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Differential levels of elastin fibers and TGF-β signaling in the skin of Caucasians and African Americans

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ABSTRACT

Background: While skin color is the most notable difference among ethnic skins the current knowledge on skin physiological and aging properties are based mainly on Caucasian skin studies.
Objective: To evaluate histological differences in elastin fiber network and differential responsiveness to TGF-β in skin of Caucasians and African Americans.
Methods: These studies were undertaken using human skin biopsies, primary dermal fibroblasts, Western blot analyses, immunofluorescence microscopy, cDNA array and quantitative real-time PCR.
Results: In Caucasian subjects, tropoelastin expression and elastin fibers in photoprotected skin was substantially less than in age-matched African American skin. Expression of tropoelastin in photoexposed skin of African American was similar to their photoprotected skin, suggesting that photoexposure did not affect elastin fibers in African American skin to the same extent as Caucasian skin. An elevated level of TGF-β1 present in media from dermal fibroblasts derived from African American skins correlated well with the higher levels of TGF-β mRNA in African American skins analyzed by cDNA array. Treatment with TGF-β1 resulted in a considerably higher induction of elastin mRNA in dermal fibroblasts from African Americans than from Caucasian fibroblasts, indicative of enhanced TGF-β signaling in African American skins. Furthermore, UVA exposure decreased levels of elastin mRNA in Caucasian fibroblasts compared to African Americans fibroblasts.
Conclusion: These results suggest that there are ethnic differences in the elastin fiber network and in TGF-β signaling in African American and Caucasian skin, and that African American have less UV dependent loss of elastin than Caucasian which may contribute to the different perceived aging phenotypes.

1. Introduction

The most apparent difference in skin across various ethnicities is color and this particular aspect is very well studied. Besides melanin content in the skin, other differences reported in the literature are primarily related to barrier function and the compactness of the corneocyte cell layers [1]. Several other studies show disagreeing amounts of skin surface flora, sebum secretion, and elastic recovery depending on the specific body site being assessed [2,3]. Most studies on skin aging parameters like structure, function, photoaging, and chronological aging have been mainly addressed to one ethnic group [4]. Few studies have examined the differences in extracellular matrix (ECM) and skin architecture with respect to chronological and photo aging and most of these studies were limited to Caucasians. Elucidating histological changes in the epidermis and dermis of different ethnicities is very important from a biological point of view in order to understand the photoaging, and chronological aging process of ethnic skin.

Histologically, skin is organized into three layers: epidermis, dermis and hypodermis. The epidermis is the outer, protective layer of the skin while the dermis lies below it and provides mechanical support. The dermis is composed of extracellular matrix primarily type I collagen, with lesser amounts of type III collagen, elastin, proteoglycans and fibronectin. The elastin fiber network is responsible for the elasticity of our skin allowing it to resume its shape after stretching or contracting. In the dermal connective tissue, the thin and sinuous elastin fibers represent approximately 5% of dermal compartment by dry weight. Functional elastin fibers are made by properly linking many soluble tropo-elastin protein molecules, in a reaction catalyzed by

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lysyl oxidase, to make a massive insoluble, cross-linked array. The synthesis of ECM molecules is regulated by specific growth factors, among which transforming growth factor-β (TGF-β) is the most prominent. TGF-β has been shown to stimulate elastin production by up-regulating the elastin promoter [5,6]. With aging, the elastic fibers progressively degenerate and eventually disappear in the area just below the dermal-epidermal junction [7].

Skin is the largest organ and its discoloration, wrinkling and sagging appearance is a primary indicator of aging. Modifications related to aging are particularly visible in human skin, which becomes wrinkled, lax, dry, and irregularly pigmented over time [8]. Aged skin is characterized by a flattening of the dermal-epidermal junction, a marked atrophy and a loss of elasticity of the dermal connective tissue which plays main role in providing structural support for skin's vasculature, appendages and epidermis [9]. This loss of firmness and elasticity is associated with a reduction and disorganization of its major extracellular matrix components, such as collagen and other elastic fibers, proteoglycans and glycosaminoglycans [10–12]. A histological characteristic of chronological aging in the epidermis is a decrease of tissue thickness [12]. Solar radiations are particularly studied as environmental factors promoting skin aging [13]. UV lights have been shown to affect both epidermis and dermis. Dermal regions of the skin and dermal damage induced by UV irradiation is principally manifested histologically as the disorganization of collagen fibrils and accumulation of non-functional elastin also referred to as solar elastosis. While the expression of these ECM in response to UV damage (photoaging) has been well documented in Caucasians, few studies have sought to investigate similar responses in different ethnic skins [14].

The purpose of the present study was to evaluate the histological differences in elastin fiber network in skin of Caucasian and African individuals living on the East Coast of the United States. The populations were determined by a visual assessment of the skin color by the clinical site and the self-declared descent of the individuals participating in the study. To elucidate the molecular mechanism of differential expression of elastin in ethnic skins, we also analyzed the expression levels of TGF-β signaling pathway components and responsiveness of dermal fibroblasts derived from Caucasian and African individuals. These results help to explain the clinical observations in skin appearance between Caucasians and African Americans during the aging process.

2. Material and methods

2.1. Subjects and skin biopsy specimens for elastin immunohistochemcial analyses

The human skin biopsy study protocol was approved by the Essex Institutional Review Board (Lebanon, NJ) and the study was performed according to the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to enrollment. Clinical Study was performed at the Education & Research Foundation (Lynchburg, VA). Caucasians and African Americans with $n = 10$/ethnic group were involved in the study. All subjects were pre-menopausal women with age range of 35–45 years and self-identified their ethnicity. Subjects were in general good heath, free of seasonal allergies/hay fever, and had not taken any over the counter or prescription medicines for cold, pain (anti-inflammatories), allergy or antibiotics for at least 2 weeks prior to the study. Subjects were non-smokers or were smoking free for at least 1 year prior to study and finally no moisturizer usage at least a week prior to the intended biopsy sites. Full thickness 2 mm punch biopsies were obtained from the inner upper arm (photoprotected site) and dorsal forearm (photoexposed site) from individuals on a single visit. Biopsies were fixed in 10% Formalin for 24 h and shipped to Paragon Bioservices Inc (Baltimore, MD) for paraffin embedding, sectioning and histological staining. The sectioned samples were immunohistochemically stained with rabbit anti-tropoelastin polyclonal antibody (ab21600) from Abcam (Cambridge, MA) at dilution of 1:50.

2.2. Expert grader assessment for histological tropoelastin staining

Five histological experts graded randomized and blinded tropoelastin stained slides on a 0–4 scale where 0 = none and 4 = high expression of tropoelastin present. Statistical significance was determined using a Student's t-test, with a significance set at $p < 0.05$.

2.3. Human skin samples for cDNA array and Western blot analyses

For cDNA array study, Human skins (9 Caucasian, 8 female, 1 male, age 45.0 ± 8.0; and 7 African American, all female, age 38.9 ± 6.4, $p > 0.11$) were obtained with informed consent from abdominal skins of healthy individuals undergoing plastic surgery (The Peer Group, Florham Park, NJ, USA). Patient identities were not disclosed to preserve confidentiality, in compliance with US HIPAA regulations.

For Western blot study, frozen human neonatal foreskins derived from Caucasian ($n = 5$) and AA ($n = 5$) were obtained from archived tissue database at Corporative Human Tissue Network (CHTN, Philadelphia, PA).

2.4. Preparation of human whole skin lysates

Human foreskin lysates were prepared by homogenizing 100–200 mg frozen foreskin in 2 ml T-Per® tissue protein extract reagent (Pierce, Rockford, IL) supplemented with the Complete™ protease inhibitor cocktail (Roche, Indianapolis, IN), 1 mM sodium vanadate and 20 mM sodium fluoride (Sigma, St. Louis, MO). Samples were homogenized on ice using a Polytron® PT 3000 (Brinkmann, Westbury, NY), centrifuged for 20 min at 4 °C and stored frozen at −80 °C until used for Western blot analysis.

2.5. Western blotting

Human neonatal foreskin whole skin extract (7 μl) was electrophoresed for 2.5 h through a 4–20% gradient denaturing SDS-polyacrylamide gel (Invitrogen Corp., Carlsbad, CA) and electro-transferred for 1 h at 25 V to a nitrocellulose membrane using the Trans-Blot SD semi-dry transfer system (Biorad, Hercules, CA). Membranes were blocked for 1 h at 37 °C in blocking solution containing 5% nonfat dry milk dissolved in TBST [1× tris-buffered saline, 0.05% Tween-20] (Biorad). Membranes were then incubated overnight at 4 °C with anti-TβR1, anti-TβR3 (both are rabbit polyclonal antibodies, Cell Signaling Technology®, Inc., Danvers, MA, diluted at 1:1000 in blocking solution), or anti-β-actin antibody (mouse monoclonal antibody, Sigma–Aldrich, St. Louis, MO, at 1:5000 dilution), washed and probed for 1 h at 37 °C with anti-rabbit or anti-mouse secondary antibody (Amersham, Buckinghamshire, UK, at 1:5000 dilution), as appropriate. Antibody binding was visualized on X-ray film by enhanced chemiluminescence using the ECL Plus Western blotting detection system (Amersham) X-ray films were scanned using a Gel Logic 100 imaging system (Kodak, New Haven, CT) and Kodak 1D version 3.6.1 scanning software.

2.6. Cell culture

Primary neonatal human foreskin fibroblasts were obtained from Dr. Zalfa Abdelmalek (The Department of Dermatology,