ANTI-AGING EFFECT OF A NEW TOPICAL CREAM DEMONSTRATED BY GENE MODULATION AND MORPHOLOGY STUDIES OF THE SKIN LAYERS

N. Chevreau[1], A. Patatian[2], L. Peno-Mazzarino[3], E. Lati[3], and P. Benech[4]

LifeVantage[1], Sandy, UT, USA, Labs Genex[2] & Bio-EC[3], Longjumeau, Aix Marseille Univ.[4] FR

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P-086 INTRODUCTION

With aging comes great wisdom and older looking skin. This study intended to demonstrate two main points: 1) The cosmetic activities of a new anti-aging cream compared to a retinol-based cream, and 2) The functional pathways involved in the anti-aging benefits of this novel cream. The experiments were conducted using skin explants (ex-vivo) of a middle age Caucasian subject rather than testing the formula in primary cell cultures where the cross talk between cells of different origins often found in living tissues is lost.

MATERIALS & METHODS

Table 1: Blend X
Brassica Juncea
Brassica Oleracea Capitata
Brassica Oleracea Italica
Brassica Oleracea Botrytis
Brassica Oleracea Acephala
Plantago Lanceolata
Bacopa Monnieri (Brahmi)
Silybum Marianum (Milk Thistle)
Curcuma Longa (Turmeric)
Black Pepper (Piperine)
Camellia Sinensis (Green Tea)
Wasabia Japonica

Tested Formula: The new anti-aging cream (TS cream) is an O/W emulsion that contains a combination of known anti-wrinkle and moisturizing bioactives and a novel patent-pending blend of 12 botanical extracts (Blend X; Table 1).

Human Skin Explants: samples of a 47 year old woman were either treated twice a day for 7 days with the TS formula or left untreated.

Sampling Time: Explants were analyzed at 3, 9 and 24 hours on Day 0 for gene expression. Histological and microscopical analyses were done at D0, D3 & D7.

Gene Expression: Total RNAs were extracted at each sampling time for each explant using the RNeasy Fibrous Tissue Kit-Qiagen after disruption and homogenization using the Tissue Lyser Kit. They were placed in an Agilent microarray to measure gene expressions. Gene expression intensity of the TS-treated samples was compared with the untreated samples. Gene expression with a fold change of at least ≥1.45 and ≤0.6 between the TS and the untreated samples was considered for submission to PredictSearch analysis. Only the genes that were modulated at least two consecutive time points were considered for analysis. This tool allows statistical co-citation analysis of annoted keywords to define relationships between genes and biological processes with a direction of effects rather than mere associations.

RESULTS

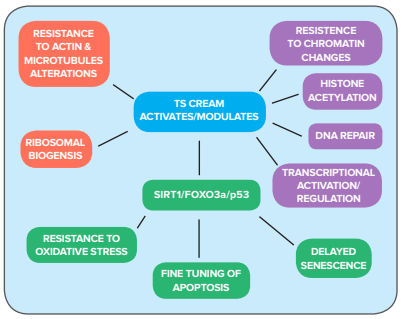
Table 3: Up-Regulated Genes		
3h9h24hUP	3h9hUP	9h24hUP
ARHGEF35	ADPGK	AHNAK
CCDC47	ARHGEF35	CTSZ
ETF1	ASPN	CXADR
FBX011	BRD3	DMTF1
IMMT	CAT	EFTUD1
LEO1	CDCP1	EFTUD1
LSM134A	GLE1	HERC2
MBTD1	HIST1H4K	MTUS1
MXI1	HOOK1	NBPF1
MXI1	HSP90AB2P	NBPF10
NAMPT	IL4R	NFIX
NCOR1	MAP3K2	NOTCH2NL
OFD1	MAT2B	NPHP3
PHACTR2	MBNL3	NXNL2
RABGAP1	MEAF6	PPFIBP1
RALBP1	MKRN1	RN18S1
C39A6	SIAH1	SMARCE1
SMARCE1	SPATA13	SRRT
SMC1A	SRRM2	UBE2E1
SMC1A	TDG	
SNX9	TJP2	
TPM4	TOP2A	
UGGT1	UTRN	
WDR3		
ZMYM6		

Gene Expression: The genes found to be significantly upregulated in the TS treated explants versus untreated ones at least at 2 consecutive time points are listed in table 3. The integration of these genes through PredictSearch led to identify 7 significant biological pathways. TS treatment may initially alter the cell shape by cellular cytoskeleton network rearrangement that also impacts chromatin remodeling. TS treatment also induced anti-oxidative activities sustained mainly by the strong induction of catalase (CAT), tropomyosin4 (TPM4), Ral-binding protein1 (RALBP1), and nicotinamide phosphoribosyl-transferase (NAMPT).

FUNCTIONAL PATHWAYS

In addition, the analysis suggests that TS treatment affects ribosomal RNA biogenesis, & strengthens mitochondria functions as well as DNA repair activities and a fine tuning of the cell cycle to avoid senescence. We should note that both LOX and LOXL2 (encoding lysyl oxidase and lysyl oxidase-like2) that are known to be overexpressed in senescence cells were significantly repressed at the 3 hour time mark (Data not shown).

Schematics of Functional Pathways

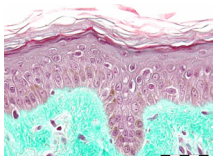


We speculate that this fine tuning of cell growth arrest and apoptosis is related to the action of TS on SIRT1, FOXO3a and p53 which contribute to inhibit senescence. The schematics representation of the functional pathways is shown here.

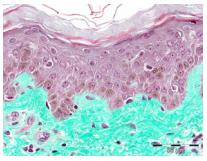
Furthermore, genes that were repressed belonged to the same family of small nucleolar RNAs (Data not shown). With the overexpression of WDR3 and the downregulation of those, we speculate that WDR3 is stimulated to reinitiate ribosome biogenesis.

SKIN CROSS SECTIONS MORPHOLOGY

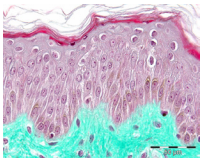
Explants treated with TS cream showed a significant thicker epidermis with a uniform stacked appearance of well differentiated cells compared to the untreated explants. No sign of inflammation or nuclear material in the stratum corneum was seen. By contrast skin samples treated with 0.2% retinol cream show significant disruptions in the epidermal cell organization at Day 7 with sign of inflammation, cellular edema and chromatin aggregation.



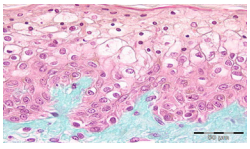
Day 0



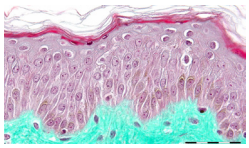
Untreated Day 7



TS Cream Day 7



0.2% Retinol Day 7



TS Cream Day 7

CONCLUSIONS

Our study suggests that the TS cream sets up protective and regenerative cellular responses. Its activities might at first affect the cytoskeleton network and chromatin structures leading to the stimulation of epigenetic events, in particular histone acetylation. Mitochondria and DNA repair activities along with ribosomal RNA biogenesis, resistance to oxidative stress and the fine tuning of the cell cycle will contribute to avoid senescence and regulate the balance between proliferation and apoptosis. These activated pathways orchestrate a continuum of activities ultimately leading to anti-aging benefits. As a consequence, seven-day treatment with the TS cream had skin restructuring effects with visible improvement at all skin layers, while no inflammation is detected.