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 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 in helping us understand the molecular biology of the skin.

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 blemmycin hydrolase (BHY) and a big increase in the more up-stream skin asparatic protease (SASP).

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, catherpin D, cathespin L2 and calpain-1, were elevated. LOX (12-LOX) did not differ between the two sites but 12-LOX was dramatically lower on the cheek.

Proteins related to SC thickness (Figure 4):
Increases in the levels of desmoglein-1, desmoplakin-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 catherpin D was elevated also (Figure 3).

Discussion
SC thickness was known to be an important parameter in the evaluation of photodamaged skin. Our data show that the SC of photo-exposed sites is thinner and has elevated serine protease activities [1, 2], reduced levels of natural moisturizing factor and a greater proportion of immature corneocyte envelopes particularly on photo-exposed sites (Figure 1) [3]. Our aim was to utilize proteomics to understand the effects of photodamage on facial Caucasian SC to explain some of these differences.

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 loricin, 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 plakoglobin-1, desmoglobin-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.

Material & Methods
Nine subsequent tape stripings 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) by a Exsigan Ultra Plus nano-UCD 2D HPLC coupled to a TripleTDF 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.

References

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