Tennessee, USA and [3]*Department of Carcinogenesis, Science Park Research Division, University of Texas MD Anderson Cancer Center, Smithville, Texas, USA*
E-mail: dkusewitt@mdanderson.org

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Effect of Aging on DNA Excision/Synthesis Repair Capacities of Human Skin Fibroblasts

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TO THE EDITOR

A key factor in the skin aging process is the cumulative effects of chronological aging and environmental-based assaults. Endogenous cellular oxidative processes generate reactive oxygen species and reactive polyunsaturated fatty acid derivatives (Lindahl, 1993; Marnett and Plastaras, 2001). These attacks on DNA cause substantial base and sugar damage, and the persistence of such lesions leads to mutations and genome instability. Skin may also suffer from chronic exposure to sun; UV radiation causes oxidative DNA damage and induces photoproducts (mainly cyclobutane pyrimidine dimers and 6-4 photoproducts (Moriwaki and Takahashi, 2008)). Age-related accumulation of somatic damage can thus be worsened by sun exposure, leading to an increased incidence of skin disorders and dramatic acceleration of skin aging (Niedernhofer, 2008).

Mammalian cells have evolved several DNA-repair pathways to remove all the categories of DNA base lesions, relying in particular on DNA excision mechanisms. One of these, nucleotide excision repair, removes bulky adducts and is thus an essential mechanism for correcting UV-induced DNA damage (Sarasin, 1999). The base excision repair pathway corrects small base modifications such as oxidized and alkylated bases (Almeida and Sobol, 2007).

The importance of repair mechanisms is demonstrated by the hazardous consequences of genetic defects in DNA repair (Friedberg, 2001), but investigating DNA repair with respect to aging remains a challenge. This is due to the complexity of the underlying repair mechanisms as well as to the varying approaches in terms of assays and end points measured (Vijg, 2008).

To better understand the relationship between aging and DNA repair, we took advantage of our newly developed multiplexed excision/synthesis assay (Millau *et al.*, 2008) to examine simultaneously, using nuclear extracts, the base excision repair and nucleotide excision repair capacities of human primary fibroblasts derived from healthy donors of different ages. In addition, we investigated changes in DNA repair attributed to chronic sun exposure.

A total of 33 healthy Caucasian women were recruited by the Dermscan Group (Lyon, France). Biopsy removal was performed in accordance with the Declaration of Helsinki Principles Guidelines after approval for the study had been given by a medical ethics committee and written consent obtained from the donors. The volunteers were classified into three groups by age (group 1: mean age = 25 years, range 20–33, $n = 9$; group 2: mean age = 46 years, range 40–50, $n = 9$; group 3: mean age = 65 years, range 61–68, $n = 15$). All subjects were nonsmokers, had phototype II or III skin, declared no excessive exposure to sun or UVA, had no cutaneous pathology, and were not receiving medical treatment. Fibroblast cultures were established from outgrowth of two 3 mm punches taken on the volar forearm (photoexposed area) and the upper inner arm (photoprotected area). Cells were harvested during the exponential phase of growth and stored frozen in liquid nitrogen at passage 5. Nuclear extracts were prepared as described by Millau *et al.* (2008). For each sample, excision/synthesis repair reactions were run for 2.5 hours at 30 °C at a final protein concentration of 0.15 mg ml^{-1}, along

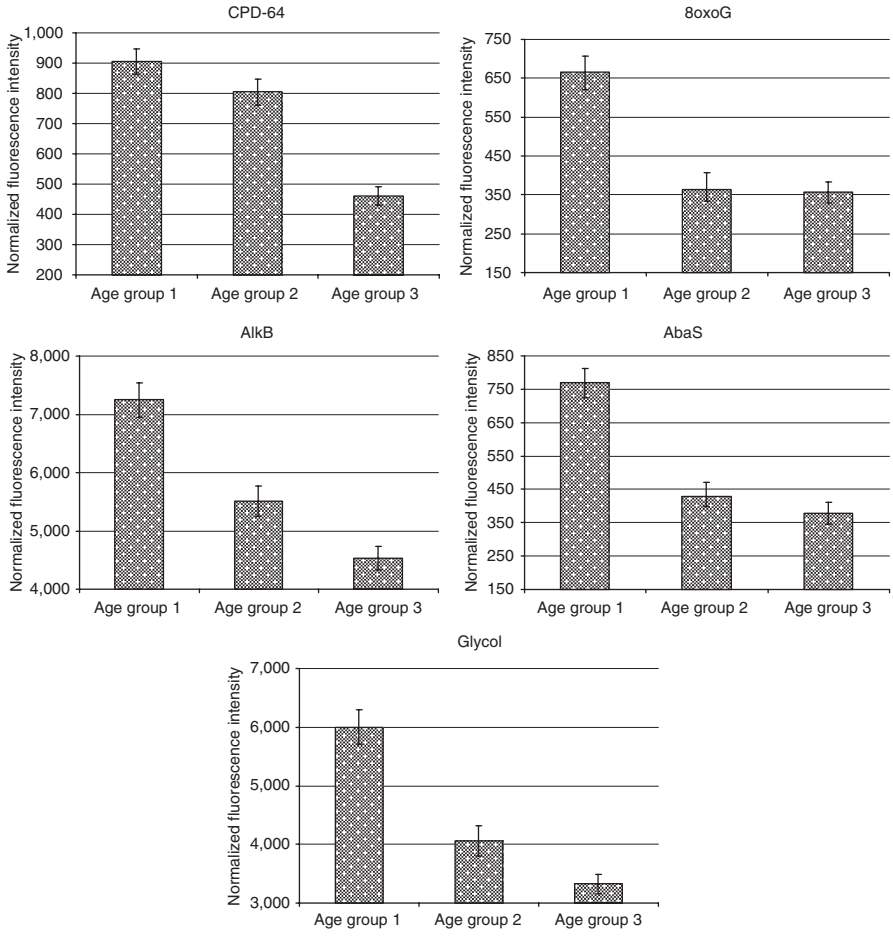

Figure 1. Effect of age on DNA repair excision/synthesis activities. Mean fluorescence intensity and standard error are reported for each lesion type (cyclobutane pyrimidine dimers and (6-4)photoproducts (CPD-64), 8-oxoguanine (8oxoG), alkylated bases (AlkB), abasic sites (AbaS), and pyrimidine glycols (Glycol)) and for each age group (1, 2, and 3). The general linear model showed that age had a significant impact on DNA repair for CPD-64 ($P=0.039$), 8oxoG ($P=0.039$), and AbaS ($P=0.027$).

with 1 mM adenosine triphosphate and 1.25 μM dCTP-Cy5 (Amersham, Little Chalfont, England), on damaged plasmid microarrays prepared as described by Millau *et al.* (2008) (see Supplementary Data and Supplementary Figures online for experimental details and nuclear extract features). The microarrays were prepared using Hydrogel slides (Perkin Elmer, Courtaboeuf, France) spotted with unmodified control plasmids together with five plasmid families containing serial amounts of typical lesions (three dilutions per plasmid family). Lesions present on the support were formed by specific physical and chemical treatments: photoproducts (cyclobutane pyrimidine dimers and (6–4)photoproducts), 8-oxoguanine, alkylated bases, abasic sites, and pyrimidine glycols. Total fluorescence intensity related to the fluorescence incorporated into plasmids was the parameter used for calculations (Genepix 4200A scanner, Axon GenePix, Molecular Devices, Sunnyvale, CA). A total fluorescence intensity value was calculated for each lesion type by adding the values for the corresponding replicates. Hence, each microarray generated one value per lesion type. The mean of the two values obtained per sample and per lesion type were used for statistical purposes. Assessment of the mean fluorescence intensity (shown with the corresponding standard error for each lesion type in Figure 1) revealed a decrease in excision/synthesis activity with age, irrespective of the repair pathway considered.

Statistical analysis (general linear model (Minitab V14 software, The MathWorks, Natick, MA) was performed on these latter data from 31 samples: photoprotected and photoexposed cells from the same subject. The general linear model was used to estimate the influence of age (three groups) and exposition (two groups) on the excision/synthesis activity. This analysis revealed that age significantly affected DNA excision/synthesis activity toward photoproducts ($P=0.039$), 8-oxoguanine ($P=0.039$), and abasic sites ($P=0.027$). The effect of age was not significant for repair of alkylated bases ($P=0.14$) and glycols ($P=0.09$).

Indeed, the fluorescent signal we measured is the end point of a multistep process that is composed of a chain of enzymatic reactions (recognition, excision, and DNA synthesis). Using this global functional approach, it is not possible to identify the steps that are most sensitive to the aging process. However, there seems to be a consensus in the literature that aging is accompanied by a decline in the rate of DNA synthesis by polymerases in mammals (Raji *et al.*, 2002; Takahashi *et al.*, 2004). Our observations could be at least partly related to a decline in polymerase-δ.

The general linear model did not show evidence of any significant effect of photoexposure on repair activities, irrespective of the lesion considered.

In conclusion, we showed that repair activities attributed to both base excision repair and nucleotide excision repair were significantly affected by aging. Conversely, we found no evidence for a significant effect of chronic photoexposure on DNA-repair pathways using this approach.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Sylvie Sauvaigo[1], Sylvain Caillat[1], Francette Odin[1], Alex Nkengne[2], Christiane Bertin[2] and Thierry Oddos[3]

[1]Laboratoire de Lésions des Acides Nucléiques, LCIB (UMR-E3 CEA-UJF), CEA Grenoble, INAC SCIB LAN, Grenoble, France; [2]Skincare

Research Institute, Johnson & Johnson Consumer France, Issy les Moulineaux, France and [3]Pharmacology Department, Johnson & Johnson Consumer France, Val de Reuil, France. E-mail: sylvie.sauvaigo@cea.fr

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Acral Peeling Skin Syndrome with *TGM5* Gene Mutations May Resemble Epidermolysis Bullosa Simplex in Young Individuals

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TO THE EDITOR

The acral peeling skin syndrome (APSS) is a rare autosomal recessive condition characterized by superficial painless peeling of the skin predominantly on the dorsal aspects of hands and feet (Shwayder *et al.*, 1997; Cassidy *et al.*, 2005). The condition is usually aggravated by heat, humidity, and exposure to water. Microscopically, the cleavage level is located in the upper epidermis, between the stratum granulosum and the stratum corneum (Garcia *et al.*, 2005). Only 15 patients with APSS have been reported since 1997 (Shwayder *et al.*, 1997; Brusasco *et al.*, 1998; Hashimoto *et al.*, 2000; Cassidy *et al.*, 2005; Garcia *et al.*, 2005; Kharfi *et al.*, 2009; Oumakhir *et al.*, 2009; Wakade *et al.*, 2009) (Table 1). In several of them, in addition to superficial peeling, acral blisters were also described (Wakade *et al.*, 2009). The genetic basis of the disease was determined in only three families, in whom two different missense mutations in the *TGM5* gene encoding transglutaminase 5 (TGase 5) were disclosed (Table 1) (Cassidy *et al.*, 2005; Kharfi *et al.*, 2009). It remains unclear whether the other patients have mutations in the same gene, or whether APSS is clinically and genetically heterogeneous (Cassidy *et al.*, 2005).

In this study, we investigated nine unrelated patients, eight children and one adult, clinically suspected to have epidermolysis bullosa simplex (EBS) because of acral skin blistering. The patients and/or diagnostic samples were referred to the Epidermolysis bullosa Center of the University Medical Center Freiburg (Volz *et al.*, 2007) for molecular diagnostics of EBS. EDTA-blood and skin samples were obtained after informed consent of the patients and, if available, of family members. EDTA-blood samples of 50 clinically unaffected Central European individuals were used as controls. The study was conducted according to the Declaration of Helsinki Principles. Immunofluorescence staining of skin cryosections was performed using a panel of antibodies to components of the epidermal basement membrane zone (Kern *et al.*, 2006), as well as antibodies to loricrin (Abcam, Cambridge, UK), filaggrin (clone 15C10; Novocastra, Newcastle, UK), involucrin (clone SY5; Sigma, Taufkirchen, Germany), cytokeratin 10 (clone DE-K10; Dako, Glostrup, Denmark), TGase 1 (clone B.C1; Biomedical Technologies, Madrid Spain), TGase 3 (Jackson Immunoresearch Laboratories, West Grove, PA), and TGase 5 (Novus Biologicals, Littleton, CO). Genomic DNA was extracted from EDTA-blood using the QiAmp DNA mini kit (Qiagen, Hilden, Germany). Amplification of all *KRT5* (NC_000012.11, National Center for Biotechnology Information (NCBI)), *KRT14* (NC_000017.10, NCBI), and *TGM5* (NC_000015.9, NCBI) exons and exon–intron boundaries, and sequencing were performed as described (Schuilenga-Hut *et al.*, 2003; Wood *et al.*, 2003; Cassidy *et al.*, 2005). Mutations were confirmed by resequencing. The mutation c.763T>C was verified in 100 control chromosomes by

Abbreviations: APSS, acral peeling skin syndrome; EBS, epidermolysis bullosa simplex; TGase, transglutaminase