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Letter to the Editor

Reduced NF- κ B activity is observed in dermal fibroblasts from black-skinned African individuals

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Abbreviations: AF, black African skin fibroblasts; CF, Caucasian skin fibroblasts.

Key words: African – ageing – Caucasian – NF- κ B – skin fibroblasts

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Background

Skin ageing is the combined result of repeated exposure to ultraviolet radiation and the passage of time, both of which directly or indirectly alter dermal fibroblast behaviour and lead to dermal homeostasis impairment (1–3). Telomere dysfunction (4), reactive oxygen species (ROS) (5) or genotoxic stresses all lead to DNA damages, which eventually cause senescence, associated with increases in p21, p16 or NF- κ B (NF- κ B) levels. Several studies have highlighted increases in NF- κ B activity with age (6,7), inducing cellular senescence (8,9, S1) and inhibiting expression of longevity genes (S2) and ECM anabolism (S3). However, skin or cellular ageing has mainly been studied on Caucasian individuals, frequently characterized by Fitzpatrick skin phototypes I, II or III; whereas skin fibroblasts from black-skinned individuals have rarely been investigated. In this study, we demonstrated, *in vitro*, that NF- κ B, a predominant regulator of ageing, was less activated in dermal fibroblasts derived from individuals of African ancestry (AF) than in Caucasian skin fibroblasts (CF).

Question addressed

Is NF- κ B differentially regulated in dermal fibroblasts from individuals of African and Caucasian ancestry?

Experimental design

Human dermal fibroblasts were sampled from sun-protected areas (i.e. breast and abdomen) in six Caucasian females with Fitzpatrick skin phototypes II or III (mean age: 36.57 \pm 9.95 years) and seven African females characterized by a Fitzpatrick

skin phototype VI (mean age: 37.14 \pm 8.17 years). Cells were provided by Johnson&Johnson Laboratories (Pharmacology department, Val de Reuil, France). Samples were used in Western blots, RT-qPCRs, electromobility shift assays (EMSA) and NF- κ B-Luc reporter assays to evaluate how fibroblasts from both origins behave regarding senescence, stress response and NF- κ B activation.

Statistics

Statistical significance was assessed using an unpaired Student's *t*-test with a Holm–Sidak correction. Western blots were performed in triplicate and representative blots are shown.

More detailed information is available in Appendix S1.

Results

Several studies have suggested, especially in fibroblasts, that NF- κ B can promote ageing, induce senescence and downregulate the expression of dermal extracellular matrix components such as type I collagen. We therefore tested whether the variation in dermal protein content, which generally depends on dermal fibroblast activities, observed between CF and AF individuals (S4), was caused by specific differential NF- κ B regulation (2).

We evaluated events that, according to the literature, depend on NF- κ B. We observed senescence-associated (SA) β -gal-positive cells in CF cultures more frequently than in AF cultures at the same passage number (Fig. 1a, Figure S1a). Furthermore, Western blot analyses revealed higher amounts of p21 and p16, two senescence-associated markers, in later passaged CF

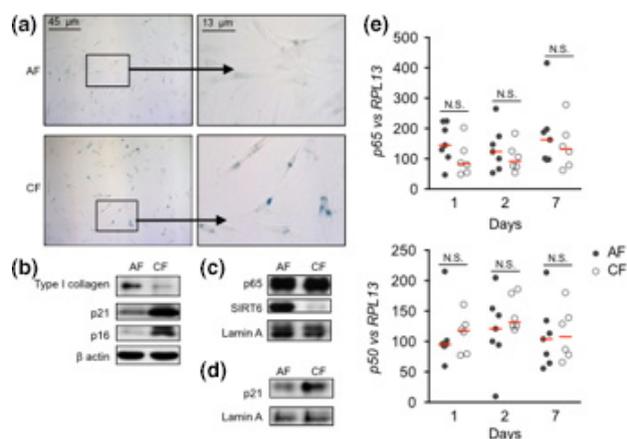


Figure 1. Dermal fibroblasts of Caucasian origin express higher levels of senescence-associated markers compared with fibroblasts of African origin. (a) Cellular senescence of cultured dermal fibroblasts from skin of African (AF) and Caucasian (CF) origin was evaluated using the SA β -gal assay at P5. Representative pictures of four experiments are shown; scale bar: 45 μ m; higher magnification bar: 13 μ m. (b) Representative Western blot analysis of whole-cell lysates of P5 dermal fibroblasts from African and Caucasian individuals (AF and CF). Relative expression of type I collagen, p21, p16 and β -actin (loading control) is shown. (c, d) Nuclear extracts of P5 dermal fibroblasts from black African and Caucasian individuals (AF and CF) were analysed by Western blotting to evaluate their Sirt6, p65 (c) or p21 (d) and Lamin A (loading control) expression levels. Representative pictures are displayed in (c) and (d). In b, c and d, each lane represents a distinct individual. (e) Analysis of the steady-state p65/p50 mRNA levels in P5 dermal fibroblasts from African (AF) and Caucasian (CF) individuals cultured for 7 days. Circles represent tested individuals. Median value is shown in red. Statistics were performed using the Student's *t*-test with a Holm-Sidak correction (N.S.: non-significant).

compared with their AF counterparts (Fig. 1b). Moreover, p21 nuclear levels were higher in CF than in AF at passage 5 (P5) (Fig. 1d). In addition, a lower turnover of type I collagen was observed in AF relative to CF (Fig. 1b, Figure S1). However, these differences in AF and CF were not associated with the level of the senescence-associated marker NF- κ B, previously reported to inhibit type I collagen expression. Comparable p65 and p50 protein and mRNA levels were observed for both fibroblast origins during the seven days of culture (Fig. 1c and e) (S5). Interestingly, Sirt6, which has been reported to prevent p65 transcriptional activity, showed higher levels in AF than in CF (Fig. 1c) (S6). Therefore, we tested whether CF and AF had different NF- κ B activation mechanisms.

I κ B α expression was analysed in the cytoplasmic compartment and a slight increase was observed in AF (Fig. 2a). However, the nuclear fraction showed higher I κ B α levels in AF than in CF, suggesting that NF- κ B DNA-binding activity could be hindered, leading to impaired NF- κ B transcriptional activities on target genes (Fig. 2b) (S7). EMSA confirmed that AF featured lower p65 and p50 DNA-binding activities than CF for the same range of passages and ages (Fig. 2c). Additionally, forced expression of the p65 or p50 subunits resulted in significantly higher NF- κ B-Luc reporter activities only in CF (Fig. 2d). AF thus appeared less responsive to stressful conditions, showing lower NF- κ B activation. Lower phospho-p38, ERK1/2 (extracellular signal-regulated kinase1/2) and phospho-ERK1/2 levels were observed in AF than in CF (Fig. 2e). Furthermore, H₂O₂ treatments drastically increased p38 and phospho-p38 levels in CF compared with AF (S8, S9).

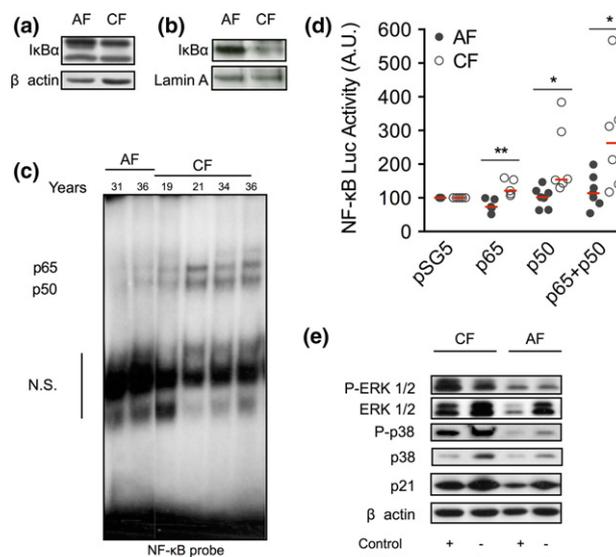


Figure 2. Dermal fibroblasts of African origin show less NF- κ B activity compared with Caucasian fibroblasts. Cytoplasmic (a) and nuclear proteins (b) from P5 dermal fibroblasts from African (AF) and Caucasian individuals (CF) were analysed using Western blots to evaluate I κ B α , β -actin and Lamin A (NF- κ B activity marker, cytoplasmic loading control and nuclear loading control, respectively). (c) Nuclear extracts from P5 dermal fibroblasts of African and Caucasian individuals were analysed using EMSA. Arrows indicate the complexes formed between the DNA probe and p50/p65 subunits. N.S.: non-specific binding. Respective ages (years) are presented above the lanes. (d) NF- κ B-Luc reporter activity from AF and CF transiently transfected with p65 and/or p50 expression vectors or with an empty vector (pSG5). Each circle represents a tested individual. Median value is shown in red. Statistics were performed using the Student's *t*-test with a Holm-Sidak correction (**P* < 0.05; ***P* < 0.01). (e) Whole-cell lysates of P5 dermal fibroblasts from African (AF) and Caucasian individuals treated with H₂O₂ (400 μ M) were analysed using Western blots to evaluate the relative expression of ERK1/2, phospho-ERK1/2, p38, phospho-p38, p21 and β -actin (loading control). In a, b, c and e, representative pictures are shown. In a, b and d, each lane represents a distinct fibroblast donor.

Conclusions

Our work demonstrates that dermal fibroblasts derived from black African skin types have weaker intrinsic abilities to activate NF- κ B compared with skin fibroblasts of Caucasian origin. In agreement with a delayed skin ageing, AF display slower collagen turnover, as NF- κ B has been described as a potent inhibitor of type I collagen expression. Finally, our results suggest that AF may have higher resistance to senescence or ageing initiation, two mechanisms depending on a NF- κ B activation.

Author contribution

NB, PG and TO designed the experiments. NB, TGL and FG performed the experiments. NB, TO and PG analysed the data. NB, MH, FL, MD and TO contributed reagents, materials and analysis tools. NB and PG wrote the manuscript.

Conflict of interest

The authors declared that they have no conflict of interest.

Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Figure S1. (a) Percentage of senescence-associated positive cells. Cultured fibroblasts at P5 from skin of African and Caucasian origin were submitted to a SA β -gal assay.

Appendix S1. Supplementary Materials and Methods.

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Letter to the Editor

Mechanisms of BRAFi-induced hyperproliferative cutaneous conditions

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Key words: BRAF inhibitors – melanoma – squamous cell carcinoma – verrucal keratosis

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Background

It is well recognized that cutaneous conditions such as verrucal keratosis (VK) and squamous cell carcinoma (cuSCC) are one of the most frequent and significant adverse events associated with the BRAF inhibitors (BRAFi), vemurafenib and dabrafenib. These agents are first line in the treatment of V600 mutant metastatic melanoma and have been shown to induce hyperproliferation of wild-type BRAF keratinocytes. (1–3).

The mechanism by which BRAFi induce hyperproliferation in keratinocytes has been postulated to be a result of paradoxical activation of the mitogen-activated protein kinase (MAPK) pathway in ^{wt}BRAF cells. Heidorn *et al.*(2) hypothesized that this was a result of the BRAFi forming a dimer with ^{wt}BRAF which then acts as a scaffold to activate CRAF, thereby activating MEK and ERK (2).

Questions addressed

In this study, we aimed to analyse the molecular interplay involved in the formation of BRAFi-induced hyperproliferative keratinocytic lesions, cuSCC and VK, seen in patients taking BRAFi for stage IV metastatic melanoma.

Experimental design

cuSCC and VK samples were collected from patients treated with BRAFi. Selected samples were previously tested for mutation using Oncocarta v1.0 panel (SequenomTM, Brisbane Australia). After histological diagnosis, tissue samples underwent immunohistochemistry analysis with antibodies directed against components of the MAPK pathway (p-BRAF, p-CRAF, p-MEK, p-ERK), Pi3K-AKT

pathway (p-AKT3, Pi3K), cell cycle control molecule cyclin D, markers of cellular proliferation (Ki67, keratin 6), keratin 10 and oncogenic protein (p53). Normal epidermis from the biopsy was used as control.

Antibodies were purchased from Abcam, Spring or Cell Marque. p-BRAF antibody was abandoned due to inability to optimize staining. Slide staining was performed using the IPX platform (Dako Autostainer Plus). Automated steps using Novocastra products were applied in sequence, protein blocking (RE7158), primary antibody application, secondary layer (RE7260-K), DAB visualization (RE7163) and a haematoxylin counterstain.

High-resolution scanned images were obtained using Leica SCN 400 Client and the expression of markers (presence, intensity and location of stain) assessed by two reviewers. Diseased skin was compared to normal skin found at the periphery of the tissue sample. Intensity was rated on a scale of 0–3 at five separate locations (basal layer, suprabasal layer, stratum spinosum, stratum granulosum and infiltrate). Scores from the two reviewers were combined and recoded in three categories: none, mild and strong stain. All results were recorded in Filemaker pro 11.0v4. Chi-square statistical analysis was performed using JMP 7.0.

Results

Twenty tissue specimens were collected from 14 patients treated with the BRAFi dabrafenib, either alone ($n = 13$) or in combination with the MEK inhibitor (MEKi) trametinib ($n = 1$) (Table S1) for stage IV metastatic melanoma. This included 10