RNA-Sequencing of Plucked Hair Follicles Identifies Gene Expression Signatures of Chemotherapy-Induced Alopecia

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Introduction

- **Chemotherapy-induced alopecia (CIA)** is one of the most common side effects experienced by cancer patients, causing ~10% of female patients to refuse treatment.[1]
- Chemotherapy induces breakdown of mitotic activity, interrupting the normal anagen growth of hair follicles.
- Severe CIA is associated with doxorubicin, paclitaxel, docetaxel, cyclophosphamide, and/or epirubicin, especially at higher doses and with combination chemotherapy.[2]
- Scalp cooling (SC) is effective at minimizing CIA and promoting hair regrowth.[3]
  - Induces **vasoconstriction** in dermal capillaries, **reducing delivery of chemotherapy** to hair follicles.[4]
  - **Reduces metabolism** in cooled region, **decreasing cellular uptake** of chemotherapy.[5]
- Skin of color (SOC) patients have been underrepresented in SC studies, and variations in hair textures may translate to different outcomes.
- Predictive biomarkers for SC and CIA are not understood, preventing the development of targeted, evidence-based therapeutics.

Methods

Scalp cooling

- Paxman machine designed to cool patient scalp (range: -1 to -4°C)
- 45 minutes prior to chemotherapy initiation, throughout infusion, and 90 minutes after infusion
- Inclusion criteria: cancer patients with Type 3 or 4 hair receiving taxane-based chemotherapy

Hair follicle (HF) analysis

- HF transcriptomics: a non-invasive method to elucidate cellular pathways by quantifying changes in global mRNA expression
- 10 HFs plucked at **baseline** (prior to chemotherapy) and **1 month** after chemotherapy completion
  - preserved in RNAlater solution at -80°C
  - total RNA extracted using RNeasy Micro Kit
  - mRNA sequencing at outside laboratory
  - Bioinformatic analysis using Kallisto and DESeq2

Discussion & Conclusions

- Principal component analysis demonstrated that patients (A and B) that had significant CIA had dissimilar gene expression between baseline and 1 month, while patient C that had minimal CIA had more similar gene expression between timepoints.
- **Upregulated** pathways included **columnar/cuboidal epithelial cell differentiation**, associated with the root sheaths of the hair follicle, and **keratinization**.
- **Downregulated** genes included **SOX2**, involved in hair shaft growth from dermal papilla cells,[6] and **Angiopoietin-1**, associated with vascular maintenance particularly during the anagen (growth) phase of the hair follicle.[7]
- These findings suggest that HF transcriptomics may be used to predict which patients are more likely to develop CIA and benefit from interventions like SC.
- Further understanding of these biomarkers may be useful to discover targeted therapies for CIA.

Results

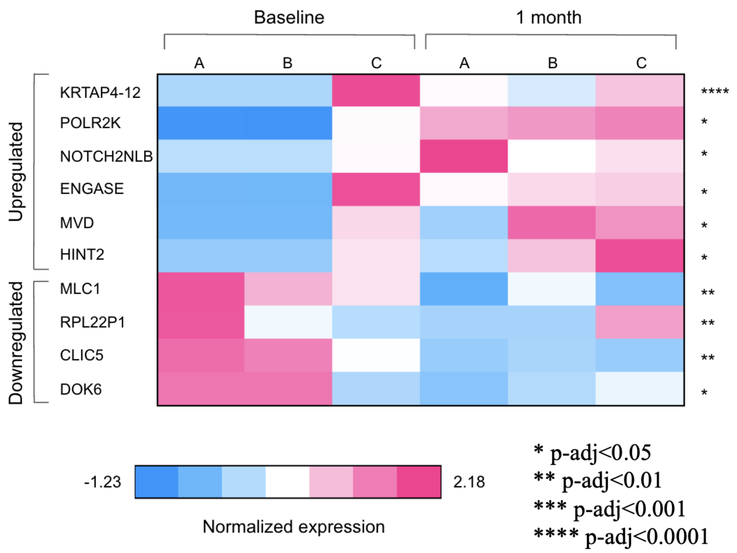

Figure 1. Heatmap of the top 10 most significant differentially expressed genes for patients A, B, and C at baseline compared to 1 month follow-up. KRTAP4-12 (keratin-associated protein) contributes to the structure of hair fibers.

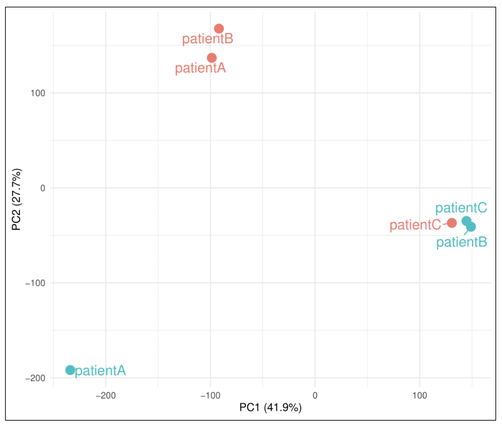

Figure 2. PCA plot demonstrating gene expression of patients A, B, and C at baseline and 1 month. **Patients A and B were clinically non-responders** to scalp cooling with CIA. **Patient C, who demonstrated minimal CIA**, had similar gene expression between time points.

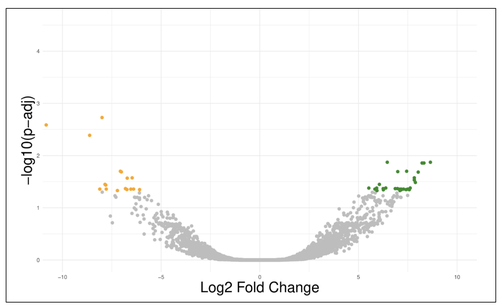

Figure 3. Volcano plot depicting the significant upregulated (green dots) and downregulated (yellow dots) genes.

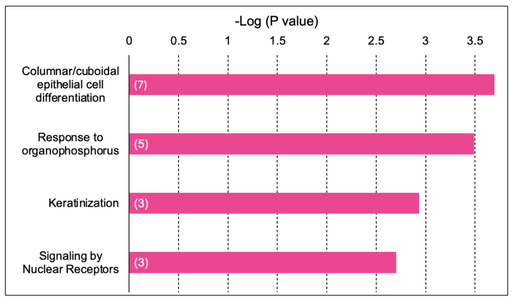

Figure 4. Top four upregulated pathways at 1 month with the corresponding number of associated significant genes.

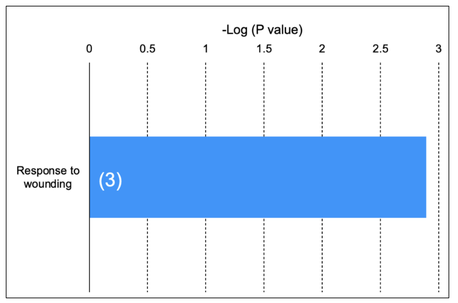

Figure 5. Top downregulated pathway at 1 month with the associated 3 significant genes (**ANGPT1**, **SOX2**, GRIN2A).

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