Benefits of infrared spectral imaging for hair follicle structure identification and glycosaminoglycans distribution

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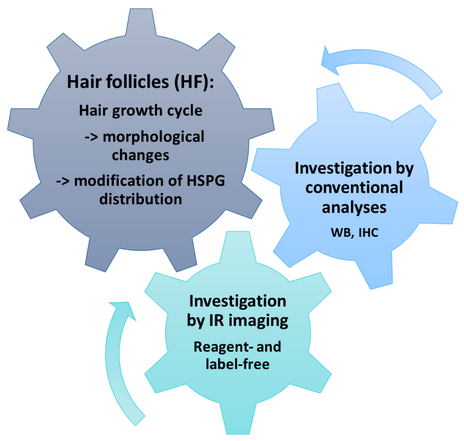
Introduction:

It has been shown that heparan sulfate proteoglycan (HSPG) plays an important role in the regulation of hair shaft growth and that a complex control of HSPG sulfation is necessary for its correct morphogenesis [1]. Previous studies have shown modifications in the distribution of various HSPGs during the hair cycle [2, 3]. However, the expression of glypicans (GPCs) in different hair follicle (HF) compartments and their potential roles during hair shaft growth are still poorly understood.

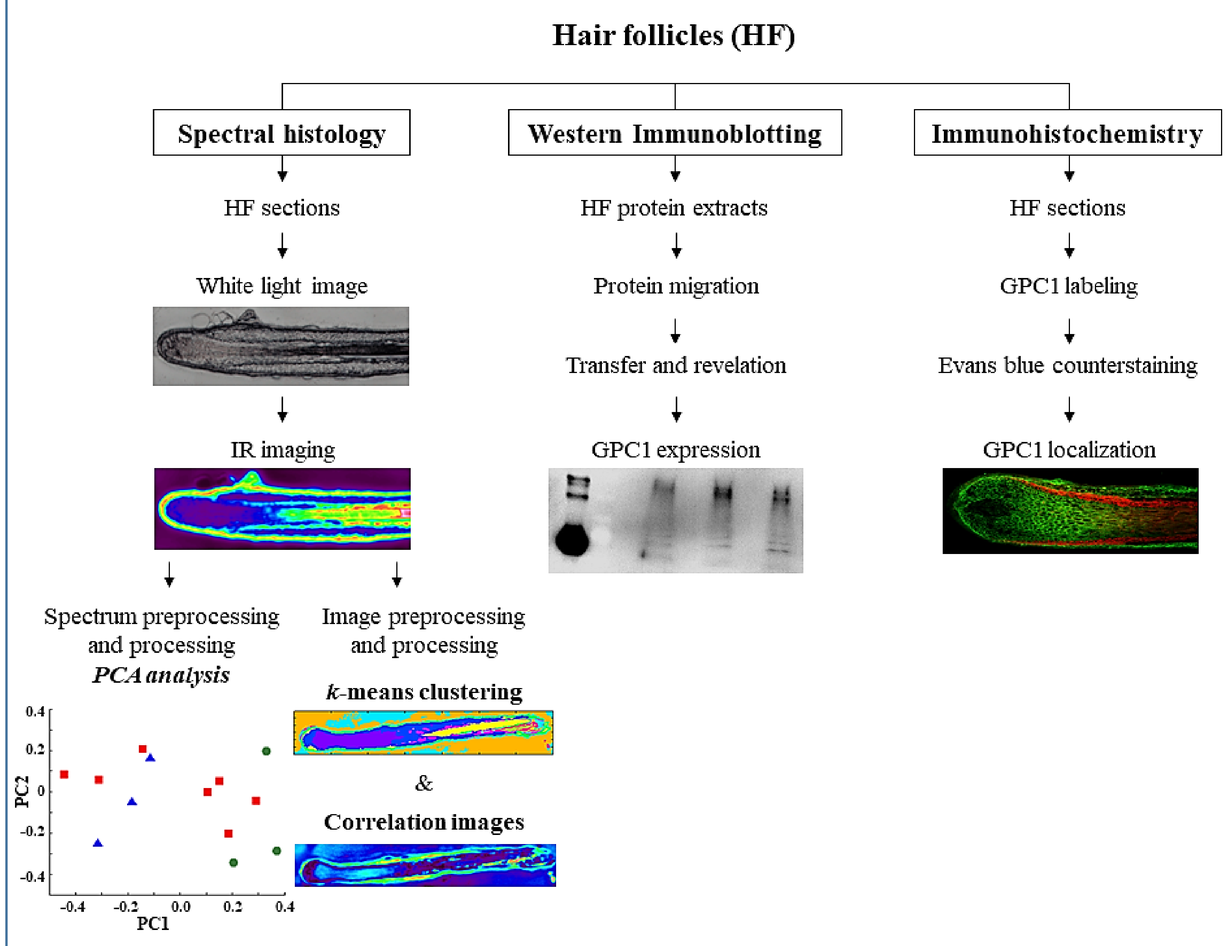
GPCs have been however shown to be essential for the formation or regeneration of many tissues and organs by regulating many pathways involved in development [4, 5]. Therefore, it appears very likely that GPCs also play a key role in the growth of a new hair shaft.

In this study, a **novel approach** is proposed to **assess hair histology and HSPG distribution changes in HFs at different phases of the hair growth cycle using InfraRed Spectral Imaging (IRSI).** IRSI provides both molecular and spatial information on biological specimens without any specific preparation [6].



IRSI was used as a non-invasive, label-, chemical-, and waste-free approach to identify the different HF tissue structures and to highlight protein, proteoglycan (PG), glycosaminoglycan (GAG), and sulfated GAG distribution in these structures.

Materials & Methods:



Workflow showing methodological approach of hair follicle analysis by label-free infrared spectral histology, Western immunoblotting and immunohistochemistry. Pictures shown here are only examples.

Results & Discussion:

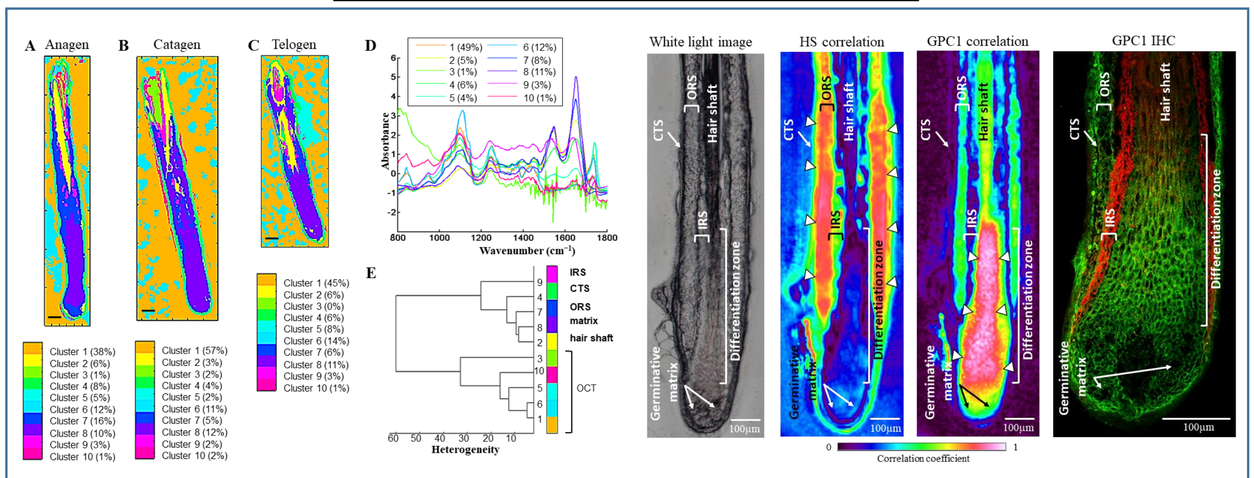


Fig. 1. Discrimination of hair follicle structures at different phases of hair growth cycle by k-means clustering.

(A-C) Representative color-coded k-means clustering images using 10 classes in the 1800-800 cm^{-1} spectral range carried out on hair follicles in anagen A1 (A), catagen C1 (B), and telogen T1 (C) phases. (D) Centroid spectra corresponding to each cluster. (E) Dendrogram of centroid spectra and assignment of corresponding hair follicle structures. CTS, connective tissue sheath; IRS, inner root sheath; ORS, outer root sheath. Scale bar: 100 µm.

Fig. 2. Characterization of hair follicle structures by different imaging approaches.

From left to right: white light image, HS and GPC1 correlated IR images, and immunohistochemical labeling of GPC1 (green) counterstained with Evans blue dye (red). CTS, connective tissue sheath; IRS, inner root sheath; ORS, outer root sheath. Arrowheads indicate high level of correlation. Scale bar: 100 µm.

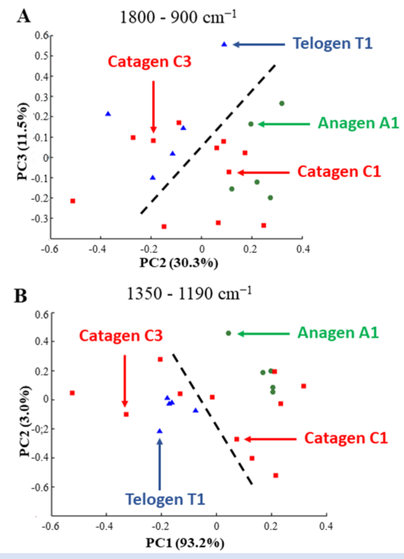


Fig. 3. Discrimination of hair follicles at different phases of hair growth cycle by PCA.

(A, B) PCA score plot performed on normalized mean spectra of ORS region 4 in the 1800-900 cm^{-1} (A) or 1350-1190 cm^{-1} (B) range and carried out on anagen A1, catagen C1/C3, and telogen T1 hair follicles.

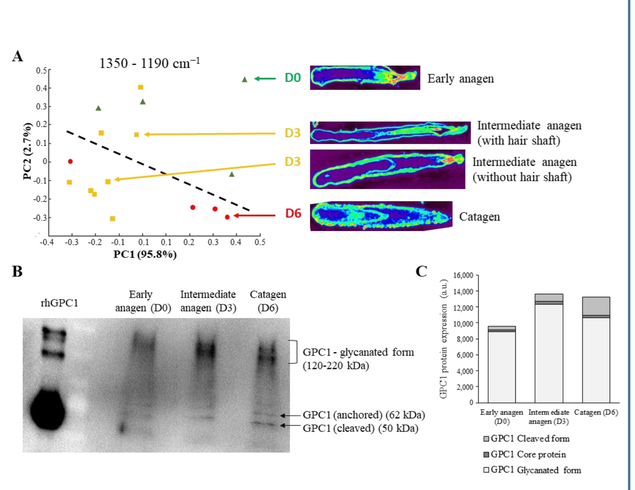


Fig. 4. Discrimination of hair follicles by PCA compared to GPC1 expression during the hair growth cycle.

(A) PCA score plot performed on normalized mean spectra of the ORS region 4 in the 1350-1190 cm^{-1} range and carried out on early anagen D0, intermediate anagen D3 and catagen D6 hair follicles. (B) GPC1 protein expression analyzed by Western immunoblotting of early anagen D0, intermediate anagen D3 and catagen D6 hair follicles. (C) Quantification of each GPC1 form.

Conclusions:

To our best knowledge, this study of hair follicles HSPGs by IRSI is the first of its kind. IRSI could identify the histological structures and the different phases of the hair growth cycle. IRSI exhibits the advantage of revealing simultaneously the location, signatures and, in a semi-quantitative manner, the content of proteins, PGs, GAGs, and sulfated GAGs in HFs. In addition, it is reagent- and label-free and corroborates with immunohistochemical and biochemical analyses. From a cosmetological point of view, IRSI appears as a promising technique to study alopecia.

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