Mass spectrometry based-proteomics of the effects of photodamage on Caucasian facial stratum corneum

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Introduction

Our understanding of stratum corneum (SC) structure, composition and function has increased tremendously over the last few decades. Initially the understanding of SC composition has generally been on an analyte by analyte basis, by two-dimensional electrophoretic methods, chromatographic methods and multiplex enzyme-linked immunosorbent assays. These approaches have been highly successful in helping us to determine the general composition of the SC but have their limitations. The use of mass spectrometry-based 'omic' approaches is on the increase for investigating skin biochemistry especially proteomics. Nevertheless, these approaches have not been used to study the proteome of facial SC. Compared with other body sites facial SC is thinner, has elevated serine protease activities [1, 2], reduced levels of natural moisturizing factor and a greater proportion of immature corneocyte envelopes particularly on photoexposed sites (Figure 1) [3]. Our aim was to utilise proteomics to understand the effects of photodamage on facial Caucasian SC to explain some of these differences.

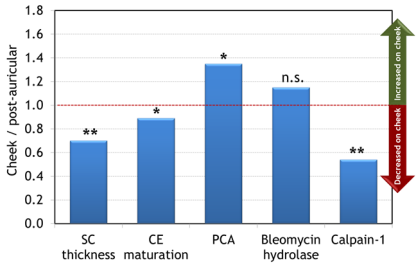


Figure 1. UV-induced changes of facial SC thickness, CE maturation, NMF biomarker PCA and proteases involved in late stage filaggrinolysis; * p<0.05, ** p<0.01, n.s., not significant.

Material & Methods

Nine subsequent tape strippings of photoexposed pre-(cheek) and photoprotected post-auricular (PA) sites of the face of six female Caucasian subjects (39.0 ± 5.3 years) 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 (particle diameter 5 µm, pore size 100Å) in trap and elute mode with ACQUITY UPLC Peptide BEH C18 nanoACQUITY Column 75µm x 250 mm (particle diameter 1.7 µm, pore size 130 Å) 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.

Results & Discussion

Proteins related to filaggrinolysis (Figure 2):
Filaggrin levels were elevated on the cheek indicating that its proteolysis was not occurring optimally. However, there were increases in the levels of the late stage filaggrinolytic enzymes caspase-14, calpain-1 and bleomycin hydrolase and a big increase in the more up-stream skin aspartic protease (SASPase).

Proteins related to corneocyte maturation (Figure 3):
SPRR 1A and 2E, and loricrin were significantly lower on the cheek compared with the PA site, whereas plakophilin-1 and transglutaminase-1 & 3 were increased. Equally enzymes associated with activation of transglutaminases, cathepsin D, cathepsin L2 and calpain-1, were elevated. eLOX-3 did not differ between the two sites but 12-R LOX was dramatically lower on the cheek.

Proteins related to SC thickness (Figure 4):
Increases in the levels of desmoglein-1, desmocollin-3 and corneodesmosin were observed but the biggest increase was that of plakophilin-1. Of the kallikreins that were found on both sites KLK7 and KLK10 were elevated. Note cathepsin D was elevated also (Figure 3).

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

Cheek (photoexposed) vs **PA** (photoprotected)
Total SC proteins
Up-regulated: **73** >2.0 fold & p<0.05
Down-regulated: **29** <0.5 fold & p<0.05
Proteins related to SC maturation
Up-regulated: **8** >2.0 fold & p<0.05
Down-regulated: **5** <0.5 fold & p<0.05

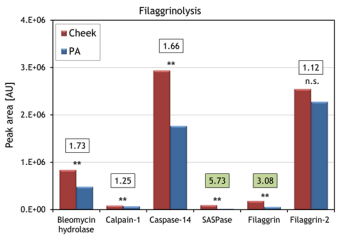
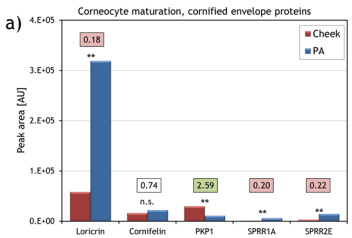
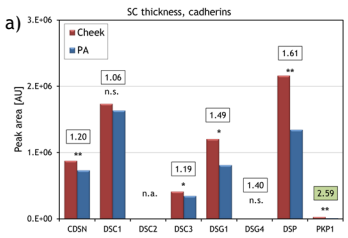
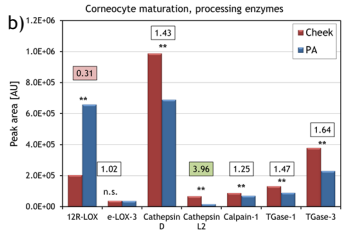


Figure 2. Comparison of SC protein quantities of cheek (red) and PA site (blue) of filaggrins and processing enzymes. Numbers in rectangles represent fold decrease (red <0.5x) or increase (green >2.0x) cheek vs. PA, respectively. Data are means, **p< 0.001, n.s., not significant.







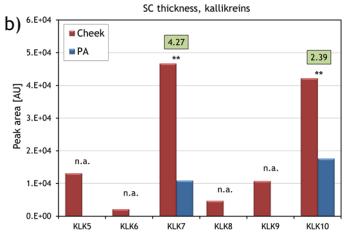


Figure 3. Comparison of SC protein quantities of cheek (red) and PA site (blue) of corneocyte envelope proteins (a) and processing enzymes (b). Numbers in rectangles represent fold decrease (red <0.5x) or increase (green >2.0x) cheek vs. PA, respectively. Data are means, *p<0.01, **p< 0.001, n.s., not significant.

Figure 4. Comparison of SC protein quantities of cheek (red) and PA site (blue) of corneodesmosomal proteins (a) and kallikreins (b). Numbers in rectangles represent fold decrease (red <0.5x) or increase (green >2.0x) cheek vs. PA, respectively. Data are means, *p<0.01, **p< 0.001, n.s., not significant, n.a., not detectable on PA site.

Conclusion

Corneocyte maturation is known to be impaired on the face and in dry skin that has been related to reduced transglutaminase activity. However, using proteomics we observed increases in the levels of transglutaminases and their activation enzymes. Reduced levels of loricrin, SPRR 1A and 2E might account for the increased corneocyte fragility. In these studies, we found a dramatic reduction in the levels of 12R-LOX in photoexposed facial skin indicating its crucial role in corneocyte envelope maturation. Facial body sites are known to have reduced levels of NMF. Yet despite increased levels of filaggrin on the cheek the mass levels of the filaggrinolysis enzymes increased. Increased mass levels of kallikrein-7 and -10 together with cathepsin D may account for the thinner SC on the face. Of the corneodesmosomal proteins contributing to the dry skin phenotype increased levels of plakophilin-1, desmoglein-1, desmocollin-3, desmoplakin and corneodesmosin were measured.
These biochemical variations highlighted new molecular pathways that need to be targeted to effectively treat or prevent photodamage of facial skin. These proteomic data are consistent with our observations on SC maturation. Although the "usual suspects" and conditions were described, various metabolic aspects may indirectly contribute as well.

References
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Acknowledgements
This study was financially supported by DSM Nutritional Products Ltd., Basel, Switzerland. We would like to thank Lebogang Kgatuke, Marlize Lategan, Caroline Moeletsi and Lee-Ann Raaff of the Photobiology Laboratory, of the Sefako Makgatho University, South Africa, for their enthusiasm in conducting the study.

2016 Annual SID Meeting, Scottsdale, AZ