CAN THE AGEING SCALP DERMAL FIBROBLAST INFLAMMATORY PHENOTYPE BE REVERSED?

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Introduction:

We have previously demonstrated significant structural age-related changes in the dermal environment of female scalp[1,2]. Such detrimental changes will reduce the ability of scalp skin to support large terminal hair follicles. Furthermore, primary scalp dermal fibroblasts from older female donors exhibited an altered phenotype in culture, including significant changes in their proteomic secretome[1].

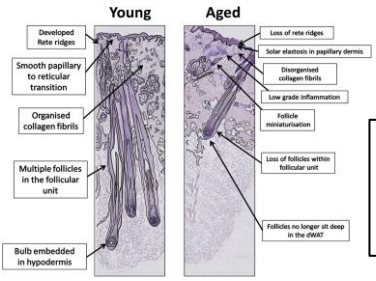


Figure 1. Structural & biochemical changes in the female scalp with age[1]

Aims:

- Determine whether conditioned medium (secretome) modulates the inflammatory phenotype of aged DFs and if a "younger secretome" can reverse it.
- Compare the intracellular proteome of cultured DFs from female scalp (<30yrs) and (>50yrs) with LC-MS.

Materials & Methods:

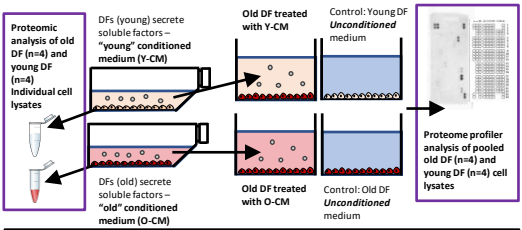


Figure 2. Workflow diagram of cytokine plasticity assay and proteomic analysis. *Old DFs (n=4) = red cells, pink CM; young DFs (n=4) = orange cells, orange CM; blue medium is unconditioned medium control.*

Cytokine Plasticity Assay:

Conditioned medium (CM) was collected from cells after 24 hours. Old DFs (n=4) were incubated in the presence of CM for 14 days (medium replaced every 3 days), to identify if the young secretome (n=4) could modulate their inflammatory profile. CM from old DFs was included for comparison, in addition to non-conditioned medium. Relative expression of 105 inflammatory cytokines were quantitated simultaneously on pooled cell lysates with a Proteome Profiler Human XL Cytokine Array Kit (R&D systems).

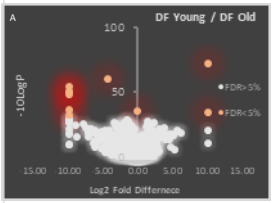
Proteomics:

Cell lysates of young (n=4; 20-29 years) and older (n=4, 53-57 years) scalp DFs were evaluated by LC-MS proteomics and compared by MS1 precursor intensities using PEAKS software, to identify age-related changes in their proteome.

Results & Discussion

Cytokine	Increase with Age (%)	Effect of O-CM (%)	Effect of Y-CM (%)
Endoglin	+27.6	+17.0	+7.3
ICAM-1	+19.4	+0.6	-23.0
Pentraxin 3	+35.3	-19.8	-26.1
MIF	+26.6	+14.2	-3.4
Thrombospondin 1	+39.9	-14.0	-18.4
DKK-1	+64.0	-17.9	+14.4
FLT-3 Ligand	+32.6	+33.8	+2.2
DPP IV	+57.2	+69.3	-7.0
uPAR	+49.5	-18.4	+1.5
Emmprin	+53.8	+14.8	-11.9
Serpin E1	+7.2	+46.6	+14.1
FGF Basic	+21.0	+51.5	+85.6
SDF-1a	+33.5	+4.0	+13.1

***Figure 3.* Table showing proteome profiler positive results, displayed as % increase in densitometry of dot blots.**
There was increased expression of 13 pro-inflammatory markers in old DFs, compared to young DFs (column 1). Some were further induced in old DFs incubated with old DF secretome (O-CM), while some were partially reduced via treatment with young DF secretome (Y-CM). Reduction of known SASP/SAASP related proteins e.g. MIF, Thrombospondin 1, DPP IV, SERPINE1 may be beneficial since these proteins can maintain proliferative arrest and contribute to other detrimental effects in the dermis.



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Protein	Log^2FC with age
Estradiol 17-beta-dehydrogenase 11	10
Retinol dehydrogenase 5	10
Tetranectin	10
CD14	10
Carboxypeptidase M	10
Plexin-B1	10
ER membrane protein complex subunit 4	10
Smoothelin	4.3
Gamma-enolase	0.2
Rho GTPase Activating Protein 21	-10
NDR1	-10

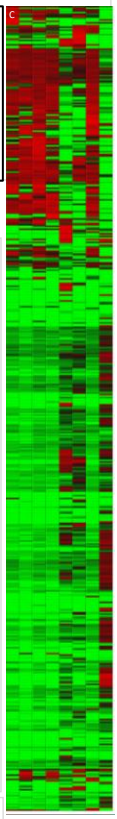

Figure 4. Proteomic comparison of intracellular protein expression between young (n=4) and old DFs (n=4). *(A) Volcano plot showing in orange the* ***consistently*** *(in all 8 donors)* ***significantly*** *(ANOVA) different proteins* ***(log²FC)*** *in a 2-way comparison (Quant threshold at 5% FDR=Significance >14.15). (B) The individual proteins increased (orange) and decreased (blue) with age. (C) Heatmap showing the 8 individual donors.*

Conclusions:

- This study provides further evidence for significant age-related changes in female scalp dermal fibroblasts
- Increased expression of proinflammatory cytokines occurs in ageing scalp dermis.
- The DF inflammatory profile was exacerbated by the secretome (CM) of other old DFs – which further elevated expression of 6 proinflammatory cytokines.
- Increased expression of some proinflammatory cytokines in old DFs was reduced by a young DF secretome.
- Proteomic analysis highlighted significant changes in 11 intracellular proteins that was consistent in all 8 donors.
- The age-related changes highlighted in this study are associated with aging-associated signaling pathways, e.g., TGF-β, thrombin/SERPIN and MMPs
- A better understanding of the changes that take place in the ageing scalp dermal environment will be important to further our understanding of hair ageing.

References:

1. Williams, R., Westgate, G. E., Pawlus, A. D., Sikkink, S. K. & Thornton, M. J. Age-Related Changes in Female Scalp Dermal Sheath and Dermal Fibroblasts: How the Hair Follicle Environment Impacts Hair Aging. *J Invest Dermatol* **141**, 1041–1051 (2021)
2. Williams, R., Pawlus, A. D. & Thornton, M. J. Getting under the skin of hair aging: the impact of the hair follicle environment. *Experimental Dermatology* **29**, 588–597 (2020)