The role of the integrated stress response in keratinocyte migration

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Background and Hypothesis:

Cutaneous wound healing involves: hemostatic, inflammatory, proliferative, and tissue remodeling phases. Re-epithelialization can be modeled in vitro using human keratinocytes and artificial wounds. Previous work showed undifferentiated keratinocytes closing wounds in vitro using individual cell migration (ICM), whilst differentiated keratinocytes utilize collective cell migration (KCCM). Therefore, we hypothesize that ICM in vitro is equivalent to keratinocyte migration during squamous cell carcinoma metastasis in vivo and KCCM is a model for wound re-epithelialization. Furthermore, we hypothesize that the integrated stress response (ISR) is important in ICM and KCCM. The ISR is activated by environmental stresses that protein kinases (GCN2 and PERK) can detect and phosphorylate translation factor, eIF2α. Our goal is to define how the ISR, specifically GCN2 and PERK, influence keratinocyte migration.

Methods:

We will evaluate in vitro wound healing and kinetic variation in protein expression and cytoskeleton remodeling. We will utilize four keratinocyte cell lines, control human keratinocyte NTERTs, and CRISPR-derived gene knockouts of GCN2, PERK, and ISR effector gene ATF4. Quantitative analysis of wound healing is accomplished using an IncuCyte ZOOM instrument. Protein expression is measured via immunoblots following high density wounding. Cytoskeletal analyses was done by immunofluorescence.

Results:

Preliminary results show PERK-KO and GCN2-KO cells have reduced expression of F-actin. Immunoblots showed actin-binding protein, phospho-cofilin, at lower levels in PERK-KO and GCN2-KO cells than in NTERT cells. Wound healing assays showed differentiated keratinocytes healing faster than undifferentiated in all cells, except GCN2-KO. GCN2-KO cells healed significantly slower than other differentiated cells and undifferentiated GCN2-KO cells. Wound healing assays showed undifferentiated PERK-KO cells healing slower than other undifferentiated cell lines.

Conclusion/Potential Impact:

The results indicate PERK and GCN2 could be key components in ICM and CCM respectfully. GCN2 and PERK could thus be potential therapeutic targets to provide cost-effective therapeutics to enhance/inhibit keratinocyte migration.