Mass spectrometry based-proteomics of facial stratum corneum of different ethnic groups to reveal novel biochemical pathways involved in photodamaged dry skin

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Introduction

Although our understanding of the ethnic differences in stratum corneum (SC) structure, composition and function has increased tremendously over the last few decades, our knowledge regarding effects of photoaging on SC composition is minimal although differences in NMF and zonal skin hydration are clearly apparent.

We have previously shown big differences in the biochemistry and physiology of facial SC among Albino Africans, Black Africans and Caucasians (Table I). Despite having elevated NMF levels Albino Africans have elevated basal TEWL and drier skin. The increased NMF levels seemed insufficient to correct the effect of photodamage on corneocyte maturation.

Our aim was to utilise proteomics to understand the effects of photodamage on facial SC (Figure 1) and to determine variations in the filaggrinolysis pathway (Figure 2) among the differently pigmented ethnic groups to explain some of these differences.

Parameter	Albino Africans	Black Africans	Caucasians	p values of comparison A vs B	A vs C	B vs C
Expert grading, facial dryness	1.9 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	<0.001	<0.005	n.s.
Expert grading, facial roughness	2.1 ± 0.2	1.1 ± 0.1	1.3 ± 0.1	<0.001	<0.005	n.s.
Capacitance [AU], cheek	34.0 ± 3.1	52.9 ± 2.2	40.2 ± 2.8	<0.001	n.s.	<0.01
Capacitance [AU], PA	25.0 ± 2.4	41.1 ± 2.1	32.5 ± 2.5	<0.001	n.s.	<0.05
Basal TEWL [g m^{-2} h^{-1}], cheek	19.5 ± 1.5	10.3 ± 0.8	11.8 ± 0.8	<0.001	<0.001	n.s.
Basal TEWL [g m^{-2} h^{-1}], PA	17.8 ± 1.3	10.6 ± 2.4	9.8 ± 0.6	<0.001	<0.001	n.s.
Skin surface pH, cheek	5.93 ± 0.09	5.68 ± 0.10	5.67 ± 0.09	n.s.	n.s.	n.s.
PCA [µmol mg^{-1} SC protein], cheek	0.50 ± 0.07	0.17 ± 0.03	0.094 ± 0.01	<0.001	<0.001	<0.01
PCA [µmol mg^{-1} SC protein], PA	0.44 ± 0.05	0.15 ± 0.02	0.070 ± 0.01	<0.001	<0.001	<0.001
CE maturity, cheek	0.59 ± 0.02	0.83 ± 0.02	0.81 ± 0.02	<0.001	<0.001	n.s.
CE maturity, PA	0.65 ± 0.02	0.93 ± 0.04	0.93 ± 0.04	<0.001	<0.001	n.s.

Table I: Expert grading, biometric data, PCA (NMF) level and cornified envelope maturity (ratio of differential Nile red and involucrin immunostaining). Data are mean ± SEM; n.s., not significant.

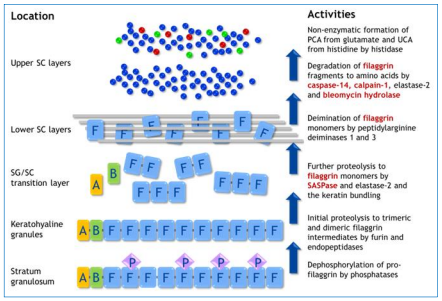

Figure 2. Processing of profilaggrin in terminal epidermal differentiation. Proteins found in our approach are shown in red font.

Methods

We enrolled 18 female subjects of different skin ethnicities including Albino Africans (40.8±6.2 years), Black Africans (43.2±4.1 years) and Caucasians (39.0±5.3 years) living in Pretoria, South Africa (Figure 3) and investigated photodamaged cheek and photoprotected post-auricular (PA) SC (Figure 4).

On each test site nine subsequent tape strippings were taken and extracted by sonication in PBS buffer containing SDS and anti-proteases. Urea and TRIS-HCl buffer soluble proteins were trypsinized and separated using a nanoACQUITY UPLC Symmetry C18 Trap Column, 180 µm x 20 mm in trap and elute mode with ACQUITY UPLC Peptide BEH C18 nanoACQUITY Column 75µm x 250 mm by a Eksigent Ultra Plus nano-LC 2D HPLC coupled to a TripleTOF® 5600 mass spectrometer interfaced to a nano spray III source. DDA spectra processing and database searching was performed with ProteinPilot (v4.5 beta, ABSciex, Framingham) using the Paragon algorithm.

Figure 3. Representative facial images of an Albino African, a Black African and a Caucasian subject.

Total SC proteins identified
Cheek (photoexposed): **473**
PA (photoprotected): **253**
Proteins related to SC maturation
Cheek (photoexposed): **57**
PA (photoprotected): **43**

Figure 1: Number of facial SC proteins identified by proteomics

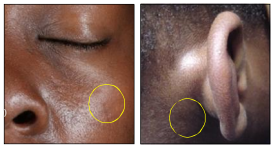

Figure 4. Location of tape strippings on photoexposed cheek and photoprotected post-auricular facial sites.

Results

Cheek, interethnic comparison

Filaggrin, filaggrin-2, bleomycin hydrolase and calpain-1 levels were highest for the Caucasian and lowest for the Albino African subjects (Table IIa and Figure 5). The level of the upstream, skin-specific retroviral-like aspartic protease (SASPase) was slightly higher in Albino African compared to Black African subjects. No significant differences for any of the proteins were found between Black African and Caucasian subjects.

Postauriclar site, interethnic comparison

As on the cheek, filaggrin-2 levels were highest in the Caucasian and lowest in the Albino African subjects (Table IIb and Figure 5). Conversely, filaggrin levels were highest in the Black African subjects. Bleomycin hydrolase and caspase-14 levels were similar across the three ethnic groups but Albino Africans had significantly lower levels of calpain-1. Albino Africans had a slightly higher levels of SASPase compared with the other two ethnic groups. Like on the cheek, no significant differences for any of the proteins were found between Black African and Caucasian subjects.

Cheek vs postauricular site, intraethnic comparison

Most of the proteins were significantly upregulated on the photoexposed site, although filaggrin-2, calpain-1 and caspase-14 were only marginally elevated in all three groups. SASPase levels were significantly increased by factor >3 in all three ethnicities and filaggrin in Albino Africans and Caucasians (Table IIc and Figure 5).

Conclusion

Presumably as a result of UV irradiation, the levels of filaggrin proteins and associated processing enzymes are increased on photoexposed facial skin in all three ethnic groups.

Obviously, the resulting elevated NMF levels are insufficient to correct the underlying biochemical abnormality in corneocyte maturation. We have previously shown that enzymes involved in corneocyte maturation are lowered in photoexposed facial skin of Caucasians. These enzyme levels are currently under investigation in the other two ethnicities.

Our results demonstrate that increased levels of filaggrin proteins, their processing enzymes and the resulting NMF are insufficient to correct the cellular and biochemical abnormalities in photodamaged skin.

Thus, we are focusing on other epidermal differentiation markers.

a) Comparison A vs B vs C, Cheek

Protein names	Gene names	Cheek A vs B p-value	A vs B q-value	A vs B Fold change	Cheek A vs C p-value	A vs C q-value	A vs C Fold change	Cheek B vs C p-value	B vs C q-value	B vs C Fold change
Filaggrin	FLG	0.824	0.619	0.87	0.520	0.540	0.68	0.673	0.580	0.78
Filaggrin-2	FLG2	0.000	0.002	0.52	0.000	0.000	0.37	0.054	0.157	0.71
Bleomycin hydrolase	BLMH	0.459	0.515	0.78	0.341	0.449	0.73	0.832	0.620	0.93
Calpain-1	CAPN1	0.026	0.101	0.67	0.001	0.009	0.56	0.289	0.417	0.83
Caspase-14	CASP14	0.953	0.646	0.99	0.511	0.537	0.85	0.550	0.551	0.86
SASPase	ASPRV1	0.381	0.470	1.49	0.677	0.580	0.83	0.196	0.349	0.55

b) Comparison A vs B vs C, PA

Protein names	Gene names	Postauricular A vs B p-value	A vs B q-value	A vs B Fold change	Postauricular A vs C p-value	A vs C q-value	A vs C Fold change	Postauricular B vs C p-value	B vs C q-value	B vs C Fold change
Filaggrin	FLG	0.033	0.067	0.31	0.420	0.351	0.64	0.184	0.219	2.09
Filaggrin-2	FLG2	0.000	0.000	0.32	0.000	0.000	0.22	0.109	0.158	0.68
Bleomycin hydrolase	BLMH	0.643	0.443	0.91	0.129	0.174	0.74	0.292	0.288	0.81
Calpain-1	CAPN1	0.000	0.000	0.63	0.000	0.000	0.54	0.149	0.191	0.87
Caspase-14	CASP14	0.413	0.348	1.13	0.972	0.545	1.01	0.434	0.353	0.89
SASPase	ASPRV1	0.138	0.184	1.74	0.294	0.289	1.48	0.664	0.451	0.85

c) Comparison Cheek vs PA

Protein names	Gene names	Albino Africans Cheek vs PA p-value	Albino Africans Cheek vs PA q-value	Albino Africans Cheek vs PA Fold change	Black Africans Cheek vs PA p-value	Black Africans Cheek vs PA q-value	Black Africans Cheek vs PA Fold change	Caucasians Cheek vs PA p-value	Caucasians Cheek vs PA q-value	Caucasians Cheek vs PA Fold change
Filaggrin	FLG	0.000	0.000	3.27	0.665	0.106	1.15	0.000	0.000	3.08
Filaggrin-2	FLG2	0.000	0.000	1.89	0.120	0.023	1.17	0.267	0.047	1.12
Bleomycin hydrolase	BLMH	0.000	0.000	1.70	0.000	0.000	1.98	0.000	0.000	1.73
Calpain-1	CAPN1	0.000	0.000	1.28	0.006	0.001	1.19	0.000	0.000	1.25
Caspase-14	CASP14	0.000	0.000	1.40	0.000	0.000	1.61	0.000	0.000	1.66
SASPase	ASPRV1	0.000	0.000	3.20	0.000	0.000	3.73	0.000	0.000	5.73

Table II. Comparison of filaggrin, filaggrin-2 and processing enzyme levels in cheek and post-auricular (PA) SC. Blue areas, p/q<0.05; red areas, fold change <0.5; green areas, fold change >2.0. A, Albino Africans; B, Black Africans; C, Caucasians.

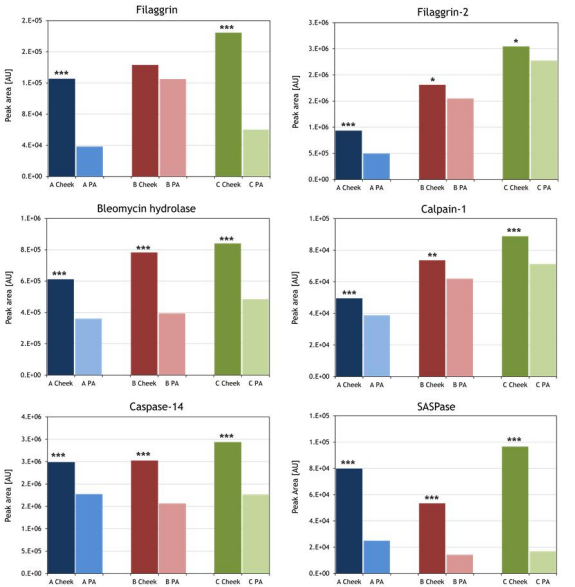

Figure 5. Comparison of sun-exposed cheek and sun-protected post-auricular site (PA) in order get an information about impact of photoaging on filaggrin and filaggrin processing. A, Albino Africans; B, Black Africans; C, Caucasians; PA, postauricular site. * q<0.05, ** q<0.01, *** q<0.001.

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