Photodynamic Therapy Effects in Skin of Color Wound Healing: an *In Vitro* Investigation

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BACKGROUND

Intercellular signaling and melanin transfer between epidermal melanocytes and keratinocytes play a critical role during cutaneous wound healing; dysregulation of these processes result in painful and pruritic fibrotic pathologies. Epidemiological studies indicate that keloids (KD) and hypertrophic scars (HS) occur more frequently in skin of color (SoC) than in lightly-pigmented skin and remain notoriously difficult to treat. Photodynamic therapy (PDT) is an emergent nonsurgical treatment that has shown clinical improvements in the management of SoC scars; however, molecular mechanisms underlying this efficacy have not been fully explored. PDT utilizes photosensitive compounds activated by varying light wavelengths that exert effects primarily through ROS generation, in turn, activating ROS pathways and ECM remodeling. Melanocytes are an integral part of intact skin and are known to scavenge ROS[1], which in turn, could potentially reduce clinical efficacy of PDT. Preclinical investigations into their role during wound healing PDT are limited. In the current study, we report on molecular mechanisms involved during monochromatic green light (MGL) activated-Rose Bengal sodium (RBS) PDT in adult human melanocytes (HEM) and keratinocytes (HEK) employing *in vitro* models of wound healing.

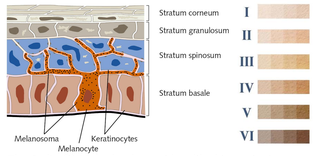

Fig. 1. (**Left**) Melanin pigment is critical to protect underlying dermis from UV radiation. Dysregulation of melanogenesis or melanin transfer may play a critical role in fibrotic pathologies. (**Right**) Fitzpatrick phototype for skin pigmentation.

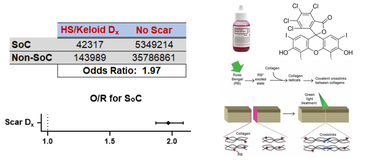

Fig. 2. (**Left**) A TriNetX database query of greater than 40M de-identified medical records show SoC patients (male/female, >6 y.o.) have significantly higher odds of being diagnosed with a hypertrophic scar or keloid compared to lighter skin patients. (**Right**) RBS photoactivation generates ROS to form covalent bonds in collagen AA residues, effectively crosslinking dermal collagen fibrils.

DESIGN & METHODS

1º adult human epidermal keratinocytes (HEK)
1º adult human epidermal melanocytes (HEM)

- 10μM Rose Bengal Sodium
- Dark conditions or diffuse LED Monochromatic Green Light (MGL) [15 mW/cm^2] irradiation for 1 hour [dose: 54 J/cm^2]
- ➢ Ki67$^+$ immunocytochemistry: proliferation status
- ➢ Scratch assays: migration capacity
- ➢ Phalloidin (fActin): morphological changes
- ➢ qRT-PCR: PDT effects on melanogenesis and ROS signaling pathways

RESULTS

Rose Bengal PDT Reduces HEK and HEM Proliferation and Migration

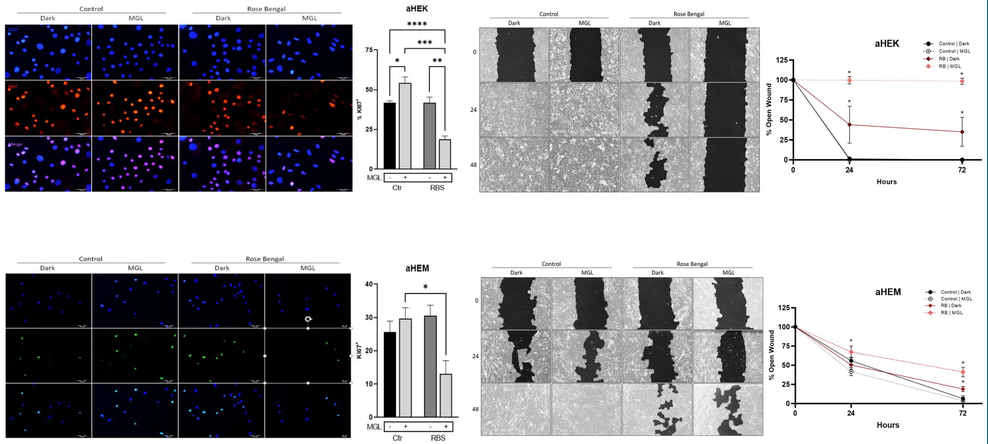

Figure 3. *In Vitro* results show RBS PDT significantly reduces cellular proliferation and migration of keratinocytes and melanocytes. Although keratinocytes were viable and metabolically active throughout the experiment, HEK proliferation was considerably reduced by RBS PDT (18.8% Ki67$^+$) compared to RBS non-PDT (42.0% Ki67$^+$) and dark controls (54.4% Ki67$^+$), *p<0.05, N=5 independent experiments (**Top** panels). Moreover, keratinocyte migration was completely halted after 1 hour RBS PDT compared to all other conditions. Melanocyte proliferation and migration rates were similarly affected (**Bottom** panels). RBS PDT significantly reduced Ki67-positive HEMs (13.1%) compared to all other treatments (>25.8%), p<0.05, N= 5 independent experiments. HEM migration was also reduced at 24 hr (67.3% open) and 72 hr (41.0% open) compared to all other treatments, <50.3% and <18.9%, respectively.

PDT Induces Rapid Morphological Changes & Increased Filamentous Actin Expression

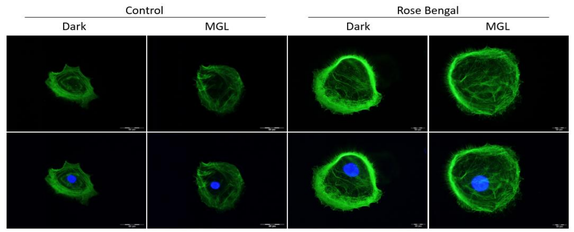

Figure 4. **Keratinocyte** Phalloidin ICC shows significantly increased filamentous actin expression and a more rigid cellular structure induced by 1 hour PDT RBS activation

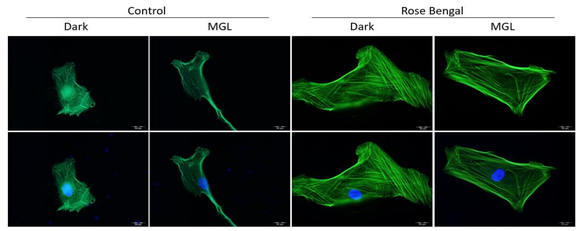

Figure 5. **Melanocyte** Phalloidin ICC shows demonstrates similarly increased filamentous actin expression and a more rigid cellular structure induced by 1 hour PDT RBS activation. Stress fibers appear more abundant compared with HEK. Additionally, actin is highly expressed with RBS alone.

Gene Expression Effects of PDT on Mitogenic and Oxidative Stress Cellular Signaling

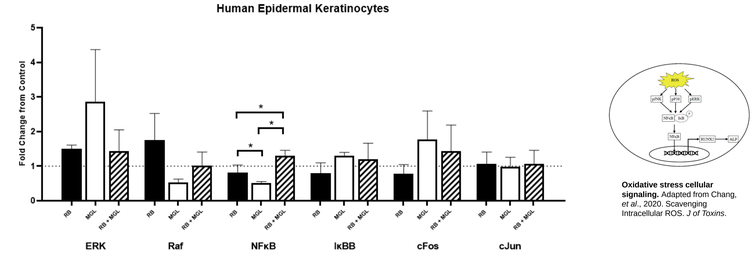

Figure 6. Gene expression analysis of mitogenic and oxidative stress pathways via qRT-PCR in keratinocytes. HEK mitogenic signaling (ERK/Raf/MAPK expression) is unaffected by 1 hr RBS PDT. Of the oxidative stress markers, the transcription factor NFκB (1.31-fold) was elevated compared to control conditions, p<0.05.

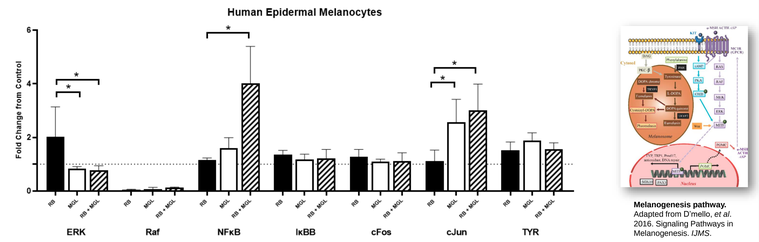

Figure 7. RBS treatment alone significantly increases mitogenic ERK expression in melanocytes. Interestingly, RBS PDT increases NFκB and cJun expression suggesting activation of oxidative stress mitigating signaling; however, melanogenesis signaling remained unaffected resulting in no change in tyrosinase (TYR) expression.

CONCLUSIONS

- ➢ Protracted RBS PDT reduces keratinocyte/melanocyte proliferation and migration.
- ➢ PDT significantly increases filamentous actin in keratinocytes and melanocytes.
- ➢ PDT does not appear to alter melanogenesis pathways which may yet provide a protective buffer against deleterious ROS and oxidative stress during photoactivation in SoC.

References & Acknowledgements

1. Zhong G , Yang X , Jiang X , Kumar A , Long H , Xie J , Zheng L , Zhao J . Dopamine-melanin nanoparticles scavenge reactive oxygen and nitrogen species and activate autophagy for osteoarthritis therapy. Nanoscale. 2019 Jun 20;11(24):11605-11616. doi: 10.1039/c9nr03060c. Erratum in: Nanoscale. 2019 Dec 28;11(48):23504-23505. PMID: 31173033; PMCID: PMC6776464.

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