



Proteomic Comparison of Sun-protected vs -Exposed and Young vs Gently-Aged Human Epithelium

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Abstract

The human skin is a complex organ, and its aging is a complicated process involving both the passage of time (chronological aging) and environmental exposure, especially to UV light (photoaging). This aging phenomenon works through various cellular mechanisms, including changes in apoptosis, alterations in cellular signaling, and an increased genetic instability. In this study, we conducted a proteomic analysis of human sun-protected vs sun-exposed epithelium from a single subject, and young vs older skin from two distinct biological sampling groups, using the same area of the body. We also tested a barocycler device vs sonication as a way to remove cells/proteins from D-square sampling discs. Some previous studies have shown higher levels of proteases in sun-damaged epithelium, while other studies have shown diminished proteasome components in aged skin. Initial results from this study revealed some proteases to be up-regulated in the sun-protected relative to sun-exposed skin, as well as higher levels of proteasome components in sun-protected skin, and also higher levels of two serpins, which inhibit serine proteases, suggesting complex regulation of protein catalysis. Few proteins showed significant differences by normalized spectral counts between the young vs aged biological samples. The differences in proteins due to sun exposure warrants further attention.

Methods

Sampling. Epidermal samples were collected using D-Square discs from healthy Caucasian human subjects. The sun-protected samples were collected from the pale inner arm of a single subject, while the sun-exposed samples were collected from a tanned/freckled outer area of the same subject's arm. Hair was removed from both areas, and the skin swabbed with isopropanol before applying discs, which were left on for 5 minutes. The young vs gently aged samples were collected from two separate groups of three subjects, gently aged (40 to 64 years of age) and young (7 to 13 years of age).

Sample Preparation. Collection discs were immersed in 50 mM ammonium bicarbonate (AMBIC), then a barocycler was employed to enhance high-pressure shearing of skin cells and removal from discs, without any detergents or organic chemicals which might release adhesives from the tape. Alternately, a sonicating device was employed for 30 min (same amount of time as barocycler run) to remove proteins from tape in AMBIC. Released proteins were digested overnight with modified Trypsin in AMBIC, then dried down on a vacuum concentrating device. Digested peptides were then resolubilized in 2% acetonitrile/ 0.1% trifluoroacetic acid for LC-MS/MS analysis.

Mass Spectrometry. Digested peptides were analyzed by LC-MS/MS on a Thermo LTQ with Michrom Paradigm LC and CTC Pal autosampler; a Michrom Advance nano-spray source was utilized at the LC-MS interface. Peptides were separated with a 120 min gradient using a Michrom 200 µm x 150 mm Magic C₁₈AQ reversed phase column at 2 µl/min. The LTQ was operated with a top 10 method. Three or four biological replicates were run for each condition (young, aged, sun-protected, sun-exposed).

Data Analysis. DATABASE SEARCHING-- Tandem mass spectra were extracted using XCalibur. All MS/MS samples were analyzed using X! Tandem and the ipiHUMAN_20100104_b6VreS database (87061 entries) assuming the digestion enzyme trypsin. X! Tandem was searched with a fragment ion mass tolerance of 0.40 Da and a parent ion tolerance of 1.8 Da. CRITERIA FOR SCAFFOLD PROTEIN IDENTIFICATION-- Scaffold (version Scaffold_2_06_01, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 80.0% probability. Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least 2 identified peptides.

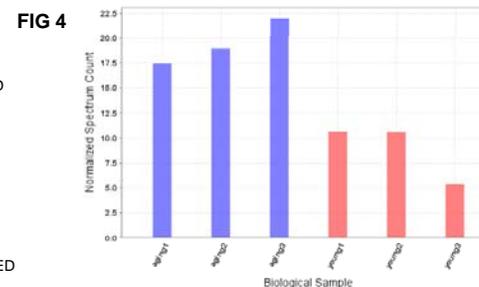
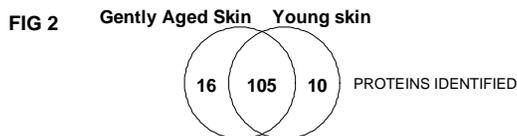
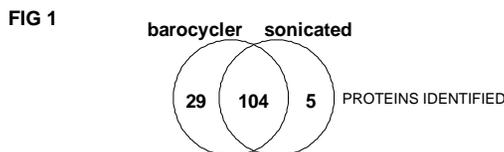
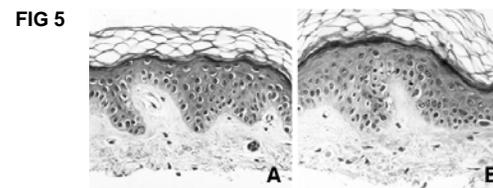


FIGURE 4. Normalized spectrum counts for Annexin A2, which appears to be up-regulated in aging skin relative to younger skin

FIGURE 3. Venn diagram showing protein IDs in samples produced by removing epithelium from the D-square discs with a Barocycler vs those produced by removing the epithelium with a sonicating device. Both were done for 30 minutes; the barocycler cycled between 20 k PSI and regular atmospheric pressure (at Davis, CA elevation).

FIGURE 3. Venn diagram showing protein IDs in young vs aged skin epithelium samples. Not only were the protein IDs very similar between the two, but few differences in protein level were detected by student t-test, using normalized spectral counts. Normalization was done by running a "preview" run of each biological sample, then using the spectral counts to determine subsequent loading amounts.



From Carpenter et al, 2004

FIGURE 5. Images of normal skin and sun-damaged skin. A. Normal epidermis is uniform in thickness and relatively flat in outside texture. B. Sun-damaged skin shows a thicker epidermis and an increase in waviness (dermal elastosis). Besides dermal changes, thickening of the epidermis and dryness result from overexposure to UVA and UVB light.

FIGURE 3. Scaffold file showing up- or down-regulation of various proteins in sun-exposed vs sun-protected adult skin determined by normalized spectral counts. Differences in relative protein amounts deemed significantly different by student t-test (p-value) are highlighted in green on Scaffold. The unweighted spectral counts for peptides identified with 95% confidence are also highlighted in green.

Conclusions

The barocycler seems to more efficiently remove epithelium from D-square discs than a sonicating device

Differences between older epithelium and younger were minor for protein up- and down-regulation

Some significant differences in protein level between the sun-exposed and sun-protected skin areas involved proteases

Normalizing by doing "preview runs" worked well, judging on the modest amount of differences, and may be less subject to interferences than normalizing by spectrometrically determined protein concentrations.