Kao J, Hall J: Skin absorption and cutaneous first pass metabolism of topical steroids: in vitro studies with mouse skin in organ culture. *J Pharmacol Exp Ther* 241:482–487, 1987

Landmann L: Epidermal permeability barrier: transformation of lamellar granule-disks into intercellular sheets by a membrane-fusion process, a freeze- fracture study. *J Invest Dermatol* 87:202–209, 1986

MacCallum DK, Lillie JH: Evidence for autoregulation of cell division and cell transit in keratinocytes grown on collagen at an air–liquid interface. *Skin Pharmacol* 3:86–96, 1990

Madison KC, Swartzendruber DC, Wertz PW, Downing DT: Presence of intact intercellular lipid lamellae in the stratum corneum. *J Invest Dermatol* 88:714–718, 1987

Nolte CJ, Oleson MA, Bilbo PR, Parenteau NL: Development of a stratum corneum and barrier function in an organotypic skin culture. *Arch Dermatol Res* 285:466–474, 1993

Regnier M, Caron D, Reichert U, Schaefer H: Barrier function of human skin and human reconstructed epidermis. *J Pharm Sci* 82:404–407, 1993

Slivka SR, Landeen LK, Zeigler F, Zimber MP, Bartel RL: Characterization barrier function, and drug metabolism of an in vitro skin model. *J Invest Dermatol* 100:40–46, 1993

Swartzendruber DC, Wertz PW, Kitko DJ, Madison KC, Downing DT: Molecular models of the intercellular lipid lamellae in mammalian stratum corneum. *J Invest Dermatol* 92:251–257, 1989

Tammi RH, Tammi MI, Hascall VC, Hogg M, Pasonen S, MacCallum DK: A preformed basal lamina alters the metabolism and distribution of hyaluronan in epidermal keratinocyte "organotypic" cultures grown on collagen matrices. *Histochem Cell Biol* 113:265–277, 2000

Vicanova J, Boelsma E, Mommaas AM, *et al*: Normalization of epidermal calcium distribution profile in reconstructed human epidermis is related to improvement of terminal differentiation and stratum corneum barrier formation. *J Invest Dermatol* 111:97–106, 1998

p53 Patches are Not Increased in Patients with Multiple Nonmelanoma Skin Cancers

To the Editor:

Mutation of p53 tumor suppressor gene is considered to be a frequent early event in nonmelanoma skin cancer (NMSC) development. NMSC includes basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Up to 40% of patients with NMSC develop a second primary tumor; however, recognition of this individual risk is currently impossible. UV irradiation is the major carcinogen for NMSC. Chronically sun-exposed epidermis harbors numerous mutated p53 clones that are detected as p53 patches by immunohistochemistry (Jonason *et al*, 1996; Ren *et al*, 1996). But whether they are precancerous remains open to debate.

We hypothesized that if p53 patches are precancerous lesions, their prevalence would increase with the number of NMSC and could serve as an individual risk marker for tumor development. In a retrospective study of 250 cases (1996–98, Hospital St-Louis, Paris, France) we scoped the presence or absence of p53 patches in 6–8 sections of normal peritumoral skin in patients treated for excision of NMSC on chronically sun-exposed skin (i.e., face, neck, and hands).

Patients were sorted into a solitary (no previous history of NMSC) or multiple group (two or more NMSC, simultaneous or successive). For each group the number of patients was 125 and the median age 71. For the solitary NMSC group, ages ranged from 26 to 97, male gender was 66%, and there was BCC in 93 patients (74%) and SCC in 32 (26%). For the multiple NMSC group, ages ranged from 32 to 100, male gender was 72%, and there was BCC in 106 patients (85%) and SCC in 19 (15%). Skin samples fixed in 10% formalin were deparaffinized and stained after antigen retrieval in a microwave oven 15 mn, at 450 W, in tris buffer pH 7.3, using monoclonal antibody D-O7 (Dako, code M7001, Denmark), with the avidin-biotin-coupled immunoperoxidase staining method and diaminobenzidine. P53 patches are defined as a well-demarcated compact pattern of strong immunostaining of basal and suprabasal keratinocytes nuclei in nondysplastic nontumoral epidermis (**Fig 1**). Mayer's hematoxylin was used for counterstaining. All slides were blind scoped for the presence of p53 patches by three independent observers (FP, AJ, NBS). Fisher's exact test was used to compare percentages between groups. Two sided tests were computed, and p values of 0.05 or less were considered statistically significant (SAS software, SAS Institute, Cary, NC).

There was no significant difference between the prevalence of p53 patches of solitary *versus* multiple NMSC groups (66% *vs* 70%, respectively, $p = 0.59$), neither was there a significant difference between the prevalence of p53 patches in the different age groups (**Table I**).

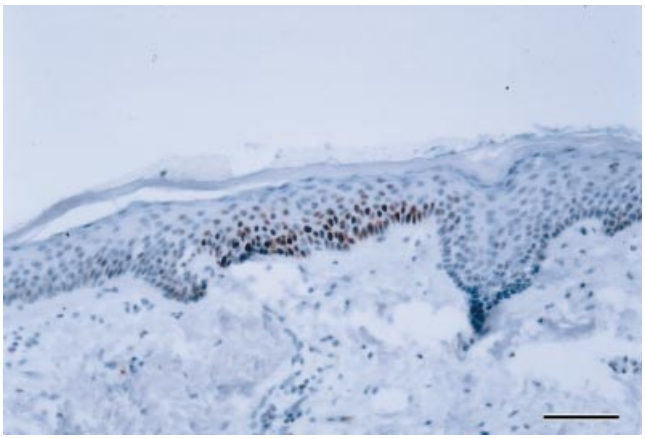

Figure 1. A typical p53 patch. P53 immunostaining × *Scale bar*: 40 μm.

Table I. P53 patch prevalences in normal skin around nonmelanoma skin cancers, a series of 250 patients

Patients' characteristics	p53 patches/total N/N	%
Age (y)		
Overall	169/250	68%
⩽50y	9/18	50%
50–59 y	24/34	71%
60–69 y	41/57	72%
70–79 y	43/63	68%
80–89 y	43/65	66%
> 90 y	9/13	69%
Tumor type		
BCC	132/199	66%
SCC	37/51	73%
Multiple NMSC	87/125	70%
Solitary NMSC	82/125	66%

Manuscript received May 23, 2001; revised July 9, 2001; accepted for publication July 17, 2001.

Reprint requests to: Dr. N. Basset-Seguin, Institut de Recherche sur la Peau, Pavillon Bazin, Hôpital Saint-Louis, 1 avenue Claude Vellefaux 75010 Paris France. Email: nbs@chu-stlouis.fr

The lack of significant difference between the p53 patches prevalence of solitary *versus* multiple NMSC suggests a lack of association between p53 patches and the number of NMSC. Previous studies suggested that p53 patches predispose keratinocytes to cancer (Jonason *et al*, 1996). They were also observed to be more common and larger in patients with BCC than in control subjects, but age difference between groups in this study could account for this difference (Tabata *et al*, 1999). What is more, p53 patches are frequent and precede NMSC-formation in cancer prone animal models (Berg *et al*, 1996).

P53 patches, however, are estimated to be 100.000 times as common as dysplasia, suggesting that most of them regress or remain stable (Ren *et al*, 1996, 1997). In our study we did not consider a NMSC-free control group but it was previously shown that p53 patches can be found in normal sun-exposed skin of patients without any history of skin cancer (Jonason *et al*, 1996). This suggests that these p53 patches could indeed be just a cognate phenomenon of UV exposure. Evaluation of risk of additional NMSC through p53 patches analysis would need more than just evaluation of their presence or absence, as p53 patches could look the same and yet have very different transformation potential. Although there was no significant difference between p53 patches prevalence in the age groups of the patients we studied, the figure observed in patients under the age of 50 was strikingly lower than in any other age group. Lack of significant increase of p53 patches after 50 y of age suggests their appearance before the age of 50, less sun-exposure, or a saturation phenomena for p53 mutation above that age. Additionally differences between cell loss and cell renewal associated with age could make the prevalence of p53 patches look rather stable. We have shown for the first time that there is no significant difference in p53 patches prevalence between BCC and SCC. This may indicate a lack of association between p53 patches and both types of NMSC.

Our study suggests that p53 patches cannot serve as a risk marker for developing multiple NMSC as the p53 patches prevalence of solitary *versus* multiple groups of patients are not significantly different. The commonness of p53 patches may mask important molecular differences in their transformation capacity. Our study underscores the importance of characterizing new markers for these molecular differences rather than relying solely on p53 protein stabilization.

Francois le Pelletier, Nadem Soufir,† Pauline de La Salmoniere,*
Anne Janin, Nicole Basset-Seguin†
Laboratoire de Pathologie EA 2378,
*Département de Biostatistique et informatique Médicale,
†INSERM U532, Institut de Recherche sur la Peau,
Hôpital Saint-Louis, France

REFERENCES

Berg RJ, van Kranen HJ, Rebel HG, *et al:* Early p53 alterations in mouse skin carcinogenesis by UVB radiation: Immunohistochemical detection of mutant p53 protein in clusters of preneoplastic epidermal cells. *Proc Natl Acad Sci USA* 93:274–278, 1996

Jonason AS, Kunala S, Price GJ, *et al*: Frequent clones of p53-mutated keratinocytes in normal human skin. *Proc Natl Acad Sci USA* 93:14025–14029, 1996

Ren ZP, Ahmadian A, Ponten F, *et al*: Benign clonal keratinocyte patches with p53 mutations show no genetic link to synchronous squamous cell precancer or cancer in human skin. *Am J Pathol* 150:1791–1803, 1997

Ren ZP, Hedrum A, Ponten F, *et al*: Human epidermal cancer and accompanying precursors have identical p53 mutations different from adjacent areas of clonally expanded non-neoplastic keratinocytes. *Oncogene* 12:765–773, 1996

Tabata H, Nagano T, Ray A, Flanagan N, Birch-Machin M, Rees JL: Low frequency of genetic change in p53 immunopositive clones in human epidermis. *J Invest Dermatol* 113:972–976, 1999

Underlying Disease Specificity of Genetic Loci in Atopic Dermatitis

To the Editor:

Atopic dermatitis is a chronic inflammatory skin disease. Chronic inflammation is a characteristic of many disorders having an immune or autoimmune component. Lee *et al* (2000) recently mapped genetic loci for what was referred to as a major susceptibility locus for atopic dermatitis to chromosome 3q21 ($p = 8.42 \times 10^{-6}$). They also identified two positive loci at 1p22 ($p = 0.0038$) and 19p13 ($p = 0.0035$) for atopic dermatitis. Recently, it has been noted that loci for autoimmune/inflammatory disorders tend to cluster or colocalize (Vyse and Todd, 1996; Becker *et al*, 1998; Teuscher *et al*, 1998; Becker, 1999; Encinas *et al*, 1999; Barnes 2000; Myerscough *et al*, 2000; Morel *et al*, 2001) suggesting, in some cases, a common or shared locus involving underlying immune regulatory pathways. **Table I** and **Fig 1** show loci from autoimmune/inflammatory disease studies that have been mapped to within 10 cM of the three loci identified in atopic dermatitis. These diseases include: 1p22, coeliac disease, systemic lupus erythematosus (SLE), Crohn's disease, multiple sclerosis; 3q21, asthma, type 1 diabetes, rheumatoid arthritis, psoriasis, multiple sclerosis; 19p13, Crohn's disease, and SLE. A locus at 3q21 has been defined in Type 1 diabetes as IDDM 9 (Mein *et al*, 1998; Cornelis *et al*, 1998). This colocalization of autoimmune/inflammatory loci at the purported dermatitis loci suggests that these loci may not be specific for atopic dermatitis *per se* but may define loci for basic immune regulatory mechanisms functional in these related immune diseases. Of particular interest is the report of linkage of asthma to 3q21 (Dizier *et al*, 2000). Asthma and atopic dermatitis share a host of systemic, cellular features common to the allergic diathesis, and coexistence of the two phenotypes is high: 60%–80% of atopic dermatitics have a history of asthma and 40% or more of asthmatics have a history of atopic dermatitis (Bergmann *et al*, 1998). The high rate of coexistence of these two clinical phenotypes complicates the search for phenotype-specific genes. The authors also suggest (Lee *et al*, 2001) two leading candidate genes for major atopic dermatitis in the region on 3q21; CD80 (B7.1) and CD86 (B7.2) (**Fig 1*A***). These candidate molecules within the atopic dermatitis locus on 3q21 interact with CD28 and CTLA4 to provide costimulatory signals to T cells leading to immune activation. The B7-CD28-CTLA4 pathway has been shown to be active in all of the immune disorders described in

Manuscript received March 14, 2001; accepted for publication March 17, 2001.

Reprint requests to: Dr. Kevin G. Becker, Gerontology Research Center, Room 4-D16, 5600 Nathan Shock Drive, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224. Email: beckerk@grc.nia.nih.gov