UV, stress and aging

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Key words: UVB, fibroblasts, keratinocytes, senescence

Abbreviations: CPD, cumulative population doubling; HDF, human diploid fibroblast; NHEK, normal human epidermal keratinocyte; ROS, reactive oxygen species; SA-βgal, senescence-associated β-galactosidase; UV, ultraviolet

Skin is a model of choice in studies on aging. Indeed, skin aging can be modulated by internal and external factors, reflecting its complexity. Two types of skin aging have been identified: intrinsic, mainly genetically determined and extrinsic—also called “photo-aging”—resulting on the impact of environmental stress and more precisely of UV rays. Simplified in vitro models, based on cellular senescence, have been developed to study the relationship between UV and aging. These models vary on the cell type (fibroblasts or keratinocytes, normal or immortalized) and the type of UV used (UVA or UVB).

Introduction

Skin is a model of choice in studies on aging. Indeed, aging of the skin can be modulated by internal and external factors, reflecting its complexity. Two different types of skin aging are identified: intrinsic, mainly genetically determined and similar to aging of other organs, and extrinsic aging, resulting on the impact of environmental stress and more precisely of UV rays (for a review see refs. 1 and 2). If these two types of skin aging present differences at the morphological and at the histological levels, they share molecular similarities (for a review see ref. 3) as the induction of matrix metalloproteinasises. In order to better understand skin photo-aging, and more precisely, the relation between UV and aging, several simplified in vitro models have been developed, based on in vitro models of cellular senescence.

Skin Aging

Skin aging can be modulated by external factors. This extrinsic aging is superimposed on intrinsic one, and is also referred as “photo-aging.” Indeed, if several exogenous factors as tobacco smoke, infrared radiation, pollution, malnutrition etc. can interfere with skin aging, the factor having the greatest impact is clearly UV rays. Naturally, intrinsic and extrinsic aging of the skin are observable in the same individual depending on whether the parts of skin were protected from the sun or not. The face and the backside of the hands are usually the most photoaged affected areas. Clinically, intrinsically aged skin is thin, smooth and presents only light wrinkles. Different subtypes can characterize extrinsic aging of the skin. Classically, it is distinguished by a thicker skin (leathery aspect), with deeply marked wrinkles and an irregular pigmentation (age spots). Histologically, both types of skin aging are characterized by change in the organization of structural components of the connective tissue. In intrinsically aged skin is marked by a decrease in epidermal and dermal thickness. The interstitial collagen and elastin content are reduced while collagen cross-links fibers content is increased. Extrinsic aged skin shows hyperplasia, with an increase of the thickness of the epidermis and dermis. There is a complete perturbation of the structural content (reduced interstitial collagen, increased elastic fibers) associated with damaged fibers leading to a severe disorganization of the connective tissue structure.

Aging of the individuals appears to be linked to internal factors as genetic predispositions (as shown for longevity), hormonal status and to environmental factors. The degree of influence of these genetic and environmental factors has not been clearly described in aging of the skin. However, several studies of cohorts of twins helped highlight the importance of these two factors. Despite their differences, evidence shows that intrinsic and extrinsic aging of the skin are probably driven by similar biological, biochemical and molecular mechanisms. Thus, the formation of reactive oxygen species (ROS) and the induction of matrix metalloproteinasises are shown to be common factors of both types of skin aging. It is assumed that ROS accumulation detected in intrinsic and extrinsic aging leads to the activation of MAPK (mitogen-activated protein kinases) pathways, ERK (extracellular signal-regulated kinases), JNK (c-Jun N-terminal kinase) and p38MAPK once activated induc the activation of AP-1 (activator protein-1) transcription factor. AP-1 induces collagen degradation by promoting the expression of matrix metalloproteinasises MMP-1, MMP-3 and MMP-9, and by preventing the expression of procollagen-1.

UV and Photo-Aging

UV are essential components of sunlight. In vivo, skin is exposed to UVB and UVA as UVC are stopped by the ozone layer. UVB
(290–320 nm) and UVA (320–400 nm) are able to cross the epidermis and to reach the dermis.23 UVB and UVA can interact with endogenous chromophores and photosensitizers resulting in the generation of ROS causing damage to DNA, proteins and lipids. Moreover, UVB can directly interact with DNA and generate dipyrimidine photoproducts such as cyclobutane pyrimidine dimers and pyrimidine (6–4) pyrimidone photoproducts (for a review see refs. 16 and 17). UVB are therefore considered as the most harmful UV rays. UV radiation activates several signal transduction pathways related to growth, differentiation, senescence and connective tissue degradation18 by the activation of several cell surface receptors. This includes cytokines or growth factors receptors as the receptors for epidermal growth factor (EGF), tumor necrosis factor (TNF), interleukin-1 (IL-1)19 and keratinocyte growth factor (KGF).19 The biological responses to UV can be immediate and transient (inflammation, sunburn cell formation, pruritus) or delayed and chronic (photo-aging, immunosuppression, carcinogenesis). Exposures to UV rays are also used in dermatology to treat many skin diseases including psoriasis, atopic dermatitis, vitiligo, etc.20

In most cases, studies on photo-aging require the participation of human volunteers. This implies ethical constraints and limits sample size. In order to investigate in vitro photo-aging, various models have been developed, based on cellular senescence.

Repressive Senescence and Stress Induced Premature Senescence

In vitro, proliferative somatic cells, as human diploid fibroblasts (HDF), have a limited capacity of cellular divisions. Replicative senescence is defined as an irreversible growth arrest. This was first described by Hayflick in the early 1960s on HDFs21 and was later extended to most proliferative cell types (for a review see ref. 22). This growth arrest is established and maintained by the p53/p21WAF1 and the p16INK4A/pRb pathways.22 Thirty years later, Harley et al.23 showed that telomeres shorten during aging of HDFs, and replicative senescence was associated to a “critical” shortening of the telomeres. Cells can remain alive for several months after the onset of replicative senescence24 and show apoptosis resistance.25 Several markers can identify senescent cells in vitro and in vivo, however, none is exclusive to the senescent state.22 Among these biomarkers are: typical enlarged and flattened morphology,24 senescence-associated β-galactosidase activity (SA-βgal),26 senescence-associated DNA-damage foci (SDFs),27,28 altered gene expression29,30 and the common mitochondrial DNA deletion of 4,977 bp.31 More recently, it was shown that senescent cells secrete growth factors, proteases, chemokines and inflammatory cytokines allowing them to interact with their cellular environment. This was termed senescence-associated secretory phenotype (SASP).32,33

Some of these biomarkers are also detected in vivo. For instance, in the skin, some of these biomarkers are also detected during aging: typical senescent morphology;2 SA-βgal activity,26 p16INK4A overexpression34 and the “common” mitochondrial DNA deletion.35

In vitro, it is possible to induce the appearance of these biomarkers by exposing human proliferative cell types, such as HDFs, endothelial cells, melanocytes, etc. to acute stress at sublethal doses of stressing agents inducing oxidative stress and/or DNA damage. This was defined as “Stress Induced Premature Senescence” (SIPS) and is detectable at 3 d after the stress,36 long before the cells reach the critical telomere length observed in replicative senescence. Cells in SIPS induced by subcytotoxic concentrations of oxidative agents such as hydrogen peroxide (H2O2)37 or tert-butylhydroperoxide (t-BHP)38 remain alive for months and display several features of replicative senescence. These features include senescence morphology,24,31 SA-βgal activity,31,38 common mitochondrial deletion31 and altered gene and protein expression.39,40

In order to study the long-term effect of UV on skin cells types, several in vitro models were developed. Indeed, if multiple studies dealt with the effects of UV very few investigate the long-term effects of UV. Here we present different models that were developed in order to study long-term effects of subcytotoxic doses of UV. Several models were set up varying on the cell type (fibroblasts or keratinocytes, normal or immortalized) and the type of UV used (UVA or UVB).

UV-Stress Induced Premature Senescence in Fibroblasts

Fibroblasts constitute the classical cell type for studies on aging. Historically, it was the first strain on which replicative senescence was detected21 and constitutes since the cell type of reference for studies of aging.22 In the skin, fibroblasts constitute the main cell type of the dermis and are responsible of the production of the different extracellular matrix (ECM) components.41

Models of premature senescence were developed by exposing dermal HDFs to sublethal doses of UVB.42,43 After ten repeated exposures to sublethal dose of UVB, HDFs display biomarkers of senescence as typical senescent morphology, SA-βgal activity, altered gene expression and the “common” mitochondrial DNA deletion.42,43 As the UV dose used must not induce mortality or apoptosis, caspase-3 activity and PARP cleavage were checked as negative in these conditions. The decreased proliferative potential observed after these UVB stresses are correlated with overexpression of p53, p21WAF1 and p16INK4A, involved in the growth arrest in replicative senescence. Concerning gene expression change observed after these repeated UVB exposures, the relative steady-state mRNA level of c-jun, c-fos, MMP-1 and MMP-2 was found to be increased. C-fos and c-jun are known to be components of the c-jun-c-fos AP-1 transcription factor. A transient increase of extracellular release of H2O2 was detected after repeated UVB exposures.44 This model has been accepted as an in vitro simplified model of dermal aging. It was used to identify potential protective effect of marine algal components.45 Moreover, it was shown that telomerase activity did not prevent premature senescence induced by UVB46 as telomerase-immortalized human foreskin fibroblasts (hTERT-BJ1) developed biomarkers of senescence after UVB exposures.

At the mechanistic level, the pathways inducing senescence are still unknown. In SIPS, the role of Transforming Growth...
Factor-β1 (TGF-β1) in the appearance of some of the biomarkers of senescence has been described. TGF-β1 is a multifunctional cytokine involved in many cellular functions like cellular division, differentiation and connective tissue synthesis. TGF-β1 is secreted in a latent form (LTGF-β), which consists of TGF-β1 noncovalently associated with its N-terminal propeptide called latency associated peptide (LAP) (for a review see ref. 48). The abundance of TGF-β1 mRNA is increased in premature senescence induced by H₂O₂, 1, β-HBP, ethanol and UVB. By using neutralizing antibodies, it was shown that TGF-β1 is responsible for the appearance of several biomarkers of senescence induced by these stresses. In premature senescence induced by H₂O₂, it was shown that H₂O₂ induces a first phase of activation of p38MAPK (ref. 50). This activation triggers an overexpression of TGF-β1, which starts a positive feedback loop allowing sustained activation of p38MAPK. P38MAPK phosphorylates and activates the transcription factor ATF-2 that interacts with hypophosphorylated pRb. This complex induces the appearance of features of replicative senescence. This regulatory loop probably also triggers UVB-induced premature senescence with concomitant activation of p53. Indeed, UVB-induced premature senescence is associated with a transient increase of p53 protein abundance and DNA-binding activity. Silencing p53 expression with small interfering RNA (siRNA) affected the basal level of SA-βgal and proliferative potential, as the expression of genes differentially expressed after repeated exposures to UVB.

Concerning long-term effects of UVA exposures on HDFs, Herrmann et al. showed that treatment of HDFs with 8-methoxypsoralen and subsequent UVA irradiation resulted in a long-term growth arrest, alterations in cell morphology (post mitotic phenotypes) and increased expression of SA-βgal. Combined treatment of psoralen and UVA, also known as pUVA therapy, is widely used in the treatment of different skin disorders as psoriasis. Psoralens act as photosensitizers via the generation of ROS. The long-term growth arrest of pUVA-treated HDFs is associated with increased protein levels of p53, p21WAF-1 and p16INK-4A (ref. 54) and change of expression of genes involved in growth arrest, stress response, modification of the extracellular matrix and senescence. However, pUVA-induced growth arrest, senescent morphology, SA-βgal increased activity and MMP-1 overexpression are fully reversible at days 100 to 130 post pUVA treatment. This suggests that pUVA-induced changes do not fully reflect replicative senescence in HDFs but rather represent a long-term transient phenocopy of senescence and was therefore presented as a SIPS model. For UVA irradiation alone, Berneburg et al. showed that the common mitochondrial deletion was detectable in HDFs exposed to 36 repeated sublethal doses of UVA radiation. This deletion was found to be mediated by singlet oxygen.

**UVB-Stress Induced Premature Senescence or Alternative Differentiation in Keratinocytes**

Normal human epidermal keratinocytes (NHEK) constitute the main cell type of the epidermis and are the first line of skin’s defense against environmental stresses. They proliferate in the basal layer before moving upwards to the suprabasal layers through a complex differentiation program that culminates in fully differentiated dead cells in the cornified superficial layer, maintaining a strong impermeable barrier. If it has been detected that keratinocytes show characteristics of replicative senescence in vivo very little was devoted to study the aging of keratinocytes in vitro. Moreover, very few are known on the long-term effect of subcytotoxic UV exposures on NHEKs. NHEKs progressively show proliferation arrest and reach a senescence plateau after about 15–25 population doublings (according to the donor) in culture. This plateau lasted only a few days to 2–3 weeks and is followed by a massive detachment of almost all cells. Some remaining cells with partially transformed and tumorigenic traits will then spontaneously emerge form senescent cultures, linked to the accumulation of ROS during senescence. Senescent keratinocytes display morphological changes as increase of cytoplasm size and of perinuclear organelle contents. They also display increased SA-βgal activity and p16INK-4A expression.

Lewis et al. demonstrated that low doses of UVB irradiation induce cellular senescence in NHEKs with increased SA-βgal activity and increased p21WAF-1 and p53 protein abundance. Activation of IGF-1R promotes this UVB-premature senescence through increased generation of ROS and by maintaining the expression of p21WAF-1.

Telomerase expression alone is not sufficient to immortalize human keratinocytes. Indeed, normal human oral keratinocytes (NHOK) expressing telomerase enter in replicative senescence after several population doublings in culture. To be immortalized, keratinocytes should express telomerase and lack p16INK-4A. As described earlier, p16INK-4A is a strong biomarker of skin aging. Immortalized keratinocytes lacking p16INK-4A and expressing telomerase retain other growth controls and keep the ability to differentiate in reconstructed epidermis in vitro. Repeated exposures to UVB of immortalized keratinocytes induce an alternative state of differentiation rather than stress-induced premature senescence. This alternative differentiation state is characterized namely by an increased abundance of involucrin, a late marker of differentiation, and cytokeratins (K) K6, K16 and K17, phosphorylation of p38MAPK and HSP27, and elevated secretion of active MMP-9, as observed in primary keratinocytes and in vivo in the epidermis. Expressions of proteins involved in keratinocyte differentiation and survival were shown to be changed after UVB exposures. Among them, TRIM 29 (TRIpartite Motif Protein 29), a survival factor, is dependent on PKCδ signaling pathway. These results suggest that p16INK-4A is essential for keratinocytes to enter into senescence.

**Conclusions**

In conclusion, several models were developed in which human fibroblasts or keratinocytes, exposed to subcytotoxic doses of UV rays, show characteristics of cellular senescence. These models can be used to better understand the relationship between UV stress and aging. Since the skin is a complex organ, consisting of different compartments with connections between them,
it would be interesting to develop in the future more complex models allowing to take into account interactions between the different cell types of the skin. In addition, aging of the skin is modulated by multiple internal (genetic, hormonal) and external (oxidative stress, UV, pollution) factors, which makes it complex to study. However, this complexity makes it an interesting model to use the skin as a picture of the overall aging of the individual.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References
1. Fisher GJ, Kang S, Varani J, Bara-Cozzo G, Wu Y, Datta S, et al. Mechanisms of photaging and chronological skin aging. Arch Dermatol 2002; 138:1462-70; PMID:12437452; http://dx.doi.org/10.1001/archderm.138.11.1462.
2. Właschek M, Tantcheva-Poir I, Nadler L, Ma W, Schneider LA, Razi-Wolf Z, et al. Solar UV irradiation and dermal photoaging. J Photochem Photobiol B 2001; 63:41-51; PMID:11684450; http://dx.doi. org/10.1016/S1010-6649(00)00220-1.
3. Kohl E, Steinbauer J, Landthaler M, Sixmiasis RM. Skin aging. J Eur Acad Dermatol Venereol 2011; 25:873-84; PMID:21261751; http://dx.doi.org/10.1111/j.1468-3083.2010.03963.x.
4. Ma W, Właschek M, Tantcheva-Poir I, Schneider LA, Nadler L, Razi-Wolf Z, et al. Chronological aging and photoaging of the fibroblasts and the dermal connective tissue. Clin Exp Dermatol 2001; 26:592-9; PMID:11690663; http://dx.doi.org/10.1046/j.1365-2230.2001.00905.x.
5. Beekman M, Blanché H, Perola M, Hervonen A, Hanneman KK, Cooper KD. Smoking and skin aging in identical twins. Arch Dermatol 2009; 145:1375-9; PMID:19819436; http://dx.doi.org/10.1001/archderm.143.12.1543.

Acknowledgments
F.D.-C. and O.T. are respectively Research Associate and Senior Research Associate of the FNRS, Belgium. We acknowledge the Direction Générale Opérationnelle de l’Economie, de l’Emploi et de la Recherche (DG06) of the Public Service of Wallonia (SPW) for the ‘Senegen’ Reseaux 2-project, no. 05/16192. We thank the European Commission for large scale Collaborative Project ‘MYOAGE’, contract HEALTH-F2-2009-223576 from the Seventh FP, for large scale Collaborative Project ‘MARKAGE’, contract HEALTH-2007-2.2.2.-3 from the Seventh FP and for Infrastructure ‘QNano’ Contract INFRASTRUCTURE-2010-1-262163.

Rittl L, Fisher GJ. UV-light-induced signal cascades and skin aging. Aging Res Rev 2002; 1:705-20; PMID:12208829; http://dx.doi.org/10.1016/S1568-1637(02)00024-7.
3. Fisher GJ, Voorhees JJ. Molecular mechanisms of photaging and its prevention by retinoid acid: ultraviolet irradiation induces MAP kinase signal transduction cascades that induce Ap-1-regulated matrix metalloproteinases that degrade collagen in vivo. J Invest Dermatol Proc 1999; 5:61-8; PMID:9732661.
4. Chang JH, Kang S, Varani J, Lin J, Fisher GJ, Voorhees JJ. Decreased extracellular-signal-regulated kinase and increased stress-activated MAP kinase activities in aged human skin in vivo. J Invest Dermatol 2000; 115:177-82; PMID:10951233; http://dx.doi.org/10.1046/j.1523-1747.2000.00099.x.
5. Rosette C, Karin M. Ultraviolet light and oesmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. Science 1999; 285:234-7; PMID:10895468; http://dx.doi.org/10.1126/science.274.5920.1194.
6. Hiraku Y, Ito K, Hirakawa K, Kawanishi S. Photosensitized DNA damage and its protection via a novel mechanism. Photochem Photobiol 2002; 83:395-12; PMID:11905181; http://dx.doi.org/10.1080/15251570210040051.
7. Peña-Bezrukov V, Sikora E, et al.; The GEHA consortium. Genome-wide linkage analysis for human longevity. J Hum Genet 2007; 52:160-7; PMID:17011230; http://dx.doi.org/10.1007/s10038-006-0628-8.
8. Schneider LA, Razi-Wolf Z, et al. Solar UV irradiation and extracellular matrix proteinases that degrade human skin in vivo. J Invest Dermatol 2000; 115:177-82; PMID:10951233; http://dx.doi.org/10.1046/j.1523-1747.2000.00099.x.
9. Kermani G, Janas A, Pociot F, et al. Polymorphic expansion of 3p22-24 to extreme longevity in humans and identifies multiple novel loci. PLoS One 2010; 5:e12432; http://dx.doi.org/10.1371/journal.pone.0012432.
10. Fishman S, Barriekova J, Niederegger H, Tariq R, Jansen-Dürr P. Differential regulation of apoptotic cell death in senescent human cells. Exp Gerontol 2004; 39:1713-21; PMID:15582287; http://dx.doi.org/10.1016/j. expger.2004.05.010.
11. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, et al. A biomarker that identifies senescent human cells in culture and in aging in vivo. Proc Natl Acad Sci USA 1999; 96:9926-31; PMID:9968133; http://dx.doi.org/10.1073/pnas.99.20.9936.
12. d’Adda di Fagagna F, Reapar PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, et al. A DNA damage checkpoint response in telomer-initiated senescence. Nat Rev Cancer 2003; 3:428-36; PMID:12866124; http://dx.doi.org/10.1038/nrc1156.
13. Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. Curr Biol 2003; 13:1549-56; PMID:12956959; http://dx.doi.org/10.1016/S0960-9822(03)00542-6.
14. Shulman DN, Chang E, Whittier PS, Choi D, Funk WD. Microarray analysis of replicative senescence. Curr Biol 1999; 9:939-45; PMID:10585881; http://dx.doi.org/10.1016/S0960-9822(99)80420-5.
15. Sook IK, Kim HK, Kim YK, Song IH, Kim W, Kim S, et al. Exploration of replicative senescence-associated genes in human dermal fibroblasts by cDNA microarray technology. Exp Gerontol 2004; 39:1369-78; PMID:15489606; http://dx.doi.org/10.1016/j. expger.2004.07.002.
16. Diament P, Burton M, Chen QM, Gnos C, Frépiat C, Mazauric JR, et al. Induction of replicative senescence biomarkers by sublethal oxidative stresses in normal human fibroblasts. Free Radic Biol Med 2006; 40:361-73; PMID:16979477; http://dx.doi.org/10.1016/j.freeradbiomed.2005.08.047.
17. Coppe JP, Park CD, Rodier F, Krolova A, Beaussjour CM, Parmigollo S, et al. A human-like senescence-associated secretory phenotype is conserved in mouse cells dependent on physiological oxygen. PLoS One 2010; 5:e12432; http://dx.doi.org/10.1371/journal.pone.0012432.
18. Coppe JP, Park CD, Rodier F, Sun Y, Muñoz DP, Goldstein J, et al. Senescence-associated secretory phenotype reveals cell‐nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 2008; 6:2853-68; PMID:19053174.
19. Resler S, Barkova J, Niederegger H, Barnek J, Scharffetter-Kochanek K, Jansen-Dürr P, et al. p16INK4A is a robust in vivo biomarker of cellular aging in human skin. Aging Cell 2006; 5:379-89; PMID:16965181; http://dx.doi.org/10.1111/j.1474-9726.2006.00251.x.
20. Berneau M, Plomteaux H, Medve-König K, Pfäfflin A, Geer-Varga H, Gefeller O, et al. Induction of the photaging-associated mitochondrial common deletion in vivo in normal human skin. Invest Dermatol 2004; 122:1277-81; PMID:15104023; http://dx.doi.org/10.1016/j.jid.2004.02.202X.2004.22502.x.
36. Brack C, Liebnow G, Osawa H, Tousi, O. EMBO WORKSHOP REPORT: Molecular and cellular gerontology Serpiano, Switzerland, September 18-22, 1999. EMBO J 2000; 19:1929-34; PMID:10793959; http://dx.doi.org/10.1093/emboj/19.9.1929

37. Chen QM, Bartholomew JC, Campus J, Acosta M, Reagan JD, Ames BN. Molecular analysis of H2O2-induced senescent-like growth arrest in normal human fibroblasts: p53 and Rb control G1 arrest but not cell replication. Biochem J 1998; 323:43-50; PMID:9576849

38. Fripriat C, Chen QM, Zdanov S, Magalhaes JP, Remacle J, Toussaint O. Subcytotoxic H2O2 stress triggers a release of transforming growth factor-beta 1, which induces biomarkers of cellular senescence of human diploid fibroblasts. J Biol Chem 2001; 276:2531-7; PMID:11060295; http://dx.doi.org/10.1074/jbc.M006809200

39. Dierick JF, Kalume DE, Wenders F, Salmon M, Dieu M, Raes M, et al. Identification of 30 protein species involved in replicative senescence and stress-induced premature senescence. FEBS Lett 2002; 531:495-504; PMID:12435600; http://dx.doi.org/10.1016/S0014-5793(02)03604-9

40. Pascal T, Debaq-Chaïniaux F, Chirénet A, Bastin C, Dubre AF, Bertholet V, et al. Comparison of replicative senescence and stress-induced premature senescence combining differential display and low-density DNA arrays. FEBS Lett 2005; 579:3651-9; PMID:15963989; http://dx.doi.org/10.1016/j.febslet.2005.05.056

41. Sorrell JM, Caplan AL. Fibroblast heterogeneity: more than skin deep. J Cell Sci 2004; 117:667-75; PMID:14754903; http://dx.doi.org/10.1242/jcs.10015

42. Chaïniaux F, Magalhaes JP, Elsiers F, Remacle J, Toussaint O. UVB-induced premature senescence of human diploid skin fibroblasts. Int J Biochem Cell Biol 2002; 34:1331-9; PMID:12200029; http://dx.doi.org/10.1016/S1357-2725(02)00022-5

43. Debaq-Chaïniaux F, Borlon C, Pascal T, Royer V, Elsiers F, Ninane N, et al. Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-beta 1 signaling pathway. J Cell Sci 2005; 118:743-58; PMID:15671065; http://dx.doi.org/10.1242/jcs.01651

44. Borlon C, Chirénet A, Debaq-Chaïniaux F, Toussaint O. Transient increased extracellular release of H2O2 during establishment of UVB-induced premature senescence. Ann N Y Acad Sci 2007; 1119:72-7; PMID:18068755; http://dx.doi.org/10.1111/j.1749-6632.2007.00207.x

45. Debaq-Chaïniaux F, Borlon C, De Hertogh B, Remacle J, Morvan PY, Vallée R, et al. Identification of potential anti-photoaging agent compounds using an in-vitro model of photoaging. J Pharm Pharmacol 2006; 58:1577-83; PMID:17331320; http://dx.doi.org/10.1211/ijpp.58.12.0003

46. de Magalhaes JP, Chaïniaux F, Remacle J, Toussaint O. Stress-induced premature senescence in BJ and hTERT-BJ1 human foreskin fibroblasts. FEBS Lett 2002; 523:157-62; PMID:12112824; http://dx.doi.org/10.1016/S0014-5793(02)02973-3

47. Massagut J. How cells read TGF-beta signals. Nat Rev Mol Cell Biol 2000; 1:169-78; PMID:11252892; http://dx.doi.org/10.1038/35043051

48. ANNES JP, MUNGER JS, RIKDIN DB. Making sense of latent TGFbeta activation. J Cell Sci 2003; 116:217-24; PMID:12482908; http://dx.doi.org/10.1242/jcs.00229

49. Debaq-Chaïniaux F, Pascal T, Boilain E, Bastin C, Bauwens E, Toussaint O. Screening of senescence-associated genes with specific DNA array reveals the role of IGFBP-3 in premature senescence of human diploid fibroblasts. Free Radic Biol Med 2008; 44:1817-32; PMID:18320988; http://dx.doi.org/10.1016/j.freeradbiomed.2008.02.001

50. Fripriat C, Dewelle J, Remacle J, Toussaint O. Signal transduction in H2O2-induced senescence-like phenotype in human diploid fibroblasts. Free Radic Biol Med 2002; 33:1354-46; PMID:12419465; http://dx.doi.org/10.1016/S0891-5849(02)01044-4

51. Borlon C, Vankoningloon S, Godard P, Debaq-Chaïniaux F, Toussaint O. Identification of p53-dependent genes potentially involved in UVB-mediated premature senescence of human skin fibroblasts using siRNA technology. Mech Ageing Dev 2008; 129:109-19; PMID:18068755; http://dx.doi.org/10.1016/j.mad.2007.01.014

52. Herrmann G, Brenneis P, Wlaschek M, Jenk F, Kaiser K, et al. Psoralen photoinactivation promotes morphological and functional changes in fibroblasts in vitro reminiscent of cellular senescence. J Cell Sci 1998; 111:759-67; PMID:9472004

53. Joshi PC, Pathak MA. Production of singlet oxygen and superoxide radicals by psoralsens and their biological significance. Biochem Biophys Res Commun 1983; 122:638-46; PMID:6303326; http://dx.doi.org/10.1016/0006-291X(83)91511-5

54. Ma W, Hommel C, Brenneis P, Peters T, Smrit N, Sedivy J, et al. Long-term growth arrest of PUVA-treated fibroblasts in G2/M in the absence of p16(INK4a) p21(CIP1) or p53. Exp Dermatol 2000; 9:629-37; PMID:11272250; http://dx.doi.org/10.1034/j.1600-0625.2000.00024.x

55. Borlon C, Debaq-Chaïniaux F, Hinrichs C, Scharffter-Kochanek K, Toussaint O, Wlaschek M. The gene expression profile of psoralen plus UVA-induced premature senescence in skin fibroblasts resembles a combined DNA-damage and stress-induced cellular senescence response phenotype. Exp Gerontol 2007; 42:911-23; PMID:17574436; http://dx.doi.org/10.1016/j.exger.2007.04.009

56. Bertrand-Vallely V, Boilain E, Ninane N, Demazy C, Friquet B, Toussaint O, et al. Repeated exposures to UVB induce differentiation rather than senescence of human keratinocytes lacking p16(INK4-A). Biogerontology 2010; 11:167-81; PMID:19554608; http://dx.doi.org/10.1007/s10522-009-9238-y

57. Bertrand-Vallely V, Belot N, Dieu M, Delaive E, Ninane N, Demazy C, Friquet B, Toussaint O, et al. Proteomic profiling of human keratinocytes undergoing UVB-induced alternative differentiation reveals TRLparticle Motif Protein 29 as a survival factor. PLoS One 2010; 5:e10462; PMID:20454669; http://dx.doi.org/10.1371/journal.pone.0010462