8th Anti-Ageing Skin Care Conference
29 - 30 November 2022
Royal College of Physicians, London

Inflammaging in human photoexposed skin: Early onset of senescence and imbalanced epidermal homeostasis across the decades

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Skin Aging Continuum Model

Daily Stress

Acute Response

Transient

Damage Repair

Chronic inflammation

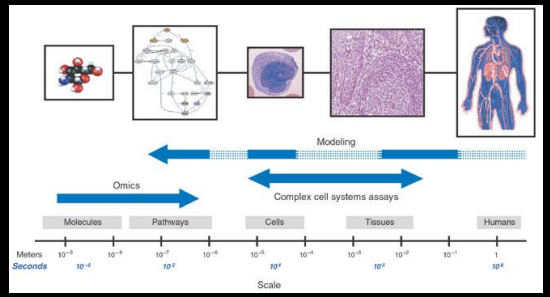
Drift

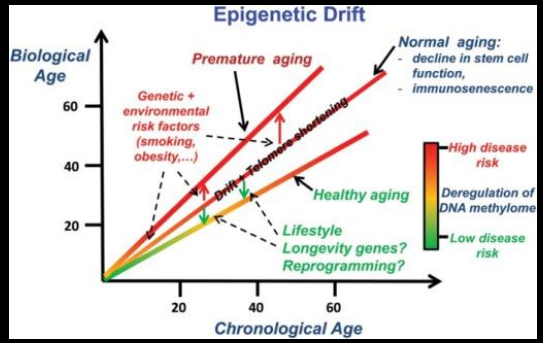
Cellular dysfunction

Tissue Dysfunction

Aged phenotype

Prematurely Aged/Less healthy



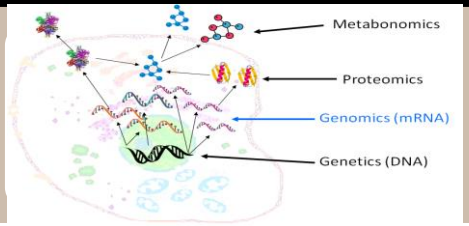


10's 20's 30's 40's 50's 60's

Multi-Decade and Ethnicity Study (MDE)

Objective:

Utilize a systems biology approach to develop a fundamental understanding of the molecular mechanisms which contribute to skin aging/photoaging



Multiple Decades

20s, 30s, 40s, 50s, 60s, 70s (n=22-25)

Multiple Body Sites

Face, outer forearm, buttocks

Systems Biology

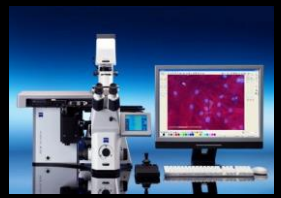
Skin structure & biology, genomics, proteomics, metabonomics, epigenetics

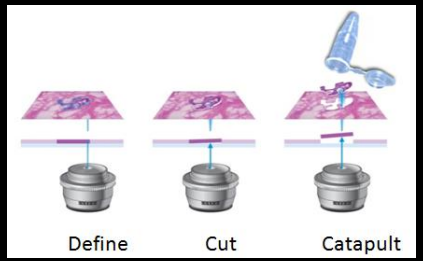
Dr. Alexa Kimball, Harvard U & Mass Gen

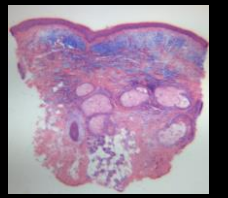
HARVARD UNIVERSITY

Laser Capture Microdissection (LCM)

Precise sampling of skin compartments and reduces complexity of signal with mixed compartments





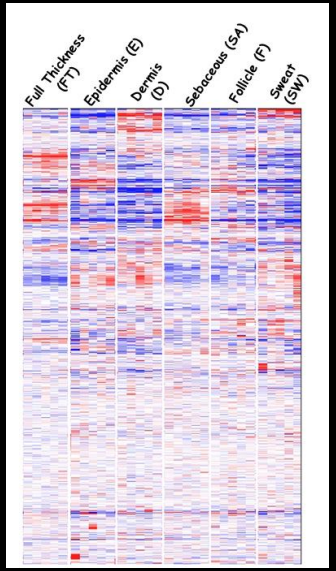


Sebaceous glands

Hair Follicles

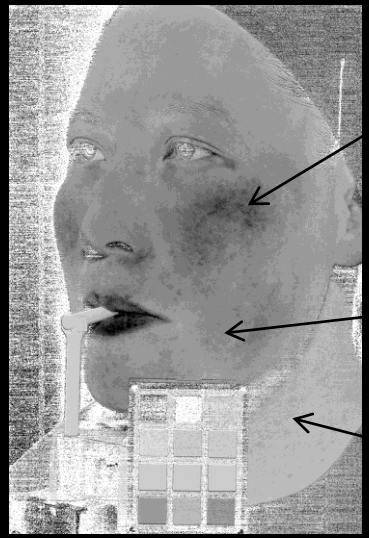
Epidermis

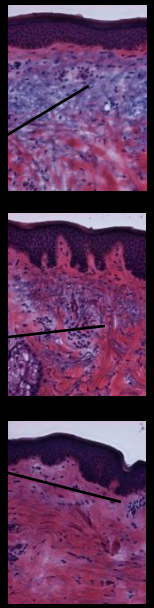
Dermis



The Multi-Decade and Ethnicity Study (MDE)

Elastosis, inflammation, and erythema: Consistent pattern of highest levels in upper cheek



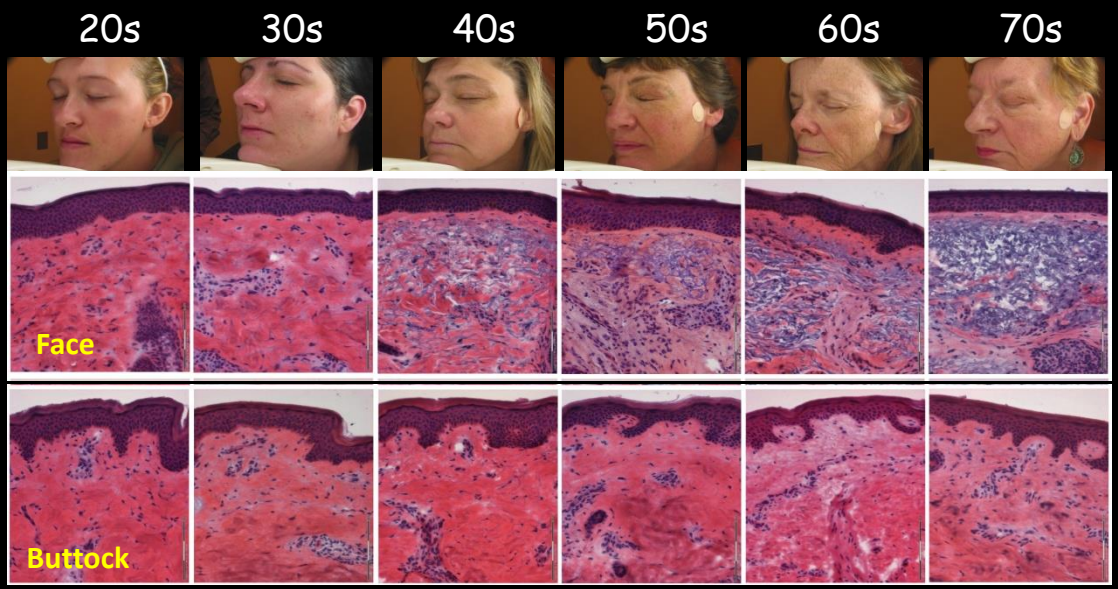


IL-1RA/IL-1α

410

188

33



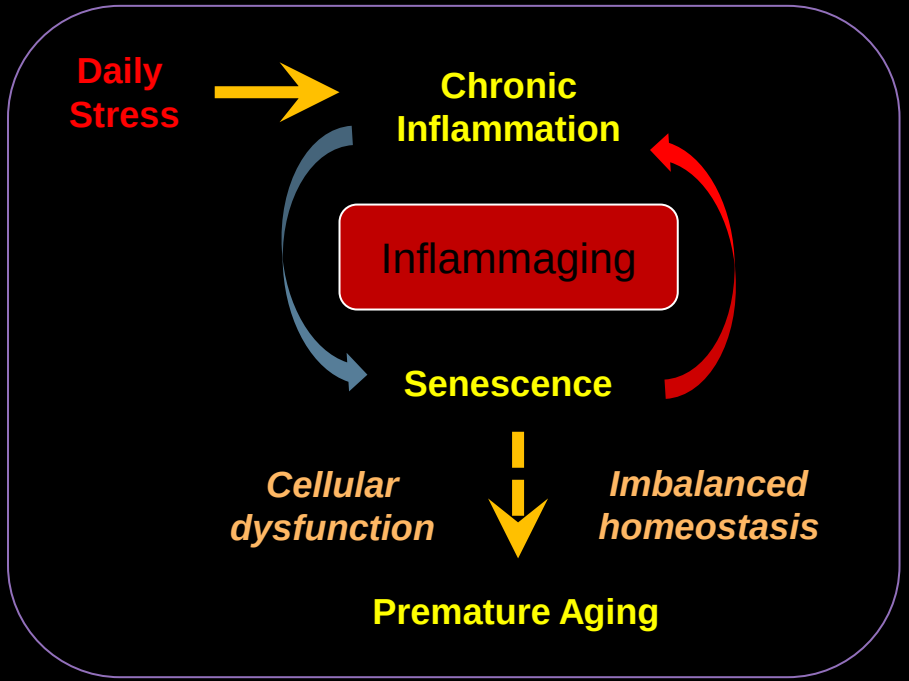
Macro structural elastosis increases with age

The Multi-Decade and Ethnicity Study (MDE)

Hypothesis: Inflammaging ecosystem in skin

Stress induced inflammation that leads to premature onset of senescence and aging

https://www.skininc.com/science/physiology/article/21880711/inflammaging-changing-the-face-of-skin-care



Markers of inflammation, photoexposure, and DNA damage and methylation elevated across age groups

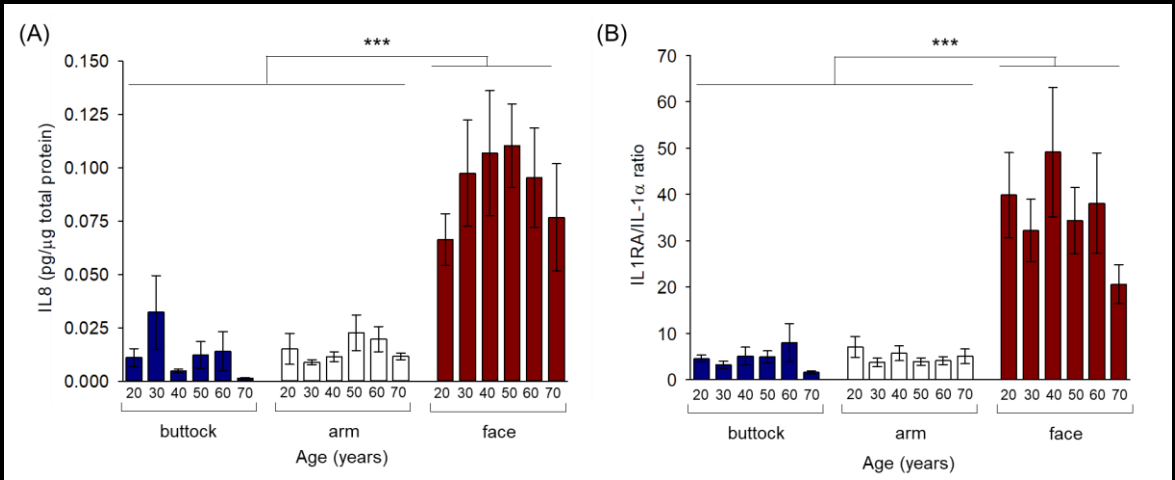
Inflammatory markers elevated in photoexposed face

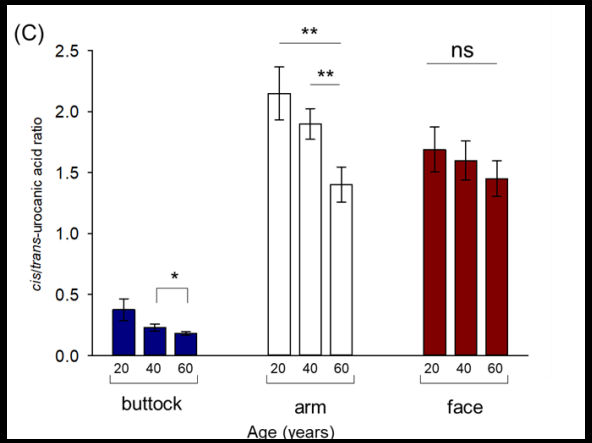
Photosensitive biomarker elevated in both photoexposed sites

IL-8

IL1RA/IL-1α

cis/trans-urocanic acid

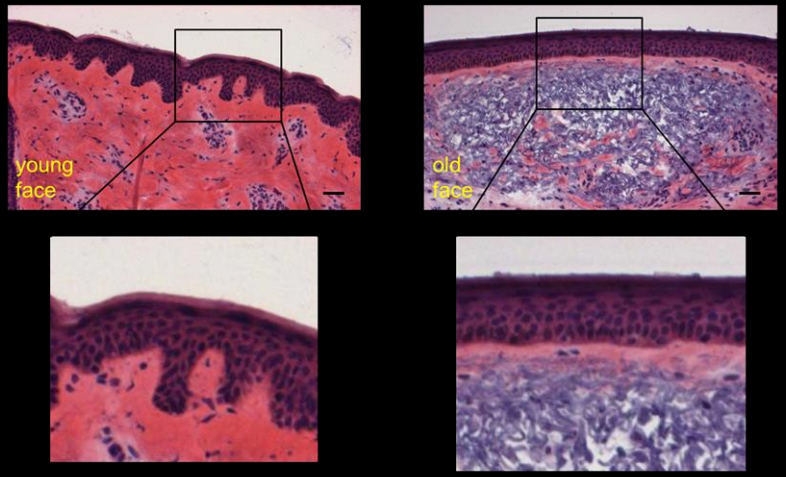


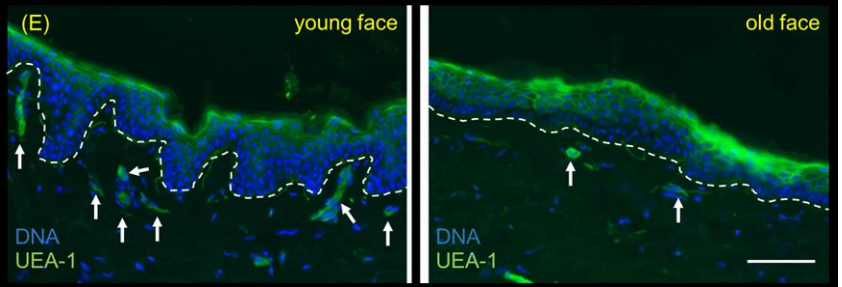


Age-associated changes in epidermal morphology

young

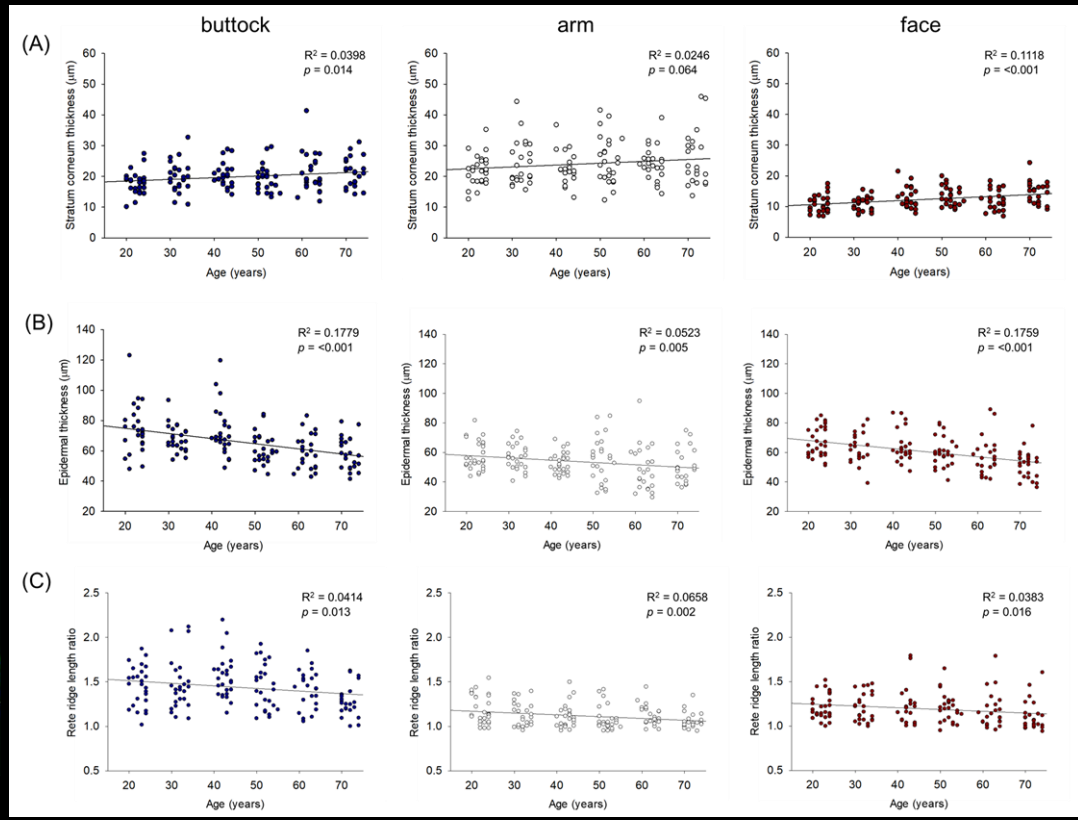
old





Loss of microcapillary vessels

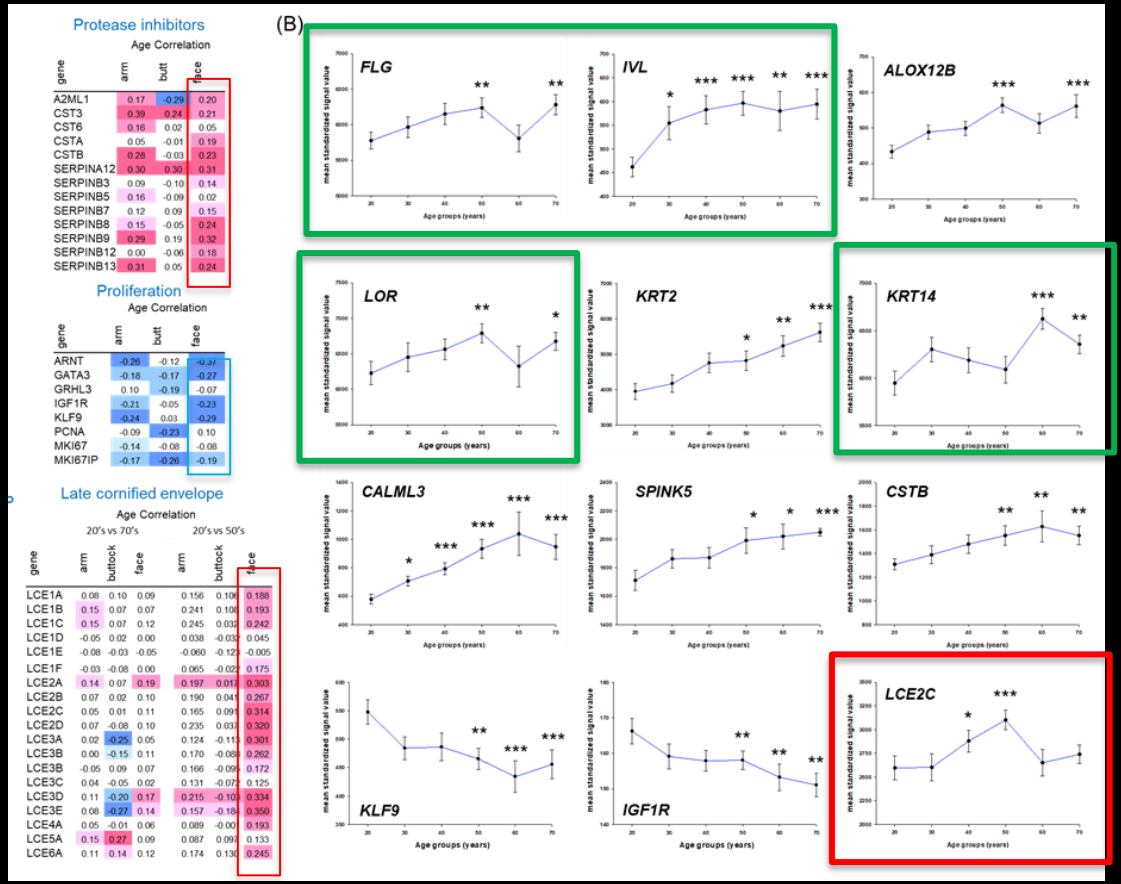
Significant changes between 20's and 60-70's



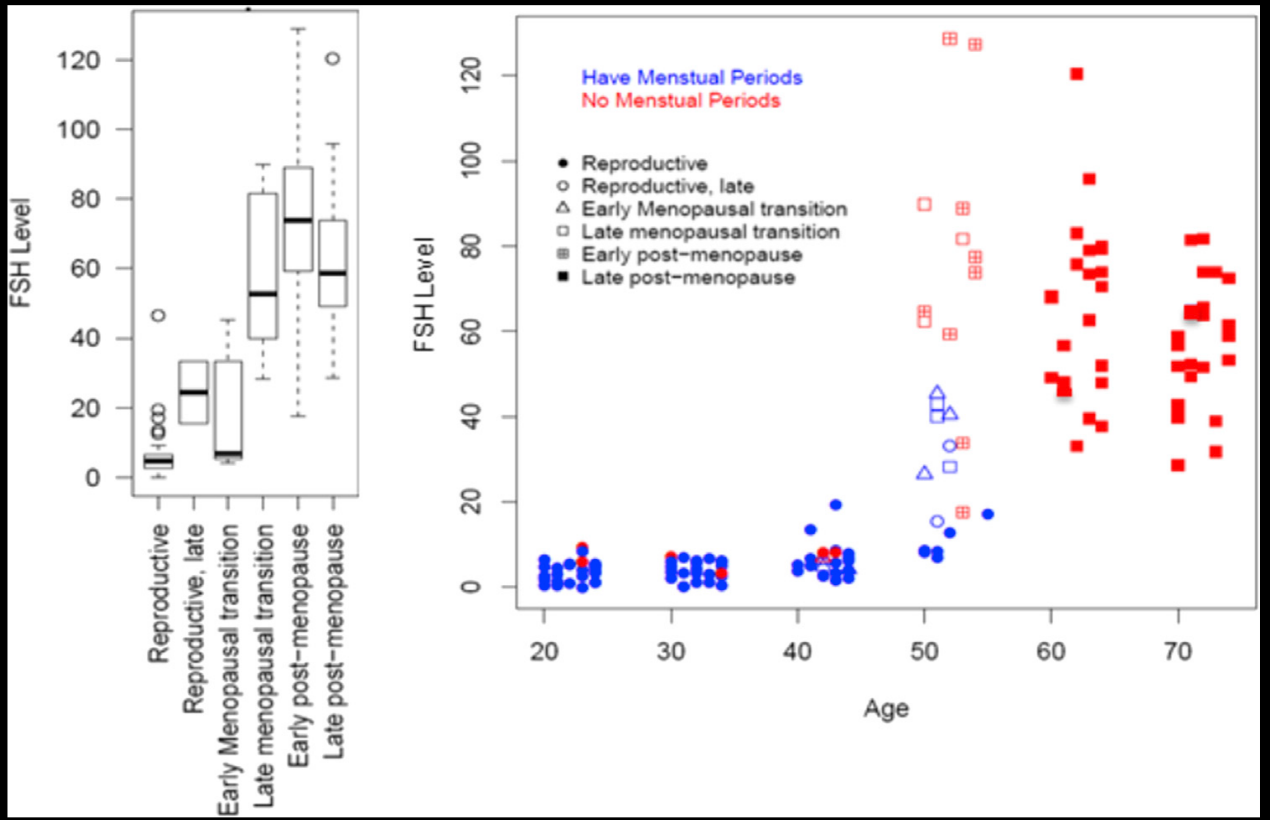
Gene expression patterns of differentiation associated proteins

Processes/components showing increased expression with photoage

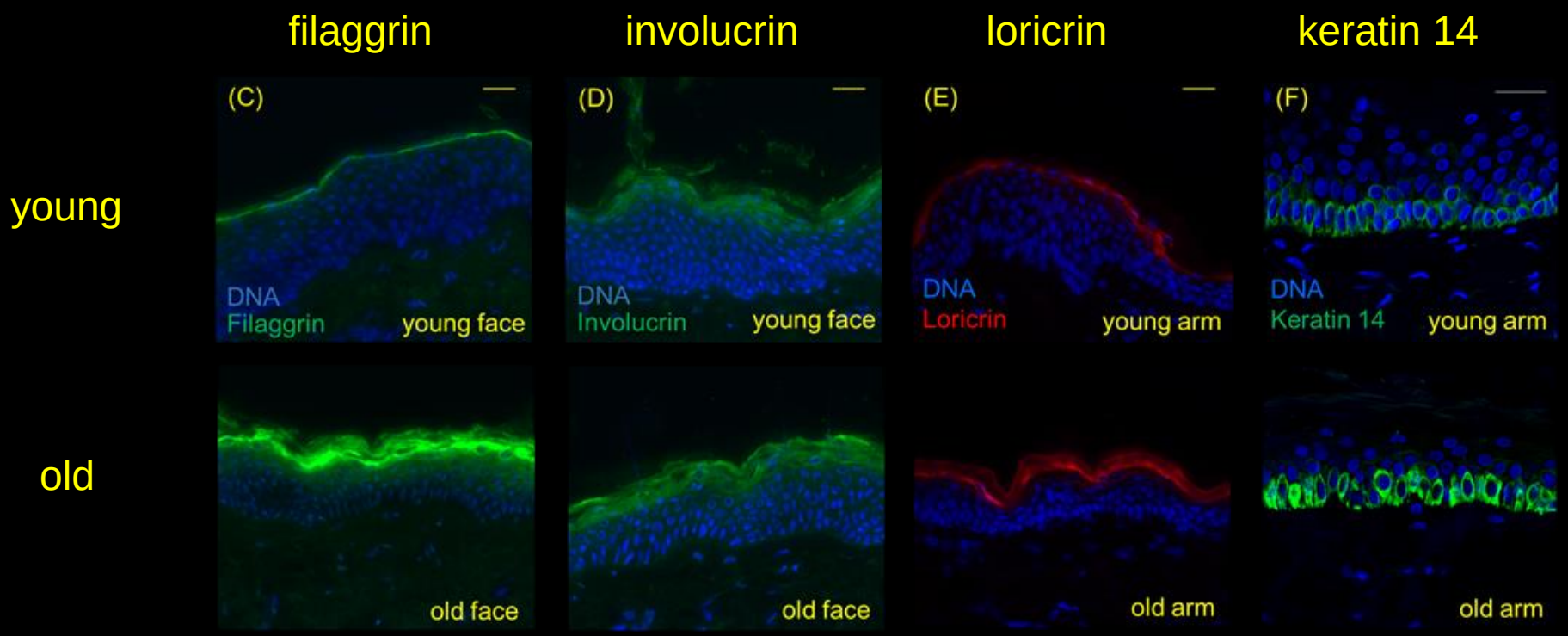
- Epidermal Differentiation Complex
- Proteases
- Keratins
- Calcium binding protein/AMP
- Protease inhibitors
- Proliferation (lower)
- Late cornified envelope (20's-50's)



Menopausal status of MDE subjects



Staining of select differentiation associated proteins in face and arms



Proteomics supports similar directional differences as expression patterns between old and young (outer forearm)

Table 2. Median fold change of detected proteins between 60's and 20's age groups from laser capture microdissection sections of epidermis from photoexposed dorsal forearms and changes in gene expression correlation.

Protein	Gene	Median fold change 60's vs 20's	*p*-value	Gene expression changes with age
Keratin 2	KRT2	1.57	<0.001	Increased
Keratin 10	KRT10	1.50	<0.001	Increased
Cystatin M	CST6	1.86	0.001	Increased
Cystatin A	CSTA	2.37	0.002	Increased
Calpain 1	CAPN1	2.33	0.009	No
Fructose-bisphosphate aldolase A	ALDOA	2.04	0.010	Increased
Arachidonate 12 lipoyxgenase 12R	ALOX12B	2.42	0.011	Increased
Bleomycin hydrolase	BLMH	1.73	0.016	Increased
Annexin A8	ANXA8	1.86	0.020	Increased
Cystatin B	CSTB	2.14	0.022	Increased
Annexin A1	ANXA1	1.41	0.026	Increased
Involucrin	IVL	1.47	0.026	Increased
Transglutaminase 1	TGM1	1.38	0.026	Increased
Annexin A2	ANXA2	1.22	0.026	Decreased
Suprabasin	SBSN	1.37	0.026	Increased
Serine protease inhibitor Kazal-type 5	SPINK5	1.43	0.028	Increased
Calmodulin-like protein	CALML3	1.71	0.030	Increased
Malate dehydrogenase 2	MDH2	0.56	0.044	Decreased
Protein S100-A14	S100A14	1.35	0.046	Increased
Pyruvate kinase M	PKM	1.23	0.049	Increased
Gelsolin	GSN	1.23	0.062	Increased
Transglutaminase 3	TGM3	1.45	0.084	Increased
Hemoglobin alpha	HBA	16.40	0.092	Increased

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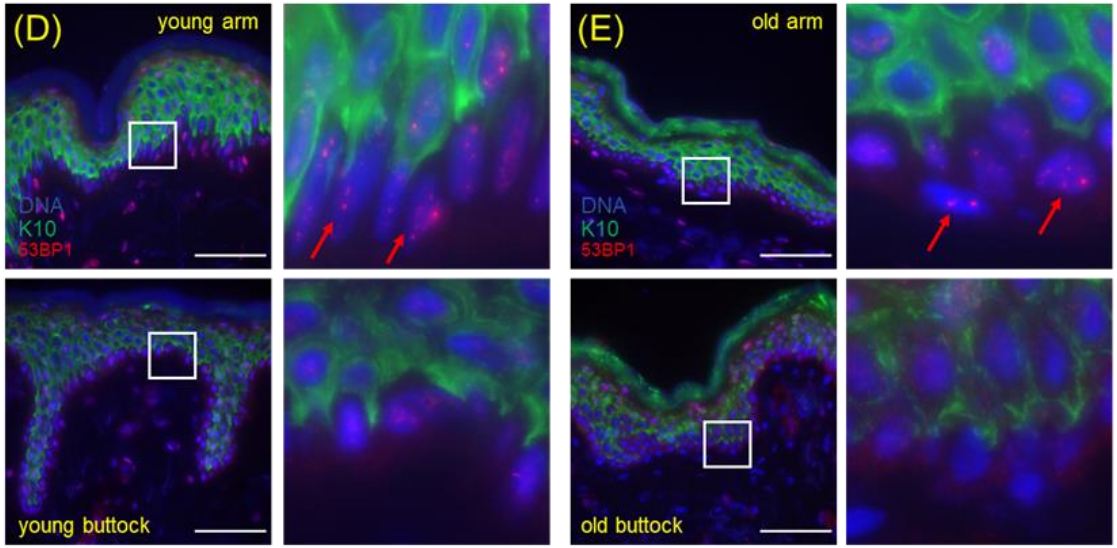
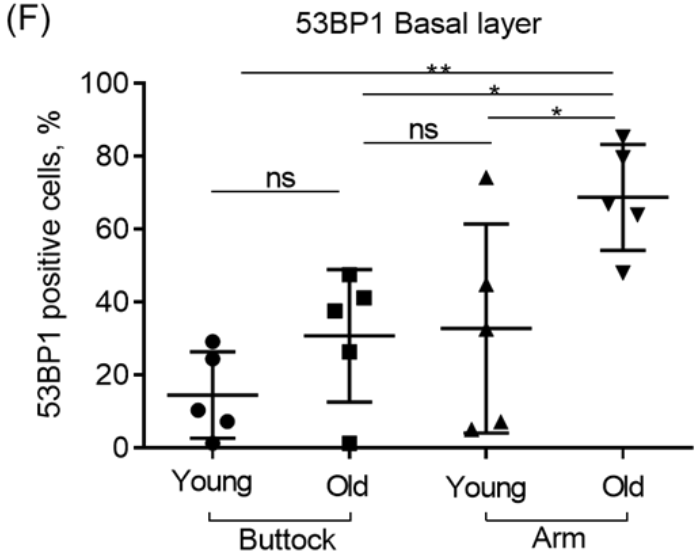
Markers of DNA damage

53BP1 – DDR marker





young old

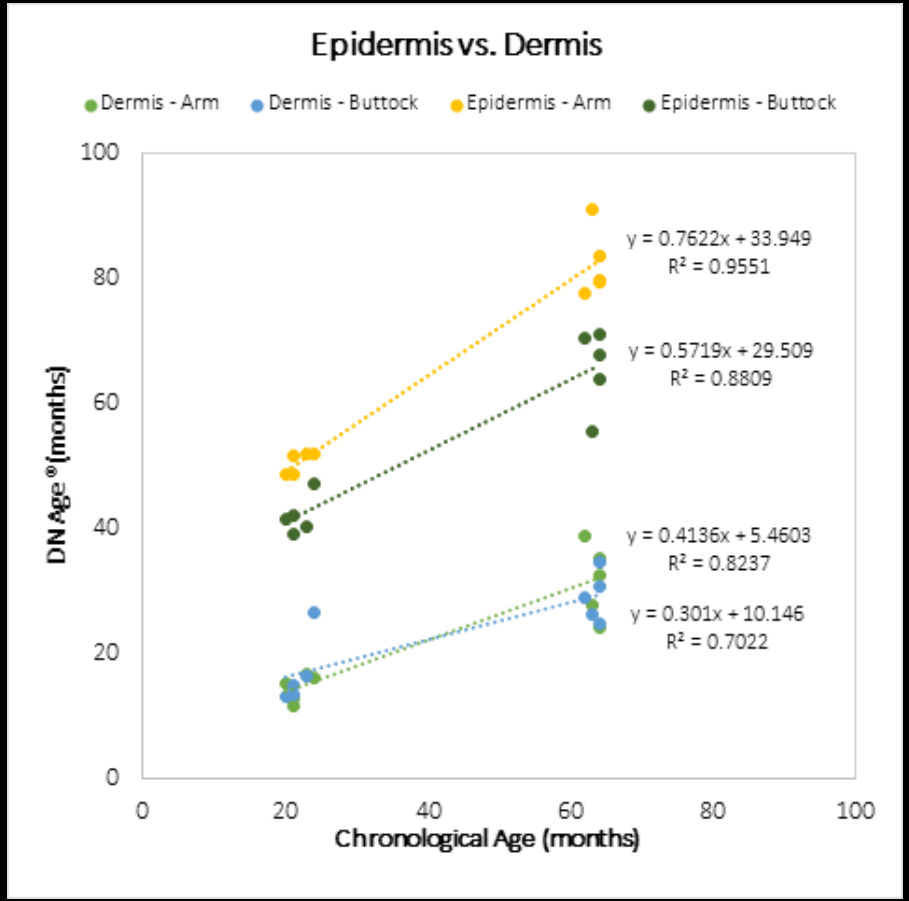
Higher in photoexposed arm compared to buttock sites and increases with age

Markers of DNA methylation

Epigenetic
methylation content

- Epidermis - Higher in photoexposed arm compared to buttock sites and increases with age
- Dermis - Patterns increase with age but not photoexposure

ZYMO RESEARCH



Senescence associated transcript profiles

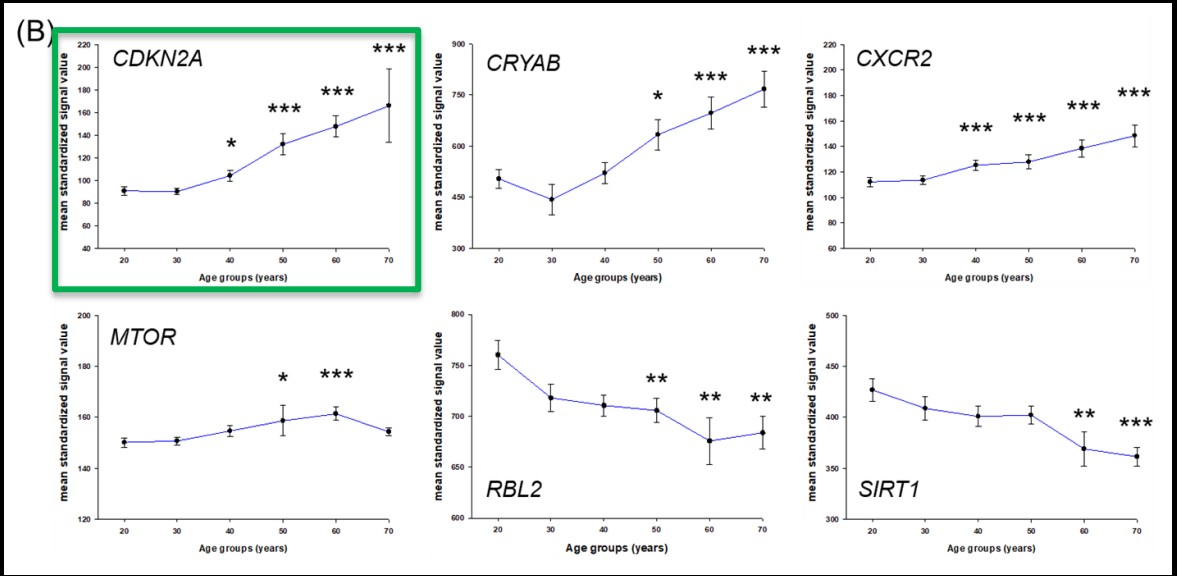
(A)

gene	Age Correlation: arm	buttock	face
CCL22	0.23	0.11	0.26
CDKN2A	0.66	0.43	0.64
CDKN2B	0.21	0.14	0.020
CD74	0.33	0.34	0.20
CREG1	-0.05	0.11	0.20
CRYAB	0.48	0.28	0.41
CXCR1	0.12	-0.26	0.26
CXCR2	0.45	0.06	0.35
FOXE1	0.48	0.04	0.30
GLB1	0.22	-0.22	0.15
IL2RG	0.14	-0.04	0.26
IL15	0.23	0.11	0.25
IL18R1	0.29	0.29	0.34
MMP9	-0.02	0.16	0.14
MTOR	0.14	-0.04	0.23
PYCARD	0.01	-0.27	0.22
SMAD3	0.31	0.09	0.20
STAT1	0.06	0.10	0.16
IL7	-0.25	-0.09	-0.33
LMNB1	-0.27	-0.17	-0.02
RBL2	-0.17	0.06	-0.24
SIRT1	-0.39	-0.10	-0.38
SIRT3	-0.29	-0.01	-0.19
SIRT4	-0.25	-0.18	-0.39
TP53	-0.20	-0.14	-0.11

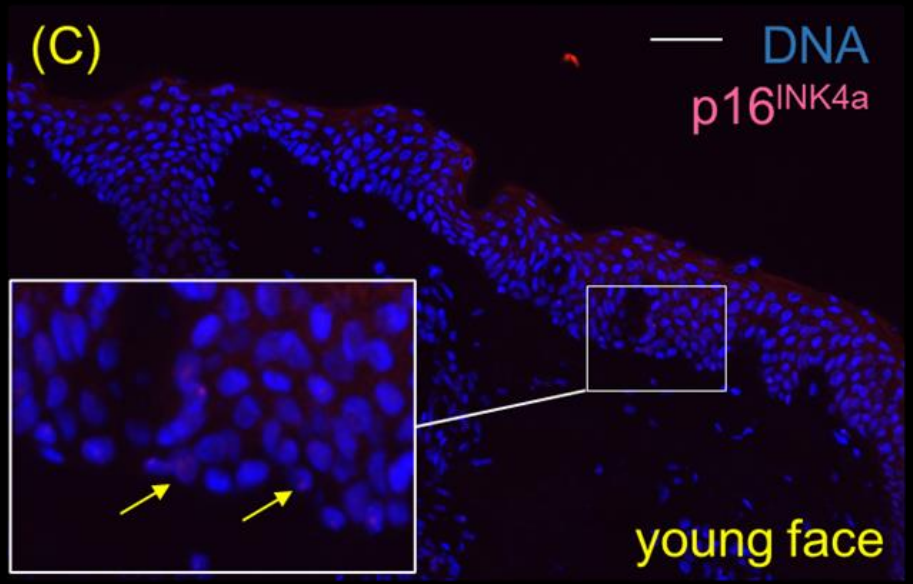
expression color legend		
p values	↑	↓
<0.05		
<0.001		
<0.0001		

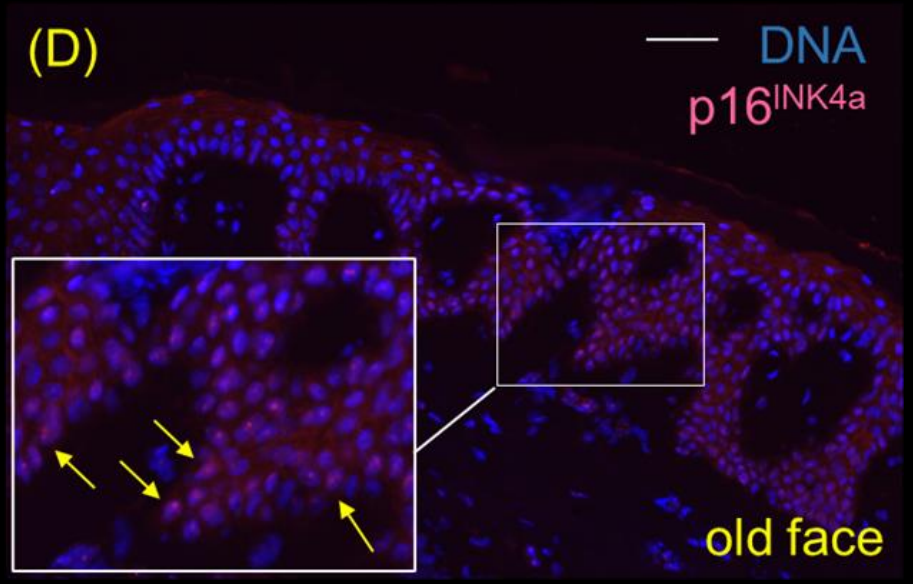
p16^{INK4a}

(B)



Elevated levels of p16^{INK4a} staining in nuclei from old face





Oxygen sensing/hypoxia and glycolysis associated transcript profiles

(A) Oxygen sensing/hypoxia

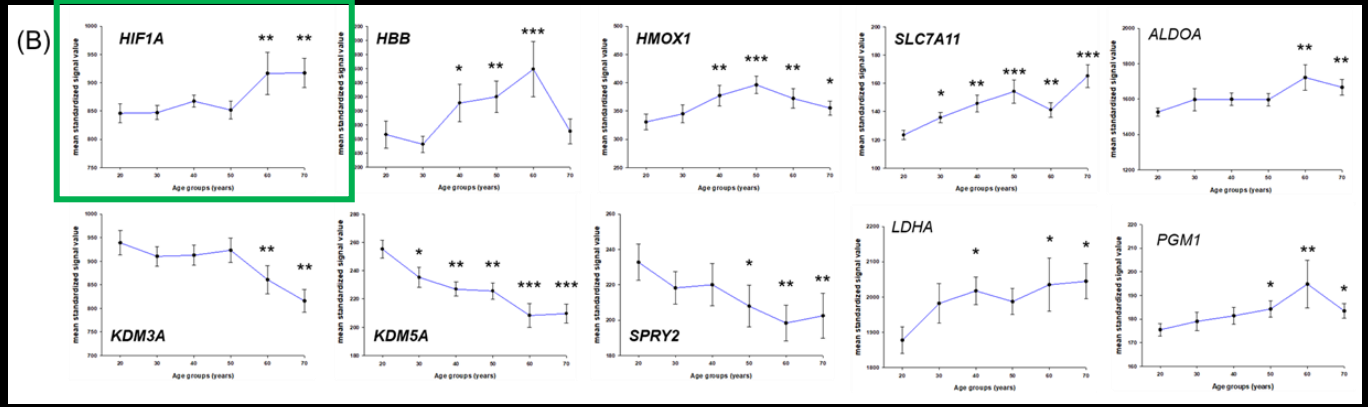
gene	Age Correlation arm	buttock	face
AK1	0.14	0.11	0.23
CDCA8	-0.12	-0.08	0.16
CXCL12	0.17	0.20	0.04
CXCL16	0.34	0.06	0.29
CXCR4	0.17	0.09	0.18
CXCR6	0.05	0.00	0.21
EGLN1	0.13	0.15	0.06
EGLN3	0.37	0.42	0.09
HBA	0.18	-0.08	0.14
HBB	0.10	-0.25	0.19
HIF1A	0.32	0.43	0.25
HMOX1	0.17	-0.04	0.17
PDSS1	-0.15	-0.07	0.17
SLC2A1	0.18	0.21	0.05
SLC2A3	0.19	0.08	0.02
SLC7A11	0.25	0.32	0.40
VHL	0.24	0.14	0.06
KDM3A	-0.05	0.20	-0.25
KDM5A	-0.04	0.01	-0.42
KDM6A	-0.24	0.02	-0.26
PPARGC1A	-0.23	-0.03	-0.15
SPRY2	-0.16	-0.24	-0.25

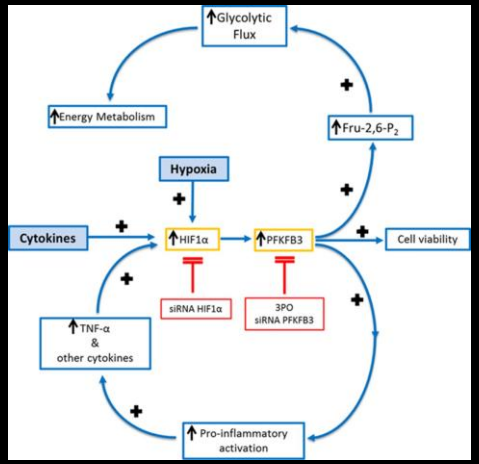
(A) Glycolysis

gene	Age Correlation arm	buttock	face
ACO1	0.17	-0.10	0.01
ACO2	0.24	0.08	0.27
ALDOA	0.19	0.27	0.25
ALDOC	0.15	0.18	0.17
ENO1	-0.04	-0.08	0.26
GAPDH	-0.07	0.12	0.10
GPI	0.00	0.10	0.06
HK1	0.13	0.09	0.18
HK2	-0.04	0.00	0.21
HK3	-0.07	0.08	0.15
LDHA	0.28	0.10	0.19
PFKFB3	0.15	0.09	0.02
PFKFB4	0.02	-0.06	0.02
PFKL	0.12	0.06	0.15
PFKP	-0.17	-0.11	0.03
PGM1	0.20	0.09	0.23
PGK1	0.06	0.21	0.28
PKM	0.20	0.30	0.23
TPI1	-0.04	-0.05	0.06

expression color legend		
p values	↑	↓
<0.05		
<0.001		
<0.0001		

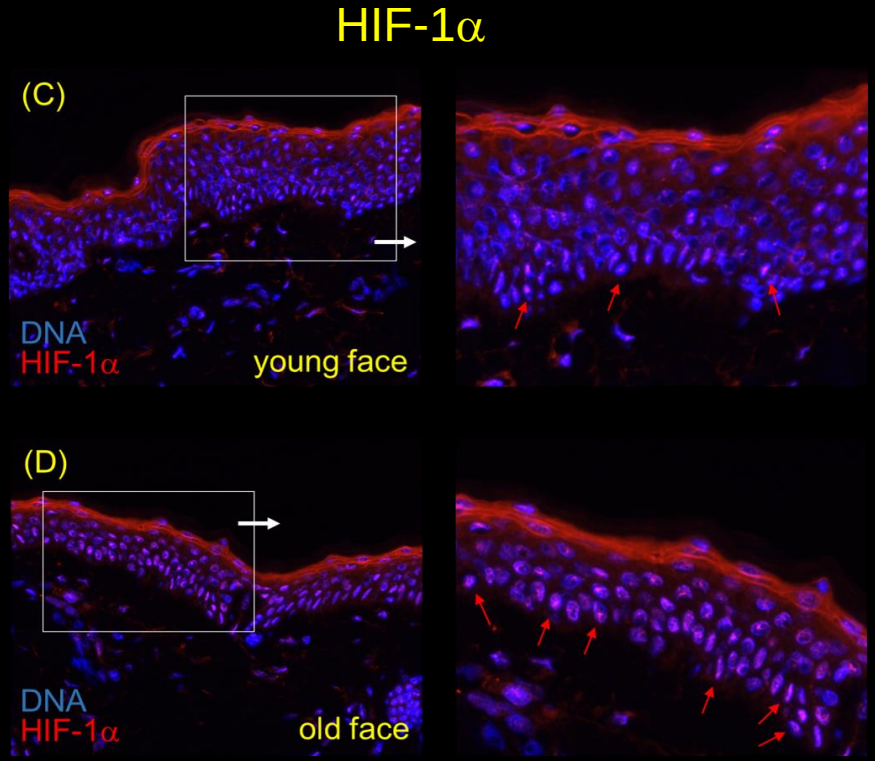
(B)





HIF-1α and PFKFB3 Mediate a Tight Relationship Between Proinflammatory Activation and Anerobic Metabolism in Atherosclerotic Macrophages
Ahmed Tawakol,et al, Arteriosclerosis, Thrombosis, and Vascular Biology. 2015;35:1463–1471

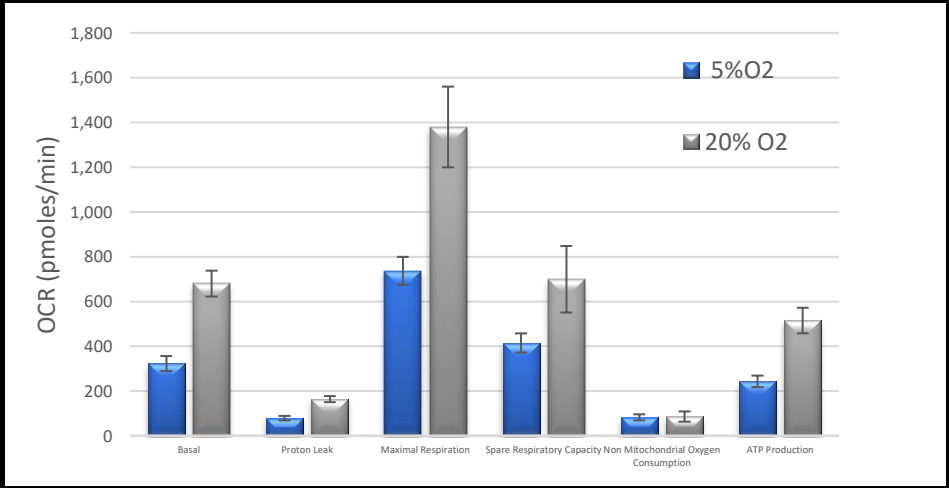
Greater staining for HIF-1α and hemoglobin α in old photoexposed sites



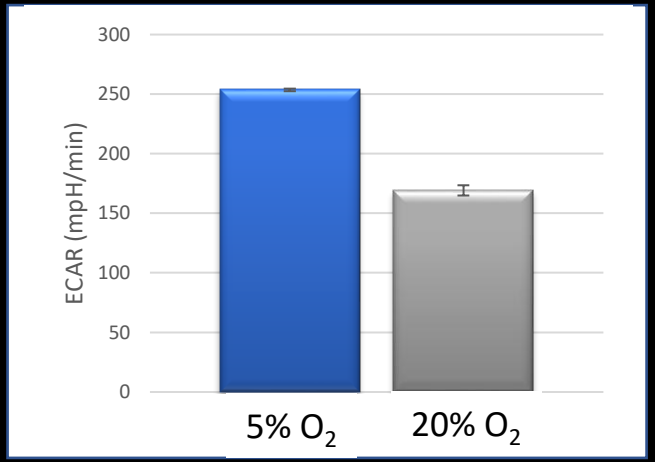
Keratinocytes shift towards glycolysis under lower oxygen tensions

- HaCaT keratinocytes grown under 5% or 20% O_2 conditions
- Mitochondrial function and glycolysis tested on Seahorse Flux Analyzer

Mitochondrial energy stress test



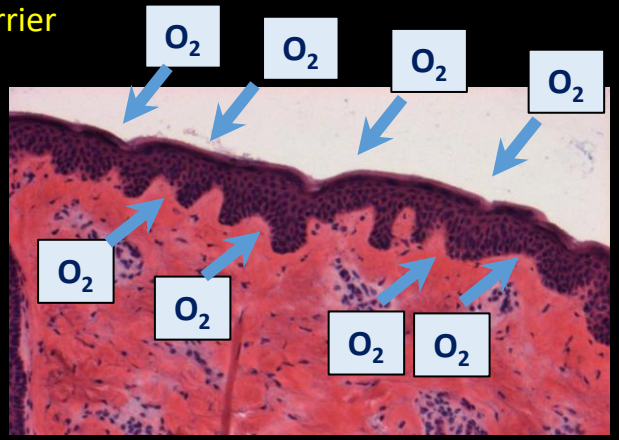
Glycolytic rates



Impact of epidermal morphology changes on oxygen availability?

Normal barrier

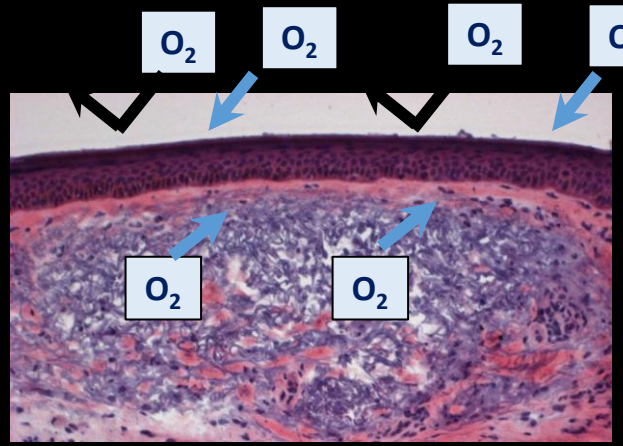
Rete ridges (high surface area)



Intact blood capillaries (normal oxygen supply)

Age

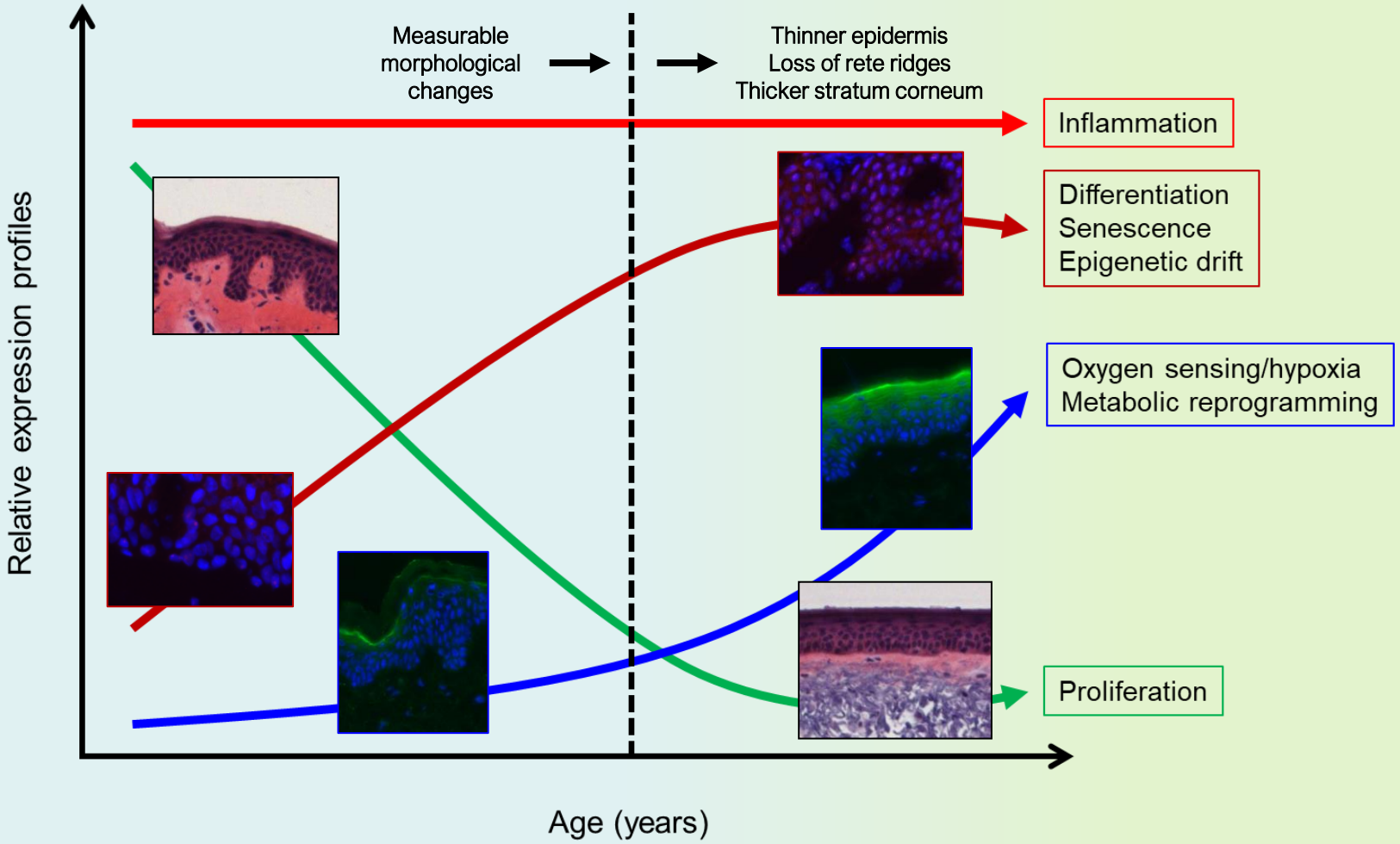
Thicker stratum corneum (longer path)



Loss of rete ridges (lower surface area)

Damaged leaky blood capillaries (lower oxygen supply)

Key biological processes changing with age in epidermis of photoexposed skin



Summary

- This systems biology-based analysis supports that photoexposed facial skin is undergoing inflammaging in the 20's and that multiple biologic pathways are affected in this process.
- Chronic presence of inflammation and senescence induction early in age may contribute to the molecular reprogramming, imbalance of epidermal homeostasis, and morphological changes.
- Heightened oxygen sensing/hypoxic response, epigenetic drift, and metabolic shift may play roles leading to this imbalance.
- Detection of non-erythroid-derived hemoglobin requires further evaluation on its function and role in skin biology and aging.
- While this work provides a body of evidence on the role of senescence and inflammaging in impacting skin aging, further work is still required.

Acknowledgements

P&G

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Yale School of Medicine (New Haven, CT, USA)

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Thank you