HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2012 December 01.

Published in final edited form as:

J Invest Dermatol. 2012 June ; 132(6): 1591–1596. doi:10.1038/jid.2012.29.

Fractionated laser resurfacing corrects the inappropriate UVB response in geriatric skin

Dan F Spandau[1,2,6], **Davina A. Lewis**[1], **Ally-Khan Somani**[1], and **Jeffrey B. Travers**[1,3,4,5]

[1]Department of Dermatology, Indiana University School of Medicine, Indianapolis, Indiana 46202

[2]Department of Biochemistry & Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana 46202

[3]Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana 46202

[4]Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana 46202

[5]Richard L. Roudebush V.A. Medical Center, Indiana University School of Medicine, Indianapolis, Indiana 46202

Abstract

Non-melanoma skin cancer is a disease primarily afflicting geriatric patients as evidenced by the fact that 80% of all non-melanoma skin cancers are diagnosed in patients over the age of 60 years. As such, geriatric skin responds to cancer-inducing UVB irradiation in a manner that allows the establishment of tumor cells. Currently, the only effective treatment for non-melanoma skin cancer is the removal of the tumors after they appear, indicating the need for a more cost-effective prophylactic therapy. Geriatric volunteers were treated with fractionated laser resurfacing therapy on either sun-protected (upper buttocks) or chronically sun-exposed (dorsal forearm) skin. Fractionated laser resurfacing therapy was demonstrated to decrease the occurrence of senescent fibroblasts in geriatric dermis, increase the dermal expression of insulin-like growth factor-1, and correct the inappropriate UVB response observed in untreated geriatric skin. These responses to fractionated laser resurfacing were equal to the effects seen previously using the more aggressive wounding following dermabrasion. Furthermore, fractionated laser resurfacing was equally effective in both sun-protected and sun-exposed skin. The ability of fractionated laser resurfacing treatment to protect against the occurrence of UVB-damaged proliferating keratinocytes indicates

[6]Corresponding author: Dan F Spandau, 975 West Walnut Street, Room 349, Medical Research and Library Building, Indianapolis, IN 46202-5121, TEL: 317 274-7115, FAX: 317 278-2815, dspanda@iupui.edu.

CONFLICT OF INTEREST

The authors state no conflict of interest.

Supporting Information. Three additional figures, a table, and specific protocols for quantitative reverse-transcription PCR, immunofluorescence, quantification of senescent fibroblasts, UVB irradiation, and immunohistochemistry can be found in the Supplementary Material. In addition, Methods and Materials for the previously presented data shown in Figures 1 and 3 are provided in the Supplementary Material.

the potential of fractionated laser resurfacing to reduce or prevent aging-associated non-melanoma skin cancer.

INTRODUCTION

The annual incidence of non-melanoma skin cancer (NMSC) is consistently the highest of all cancers worldwide [American Cancer Society, 2010; Rogers et al, 2010]. However, despite intensive education efforts instructing individuals to avoid or protect themselves against NMSC-inducing sun exposure, the number of newly diagnosed NMSC lesions is still growing each year [American Cancer Society, 2010]. As such, the only currently used effective treatment for NMSC is the removal of the tumors after they appear. This type of reactive treatment of NMSC is prohibitively expensive and can be traumatic and disfiguring to patients. In fact, while the occurrence of NMSC is only rarely life-threatening in the general population, it is still the fifth most costly cancer to treat [Bickers et al, 2006; Housman et al, 2003]. Therefore, there is an exquisite need for a prophylactic therapy which could reduce the occurrence of NMSC, especially in highly susceptible geriatric populations.

Clearly, NMSC is a disease primarily afflicting geriatric patients as evidenced by the fact that only 20% of all NMSC are diagnosed in patients under the age of 60 years old [Kraemer, 1997; National Institutes of Health, 2010]. Furthermore NMSC, especially squamous cell carcinoma and actinic keratosis, tend to only develop in areas of significant sun exposure and significant solar damage [Albert & Weinstock, 2005; Lewis & Weinstock, 2004; Weinstock et al, 2009; Ciscione et al, 2009; de Berker et al, 2007; Higashi et al, 2004]. The use of sunscreen and sun avoidance has been demonstrated to protect against actinic neoplasia in geriatric populations, indicating that this is an ongoing process, not just the end result of previous sun exposure many years earlier [Thompson & Marks, 1993; Naylor et al, 1995]. Recently our laboratories have provided substantial evidence which explains why geriatric patients have an increased susceptibility to NMSC [Lewis et al, 2008a; Lewis et al, 2010a; Lewis et al, 2011b; Lewis et al, 2011]. The manner in which keratinocytes respond to UVB irradiation is dependent on the activation of the insulin-like growth factor-1 receptor (IGF-1R) on epidermal keratinocytes [Kuhn et al, 1999; Lewis et al, 2008b]. Ligand-bound activated IGF-1R is required in order for keratinocytes to respond appropriately to UVB exposure [Kuhn et al, 1999; Lewis et al, 2008b]. Because human epidermal keratinocytes do not produce IGF-1, the IGF-1R is primarily activated by IGF-1 produced and secreted by adjacent fibroblasts in the papillary dermis. Unfortunately, the expression of IGF-1 in the dermis diminishes with age due to an increasing population of senescent fibroblasts [Lewis et al, 2010a]. Therefore, the skin of geriatric individuals is often deficient in IGF-1 leading to insufficient activation of the IGF-1R in geriatric epidermal keratinocytes [Lewis et al, 2010a; Lewis et al, 2011]. When exposed to UVB radiation, this IGF-1 deficiency results in an inappropriate response in epidermal keratinocytes which could permit the establishment of UVB-induced mutations in geriatric skin, the initial step in the progression to NMSC [Albert & Weinstock, 2005]. In this regard, therapies that correct the aging-associated silencing of IGF-1 expression should restore the appropriate UVB response in geriatric skin and correspondingly reduce the incidence of NMSC in this susceptible population [Lewis et al, 2008a; Lewis et al, 2010a].

Photorejuvenation techniques such as dermabrasion and fractionated laser resurfacing (FLR) have been employed for many years to create more youthful-appearing skin [Ramos-e-Silva & da Silva Carneiro, 2007; Friedman & Lippitz, 2009]. We have previously demonstrated that dermabrasion of geriatric sun-protected skin can restore young adult levels of IGF-1 expression and correct the inappropriate UVB response that occurs in geriatric skin. These encouraging results are tempered by the short-term traumatic effects of dermabrasion on patient's skin. In contrast, FLR is dramatically less distressful to patients; in fact, the downtime in patient activity following FLR is negligible. The present studies examine the ability of wounding by FLR to decrease the numbers of senescent fibroblasts, increase dermal IGF-1 levels and protect against the production of keratinocytes proliferating with UVB-induced DNA damage twenty-four hours following exposure to a minimal erythema dose of UVB. Furthermore, we have tested the effect of FLR on both aged sun-protected skin and the more clinically relevant sun-exposed skin. As these studies indicate that these photorejuvenation procedures result in the complete reversal of the geriatric inappropriate UVB response, they hold the potential of protecting against actinic neoplasia.

RESULTS

Similar to dermabrasion, treatment of geriatric skin with fractionated laser resurfacing increases collagen expression

One of the biological features of aging skin is the reduction of collagen expression in geriatric skin as compared to young adult skin [Farage et al, 2010; Mine et al, 2008; Fisher et al, 2008]. Previously it has been demonstrated that treating geriatric skin with dermabrasion greatly increases the expression of type I collagen [Lewis et al, 2011].

However, dermabrasion therapy with removal of the entire epidermis and the superficial papillary dermis, has considerable morbidity and involves a recovery period (Fig. 1A) until the treated area has healed. In addition, dermabrasion can result in scarring and have long term pigmentary changes. Fractionated laser resurfacing (FLR) is a less ablative cosmetic therapy that avoids both of these drawbacks associated with dermabraded skin [Alexiades-Armenakas et al, 2008] (Fig. 1A). To determine whether treatment of geriatric skin with FLR increases collagen 1 expression, geriatric volunteers were treated with FLR on either sun-protected skin (upper buttocks) or chronically sun-exposed skin (dorsal forearm) as described in the Methods. Three months following FLR treatment, the patients returned and punch biopsies were obtained from FLR-treated skin as well as adjacent untreated skin. Collagen 1 expression was determined by QRT-PCR analysis of each biopsy. As shown in Fig. 1B, the less invasive FLR treatment increased collagen 1 expression in a manner similar to that observed in dermabraded skin.

Fractionated laser resurfacing decreases the relative number of senescent fibroblasts in both sun-protected and sun-exposed geriatric skin

As skin ages, the papillary dermis in sun-protected skin has an increasing proportion of senescent fibroblasts as compared to young adult skin [Mine et al, 2008; Coppe et al, 2010]. To determine if chronic sun-exposure influenced the accumulation of senescent fibroblasts, biopsies from geriatric sun-protected skin and sun-exposed skin were assayed for markers of

senescent fibroblasts (as shown in Fig. 2A). Previously, histologic changes in photodamaged skin included an increase in the proportion of senescent fibroblasts [Fisher et al, 2008]. Surprisingly in our studies, no differences in the proportion of senescent fibroblasts were observed in untreated sun-protected geriatric skin versus sun-exposed geriatric skin indicating that intrinsic aging appears to be as important as extrinsic aging in the studied population (Fig. S1A). To determine if FLR could reduce the percentage of senescent fibroblasts in geriatric skin, volunteers were treated as described above (both sun-protected and sun-exposed skin) and the treated biopsies were assayed for markers of senescent fibroblasts (Fig. 2A). Following FLR treatment, the percentage of senescent fibroblasts was significantly reduced in both sun-protected and sun-exposed skin. In fact, the reduction in senescent fibroblasts in FLR-treated sun-protected skin was significantly greater than that seen in dermabraded sun-protected skin (Fig. 2B; Geriatric DA vs Geriatric FLR (SP), p=0.013, student ttest).

Fractionated laser resurfacing restores the appropriate UVB response in geriatric skin while increasing dermal IGF-1 expression

As previously described, sun-protected geriatric skin exhibits an aberrant pro-carcinogenic UVB response due to IGF-1 silencing in senescent geriatric keratinocytes [15–18]. To date, we have only examined the effects of chronological aging on sun-protected skin so that actinic damage did not influence our interpretation of those results. However, UVB-induced skin cancer only occurs on sun-exposed skin [Albert & Weinstock, 2005] which necessitated a comparison of the UVB response on sun-protected and sun-exposed skin. Geriatric volunteers were UVB-irradiated either on their upper buttocks (sun-protected skin) or on their dorsal forearm (sun-exposed skin). Twenty-four hours post-UVB irradiation, biopsies of the irradiated skin, as well as adjacent unirradiated skin, were collected and assayed for IGF-1 expression and the UVB response [Lewis et al, 2010a]. No significant differences were observed for IGF-1 expression, UVB-induced DNA damage (a crude marker of DNA repair), or in the inappropriate UVB response between sun-exposed and sun-protected skin (Fig. S1), indicating based on these parameters the UVB response of sun-protected and sun-exposed geriatric skin is equivalent.

Treatment of geriatric skin with dermabrasion corrects the inappropriate UVB response and increases IGF-1 signaling in geriatric papillary dermis [Lewis et al, 2011]. In addition, dermabrasion has been shown to be an effective treatment and prophylactic therapy for NMSC [Benedetto et al, 1992; Coleman et al, 1996]. However, the physical and psychological consequences of dermabrasion treatment render it impractical for widespread usage in NMSC therapy. Therefore, the ability of the less ablative FLR treatment to influence the UVB response in geriatric skin was evaluated. Using the subject biopsies described above, the expression of IGF-1 was measured via QRT-PCR (Fig. 3A; [Lewis et al, 2010a]) and the number of basal layer keratinocytes containing both UVB-induced DNA damage and replication proteins (Fig. 3B; [Lewis et al, 2010a]) were determined at 24 hours post-UVB irradiation [Lewis et al, 2010a; Lewis et al, 2011]. FLR treatment on either sun-protected or sun-exposed skin significantly increased IGF-1 expression in the geriatric dermis. In fact, the level of IGF-1 expression in FLR-treated skin was indistinguishable from that of young adult controls (Fig. 3A; [Lewis et al, 2010a]). Furthermore, FLR-treatment

virtually eliminated the inappropriate UVB response shown in matched geriatric control tissue. This reduction in the inappropriate UVB response exceeds that previously observed in dermabraded geriatric skin (Fig. 3B; [Lewis et al, 2011]). FLR treatment does not significantly increase the volume of epidermal or papillary dermis tissue (Fig. S2A), but similar to dermabrasion, FLR treatment does increase the density of fibroblasts in the papillary dermis of sun-exposed skin (Fig. S2B; [Lewis et al, 2011]). Furthermore, FLR treatment appears to reverse the age-dependent decrease in UVB-induced DNA repair response seen in geriatric skin (Fig. S3). The number of basal layer keratinocytes containing UVB-induced DNA lesions at twenty-four hours post-irradiation in dermabraded skin is statistically the same as the elevated number observed in untreated geriatric epidermis (Fig. S3). In contrast, FLR-treated geriatric skin has similar low numbers of keratinocytes containing UVB-damaged DNA at the same timepoint as found in young adult skin. These data indicate that FLR treatment more than exceeds the previously described corrective ability of dermabrasion in restoring the appropriate UVB response in geriatric skin. However, FLR has the added benefit of obtaining the desired results without the extensive tissue damage associated with dermabrasion treatment (Fig. 1A; [Friedman & Lippitz, 2009]).

The number of photodamaged keratinocytes in chronically sun-exposed skin is reduced by fractionated laser resurfacing

Chronically sun-exposed skin in geriatric patients has been shown to have actinically damaged keratinocytes, which can be identified by overexpression of the p53 protein [El-Domyati et al, 2007]. To determine the effect of FLR treatment on these damaged keratinocytes, matched pairs of control and FLR-treated geriatric skin biopsies were analyzed for p53 expression (example shown in Fig. 4A). As seen in Fig. 4B, FLR treatment decreased the expression of p53 as compared to control untreated tissue. These data indicate that in addition to correcting the inappropriate UVB response in geriatric skin, FLR also reduces the proportion of endogenous actinically-damaged keratinocytes in sun-exposed geriatric epidermis.

DISCUSSION

Previously, we have demonstrated that geriatric skin lacks the ability to respond appropriately when exposed to the wavelengths of UVB found in sunlight, as compared to the ability of younger skin [Lewis et al, 2010a]. Almost certainly, this failure to adequately cope with UVB-damaged keratinocytes in geriatric skin contributes to the increased susceptibility of elderly individuals to NMSC by enhancing the likelihood of UVB-induced mutations in epidermal keratinocytes [Lewis et al, 2008a; Lewis et al, 2010b]. As this aberrant response to UVB exposure is due to the accumulation of senescent fibroblasts in geriatric dermis [Lewis et al, 2010a], a reduction in the proportion of senescent fibroblasts should correct the inappropriate UVB response in geriatric skin and therefore could reduce the incidence of aging-associated NMSC [Lewis et al, 2008a; Lewis et al, 2010b]. We now report that the treatment of geriatric skin with FLR therapy reduces the percentage of senescent fibroblasts in geriatric papillary dermis and, more importantly, restores the appropriate UVB response of geriatric keratinocytes. Furthermore, we have demonstrated

that FLR therapy is effective on both sun-protected and chronically sun-exposed geriatric skin. By inducing a mild wound healing response, FLR therapy increases the percentage of replicating fibroblasts in the papillary dermis. These newly recruited youthful fibroblasts actively produce new collagens which partially accounts for the cosmetic benefits of FLR on geriatric skin. Additionally, the new fibroblasts also have increased expression of IGF-1 which serves to correct the IGF-1 deficiency seen in untreated geriatric skin. This restoration of adequate IGF-1 expression in geriatric skin is responsible for correcting the inappropriate UVB response in geriatric keratinocytes.

These findings are important because treating NMSC, particularly highly susceptible geriatric populations, has a high economic burden on our healthcare system, in part due to the need for multiple surgical treatments over many years [Bickers et al, 2006; Housman et al, 2003]. The development of a prophylactic therapy which could be used on at-risk actinically damaged skin could substantially reduce the overall treatment costs of NMSC. Interestingly, there have been isolated reports and small studies which indicate that cosmetic wounding techniques, including FLR and dermabrasion, have been used to treat existing NMSC [Halachmi & Lapidoth, 2008; Choudhary et al, 2011]. In fact, dermabrasion has also been reported to successfully prevent the recurrence of actinic keratosis and NMSC [Field, 2007]; however, the extensive cutaneous damage inherent with dermabrasion therapy is often unacceptable [Field, 2007]. In contrast, the specific FLR treatment used in this study has none of the debilitating secondary effects seen in dermabrasion therapy. More importantly, in our studies FLR treatment was equal if not superior to dermabrasion in correcting the inappropriate UVB response in geriatric skin.

While our previous studies have provided a potential mechanism for the protective effects of dermabrasion treatment on the prevention of actinic keratosis and NMSC, we can now suggest that other wounding modalities, such as FLR, may have similar protective effects by decreasing the proportion of senescent fibroblasts in geriatric dermis, upregulating IGF-1 expression, and reversing the inappropriate UVB response. While the benefits of FLR on at-risk geriatric skin will need to be studied further, these data indicate the potential of relatively benign FLR treatment to reduce or prevent aging-associated NMSC.

MATERIALS AND METHODS

Human volunteers

Geriatric volunteers (aged 65 years old and older) were recruited from patients treated at dermatology clinics within the Indiana University medical center (Table SI). The studies have the approval of the Indiana University School of Medicine Institutional Review Board in accordance with the Declaration of Helsinki protocols. Specific requirements for inclusion and exclusion criteria can be found in the Supplemental Materials.

Fractionated Laser Resurfacing

All subjects were thoroughly briefed on the risks and benefits of participating in the study and they signed an informed consent statement attesting to their willful participation. Geriatric volunteers who met the criteria for inclusion in this study were divided into two

cohorts, one group was be treated with FLR on sun-protected skin (upper buttocks) while the second group was treated on chronically sun-exposed skin (dorsal forearm). Members of each cohort underwent wounding of a small 5 × 5 cm area of either the upper buttocks or dorsal forearm with two passes at 12% coverage using 120 mJ of energy per microspot (Pearl Fractional Laser, Cutera, Inc., Brisbane, CA) without anesthesia. The Pearl Fractional Laser is a 2790nm yittrium scandium gallium garnet ablative fractional resurfacing device that thermally ablates microscopic columns of epidermal and dermal tissue in regularly spaced arrays. Patients were given wound care instructions and asked to return in three months.

Human UVB response assay

On return, a localized area 1 × 1 cm of either FLR-treated skin or untreated normal skin (on the opposite hip/buttock or forearm) was irradiated with dose of 350 J/m^2 of UVB. In Fitzpatrick Skin Types I and II, this dose of UVB is sufficient to cause a minimal erythematous reaction. Permanent marker was used to outline the areas of skin that was irradiated. Twenty-four hours following UVB exposure, photographs were taken of the skin to document the extent of the UVB reaction. A portion of the irradiated skin, as well as unirradiated adjacent skin, was removed by punch biopsy, (4 mm punch biopsies of the UVB-treated skin and 3 mm punch biopsies of unirradiated skin; 4 biopsies per individual). The epidermal response to UVB irradiation was assayed as previously described [Lewis et al, 2010a; Lewis et al, 2011]. Briefly, thin paraffin-embedded sections from unirradiated and UVB-irradiated biopsies were simultaneously stained with antibodies to Ki67 and thymine dimers. Secondary antibodies that specifically detect only one of the primary antibodies are conjugated to the fluorescent dyes AlexaFluor 488 (detecting Ki67, emitting green wavelengths), and AlexaFluor 568 (detecting thymine dimers, emitting red wavelengths). Images were captured sequentially along the entire length of the biopsy specimen (3mm non-irradiated, 4mm irradiated) using a Nikon Eclipse 80i microscope with Intensilight epifluorescence. These images were analyzed by counting the number of keratinocytes in contact with the basement membrane that are Ki67(+), thymine dimer(+), and Ki67(+):thymine dimer(+). These numbers were expressed as a percentage of total basal layer keratinocytes in the biopsy specimen (determined by counting basal layer keratinocytes for each specimen on H&E-stained slides).

Statistical analysis

Statistical analyses were done by two-tailed Student’s t test. Statistical significance was defined as $p < 0.05$ unless otherwise noted in the figure legend. ANOVA analysis was used to compare multiple cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to Dr. Raymond Konger (Indiana University Departments of Pathology and Dermatology) for his valuable suggestions. This work was supported by grants from the National Institutes of Health (R21CA131901 to

DFS; R01HL062996; U19A1070448 to JBT), VA Merit Award (JBT), and a Dermatology Foundation Career Development Award (AKS). The authors have no conflicts of interest in regard to the data presented in this manuscript.

References

Albert MR, Weinstock MA. Keratinocyte carcinoma. CA Cancer J Clin. 2005; 53:292–302. [PubMed: 14570228]

American Cancer Society. Cancer Facts and Figures 2010. www.cancer.org/downloads/STT/Cancer_Facts_and_Figures_2010.pdf

Alexiades-Armenakas MR, Dover JS, Arndt KA. The spectrum of laser skin resurfacing: nonablative, fractional, and ablative laser resurfacing. J Amer Acad Derm. 2008; 58:719–737. [PubMed: 18423256]

Benedetto AV, Griffin TD, Benedetto EA, et al. Dermabrasion: therapy and prophylaxis of the photoaged face. J Am Acad Dermatol. 1992; 27:439–447. [PubMed: 1401281]

Bickers DR, Lim HW, Margolis D, et al. The burden of skin diseases. J Am Acad Dermatol. 2006; 55:490–500. [PubMed: 16908356]

Choudhary S, Tang J, Elsaie ML, et al. Lasers in the treatment of nonmelanoma skin cancer. Dermatol Surg. 2011; 37:409–425. [PubMed: 21414072]

Coleman WP, Yarborough JM, Mandy SH. Dermabrasion for prophylaxis and treatment of actinic keratoses. Dermatol Surg. 1996; 22:17–21. [PubMed: 8556252]

Coppe JP, Desprez PY, Krtolica A, et al. The senescence-associated secretory phenotype: the dark side of tumor suppression. Ann Rev Path Mech Dis. 2010; 5:99–118.

Criscione VD, Weinstock MA, Naylor MF, et al. Actinic keratoses. Natural history and risk of malignant transformation in the Veterans Affairs topical tretinoin chemoprevention trial. Cancer. 2009; 21:2523–2530. [PubMed: 19382202]

de Berker D, McGregor J, Hughes BR. Guidelines for the management of actinic keratoses. Br J Dermatol. 2007; 156:222–230. [PubMed: 17223860]

El-Domyati MM, Attia DK, Esmat AM, et al. Effect of laser resurfacing on p53 expression in photoaged facial skin. Dermatol Surg. 2007; 33:668–678. [PubMed: 17550442]

Farage, MA.; Miller, KW.; Maibach, HI. Degenerative changes in aging skin. In: Farage, MA.; Miller, KW.; Maibach, HI., editors. Textbook of Aging Skin. Springer; Berlin Heidelberg: 2010. p. 25-35.

Field LM. The superiority of dermabrasion over laser abrasion in the prophylaxis of malignant and premalignant disease. Dermatol Surg. 2007; 33:258–259. [PubMed: 17300618]

Fisher GJ, Varani J, Voorhees JJ. Looking older: fibroblast collapse and therapeutic implications. Arch Dermatol. 2008; 144:666–672. [PubMed: 18490597]

Friedman S, Lippitz J. Chemical peels, dermabrasion, and laser therapy. Dis Mon. 2009; 55:223–235. [PubMed: 19286056]

Halachmi S, Lapidoth M. Lasers in skin cancer prophylaxis. Exp Rev Anticancer Therapy. 2008; 8:1713–1715.

Higashi MK, Veenstra DL, Langley PC. Health economic evaluation of non-melanoma skin cancer and actinic keratosis. Pharmacoeconomics. 2004; 22:83–94. [PubMed: 14731050]

Housman TS, Feldman SR, Williford PM, et al. Skin cancer is among the most costly of all cancers to treat for the Medicare population. J Am Acad Dermatol. 2003; 48:425–429. [PubMed: 12637924]

Kraemer KH. Sunlight and skin cancer: another link revealed. Proc Natl Acad Sci USA. 1997; 94:11–14. [PubMed: 8990152]

Kuhn C, Kumar M, Hurwitz SA, et al. Activation of the insulin-like growth factor-1 receptor promotes the survival of human keratinocytes following ultraviolet B irradiation. Intl J Cancer. 1999; 80:431–438.

Lewis DA, Travers JB, Spandau DF. A new paradigm for the role of aging in the development of skin cancer. J Invest Dermatol. 2008a; 129:787–791. [PubMed: 18818672]

Lewis DA, Yi Q, Travers JB, et al. UVB-induced senescence in human keratinocytes requires a functional IGF-1R and p53. Mol Biol Cell. 2008b; 19:1346–1353. [PubMed: 18216278]

Lewis DA, Travers JB, Somani AK, et al. The IGF-1/IGF-1R signaling axis in the skin: a new role for the dermis in aging-associated skin cancer. Oncogene. 2010a; 29:1475–1485. [PubMed: 19966862]

Lewis, DA.; Travers, JB.; Spandau, DF. Aging-associated non-melanoma skin cancer: a role for the dermis. In: Farage, MA.; Miller, KW.; Maibach, HI., editors. Textbook of Aging Skin. Springer; Berlin Heidelberg: 2010. p. 588-599.

Lewis DA, Travers JB, Machado C, et al. Reversing the aging stromal phenotype prevents carcinoma initiation. AGING. 2011; 3:407–416. [PubMed: 21515933]

Lewis KG, Weinstock MA. Nonmelanoma skin cancer mortality (1988–2000): The Rhode Island follow-back study. Arch Dermatol. 2004; 140:837–42. [PubMed: 15262694]

Mine S, Fortunel NO, Pageon H, et al. Aging alters functionally human dermal papillary fibroblasts but not reticular fibroblasts: a view of skin morphogenesis and aging. PLoS ONE. 2008; 3:e4066, 1–13. [PubMed: 19115004]

National Institutes of Health. Sun Protection in Cancer Trends Progress Report – 2009/2010. www.cancer.gov

Naylor MF, Boyd A, Smith DW, et al. High sun protection factor sunsreens in the suppression of actinic neoplasia. Arch Dermatol. 1995; 131:170–175. [PubMed: 7857113]

Ramos-e-Silva M, da Silva Carneiro SC. Elderly skin and its rejuvenation: products and procedures for the aging skin. J Cosmetic Dermatol. 2007; 6:40–50.

Rogers HW, Wenstock MA, Harris AR, et al. Incidence estimate of nonmelanoma skin cancer in the United States, 2006. Arch Dermatol. 2010; 146:283–287. [PubMed: 20231499]

Thompson SC, Marks R. Reduction of solar keratoses by regular sunscreen use. New England J Med. 1993; 329:1147–1151. [PubMed: 8377777]

Weinstock MA, Lee K, Chren MM, et al. Quality of life in the actinic neoplasia syndrome: The VA topical tretinoin chemoprevention trial. J Amer Acad Dermatol. 2009; 61:207–215. [PubMed: 19398145]

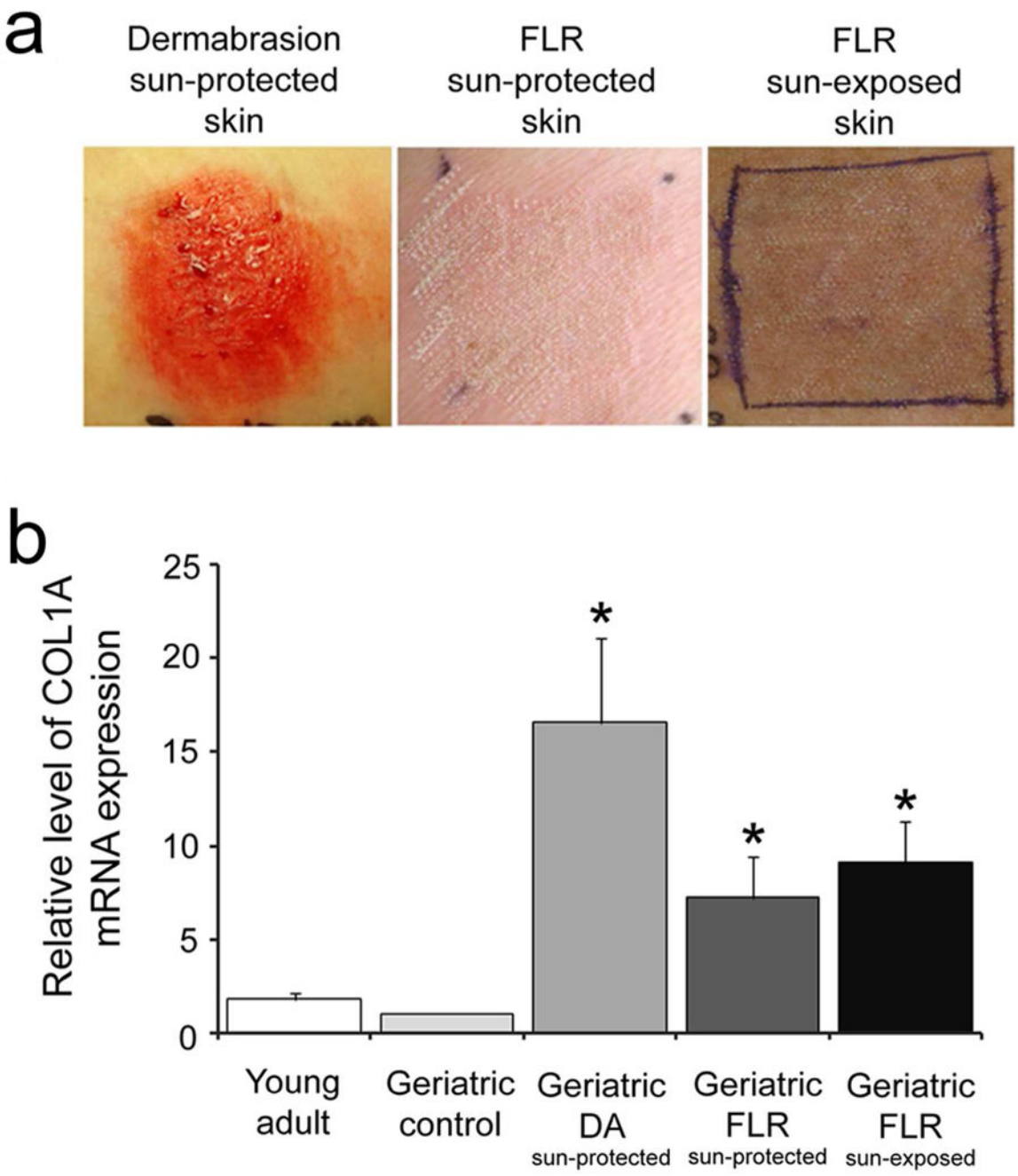

Figure 1. Fractionated laser resurfacing increases collagen expression in geriatric skin

(a) Sun-protected (upper buttocks) or sun-exposed (dorsal forearm) on geriatric (>65 years old) volunteers were treated with dermabrasion (DA) or fractionated laser resurfacing (FLR). Images shown were obtained immediately following treatment. *(b)* Relative *COL1A* expression was assayed by QRT-PCR (standardized by actin expression)from biopsies obtained from sun-protected skin from young adult (20–28 years old) volunteers, sun-protected skin from geriatric volunteers, healed (3 months) sun-protected skin from geriatric patients treated with DA, healed (3 months) sun-protected skin from geriatric patients treated with FLR, and healed (3 months) sun-exposed skin from geriatric patients treated

with FLR. Error bars indicate SEM; asterisks indicate significant difference from geriatric control values ($p<0.05$, student t-test). Young adult (n=4); geriatric control (n=23); geriatric DA (n=6); geriatric FLR sun-protected (n=6); geriatric FLR sun-exposed (n=7). NOTE: Young adult and geriatric DA data are presented in this figure for comparison; similar data have been previously reported in another format [Lewis et al, 2008a; Lewis et al, 2010b].

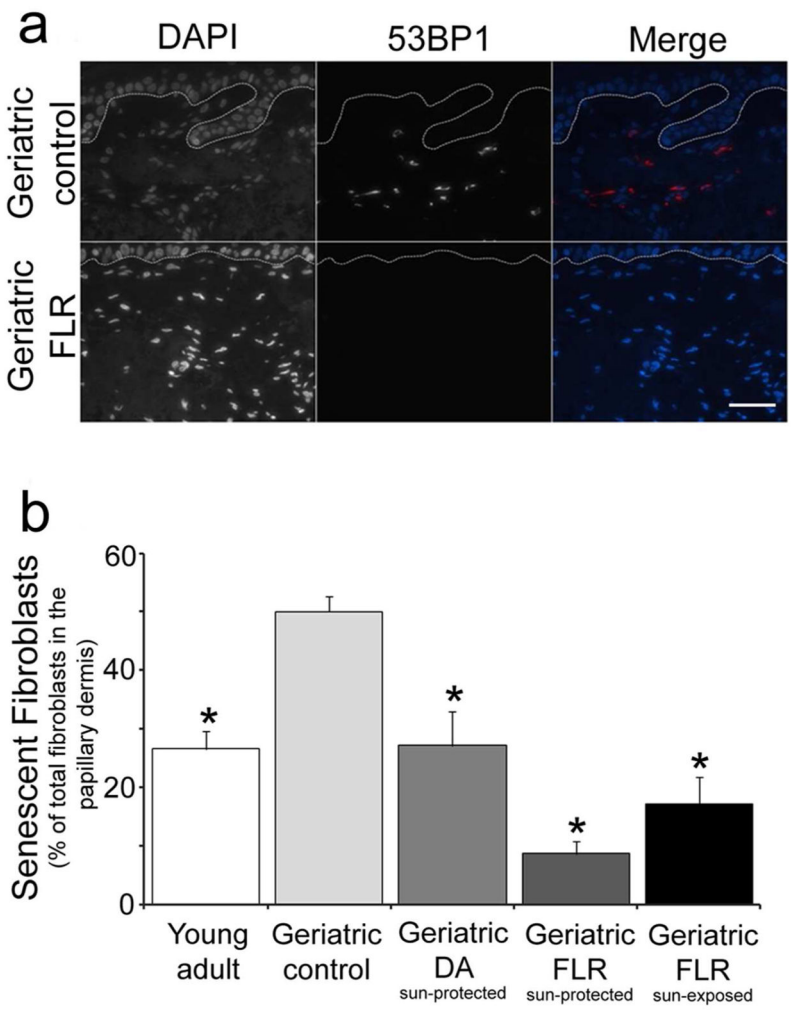

Figure 2. Fractionated laser resurfacing decreases the proportion of senescent fibroblasts found in geriatric skin

(a) Biopsies from untreated control geriatric skin and geriatric skin treated with fractionated laser resurfacing (following 3 months of healing) were stained with antibodies to 53BP1 and DAPI. Senescent fibroblasts in the dermis are indentified by their persistent expression of 53BP1. Bar = 50 μm. *(b)* The percentage of senescent fibroblasts in the papillary dermis of the indicated skin samples were determined by quantifying the number of 53BP1(+) cells in relation to the total number of DAPI(+) fibroblasts. Image analysis was performed as described in the Materials and Methods using Nikon Elements software. Error bars indicate SEM; asterisks indicate significant difference from geriatric control values ($p<0.006$, student t-test). Young adult (n=3); geriatric control (n=22); geriatric DA (n=6); geriatric FLR sun-protected (n=6); geriatric FLR sun-exposed (n=7).

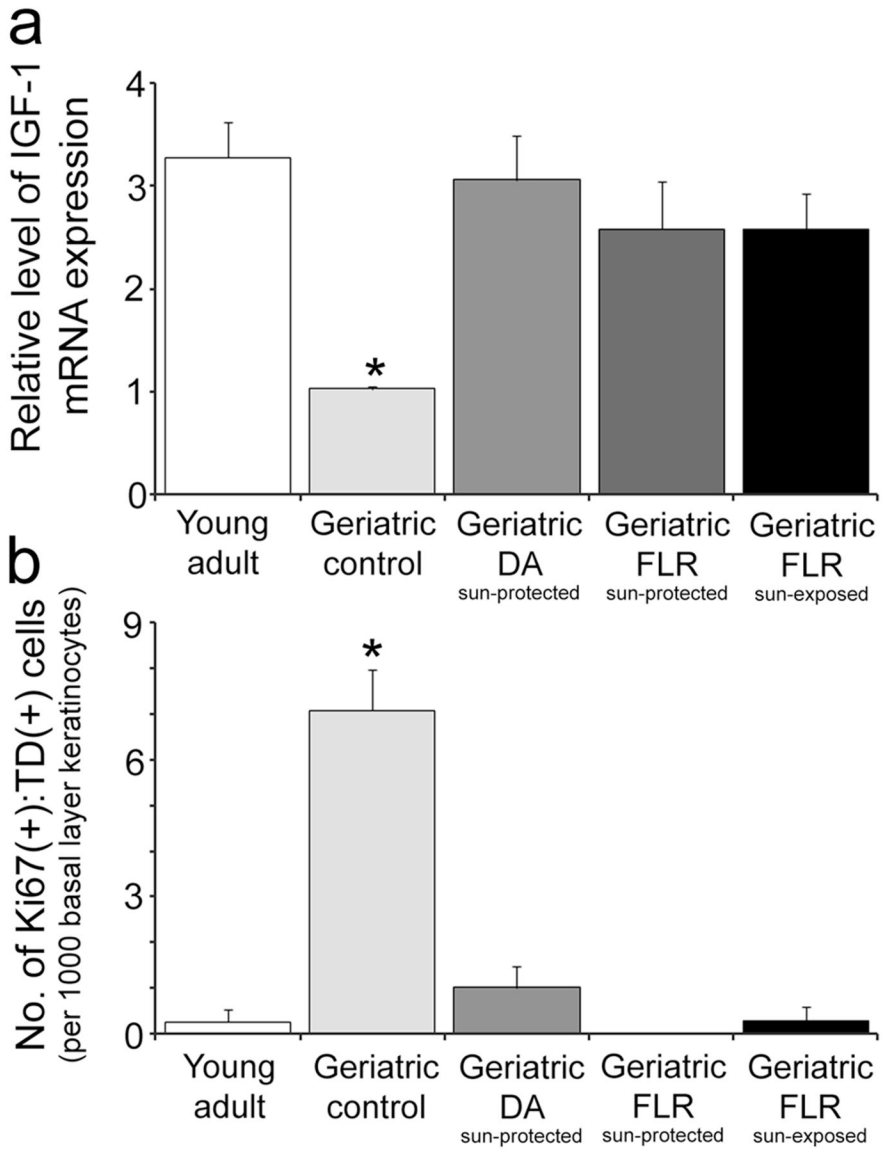

Figure 3. Fractionated laser resurfacing increases dermal IGF-1 expression and restores the appropriate UVB response in geriatric skin

(a) IGF-1 gene expression was measured by QRT-PCR analysis of biopsies from volunteers described in Fig 1B and standardized to actin expression. Error bars indicate SEM; asterisk denotes statistical significance of Geriatric Control values from all other cohorts ($p<0.02$; individual paired t-test). In addition, ANOVA analysis detected no significant difference between Young Adult, Geriatric DA, and Geriatric FLA cohorts. Young adult (n=4); geriatric control (n=27); geriatric DA (n=6); geriatric FLR sun-protected (n=8); geriatric FLR sun-exposed (n=8). *(b)* The skin of the patient cohorts described in Fig. 1 were assayed for their response to UVB irradiation by quantifying the level of basal layer keratinocytes in the biopsies that had detectable UVB-induced DNA damage (TD+) and cellular markers of proliferation (Ki67+) at 24 hours following an exposure to UVB. Error bars indicate SEM; asterisk denotes statistical significance of Geriatric Control values from all other cohorts

($p<0.001$; individual paired t-test). In addition, ANOVA analysis detected no significant difference between Young Adult, Geriatric DA, and Geriatric FLA cohorts. Young adult (n=6); geriatric control (n=27); geriatric DA (n=7); geriatric FLR sun-protected (n=7); geriatric FLR sun-exposed (n=8). NOTE: Young adult and geriatric DA data are presented in this figure for comparison; similar data have been previously reported in another format [Lewis et al, 2008a; Lewis et al, 2010b].

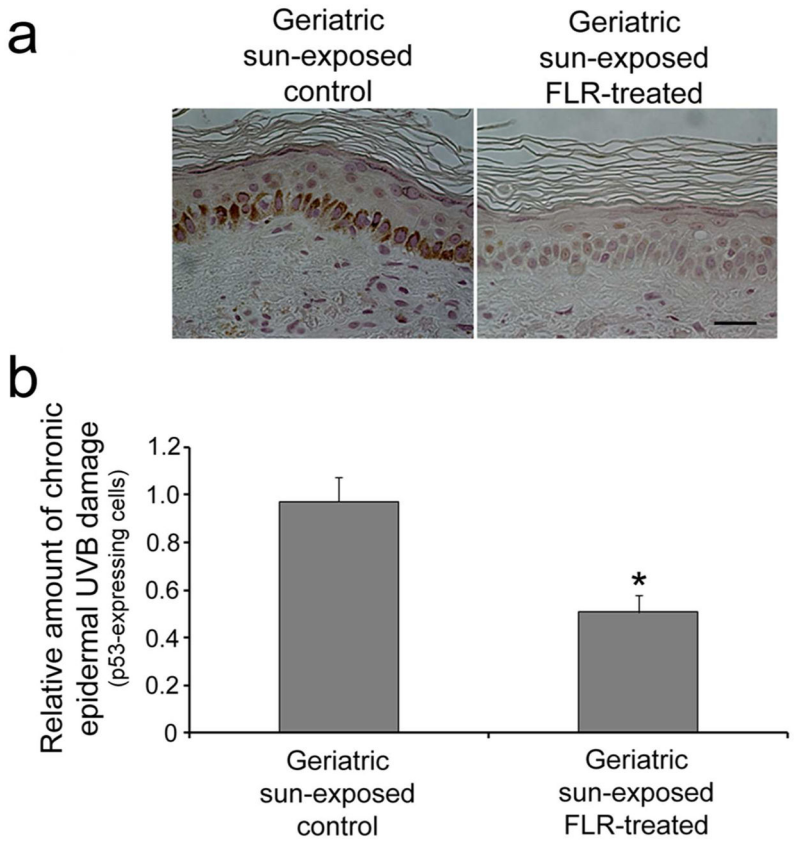

Figure 4. Fractionated laser resurfacing reduces the number of chronically photodamaged keratinocytes in sun-exposed geriatric skin

The expression of the tumor suppressor p53 in the keratinocytes of sun-exposed skin was used as a surrogate marker of chronic photodamage often found in geriatric patients. Matched tissue biopsies from individual patients, one from untreated dorsal forearm and a second from dorsal forearms treated with fractionated laser resurfacing (3 months following the procedure) were assayed for the expression of p53 using immunohistochemistry, *(a)* is an example of one of these matched biopsy sets. Bar = 50 μm. *(b)* The relative expression of p53 was determined for each set of biopsies described in *(a)* using Nikon Elements Image Analysis software. Error bars indicate SEM; asterisks indicate significant difference from untreated geriatric control values ($p<0.002$, student t-test; $n=12$).