

Chronic Inflammation and Vascular Density in Sun-Exposed Skin

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Abstract

Introduction: Prior studies have identified increased chronic inflammation in sun-exposed sites compared to sun-protected sites. Ultraviolet radiation has also been found to promote angiogenesis. We propose a possible relationship between inflammation, angiogenesis and photocarcinogenesis. **Materials and Methods:** Two elliptical biopsies from sun-exposed skin and sun-protected skin were taken from 13 Caucasian cadavers. Dermal vessels and inflammatory cells were counted in H&E stained slides per 10 consecutive high-power fields (400 X). **Results:** Sun-exposed biopsies showed a significant increase in mean numbers of both chronic inflammatory cells and vessels compared to sun-protected biopsies ($p < 0.001$). No statistically significant correlation was found between mean number of vessels and mean number of chronic inflammatory cells in either exposed and protected specimens ($r = -0.37$; $p=0.21$ and $r = 0.24$; $p=0.43$, respectively). **Conclusion:** Sun-exposed skin demonstrates an increase in chronic inflammatory cells and vessels compared to sun-protected skin.

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Introduction

Cutaneous photoaging and carcinogenesis induce microscopic and macroscopic changes resulting from short-term and long-term exposure to ultraviolet light type B (UVB). Clinically, chronic exposure may result in fine lines, wrinkles, fragility, and malignant neoplasms. Acute exposure to UVB radiation has been found to cause epidermal hyperplasia, dilation and enhancement of the dermal vasculature.^{1,2} In addition, biochemical alterations with decreased levels of IFN-beta, an anti-angiogenic mediator, and increased levels of vessel-endothelial-growth factor, TNF-alpha, IL-8, and fibroblast growth factor have been reported following UV radiation.³⁻⁵ The most commonly documented histological changes in chronic sun-exposure include accumulation of glycosaminoglycan, loss of collagen fibers, and production and laying down of abnormal elastin fibers, resulting in solar elastosis.^{6,7} These changes may be due to UVB induction of fibroblasts, mast cells, keratinocytes, endothelial cells, and infiltrating inflammatory cells.^{7,8}

The goal of our study was to observe inflammatory and vascular changes in sun-exposed versus sun-protected skin areas, and determine if these findings of chronic inflammation and vascular changes associated with chronic sun-exposure are consistent in a simple model. The use of cadaver skin allowed for larger specimen sampling. Based on this information and data from other studies, the implications of chronic inflammation and angiogenesis in chronic sun exposure and associated lesions will be discussed. We used a quantitative approach with hematoxylin and eosin (H&E) stained specimens from cadavers.

Materials and Methods

Institutional review board approval for exempt status based on work with cadavers was obtained.

From each of 15 randomly selected Caucasian cadavers, two 2-inch elliptical skin biopsies were obtained: one from a sun-exposed site, defined as the area from the face anterior to the ear, inferior to the hairline and superior to the chin and jaw

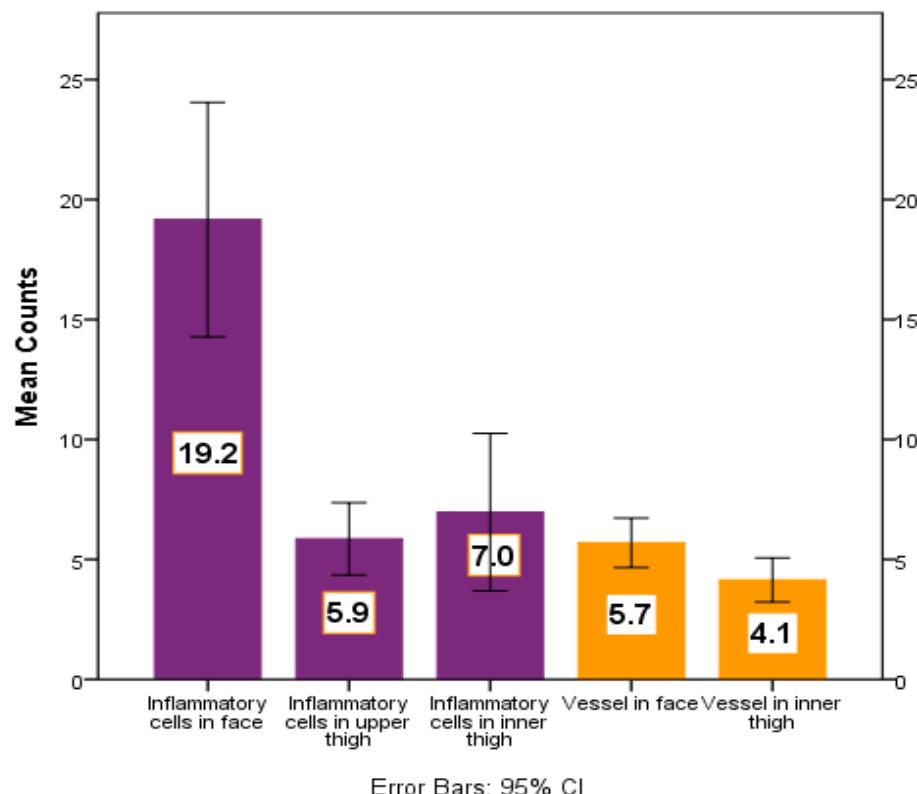
line; and one from a sun-protected site, designated as the upper and inner thigh regions. The tissue underwent routine processing and staining with H&E. Only data from 13 cadavers were statistically analyzed due to poor tissue preservation from two cadavers.

Chronic inflammatory cells (lymphocytes, macrophages, and plasma cells) were manually counted from 10 consecutive high-power fields at 400x magnification, with field placement immediately below the basal layer of the epidermis. In addition, superficial dermal vessels including arterioles, veins, and lymphatic vessels were counted

within each field at the same magnification. In sun-exposed skin, we observed solar elastosis as evidence of UV exposure. We were able to perform multiple field counts due to the amount of tissue available.

Statistical evaluation was performed with IBM SPSS Grad Pack 22. A paired t-test was used to compare mean numbers of inflammatory cells between sun-exposed and sun-protected specimens. A paired t-test was also used to compare the mean numbers of dermal vessels from sun-exposed and sun-protected specimens. A Pearson's correlation was performed to assess the relationship between mean vessel quantities and mean number

Figure 1. Mean inflammatory cells and mean vessels in sun-exposed vs. sun-protected skin.



of inflammatory infiltrates of the two cohorts. For both statistical analyses, $p < 0.05$ was used to designate statistical significance.

Results

Determination of age and gender as covariates was not statistically significant, allowing for justified comparison. Paired samples t-test between the mean inflammatory cell counts in the upper thigh (5.86, [2.50]) and the inner thigh (6.98, [5.43]) revealed no statistical significance ($p = 0.40$). Based on this, the mean value of both inner thigh and upper thigh were used to represent the sun-protected value. Comparison of the mean number of inflammatory infiltrates between face (sun-exposed) (19.17, [8.08]) and the average of upper thigh and inner thigh (sun-protected) (6.42, [3.56]) biopsy specimens was statistically significant ($p < 0.001$). More chronic inflammatory cells were present in sun-exposed skin versus sun-protected skin (Figures 1-3).

Comparison of the mean vessel quantity between face (5.69 [1.71]) and inner thigh (4.15 [1.52]) biopsy specimens was statistically significant ($p = 0.011$), with a greater number of vessels in sun-exposed versus sun-protected skin (Figures 1-3).

A direct relationship between inflammatory-cell quantity and dermal-vessel quantity was not established. Correlation of mean inflammatory cell count and mean dermal vessel count in sun-exposed specimens was not statistically significant ($r = -0.37$, $p = 0.21$), nor was correlation of mean dermal vessel count and mean inflammatory cell count in sun-protected specimens ($r = 0.24$, $p = 0.43$).

Discussion

A limited number of studies have examined the relationship between chronic sun exposure and inflammation. A French study examined specimens of pre-auricular (sun-exposed) and post-auricular (sun-protected) skin for comparative differences in inflammation.⁹ They identified a greater number of mononuclear cells in the dermis of pre-auricular specimens, specifically around areas of elastolysis and perifollicular areas, whereas inflammatory infiltrates of post-auricular skin showed greater evidence of intrinsic aging and were perivascular, perifollicular, and interfollicular in nature.⁹ Immunohistochemical studies with anti-CD68 antibodies to detect macrophages, and tryptase to detect mast cells, have identified a greater number of dermal macrophages and mast cells in sun-exposed skin compared to sun-protected skin.⁹ Specific T-cell antibodies identified a greater number of CD4+ T cells and fewer CD8+ T cells in sun-exposed skin sites compared to sun-protected skin sites. These results, combined with our results, support an association between chronic UV exposure and inflammation. In our study, we designated sun-protected skin as the upper and inner thigh rather than post-auricular skin. It's possible post-auricular skin may have some degree of sun exposure, whereas the inner and upper thigh were less likely to be sun-exposed in a general elderly population.

Inflammatory cells play many roles in the skin, covering both destruction and repair. One study found a greater number of mast cells in sun-exposed skin specimens.⁹ These cells, along with other inflammatory cells, secrete cytokines and metallomatrix proteinases (MMP), specifically

interstitial collagenase (MMP-1). MMP-1 is primarily responsible for the collagen damage found in photodamaged skin.^{10,11} One study employed the use of sunscreen, compared to sunscreen and anti-oxidants, and noted a 43% and 60% decrease in MMP-1 expression, respectively.¹² Of greater clinical significance is the role of inflammatory cells, MMPs, and photocarcinogenesis. MMP gene expression is evident in many cell types, including macrophages, T-cells, monocytes, fibroblasts, keratinocytes, and endothelial cells.¹³ Ultraviolet radiation stimulates growth factor and cytokine receptors located on keratinocytes and fibroblasts, further upregulating transcription of AP-1, a nuclear transcription factor, which then stimulates the production of collagenase (MMP-1), stromelysin 1 (MMP-3), and 92-kda gelatinase (MMP-9).¹⁰ Metallomatrix proteinases act to degrade the extracellular matrix basement membrane, altering cellular architecture and facilitating tumor invasion and metastasis.¹⁴

A Chinese study compared MMP-12 expression in 298 melanoma specimens to MMP-12 expression in 60 normal skin specimens, and found elevated levels of MMP-12 expression in melanoma specimens with a significant association with tumor invasion and metastatic potential.¹⁵ It has been reported that increased expression of MMP-1 and MMP-3 in melanoma metastases correspond with significantly shorter disease-free survival periods.¹⁶ Although the relationship between inflammatory-cell counts/types, MMP expression, and cutaneous progression have not been completely ascertained, together these individual findings may point to a clearer mechanism for photoaging and

photocarcinogenesis in chronic inflammation.

NSAIDs target a group of pro-inflammatory enzymes known as cyclooxygenases, COX-1 and COX-2.¹⁷ COX-2, although not present in normal skin, can be produced in the presence of UVB radiation.¹⁸ UVB is a known environmental carcinogen that allows for the formation of (6-4) pyrimidine-pyrimidine cyclobutane pyrimidine dimers, which initiate and promote photocarcinogenesis.^{19,20} UV-induced COX-2 has been shown to induce prostaglandin-E2 synthesis (PGE-2), resulting in elevated PGE-2, which is able to bind various EP receptors on the surface of cells. EP-1, EP-2, and EP-4 have been identified as playing a role in photocarcinogenesis in murine models.²¹ Through its diverse action on various receptors, PGE-2 has been determined to cause an inflammatory response, aide in tumoral invasion, and inhibit apoptosis.²² COX-2 has been identified in epithelial cells of UVB-induced SKH-1 tumors in mice, in addition to dermal fibroblasts and macrophages within the tumor stroma.²³ One study found COX-2 expression significantly increased in actinic keratoses, Bowen's disease, and squamous cell carcinoma lesions, compared to normal skin, with normal skin having no expression based on study-specific staining standards.²⁴

Another study found COX-2 expression in 90%, 100%, and 88.9% of basal cell carcinomas, squamous cell carcinomas, and actinic keratoses, respectively, with expression not only in the epithelial components of the tumors but also in vessel walls and inflammatory cells.²⁵ The presence of COX-2 within inflammatory cells may be

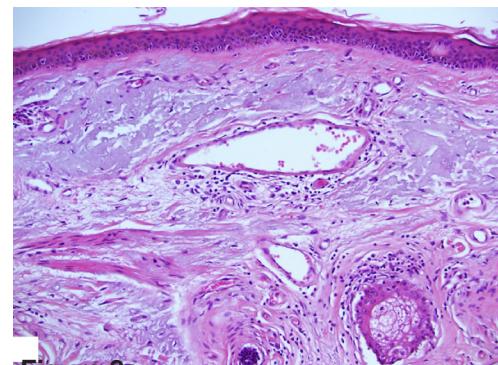


Figure 2a

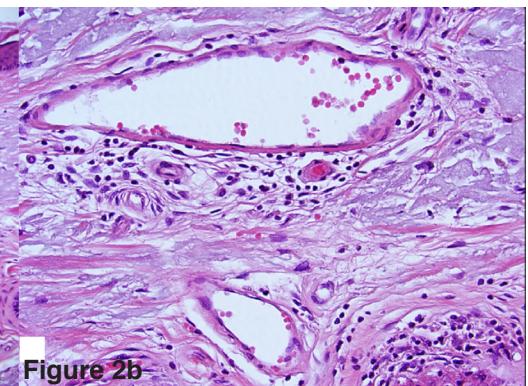


Figure 2b

Figures 2a, 2b. H&E of sun-exposed specimens, demonstrating: a) increased inflammatory-cell and dermal-vessel counts (200x); b) increased perivascular inflammation, dilated vessels with increased number of smaller surrounding vessels (400x).

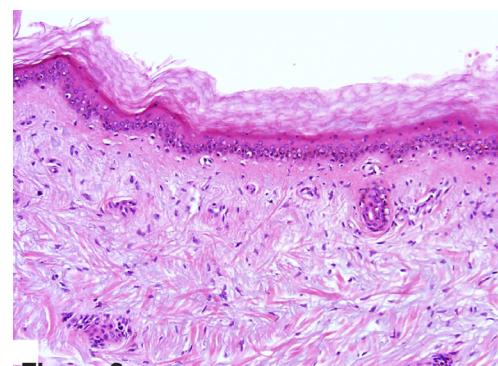


Figure 3a

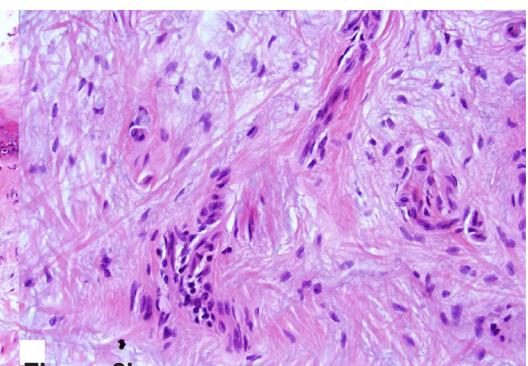


Figure 3b

Figures 3a, 3b. H&E of sun-protected specimens, demonstrating: a) decreased dermal-vessel count and comparatively less inflammation (200x); b) few focal inflammatory cells and vessels (400x).

related to tumorigenesis. The cellular presence and role of COX-2 remains questionable, as studies have shown that mice with COX-2 deletions in epithelial cells are not devoid of UVB-induced skin tumors.²³

Diclofenac sodium 3% gel, an NSAID with preferential activity on COX-2, has been FDA-approved in the United States for actinic keratoses.²⁶ A review of 18 articles studying diclofenac 3% gel in the treatment of actinic keratoses has suggested it is effective.²⁷ The evidence for NSAIDs in the management of basal cell carcinoma is weaker; however, a recent meta-analyses identified a 10% risk reduction in basal cell cancer in patients deemed high-risk (history of skin cancer and/or high prevalence of AKs) who were taking any oral NSAID.²⁸ Another meta-analysis determined, despite significant study heterogeneity, a significantly reduced risk of SCC among people taking any NSAIDs.²⁹ Our results bolster these findings, as inflammation resulting from chronic sun exposure may play a role in inflammatory and angiogenic changes of photocarcinogenesis. The use of NSAIDs has helped elucidate the possible roles of chronic inflammation in chronic sun-exposed skin lesions, further lending support to their pathogenetic role.

Vascular changes occur, as well, both in photoaging and carcinogenesis. Traditionally, a noted reduction in vasculature has been described in elderly skin.³⁰ Most elderly individuals have some evidence of cumulative sun damage in commonly sun-exposed regions, such as the head and neck, compared to relatively sun-protected areas like the inner thigh. One case series from South Korea examined biopsies from 21 patients from the face and buttocks, performing immunohistochemical and computer-assisted morphometric analyses of dermal vessels.³¹ The authors identified a significant reduction in the number of dermal vessels in photodamaged skin compared to sun-protected skin in patients 70 years of age and

older.³¹ A reduction in vessel size was noted in patients 40 years of age and older.³¹ Linear regression revealed a negative correlation between age and vessel density, vessel size, and vessel area.³¹ They theorized that repeated, acute exposure to UV radiation causes inflammation, angiogenesis, and extracellular matrix degradation, cumulatively causing an unfavorable physical environment for dermal vessels.³¹ However, it should be noted that sun exposure may be avoided in many Asian countries as an effort to prevent tanning.³²

In contrast, a murine study subjected skh-1 hairless mice to UVB radiation, gradually increasing minimal erythema doses over a 10-week period to reach 4.5, in an effort to examine the angiogenic changes that occur with chronic UVB exposure in actinically sun-damaged skin.³³ Irradiated mice had evidence of UV exposure, with wrinkling of skin compared to non-irradiated mice. CD31 immunostaining of irradiated skin specimens revealed not only an increased number of vessels, along with significantly increased size and density, but also an inflammatory infiltrate in the upper dermis.³³ This may indicate a direct relationship between inflammatory cells and dermal vasculature changes that occur in response to chronic UVB exposure. The authors subjected transgenic mice with skin-specific overexpression of thrombospondin-1 (TSP-1), an angiogenic inhibitor, to the same UVB radiation regimen. They reported an absence of clinical wrinkling in transgenic mice subjected to the same UVB regimen as wild type mice, in addition to reduced numbers of dermal inflammatory cells and vessels and a reduction of more than 55% in average vessel size of dermal vessels compared to wild-type mice.³³ Of note, ki-67 and CD31 immunostaining of specimens of irradiated transgenic mice revealed a reduced number of proliferating dermal endothelial cells compared to exposed wild type mice.³³ It has been reported that thrombospondins may inhibit zymogens of MMP-2 and MMP-9.³⁴ This may help further explain the multiple actions of these MMPs in cutaneous carcinogenesis from not only

an inflammatory perspective, but from a vascular component as well.

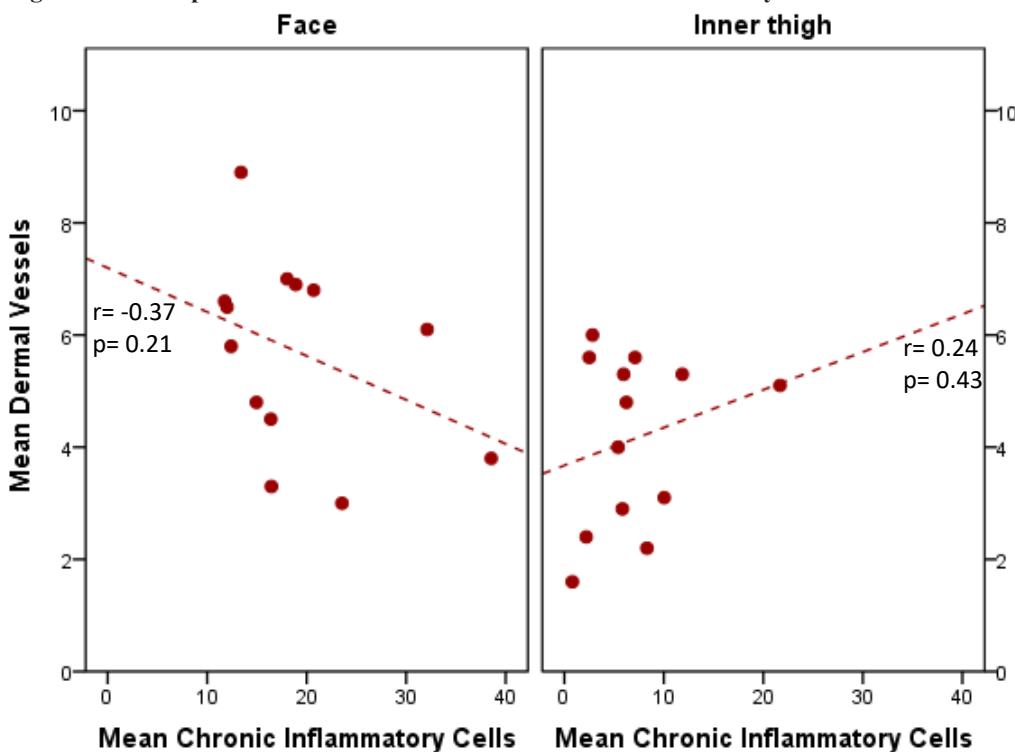
Studies have recently identified a significant association between COX-2 immunoreactivity and proliferating endothelial cell fractions in actinic keratoses, Bowen's disease, and squamous cell carcinoma lesions compared to normal skin. However, this is not true for microvessel density.²⁴ This may indicate COX-2 plays an indirect role in angiogenesis in skin cancers without increasing vessel numbers. However, COX-2 expression has been significantly associated with microvessel density in colorectal and breast cancers.^{35,36}

Our subjects were from any elderly Caucasian population, representative of an at-risk skin cancer population in the general U.S. population. Limitations of our study include a small sample size and lack of data on actual cumulative sun-exposure, personal and family history of pre-cancer and cancerous skin, and presence of risk factors for increased incidence of skin cancer. Skin cancer was not a cause of death in any of the subjects. Prospective studies examining pre-cancer-prone skin and inflammation might help determine an effective time to intervene with topical NSAID therapy to treat and prevent photocarcinogenesis.

Conclusion

Vessel density and chronic inflammation were increased in sun-exposed skin compared with sun-protected skin. These changes could play a role in photoaging and photocarcinogenesis.

Figure 4. Scatter-plot and linear correlation between mean inflammatory cells and mean vessels.



References

1. Kripke ML. Ultraviolet radiation and immunology: something new under the sun—presidential address. *Cancer Res.* 1994;54:6102–5.
2. Cox NH, Diffey BL, Farr PM. The relationship between chronological age and the erythema response to ultraviolet B radiation. *Br J Dermatol*. 1992;126:315–9.
3. Kramer M, Sachsenmaier C, Herrlich P, et al. UV irradiation induced interleukin-1 and basic fibroblast growth factor synthesis and release mediate part of the UV response. *J Biol Chem.* 1993;268:6734–41.
4. Bielenberg DR, Bucana CD, Sanchez R, et al. Molecular regulation of UVB-induced cutaneous angiogenesis. *J Invest Dermatol.* 1998;111:864–72.
5. Strickland I, Rhodes LE, Flanagan BF, et al. TNF-alpha and IL-8 are upregulated in the epidermis of normal human skin after UVB exposure: correlation with neutrophil accumulation and E-selectin expression. *J Invest Dermatol.* 1997;108:763–8.
6. Kligman LH. The ultraviolet-irradiated hairless mouse: a model for photoaging. *J Am Acad Dermatol.* 1989;21:623–31.
7. Fisher GI, Wang ZQ, Datta SC. Pathophysiology of Premature Skin Aging Induced by Ultraviolet Light. *N Eng J Med.* 1997;337(20):1419–28.
8. Gonzalez S, Moran M, Kochevar IE. Chronic photodamage in skin of mast cell-deficient mice. *Photochem Photobiol.* 1999;70:248–53.
9. Bosset S, Bonnet-Duquennoy M, Barré P, Chalon A, et al. Photoaging shows histological features of chronic skin inflammation without clinical and molecular abnormalities. *BJD.* 2003;149:826–35.
10. Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S, Voorhees JJ. Mechanisms of photoaging and chronological skin aging. *Arch Dermatol.* 2002;138(11):1462–70.
11. Brennan M, Bhatti H, Nerusu KC, Bhagavathula N, Kang S, Fisher GJ, et al. Matrix metalloproteinase-1 is the major collagenolytic enzyme responsible for collagen damage in UV-irradiated human skin. *Photochem Photobiol.* 2003;78:43–8.
12. Matsui MS, Hsia A, Miller JD, Hanneman K, Scull H, Cooper KD, Baron E. Non-sunscreen photoprotection: antioxidants add value to a sunscreen. *J Invest Dermatol Symp P.* 2009;14(1):56–9.
13. Caley MP, Martins VLC, O'Toole EA. Matrix metalloproteinases and Wound Healing. *Adv Wound Care.* 2015;4(4):225–34.
14. Kerkela E, Saarialho-Kere U. Matrix metalloproteinases in tumour progression: focus on basal and squamous cell skin cancer. *Exp Dermatol.* 2003;12:109–25.
15. Zhang Z, Zhu S, Yang Y, Ma X, Guo S. Matrix metalloproteinase-12 expression is increased in cutaneous melanoma and associated with tumor aggressiveness. *Tumour Biol.* 2015;36(11):8593–600.
16. Nikkola J, Vihinen P, Vlaykova T, Hakka-Kemppinen M, Kähäri VM, Pyrhönen S. High expression levels of collagenase-1 and stromelysin-1 correlate with shorter disease-free survival in human metastatic melanoma. *Int J Cancer.* 2002;97(4):432–8.
17. Rundhaug JE, Fischer SM. Cyclooxygenase-2 plays a critical role in UV-induced skin carcinogenesis. *Photochem Photobiol.* 2008;84(2):322–9.
18. Buckman SY, Gresham A, Hale P, Hruza G, Anast J, Masferrer J, Pentland AP. COX-2 expression is induced by UVB exposure in human skin: implications for the development of skin cancer. *Carcinogenesis.* 1998;19(5):723–9.
19. Ikehata H, Ono T. The mechanisms of UV mutagenesis. *J Radiat Res.* 2001;52:115–25.
20. Rastogi, Rajesh P, Richa, Ashok Kumar, Madhu B. Tyagi, and Rajeshwar P. Sinha. Molecular Mechanisms of Ultraviolet Radiation-Induced DNA Damage and Repair. *J Nucleic Acids.* 2010 (2010), Article ID 592980, 32 p.
21. Rundhaug JE, Simper MS, Surh I, et al. The role of the EP receptors for prostaglandin E2 in skin and skin cancer. *Cancer Metastasis Rev.* 2011;30:465–80.
22. Elmets CA, Ledet J, Athar M. Cyclooxygenases: Mediators of UV-induced Skin Cancer and Potential Targets for Prevention. *J Invest Dermatol.* 2014 Oct;134(10):2497–502.
23. Jiao J, Mikulec C, Ishikawa TO, Magyar C, Dumlaor DS, Dennis EA, Fischer SM, Herschman H. Cell-type-specific roles for COX-2 in UVB-induced skin cancer. *Carcinogenesis.* 2014 Jun;35(6):1310–9.
24. Nijsten T, Colpaert CG, Vermeulen PB, Harris AL, Van Marck E, Lambert J. Cyclooxygenase-2 expression and angiogenesis in squamous cell carcinoma of the skin and its precursors: a paired immunohistochemical study of 35 cases. *Br J Dermatol.* 2004;151(4):837–45.
25. Karagece Yalçın U, Seçkin S. The expression of p53 and COX-2 in basal cell carcinoma, squamous cell carcinoma and actinic keratosis cases. *Turk Patoloji Derg.* 2012;28(2):119–27.
26. PharmaDerm [Internet]. SOLARAZE® Gel. SOLARAZE® Gel (diclofenac sodium 3%). 2015 Nov 22. Available from: http://www.solaraze.com/pdsol_web_5_benefitsOfSolaraze.shtml.
27. Martin GG, Stockfleth E. Diclofenac sodium 3% gel for the management of actinic keratoses: 10+ years of cumulative evidence of efficacy and safety. *J Drugs Dermatol.* 2012;11(5):600–608.
28. Muranushi C, Olsen CM, Green AC, Pandeya N. Can oral nonsteroidal antiinflammatory drugs play a role in the prevention of basal cell carcinoma? A systematic review and metaanalysis. *J Am Acad Dermatol.* 2016 Jan;74(1):108–119.e1.
29. Muranushi C, Olsen CM, Pandeya N, Green AC. Aspirin and nonsteroidal anti-inflammatory drugs can prevent cutaneous squamous cell carcinoma: a systematic review and meta-analysis. *J Invest Dermatol.* 2015 Apr;135(4):975–83.
30. Norman RA, Young EM. *Atlas of Geriatric Dermatology.* London: Springer; 2013. p. 4–6.
31. Chung JH, Yano K, Lee MK, Youn CS, Seo JY, Kim KH, Cho KH, Eun HC, Detmar M. Differential effects of photoaging vs intrinsic aging on the vascularization of human skin. *Arch Dermatol.* 2002 Nov;138(11):1437–42.
32. Day AK, Wilson CJ, Hutchinson AD, Roberts RM. Sun-related behaviours among young Australians with Asian ethnic background: differences according to sociocultural norms and skin tone perceptions. *Eur J Cancer Care.* 2015 Jul;24(4):514–21.
33. Yano K, Oura H, Detmar M. Targeted overexpression of the angiogenesis inhibitor thrombospondin-1 in the epidermis of transgenic mice prevents ultraviolet-B-induced angiogenesis and cutaneous photo-damage. *J Invest Dermatol.* 2002 May;118(5):800–5.
34. Bein K, Simons M. Thrombospondin type 1 repeats interact with matrix metalloproteinase 2. Regulation of metalloproteinase activity. *J Biol Chem.* 2000 Oct 13;275(41):32167–73.
35. Cianchi F, Cortesini C, Bechi P, et al. Up-regulation of cyclooxygenase 2 gene expression correlates with tumor angiogenesis in human colorectal cancer. *Gastroenterology.* 2001;121:1339–47.
36. Costa C, Soares R, Reis-Filho JS, et al. Cyclo-oxygenase 2 expression is associated with angiogenesis and lymph node metastasis in human breast cancer. *J Clin Pathol.* 2002;55:429–34.