Loss of EphA2 inhibits GATA3 transcriptional function leading to a terminal differentiation defect

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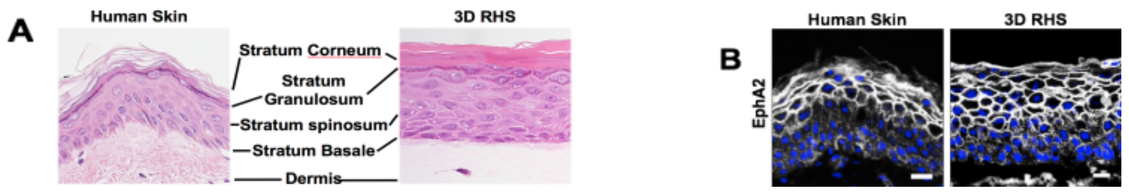
ABSTRACT

Epidermal differentiation involves complex signal transduction networks. Often, these signal relays depend on the transmission of cues from the plasma membrane to the nucleus to control gene expression. Receptor tyrosine kinases (RTKs) are integral in orchestrating intracellular communication cascades to induce differentiation. We have shown loss of EphA2 RTK causes a differentiation defect in 3D reconstituted human skin (3D RHS). This defect is characterized by ablation of the granular and cornified layers. In EphA2-deficient (shEphA2) 3D RHS we show significant loss of loricrin, filaggrin, and involucrin at protein and mRNA levels, indicating EphA2 signaling impacts keratinocyte differentiation at the transcriptional level. GATA3 is a key driver of differentiation-associated gene expression in epidermis. Yet, the upstream signaling leading to GATA3 transcriptional activity is unknown. We show by immunofluorescence that GATA3 is mainly expressed in the nuclei of suprabasal keratinocytes in mature 3D RHS, mimicking the expression pattern of normal human skin. This expression of GATA3 is lost in shEphA2 3D RHS. This loss of expression in shEphA2 cells results in a loss of GATA3-driven transcription. Taken together, these results indicate that EphA2 promotes GATA3 nuclear accumulation and positively regulates the transcription of terminal epidermal differentiation genes.

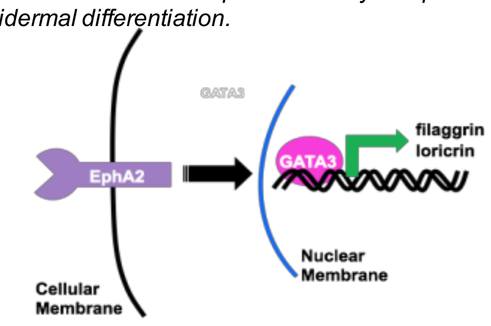
BACKGROUND

- The outermost layer of the skin is the epidermis, which is composed of a stratified squamous epithelium and protects the body.
- Progenitor cells in the stratum basale differentiate as they move into the stratum spinosum, stratum granulosum, and the stratum corneum (**Fig. A**)
- EphA2 is prominent in the cell borders of the suprabasal layers of the epidermis.
  - Because expression of this receptor in 3D reconstituted human skin (3D RHS) mimics expression pattern in human epidermis, it is an accurate model to study this RTK. (**Fig. B**)



HYPOTHESIS

EphA2 promotes GATA3 transcriptional activity and positively regulates terminal epidermal differentiation.



METHODOLOGY

- Purpose: To determine GATA3 and EphA2 expression patterns in control (pLKO) and EphA2 knockdown (shEphA2) in keratinocyte cultures.
- Expected Results: pLKO will show expression of GATA3 and EphA2 while shEphA2 will have little to no staining for GATA3 or EphA2.
- Methods: Fluorescent immunohistochemistry and fluorescent immunocytochemistry.
- These are human skin cell cultures, so we fixed it in 4% paraformaldehyde in PBS (Phosphate Buffered Saline).
- We incubated the specimen in a blocking buffer to prevent non-specific binding.
- After applying the primary antibody (for GATA3: D13C9 Rabbit Monoclonal and for EphA2: AF3035 Goat Monoclonal), we let the samples incubate in 4°C overnight.
- We rinsed in PBS, and added the secondary antibodies (for GATA3: Alexa Fluor 488 donkey anti-mouse and for EphA2: Alexa Fluor 555 donkey anti-goat) diluted (1:300) in 1% donkey serum in cell signaling buffer.
- We then incubated in 4',6-Diamidino-2-Phenylindole (DAPI), blue dye for staining the nuclei of cells, and mounted the specimens with Gelvatol.
- To acquire images, we used an epifluorescence microscope system, Zeiss Axio Imager.Z1, to analyze the effects of EphA2 knockdown.

RESULTS

Loss of EphA2 causes a terminal differentiation defect in 3D RHS.

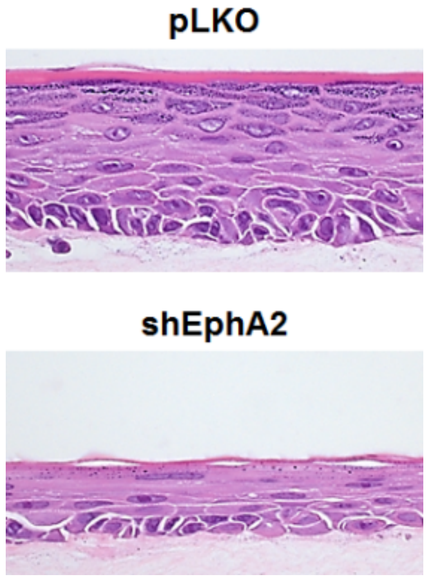


Fig. 1 : A. Hematoxylin and eosin staining of 3D RHS empty vector control (pLKO) and EphA2 short hairpin (shEphA2) knockdown cultures.

GATA3 is expressed in the suprabasal layers of epidermis and 3D RHS.

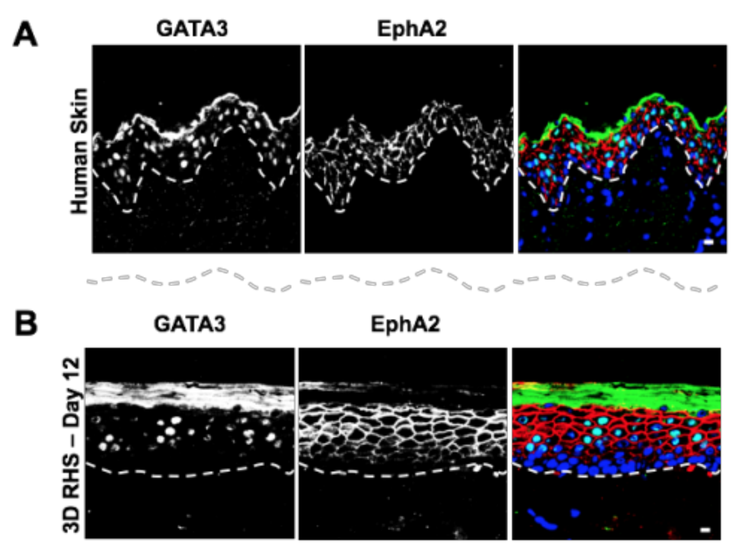


Fig. 2 : Immunostaining of GATA3 (green) and EphA2 (red) in A. human skin and B. 3D RHS at Day 12 after lifting to the air-liquid interface. Scale bars = 10 µm. Dashed line demarcates the epidermal-dermal junction. DAPI (blue) was used as a counterstain.

GATA3 nuclear expression is reduced in EphA2-deficient 3D RHS.

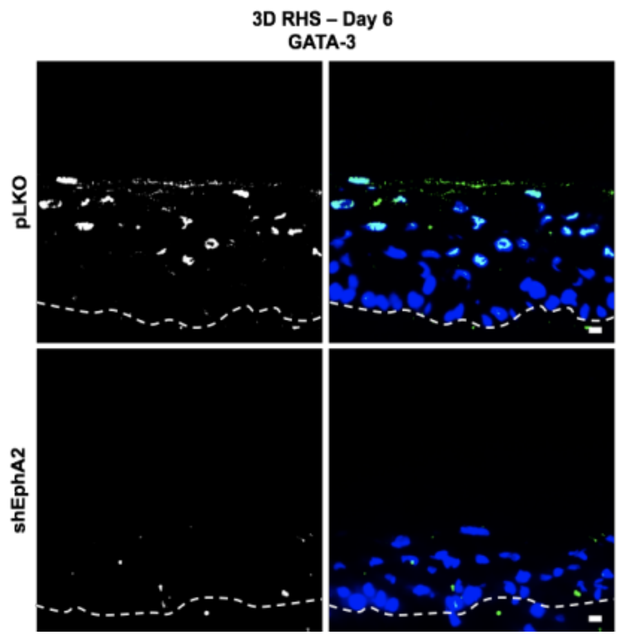


Fig. 3: Immunostaining of GATA3 (green) in pLKO and shEphA2. Scale bars = 10 µm. Dashed line marks the epidermal-dermal junction. DAPI, blue dye, was used as a counterstain.

Loss of EphA2 results in inhibition of GATA3 in 2D Cultures

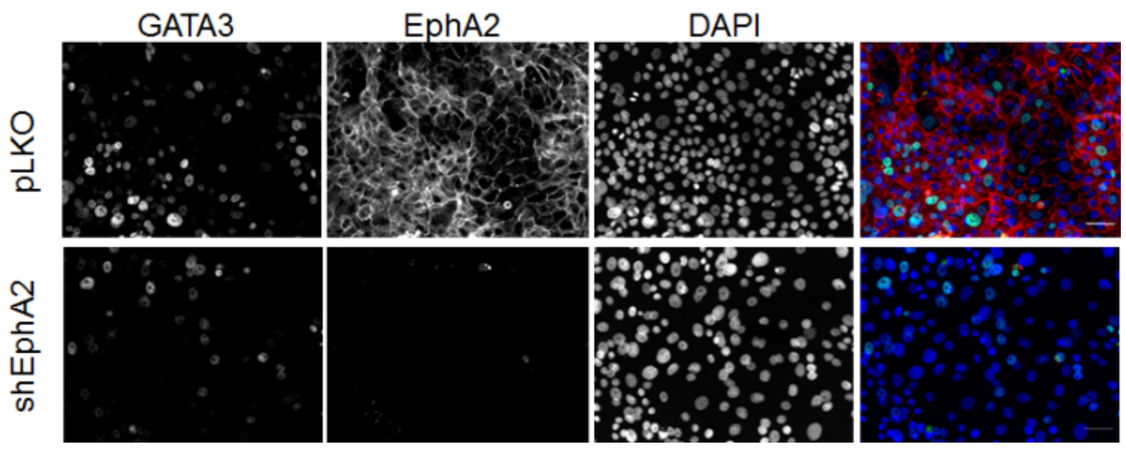


Fig. 4 : Immunostaining of GATA3 (green) and EphA2 (red) in pLKO and shEphA2 at Day 1 exposure to 1.2 mM calcium (to induce differentiation). Scale bars = 50 µm. DAPI (Blue) was used as a counterstain.

Semi-Quantitative Density of Immunostaining of GATA3 and EphA2

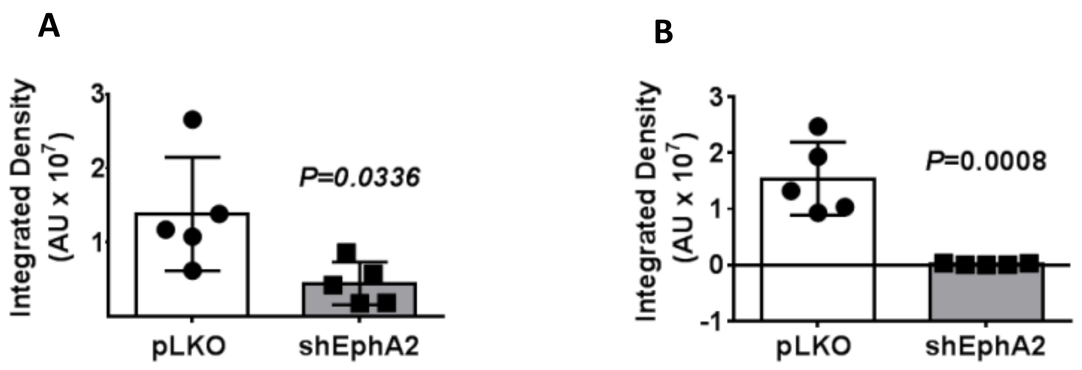


Fig. 5 : Quantification of integrated density of 2D culture imaging with t-test statistical analysis. Panel A displays the integrated densities of GATA3 in pLKO and shEphA2, with a statistically significant difference after knockdown of EphA2 in a t-test (P=0.0336). Panel B displays EphA2 in pLKO and shEphA2 with a statistically significant difference after knockdown of EphA2 in a t-test (P=0.0008). AU is arbitrary units.

CONCLUSIONS

- Loss of EphA2 Inhibits epidermal differentiation (Fig. 1)
- There is a clear expression of both GATA3 and EphA2 in the suprabasal layers of human skin, as seen in the merged image. (Fig. 2A)
- GATA3 is expressed in mature Day 12 3D RHS, similar to human skin. (Fig. 2B)
- The knockdown of EphA2 (shEphA2) results in a decrease of GATA 3 expression in the suprabasal layers of 3D RHS. (Fig. 3)
- Compared to pLKO, EphA2 knockdown showed a decrease in both the GATA3 and EphA2 expression, indicating that EphA2 expression is related to GATA3 expression (Fig. 4)
- The p values below 0.05 suggest that there is a statistically significant difference in the immunostaining of GATA3 and EphA2 under the shEphA2 knockdown when compared to the pLKO. (Fig. 5A,5B)
- **Taken together, loss of EphA2 inhibits epidermal differentiation and decreases GATA3 protein expression in 2D and in 3D keratinocyte cultures.**

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