Shifts to Aerobic Glycolysis Produce Physiological Responses to Injury Resulting in Diverse Wound Repair

Texas Children's Hospital, Department of Surgery and Division of Pediatric General Surgery, Baylor College of Medicine

Fayiz Faruk
Walker D. Short
Swathi Balaji

Baylor College of Medicine

BACKGROUND

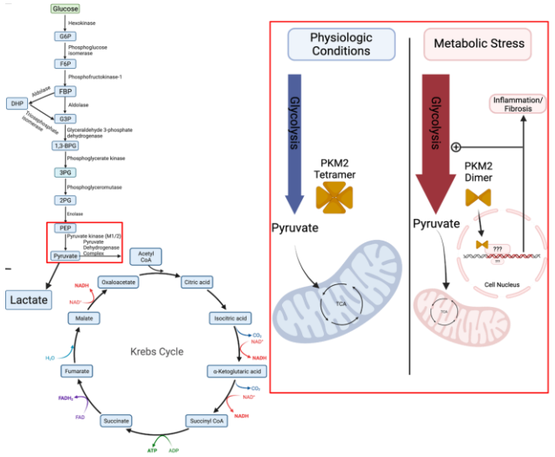
- Normal postnatal wound healing invariably results in scar formation
- There is extreme inter-individual variability in the degree of scarring to similar injuries
- The mechanisms underlying these variable scarring responses are unknown
  - Dermal fibroblasts from fibrotic models such as radiation-induced fibrosis and scleroderma show an increased dependence on glycolysis associated with increased ECM accumulation[1,2]
  - Warburg effect: Diseased cells (tumor) increase their dependence on aerobic glycolysis
  - **Does Warburg's aerobic glycolysis underlie why we scar differently after wounding?**

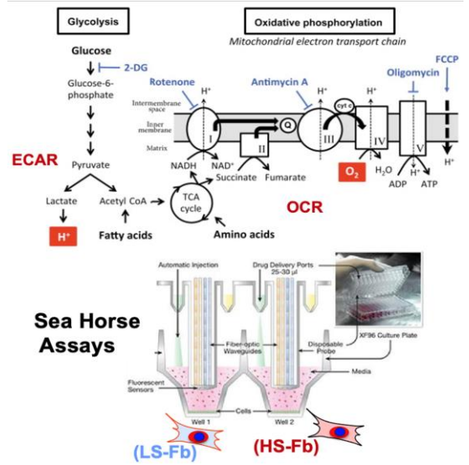
HYPOTHESIS

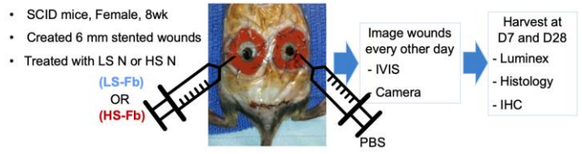
Intrinsic differences in how patients' dermal fibroblasts respond to wound healing by engaging in differential energy metabolism, that resembles Warburg's aerobic glycolysis, predicts the degree of fibrosis leading to either a low or high scarring phenotype.

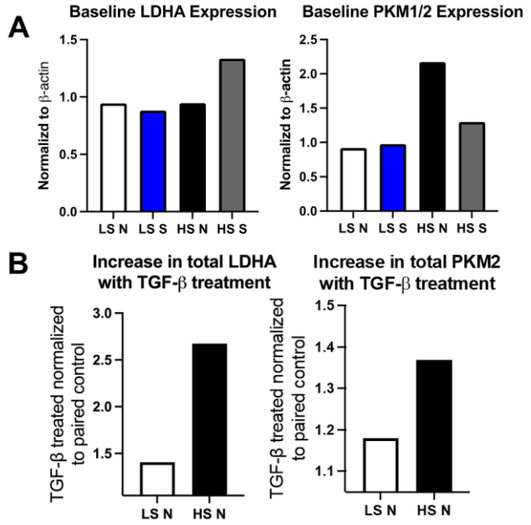
METHODS

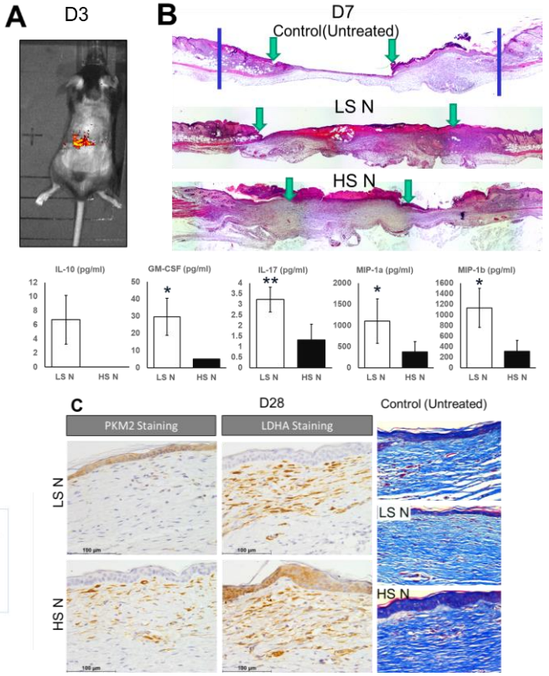
- **Vancouver Scar Scale** used to classify Caesarian scars in-situ pre-operatively
- Low scarring (LS): score 1-3
- High scarring (HS): score 6-9
- **Dermal Fibroblasts (Fb)** were harvested from normal skin (N) and C-section scar (S) tissue.
- Energy metabolism profiling by XF96 flux analyzer Sea Horse assays
  - Oxygen Consumption Rate (OCR): measure of mitochondrial Oxidative Phosphorylation Pathway
  - Extracellular Acidification Rate (ECAR): measure of Glycolysis Pathway











RESULTS

- **High scarring patient fibroblasts have more LDHA and total PKM protein expression**
  - **A:** Protein expression of PKM1/2 was highest in HS.
  - B: There was a significant increase in total LDHA and total PKM2 expression with TGF-β (10ng/ml for 24hr) treatment in HS N compared to LS N.
- **Murine wounds grafted with normal skin fibroblasts from high scarring patients heal faster and with increased granulation tissue, collagen scarring, and PKM2 and LDHA expression**
  - A. IVIS imaging of fluorescently labeled-cells in wounds at Day 3.
  - B: Wounds treated with HS N fibroblasts healed faster, with decreased epithelial gap and increased granulation tissue at Day 7 as compared to wounds treated with LS N fibroblasts.
  - C. Immuno-staining revealed that wounds with HS N fibroblasts had higher LDHA and PKM2 expression in the scar area, with an increased collagen packing and thicker epidermis at Day 28.

CONCLUSION

- Fibroblasts of different scarring phenotypes display characteristic bioenergetic metabolism profiles, with a shift to aerobic glycolysis (Warburg effect) associated with increased fibrosis (high scar).
- High scarring patient fibroblasts show increased LDHA and PKM2, with increase in PKM2 phosphorylation and dimerization when treated with TGF-β.
- Low vs. high scarring patient fibroblasts produce distinct scarring in murine wounds.
- These data suggest that wound-driven oxidative stress transduces individually imprinted bioenergetic responses, which may underscore wound repair heterogeneity and scarring outcomes.

REFERENCES

1. Zhao et al., Nature Metabolism 2019, 1:147-157;
2. Park et al., Nature. 2020 February ; 578(7796): 621–626