schegget J. Human papillomavirus infection and skin cancer risk in organ transplant recipients. *J Invest Dermatol Symp Proc* 2001;6(3):207–11.
- [6] Pfister H. Human papillomavirus and skin cancer. *J Natl Cancer Inst Monogr* 2003;31:52–6.
- [7] Weissenborn SJ, Nindl I, Purdie K, Harwood C, Proby C, Breuer J, et al. Human papillomavirus-DNA loads in actinic keratoses exceed those in non-melanoma skin cancers. *J Invest Dermatol* 2005;125(1):93–7.
- [8] Struijk L, Hall L, van der Meijden E, Wanningsen P, Bavinck JN, Neale R, et al. Markers of cutaneous human papillomavirus infection in individuals with tumor-free skin, actinic keratoses, and squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev* 2006;15(3):529–35.
- [9] Plasmeyer EI, Neale RE, de Koning MN, Quint WG, McBride P, Feltkamp MC, et al. Persistence of betapapillomavirus infections as a risk factor for actinic keratoses, precursor to cutaneous squamous cell carcinoma. *Cancer Res* 2009;69(23):8926–31.
- [10] Bouwes Bavinck JN, Stark S, Petridis AK, Marugg ME, Ter Schegget J, Westendorp RG, et al. The presence of antibodies against virus-like particles of epidermodysplasia verruciformis-associated human papillomavirus type 8 in patients with actinic keratoses. *Br J Dermatol* 2000;142(1):103–9.

- [11] Masini C, Fuchs PG, Gabrielli F, Stark S, Sera F, Ploner M, et al. Evidence for the association of human papillomavirus infection and cutaneous squamous cell carcinoma in immunocompetent individuals. *Arch Dermatol* 2003;139(7):890–4.
- [12] Schaper ID, Marcuzzi GP, Weissenborn SJ, Kasper HU, Dries V, Smyth N, et al. Development of skin tumors in mice transgenic for early genes of human papillomavirus type 8. *Cancer Res* 2005;65(4):1394–400.
- [13] Marcuzzi GP, Hufbauer M, Kasper HU, Weissenborn SJ, Smola S, Pfister H. Spontaneous tumor development in human papillomavirus type 8 E6 transgenic mice and rapid induction by UV-light exposure and wounding. *J Gen Virol* 2009;90(Pt 12):2855–64.
- [14] Pfeifferle R, Marcuzzi GP, Akgül B, Kasper HU, Schulze F, Haase I, et al. The human papillomavirus type 8 E2 protein induces skin tumors in transgenic mice. *J Invest Dermatol* 2008;128(9):2310–5.
- [15] Hufbauer M, Lazić D, Akgül B, Brandsma JL, Pfister H, Weissenborn SJ. Enhanced human papillomavirus type 8 oncogene expression levels are crucial for skin tumorigenesis in transgenic mice. *Virology* 2010;403(2):128–36.
- [16] Durchdewald M, Guinea-Viniegra J, Haag D, Riehl A, Lichter P, Hahn M, et al. Podoplanin is a novel fos target gene in skin carcinogenesis. *Cancer Res* 2008;68(17):6877–83.
- [17] Akgül B, Karle P, Adam M, Fuchs PG, Pfister HJ. Dual role of tumor suppressor p53 in regulation of DNA replication and oncogene E6-promoter activity of epidermodysplasia verruciformis-associated human papillomavirus type 8. *Virology* 2003;308(2):279–90.
- [18] Proby CM, Purdie KJ, Sexton CJ, Purkis P, Navsaria HA, Stables JN, et al. Spontaneous keratinocyte cell lines representing early and advanced stages of malignant transformation of the epidermis. *Exp Dermatol* 2000;9(2):104–17.
- [19] Akgül B, Ghali L, Davies D, Pfister H, Leigh IM, Storey A. HPV8 early genes modulate differentiation and cell cycle of primary human adult keratinocytes. *Exp Dermatol* 2007;16(7):590–9.
- [20] Duffy CL, Phillips SL, Klingelutz AJ. Microarray analysis identifies differentiation-associated genes regulated by human papillomavirus type 16 E6. *Virology* 2003;314(1):196–205.
- [21] Vasilopoulos Y, Walters K, Cork MJ, Duff GW, Sagoo GS, Tazi-Ahnini R. Association analysis of the skin barrier gene cystatin A at the PSORS5 locus in psoriatic patients: evidence for interaction between PSORS1 and PSORS5. *Eur J Hum Genet* 2008;16(8):1002–9.
- [22] Takahashi H, Komatsu N, Ibe M, Ishida-Yamamoto A, Hashimoto Y, Iizuka H, et al. suppresses ultraviolet B-induced apoptosis of keratinocytes. *J Dermatol Sci* 2007;46(3):179–87.
- [23] De Heller-Milev M, Huber M, Panizzon R, Hohl D. Expression of small proline rich proteins in neoplastic and inflammatory skin diseases. *Br J Dermatol* 2000;143(4):733–40.
- [24] Kainu K, Kivinen K, Zucchelli M, Suomela S, Kere J, Inerot A, et al. Association of psoriasis to PGLYRP and SPRR genes at PSORS4 locus on 1q shows heterogeneity between Finnish, Swedish and Irish families. *Exp Dermatol* 2009;18(2):109–15.
- [25] Iizuka H, Takahashi H, Honma M, Ishida-Yamamoto A. Unique keratinization process in psoriasis: late differentiation markers are abolished because of the premature cell death. *J Dermatol* 2004;31(4):271–6.
- [26] Lehr E, Hohl D, Huber M, Brown D. Infection with human papillomavirus alters expression of the small proline rich proteins 2 and 3. *J Med Virol* 2004;72(3):478–83.
- [27] Patel S, Kartasova T, Segre JA. Mouse Sprr locus: a tandem array of coordinately regulated genes. *Mamm Genome* 2003;14(2):140–8.
- [28] Roberson, Bowcock. Psoriasis genetics: breaking the barrier. *Trends Genet* 2010;26(9):415–23.
- [29] Pasić A, Lipožencić J, Ceović R, Kostović K. The genetics of psoriasis—selected novelties in 2008. *Acta Dermatovenol* 2009;17(3):176–81.
- [30] O'Shaughnessy RF, Akgül B, Storey A, Pfister H, Harwood CA, Byrne C. Cutaneous human papillomaviruses down-regulate AKT1, whereas AKT2 up-regulation and activation associates with tumors. *Cancer Res* 2007;67(17):8207–15.
- [31] Proksch E, Holleran WM, Menon GK, Elias PM, Feingold KR. Barrier function regulates epidermal lipid and DNA synthesis. *Br J Dermatol* 1993;128(5):473–82.