# Transcriptomic Differences between Oral and Skin Keratinocytes

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## Background

In comparison to skin wounds, oral mucosal wounds heal more rapidly with significantly less inflammation, faster re-epithelialization, and minimal scarring. Our lab has previously shown that skin and oral excisional biopsy mucosal wounds exhibit site-specific differences in their genetic response to injury and that intrinsic keratinocyte characteristics may be one differentiating factor. The aim of this study was to investigate whether the intrinsic differences between oral and skin keratinocytes would be reflected in their transcriptome at baseline and after injury.

## HYPOTHESIS/OBJECTIVE

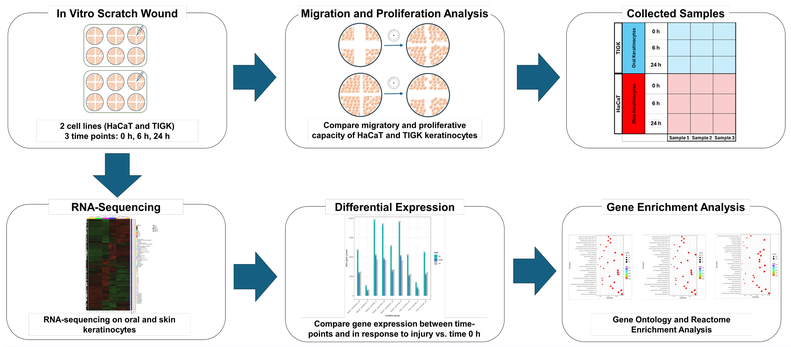
We hypothesize that oral and skin keratinocytes have intrinsic differences at baseline and in their response to injury, and that such differences would be reflected in their transcriptome.

## METHODS

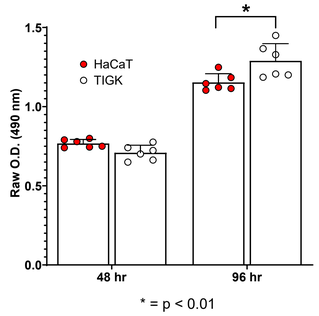
In this study, we used two keratinocyte cell lines: 1) hTERT-immortalized gingival keratinocytes (TIGK) and 2) spontaneously immortalized skin keratinocytes (HaCaT). RNA was isolated from HaCaT and TIGK at 0-, 6-, and 24-hours post-scratch (N=3). Following DNase treatment, RNA-sequencing was performed and transcriptomic changes in response to injury were compared between HaCaT and TIGK. Genes that were significantly downregulated ($p<0.05$) in HaCaT versus TIGK underwent gene ontology (GO) enrichment analysis and Reactome pathway analysis. GO terms were annotated to biological processes (BP). Additionally, HaCaT were stably transfected to overexpress Basic Leucine Zipper ATF-Like Transcription Factor 3 (BATF3-OvExp) or with an empty vector control (EV-Ctrl). Cellular migration and proliferation were assessed for BATF3-OvExp and EV-Ctrl (N=8-10). Expression of genes down-stream of BATF3 were assessed via RT-PCR (N=3-4).HaCaT migration was also assessed following pre- and post-scratch treatment with Interferon (IFN) Type I (N=10-12). Two-way ANOVA with two-stage linear step-up procedure of Benjamini, Krieger and Yekutielipost-hoc testing or unpaired two-sided t test with Welch's correction was used for statistical testing.

## RESULTS

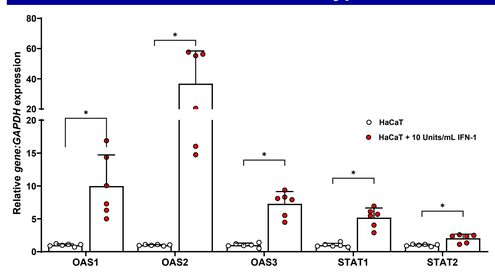
### Schematic of study overview

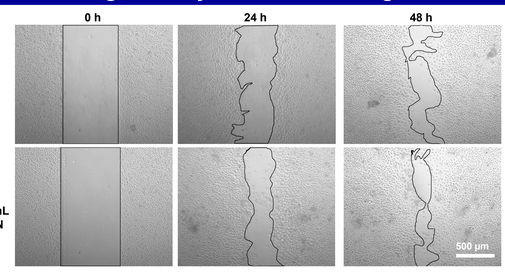



### HaCaT exhibit significantly reduced proliferative capacity as compared to TIGK

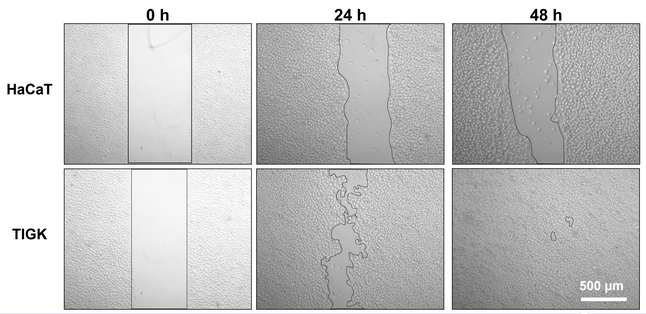


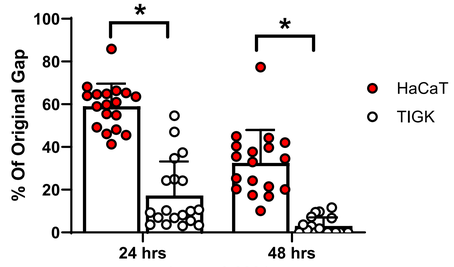

### Type I IFN stimulation of HaCaT significantly enhances cell migration



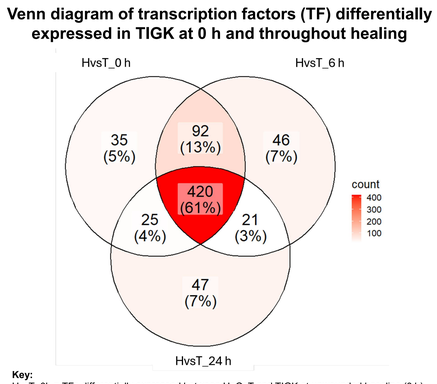



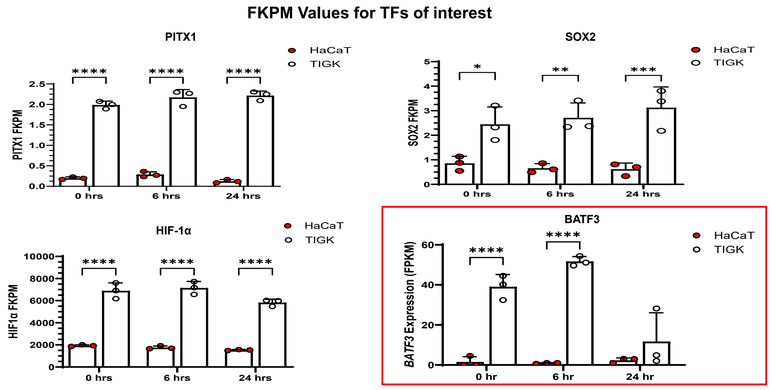

### HaCaT exhibit significantly reduced rates of migration post-wounding as compared to TIGK



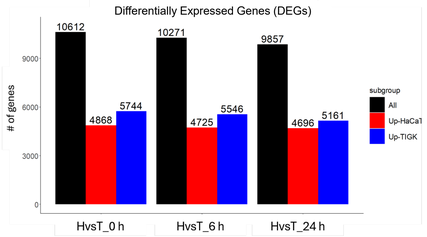




### HaCaT and TIGK exhibit differential expression of transcription factors at baseline and throughout injury



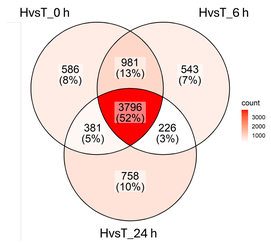




### Major transcriptomic differences exist between HaCaT and TIGK at baseline and throughout *in vitro* wound healing




**Key:**
HvsT_0h = DEGs between HaCaT and TIGK at unwounded baseline (0 h)
HvsT_6h = DEGs between HaCaT and TIGK at 6 h post-wounding (6 h)
HvsT_24h = DEGs between HaCaT and TIGK at 24 h post-wounding (24 h)
All = All DEGs between HaCaT and TIGK
Up-HaCaT = DEGs upregulated in HacaT compared to TIGK
Up-TIGK = DEGs upregulated in TIGK compared to HaCaT

### Many genes are constitutively upregulated in TIGK at baseline and throughout wound healing

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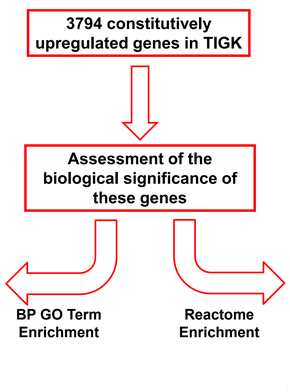
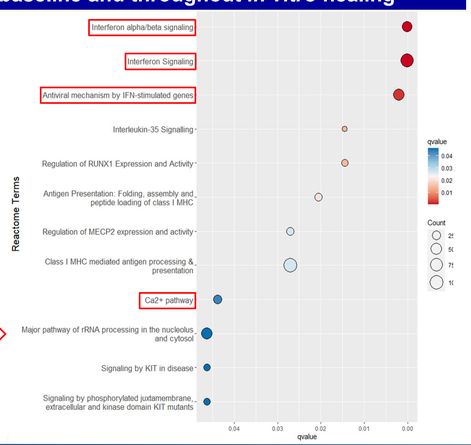
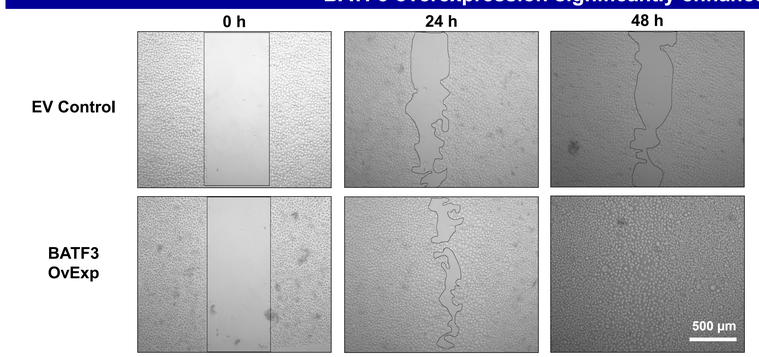
### TIGK exhibit enhanced activation of wound healing signal pathways at baseline and throughout *in vitro* healing

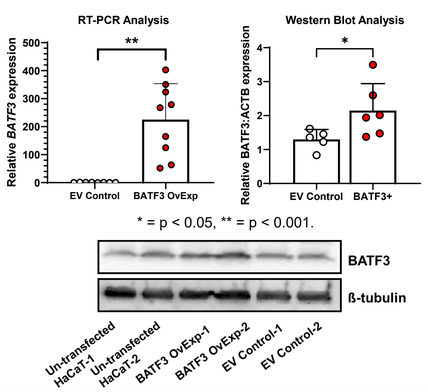



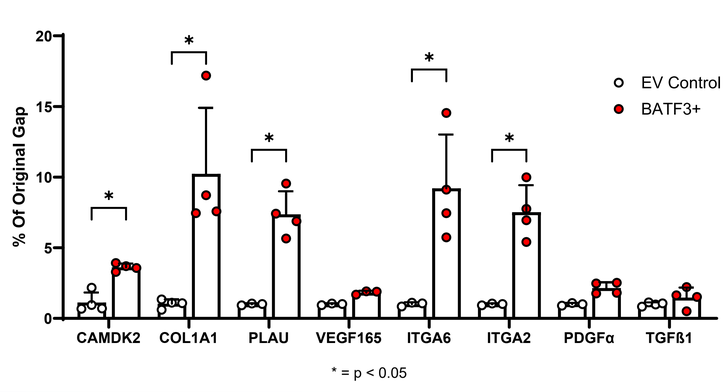

### BATF3 overexpression significantly enhances HaCaT migration




### RT-PCR and Western Blot Analysis to Confirm Overexpression




### RT-PCR Analysis for Genes Downstream of BATF3




## Conclusions

Our results demonstrate that HaCaT and TIGK exhibit differences in baseline behavior and transcriptomic responses to injury. Both Type I IFN stimulation and BATF3 overexpression in HaCaT enhanced cellular migration, which may be beneficial to wound healing. The results suggest that transcriptomic differences between oral and skin keratinocytes at baseline and in response to injury underlie the distinct wound healing phenotypes observed in skin and mucosal wounds.

## Future Directions

- Assess which IFN-stimulated genes are related to enhanced migration
- Utilize *in vivo* mouse models to validate whether inherent transcriptomic differences explain physiological differences in skin and oral mucosa healing

## ACKNOWLEDGMENTS

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