Characterization of epidermal lipidome during the maturation of keratinocytes using LC/HRMS^n

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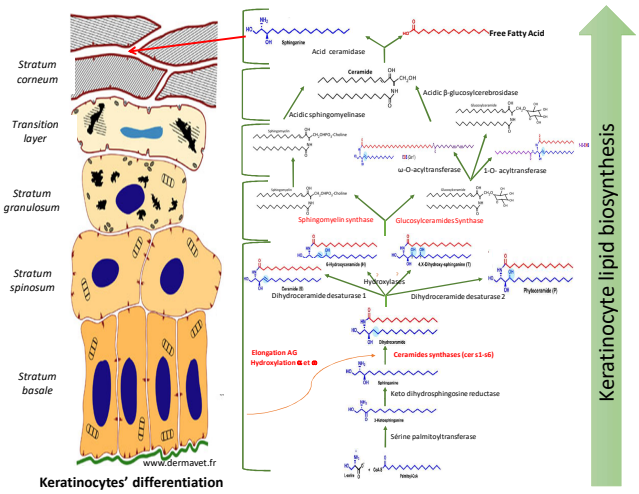
INTRODUCTION / OBJECTIVE

Stratum Corneum lipids play a key role in the barrier function of the skin. This is due to the complex composition and organization of lipid classes and subclasses.
Stratum corneum lipid biosynthesis is initiated at the beginning of keratinocytes' differentiation. During this process, there is an evolution of the simple lipid biosynthesis towards more complex lipids. At the terminal differentiation stage, ceramides, fatty acids and cholesterol molecules are synthesized to fill the intercellular region of *Stratum Corneum* and to surround the corneocytes. These molecules are known to be responsible of the barrier function in the outermost layers of the skin. **A deeper knowledge of lipids' metabolism would allow a better understanding of physiological and pathological factors behind the disturbances of the cutaneous barrier function** (atopy, xerosis, ichthyosis).

Based on **lipidomic approach,** our work aims in order to study lipid biosynthesis at the molecular level during the keratinocytes' differentiation process. Thus, we developed **a new analytical method allowing us to analyze and characterize all lipid molecules issued from keratinocytes' culture with a single analysis run.** In fact, these lipids represent a complex mixture with highly variable polarities inter- and intra-lipid class. In addition, there is an important structural micro-heterogeneity between the compounds due to variations in the alkyl chain length, the hydroxyl group number, the degree of unsaturation and the isomerism. This approach allowed us to follow the lipid profile evolution at different steps of keratinocytes' maturation.



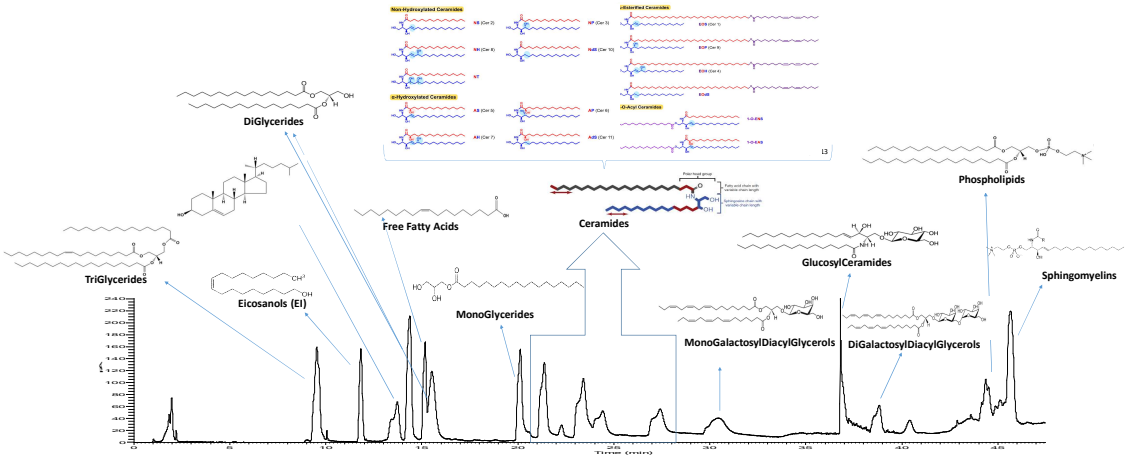
Rabionet et al, 2013

ANALYTICAL DEVELOPMENT

Method

In this study, two keratinocyte models have been studied: the spontaneously immortalized human keratinocyte cell line HaCaT (2D model) and Reconstructed Human Epidermis (RHE 3D model).
At different steps of the keratinocytes' differentiation, cell lipids have been extracted and analyzed using Normal Phase High-Performance Liquid Chromatography (NPLC) coupled simultaneously to charged aerosol detection (Corona-CAD®) and to High-Resolution Mass Spectrometry (HR-MS^n) (LTQ Velos Pro®).
Lipids' profiles have been compared and two differentiation markers have been studied: ceramides (as marker of the corneum layer) and glucosylceramides (as marker of the living epidermis).

Results

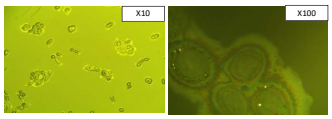


Chromatogram obtained for analytical standard mixture by NPLC coupled simultaneously to Corona-CAD® and to HR-MS^n (LTQ Velos Pro®)

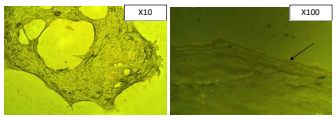
BIOLOGICAL APPROACH

2D MODEL - HaCaT

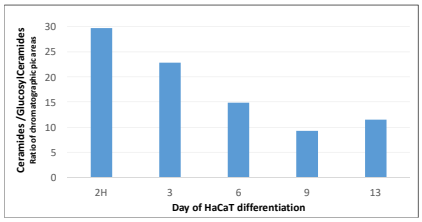
Study and detection of epidermis layers between *S. Basale* & *S. Granulosum*



White light image: HaCaT
Incubation: 2 hours
Concentration of calcium: 2.8mM



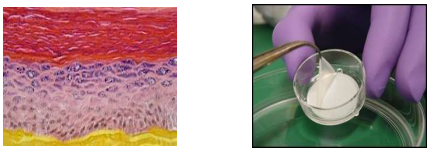
White light image: HaCaT
Incubation: 13 days
Concentration of calcium: 2.8mM



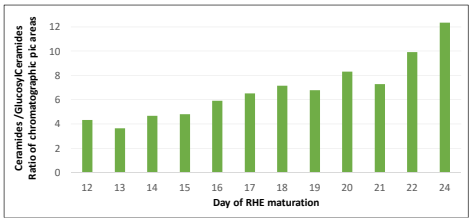
In HaCat study, epidermal lipidom corresponds to *S. Granulosum* where an important amount of ceramides is converted into GlucosylCeramides

3D MODEL - RHE

Study and detection of epidermis layers between *S. Spinosum* & *S. Corneum*



Reconstructed Human Epidermis-Episkin®



In RHE study, epidermal lipidom covers the overal maturation of keratinocytes, showing the highest formation of ceramides in *S. Corneum*. *Ceramides* are regenerated from their precursor GlucosylCeramides

CONCLUSION

This analytical method represents an efficient methodology for studying epidermal lipids at molecular level. We can observe that ceramide classes underwent an important quantitative and structural evolution during keratinocytes' differentiation. This information could be obtained thanks to the High Resolution Mass Spectrometry. Analytical results could be explored using chemometric approach in order to set up a "molecular link" between the abnormality in lipid biosynthesis and the symptoms of skin's pathology.

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