

Stratum Corneum Lipid Metabolism Pathways Associated with Intrinsic and Extrinsic Aging

Bradley B. Jarrold, Robert L. Binder, Michael K. Robinson, Jay P. Tiesman, Kenton D. Juhlin, Deborah R. Finlay and Rosemarie Osborne
P&G Beauty & Grooming and Global Biotechnology, Cincinnati, Ohio USA

INTRODUCTION

Skin aging is a cumulative process resulting from unrepaired damage and age-related physiological changes due to intrinsic (e.g., free radicals) and extrinsic (e.g., UV exposure) factors. In the current study we examined the effects of intrinsic and photoaging on stratum corneum (SC) lipid metabolism pathways of human skin barrier *in vivo*. Human skin equivalent cultures (SE) were utilized to examine how a cosmetic compound, known to improve skin barrier function, affects these same biomarkers *in vitro*.

OBJECTIVE

The goal of the current work was to 1) evaluate the effects of both intrinsic and photoaging on the gene expression profiles of pathways involved in SC lipid metabolism; and 2) evaluate *in vitro* how a cosmetic compound affects these biomarkers.

BACKGROUND

The major lipids of the human SC are ceramides, cholesterol, and fatty acids, comprising approximately 50%, 25%, and 10% of the total lipid mass, respectively¹. These mature SC lipids are generated from precursor lipids^{2,3} which are synthesized, packaged into lamellar bodies (LB), and then released into the SC extracellular space following LB fusion with the plasma membrane of granular keratinocytes⁴. Subsequent lipid processing yields the mature SC lipids [Figure 1]^{2,3}.

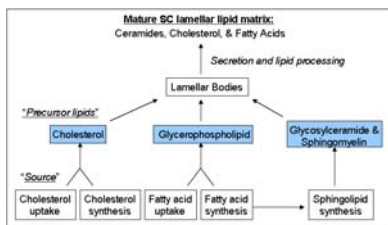


Figure 1. Summary of the *in vivo* pathways to form mature SC lamellar lipids.

METHODS

RNA was purified from: (1) Full thickness skin biopsies from individual study subjects' [young (18-20 yrs of age) or older (60-67 yrs of age) females] buttocks (sun-protected) and outer forearm (sun-exposed); and (2) SE (constructed from cells donated by 54yr old females) were treated typically for 32hrs with vehicle or a cosmetic compound [Figure 2]. Labeled target cRNA was hybridized to Affymetrix U133 Plus 2.0 microarrays; bioinformatics focused on SC lipid metabolism genes.

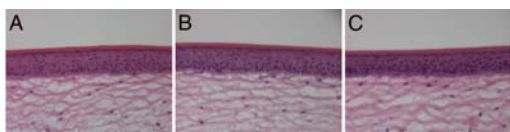


Figure 2. Representative images illustrating the histological appearance of SE cultures, (A) non-treated, (B) vehicle treated for 32hrs, and (C) cosmetic compound treated for 32hrs.

RESULTS

Figure 3 illustrates the gross appearance of skin samples analyzed, demonstrating that both intrinsically and photoaged skin show increased uneven texture and discoloration. Figures 4, 5 and 6 highlight the biochemical pathways by which SC cholesterol, fatty acids and ceramides are produced. The key intermediates are given in yellow, while the enzymes responsible for their formation are given in red.

Tables I, II, and III highlight the expression of these genes in intrinsic, photoaging and SE treated with a cosmetic compound.

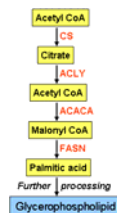


Figure 4.

Pathway for the production and metabolism of fatty acids.

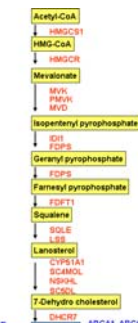


Figure 5.

Pathway for the production and metabolism of cholesterol.

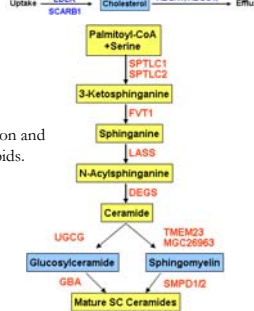


Figure 6.

Pathway for the production and metabolism of sphingolipids.



Figure 3. Representative images depicting the gross appearance of buttock (left panel) and forearm (right panel) samples used to analyze intrinsic and extrinsic aging, respectively.

Table I. Expression of genes responsible for fatty acid formation.
Red= up-regulation (p<0.05). Blue=down-regulation (p<0.05).

Acronym	Gene Name	Fold Change		Fold Change Veh vs. Treated
		Intrinsic aging (Buttock samples)	Photoaging (Forearm samples)	
CS	Citrate synthase	-1.2	-1.23	
ACLY	ATP citrate lyase	-1.57	-1.44	1.69
ACACA	Acetyl-Coenzyme A carboxylase alpha	-1.32	-1.52	1.68
FASN	Fatty acid synthase	-2.37	-2.26	1.57

Table II. Expression of genes involved in cholesterol production.
Red= up-regulation (p<0.05). Blue=down-regulation (p<0.05).

Acronym	Gene Name	Fold Change		Fold Change Veh vs. Treated
		Intrinsic aging (Buttock samples)	Photoaging (Forearm samples)	
Cholesterol Biosynthetic Genes				
HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	-1.87	-1.8	1.83
HMGCR	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	-1.32	-1.32	1.36
MVK	Mevalonate kinase	-1.57	-1.53	
PMVK	Phosphomevalonate kinase	-1.47	-1.69	
MVD	Mevalonate decarboxylase	-1.72	-2.18	
ID1	Isopentenyl-diphosphate delta isomerase 1	-1.36	-1.24	2.22
FDPS	Farnesyl diphosphate synthase	-1.41	-1.67	1.47
FPP1T1	Farnesyl-diphosphate farnesyltransferase 1	-1.35	-1.37	2.04
SOLE	Squalene epoxidase	-1.39	-1.82	1.43
LSS	Lanosterol synthase		1.13	
CYP51A1	Cytochrome P450, family 51, subfamily A, polypeptide 1	-1.48		1.36
SCAMOL	Sterol-C4-methyl oxidase-like	-1.83	-1.71	1.48
SC5DL	Sterol-C5-desaturase			1.76
NSDHL	NAD(P)-dependent steroid dehydrogenase-like	-1.66	-1.71	1.33
DHCR7	7-dehydrocholesterol reductase	-1.65	-1.65	1.53
Cholesterol Uptake and Efflux Genes				
LDLR	Low density lipoprotein receptor	-1.54		2.96
SCARB1	Scavenger receptor class B, member 1	-1.28		-1.60
ABCA1	ATP-binding cassette, sub-family A, member 1	1.43	1.56	1.52
ABCG4	ATP-binding cassette, sub-family G, member 4			-2.21

Table III. Expression of genes responsible for sphingolipid formation.
Red= up-regulation (p<0.05). Blue=down-regulation (p<0.05).

Acronym	Gene Name	Fold Change		Fold Change Veh vs. Treated
		Intrinsic aging (Buttock samples)	Photoaging (Forearm samples)	
Sphingolipid Biosynthetic and Processing Genes				
SPTLC1	Serine palmitoyltransferase, long chain base subunit 1	-1.23	1.41	1.12
SPTLC2	Serine palmitoyltransferase, long chain base subunit 2	-1.35	1.11	1.94
FVT1	Follicular lymphoma variant translocation 1	1.12		1.70
LASS2	LAG1 longevity assurance homolog 2		1.37	
LASS4	LAG1 longevity assurance homolog 4	-1.51	-1.54	-1.50
LASS5	LAG1 longevity assurance homolog 5			
LASS6	LAG1 longevity assurance homolog 6		-1.44	-1.60
DEGS1	Degenerative spermatocyte homolog 1, lipid desaturase		-1.32	-1.70
DEGS2	Degenerative spermatocyte homolog 2, lipid desaturase	-1.69	-1.52	-1.59
UGCG	UDP-glucose ceramide glucosyltransferase	-1.23	-1.94	1.45
MGC26963	Hypothetical protein MGC26963			
TMEM23	Transmembrane protein 23		1.31	1.76
GBA	Glucosidase, beta, acid	-1.32	-1.17	
SMPD1	Sphingomyelin phosphodiesterase 1			1.42
SMPD2	Sphingomyelin phosphodiesterase 2			

SUMMARY

Both intrinsically and photoaged skin show increased uneven texture and discoloration, both more pronounced in photoaging, suggesting common underlying pathways. In both intrinsic and photoaging there was a coordinated down-regulation of genes involved in SC lipid metabolism pathways. In contrast, treatment of SE cultures with the cosmetic compound led to increased expression of lipid metabolism genes as biomarkers of an improved skin barrier.

- As compared to younger skin, the expression of genes involved in fatty acid production was down-regulated in both intrinsic and photoaging. In contrast, treatment of SE with a cosmetic compound led to increased expression of these genes as biomarkers of an improved skin barrier [Table I].
- Similarly, the expression of cholesterol synthesis pathway genes was down-regulated in both intrinsic and photoaging. Treatment of SE with a cosmetic compound led to increased expression of these genes as biomarkers of an improved skin barrier [Table II].
- Sphingolipid biosynthesis and processing were also down-regulated, however this was more pronounced in intrinsically than photoaged skin. Treatment of SE with a cosmetic compound led to a moderate increase in expression of these barrier biomarkers [Table III].

CONCLUSIONS

The coordinated down-regulation of SC lipid pathways likely contributes to the decreased capacity of aged skin to maintain and repair the SC barrier, although the SE data suggest this state can be improved by cosmetic compounds.

This work was funded by P&G Beauty & Grooming.

- References
- Wenz P, Norder L. "Confidence intervals" for the "true" lipid composition of the human skin barrier? In: Fortlind B, Lindberg M, eds. Skin, Hair, and Nails. Structure and Function. New York: Marcel Dekker Inc, 2003:85-106.
 - Mao-Qiang M, Feingold KR, Jain M, Elias PM. Extracellular processing of phospholipids is required for permeability barrier homeostasis. J Lipid Res. 1995 Sep;36(9):1925-35.
 - Uchida Y, Hara M, Nishio H, Sidransky E, Inoue S, Otsuka F, Suzuki A, Elias PM, Holleran WM, Hamanaka S. Epidermal sphingomyelins are precursors for selected stratum corneum ceramides. J Lipid Res. 2000 Dec;41(12):1071-82.
 - Rastner U, Feingold KR, Curnie DA, Elias PM. Coordinate assembly of lipids and enzyme proteins into epidermal lamellar bodies. Trans GCB Res Clin. 1999; 498.

