Investigation into the role of HIF1A stabilisation in hair follicle metabolism

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Background

The aim of this study is to determine whether stabilization of HIF-1A is able to regulate hair follicle (HF) aerobic glycolysis and understand what impact this has on HF signaling pathways.

Hypoxia-inducible factor 1 (HIF-1) is a transcription factor that functions as a master regulator of oxygen homeostasis. It consists of two subunits: an inducibly-expressed HIF-1alpha (HIF-1A) subunit and a constitutively-expressed HIF-1beta (HIF-1B) subunit. Under normoxia, HIF-1A undergoes hydroxylation at specific prolyl residues which leads to an immediate ubiquitination and subsequent proteasomal degradation of the subunit. In contrast, under hypoxia, HIF-1A subunit becomes stable and interacts with coactivators such as p300/CBP to modulate its transcriptional activity. Eventually, HIF-1 acts as a master regulator of numerous hypoxia-inducible genes under hypoxic conditions. The target genes of HIF-1 encode proteins that increase O₂ delivery and mediate adaptive responses to O₂ deprivation. Despite its name, HIF-1 is induced not only in response to reduced oxygen availability but also by other stimulants, such as nitric oxide, or various growth factors.

The hair follicle and metabolism

Adapted from Paus, 2003

1. Early studies show hair follicles to engage in aerobic glycolysis
2. HIF-1A stabilization promotes glycolysis
3. Therefore, if we can stabilize HIF-1A in HFs we can promote glycolysis over TCA, oxidative phosphorylation - preventing reactive oxidative species (ROS) damage

Results

HIF protein expression in human skin sections

Figure 1: Immunohistochemical (IHC) staining of HIF proteins show differential expression in human hairy skin sections with highest expression seen within the outermost layer of epidermis, hair follicles (HF) and sebaceous glands. Scale bar represent 100µm

Co-localization of HIF-2A with Keratin 6, marker of suprabasal layer of outer root sheath (ORS) in human skin -

Effect of HIF proteins in isolated anagen and catagen hair follicles (HF)

Figure 2: IHC staining of HIF proteins in isolated anagen and catagen HFs. **Anagen - HIF1A** staining shows strong nuclear staining in the hair matrix region and ORS. **HIF1B** and **HIF2A** was seen throughout the HF with cytoplasmic signal in different compartments. Strong staining in the cuticle region of the HF and positive nuclear expression can be seen in the ORS. **Catagen** - Staining is more prominent within the inner root sheath (IRS) for both HIF1A and HIF2A. Scale bar represent 100µm

In vitro stabilization in isolated hair follicles (HF) using HIF stabilizers

Figure 4: A schematic representation of imaging a whole follicle using the INCA 2200 microscope

HIF-1A stabilization using small molecule inhibitors

- Immortalized non balding (BAN) and balding (BAB) dermal papilla (DP) cells were treated for 24 hours with varying concentration solution of Deferoxamine (DFO).
  - DFO stabilizes HIF-1A through the inhibition of Prolyl Hydroxylases (PHDs) which target HIF-1A through degradation.
  - The mechanism of DFO inhibition is likely through the chelation of Fe²⁺ bound to the active site of PHD which is required for enzymatic activity.
- **Figure 5:** Primary outer root sheath keratinocytes (ORS) were treated for different time points with varying concentration solution of unknown Procter Gamble (P&G) HIF stabilizers, PG2.
- HIF-1A expression can be seen at 4 hours from concentrations starting at 10uM
- HIF-1B is present at the highest in untreated ORS at 6 hours.

Initial cellular metabolism experiment using Seahorse Analyser

Figure 6: Non-balding (BAN) cells were seeded a day before onto a seahorse specific 24 well plate. Half the wells were treated with 100uM DFO for 24hrs and the rest were untreated. Using a specific mitochondria-stress kit (A), by the addition of inhibitors, we were able to measure various components of mitochondrial respiration. Metabolic analysis using this Seahorse analyzer showed cells treated with DFO exhibited a shift to a more glycolytic phenotype compared to untreated cells (B).

Conclusion

- Positive nuclear staining for HIF-1A, HIF-1B HIF-2A proteins is present in outer root sheath (ORS) of anagen hair follicles - no expression seen in the DP cells
- Therefore, suggest HF and especially the ORS is hypoxic.
- However, HIF-1A protein is stabilized in immortalized balding and non blading DP cell lines when treated with a HIF-1 degradation inhibitor, DFO.
- In vitro stabilization of treated HFs with HIF stabilizer, PG3 - positive nuclear staining is increased in the ORS.
- Metabolic analysis shows DFO causes a glycolytic switch in BAN cells

Future work will focus on the downstream signalling pathway after HIF stabilization in intact hair follicles and primary ORS

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