Age-related changes in human scalp extracellular matrix– implications for the hair follicle?

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Introduction

Human skin is routinely used to study age-related changes in structure, gene and protein expression, however ageing in the human scalp still needs further exploration. It is known that facial skin integrity is diminished with age due to intrinsic and extrinsic factors including UV exposure, lifestyle and hormonal changes. Dermal fibroblasts (DFs) are responsible for the production and maintenance of the extracellular matrix (ECM), a network of collagen and elastin fibrils interwoven with proteoglycans. The main age-related changes within the dermis result in alterations to the structure of the ECM. Therefore, we hypothesise that these ECM changes will also influence the hair follicle dermal environment. The aim of this study was to identify age-related changes within adult female hair-bearing scalp dermis with increasing age.

Methods

Cell culture

Primary cultures of DFs derived from normal healthy female scalp aged 19-81yrs (n=12) were established and passaged no more than 3 times. Quantitative-RT-PCR comparisons were made between DFs cultured from women under 40yrs (n=4) and over 40yrs (n=8).

Histology

Cryosections from 13 donors, 12 of which were matched to the qPCR donors, were stained with H&E and imaged with a Phillip’s digital pathology scanner. Changes within structure were noted and where applicable measured (i.e. area of papillary dermis and hair follicle depth) with the Phillip’s digital pathology software.

Quantitative RT-PCR

Confluent cultures of DFs were harvested from T75 flasks, with RNA extracted and converted to cDNA. Expression of mRNA was calculated as the relative gene expression in relation to the housekeeping gene RPS18 and analysed using the 2-ΔΔCT (Livak) Method.

Statistical Analysis

Statistical analysis was conducted using PRISM software. For the comparison of under 40yrs vs. over 40yrs the Mann Whitney T-test was used with a level of significance p<0.05.

The ageing hair follicle environment

Female age 20

Female age 81

Female age 21 years

Female age 81 years

A flatter epidermal-dermal junction with less prominent rete ridges was seen in scalp with increasing age. The papillary & reticular dermis was indistinct under 40yrs (n=4) but clearly distinguishable over 40yrs (n=9). Hair follicles appeared to penetrate deeper into the subcutis in scalp from the younger group, whereas with age the collagen fibrils appeared disorganised.

Changes in DF ECM biomarkers with age

Biomarker (mRNA)	Associated function	Change with age
MMP1	Increased levels associated with fragmented and dissociated collagen fibrils	Increases with age p<0.05 between under 60 and over 60 age groups
MMP9	Increased levels associated with elastin & collagen degradation	No difference
TIMP1	Maintains homeostasis of ECM	No difference
Collagen XVI	Connects ECM proteins to cells – ensures mechanical anchorage	Decreases with age P< 0.05 between under 40s and over 40s
Versican (VCAN)	Proteoglycan disappears from the dermal papilla in androgenetic alopecia	Decreases with age P< 0.05 between under 40s and over 40s
Hyaluronic acid synthase type 2 HAS2	Enzyme responsible for synthesis of the proteoglycan hyaluronic acid	Decreases with age P< 0.05 between under 40s and over 40s
Protease nexin1 (Serpine 2)	Levels in dermal papilla cells are reduced by 5α-dihydrotestosterone	Decreases with age P< 0.05 between under 40s and over 40s
Cartilage oligomeric matrix protein (COMP)	A glycoprotein that functions as an adaptor protein organizing collagen fibrils	No difference
Cysteine-rich protein 61 (CYR61)	Elevated levels inhibit collagen I and III synthesis	No difference
Lysyl oxidase -like protein 2 (LOXL2)	Important in the biosynthesis and renewal of elastic tissue	No difference
Integrin	Important in remodelling of ECM and also transducing signals to cells	No difference
Kallistatin (Serpina 4)	A serine protease inhibitor that has anti-inflammatory effects	Not present in any of the samples (confirmed with a positive primer control)

Significant alterations in ECM gene expression with age

Quantitative RT-PCR comparing the mRNA expression of MMP1 in cultured human female scalp DFs from two different age groups; 19-59yrs (n=7) and 60-81yrs (n=5). The mRNA expression of Collagen XVI, Versican, Hyaluronan synthase 2, Protease nexin-1 was compared in cultured human female scalp DFs from two different age groups; 19-38yrs (n=4) and 43-81yrs (n=8). All were assayed between passage 0 and 3. Data presented as donor mean ± SEM. *p<0.05

Conclusion

With increasing age, striking histological changes in scalp dermis were observed, including alterations in papillary and reticular dermis, collagen organisation and anagen hair follicle depth. Age-related changes in mRNA expression in 5 of the 12 ECM markers selected, i.e. **MMP1**, **collagen 16**, **versican**, **hyaluronic acid synthase type 2** and **protease nexin 1** were confirmed in cultured DFs. This confirms that similar to facial skin, ECM composition alters with ageing in the scalp dermis. However, age-related ECM changes in scalp interfollicular dermis supporting the hair follicle environment may significantly impact on maintenance of the scalp anagen hair follicle. Studies are currently underway to expand these data by comparing the protein expression of these markers *in situ*, including the hair follicle, to determine how age-related changes in the dynamic dermal environment impact on the ageing hair follicle.