Transcriptome profiling of laminin $\alpha 3$ inhibited primary keratinocytes reveals the regulatory effect of laminin $\alpha 3$ -binding on keratinocyte differentiation

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Introduction:

Laminin 332 is a principal component of the dermal-epidermal basement membrane zone. It is a heterotrimer containing a $\alpha 3$, $\beta 3$, and $\gamma 2$ subunit. The $\alpha 3$ subunit is known to bind to both integrin $\alpha 3\beta 1$ and $\alpha 6\beta 4$. Interaction of the laminin $\alpha 3$ domain with integrin $\alpha 6\beta 4$ is known to lead to phosphorylation of tyrosine residues on the $\beta 4$ endodomain with myriad signaling events thereafter. Likewise, laminin $\alpha 3$ can regulate cell migration and polarity in an integrin dependent manner. Laminin $\alpha 3$'s effect on keratinocyte differentiation, however, is unclear. To investigate the regulatory role of laminin $\alpha 3$ on keratinocyte signaling, we treated primary human keratinocytes with blocking antibodies specific to the $\alpha 3$ domain of laminin 332.

Methods:

- Primary keratinocytes were grown in serum free, low calcium media to sub-confluence, and subsequently treated for 24 hours with a mouse monoclonal IgG1 antibody specific to human laminin $\alpha 3$ (P3H9-2) or isotype controls in triplicate
- Following treatment, RNA was extracted, with subsequent quality control, enrichment, and library preparation
- Sequencing was performed on an Illumina HiSeq PE150
- A mean number of 27,279,262 clean reads were attained, with mean percentage $\geq Q30$ of 94.2. Filtered clear reads were mapped to the reference genome using HISAT2, yielding a mean of 52,145,923 (95%) mapped reads per sample. Reads were normalized to the number of mapped reads (Fragments Per Kilobase of transcript per Million mapped reads)
- DESeq was used to analyze differentially expressed genes, using a fold change ≥ 2 and false discovery rate < 0.01 as screening criteria
- Additionally Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis was performed

Results:

Blockade of laminin $\alpha 3$ resulted in 3,394 differentially expressed genes. GO analysis demonstrated significant impact on epidermis development, skin development, extracellular structure organization, extracellular matrix organization, keratinocyte differentiation, and cornification (**Figure 1**).

Results Continued:

RT-PCR was used to confirm several differentially expressed genes from RNA-seq experiments. We further investigated specific genes differentially expressed in various keratinocyte processes. Of particular interest, we noted significant upregulation of desmosomal genes (DSC1, DSC3, DSG1, DSG3, JUP, PKP1, DSP, and EVPL), with the greatest log2fold change of 3.54 for DSC1. Keratin expression profile additionally demonstrated several changes including downregulation of keratins 7, 8, 18, 19, 34, and upregulation of keratins 1, 2, 3, 4, 6a, 6c, 9, 10, 15, 16, 74, 77, and 80 with the most striking upregulation of keratin 10 (log2fold 4.3), consistent with differentiation.

Figure 1: Dotplot of a gene ontology over representation analysis on a dataset of primary keratinocytes treated with a mouse monoclonal IgG1 antibody specific to human laminin- $\alpha 3$

Conclusion:

Our results demonstrate a significant role of laminin $\alpha 3$ binding in altering the keratinocyte transcriptome, particularly as it pertains to differentiation. Elucidating the mechanism by which laminin $\alpha 3$ binding regulates this process remains to be determined.