Metabolic Mutations Drive *Staphylococcus aureus* Adaptation to the Skin

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### INTRODUCTION

- *Staphylococcus aureus* is the most common pathogen causing skin and soft tissue infection, and poses a particular problem to patients with atopic dermatitis who have increased rates of colonization and infection.
- Human skin provides a relatively hypoxic environmental niche requiring bacterial metabolic adaptation.
- *S. aureus* adaptation to skin may be mediated by immunometabolic mechanisms including Hif1α signaling and fumarate accumulation, which have been shown to be linked to glycolysis and epigenetic changes resulting in trained immunity.

### METHODS

- Ten representative *S. aureus* strains isolated from pediatric atopic dermatitis patients were analyzed by whole genome sequencing, selective transcriptional analysis, metabolic analysis, immunophenotyping, and in vivo characterization in a murine model of skin infection.

### RESULTS

**Figure 1. Colonizing diversity.** (A) Diagram of glycolysis and TCA cycle. (B) Heatmap of nonsynonymous SNPs in atopic dermatitis (AD) isolates in TCA cycle, glycolytic, and small colony variant (SCV) genes. Reference strain FPR3757 protein sequences were BLASTed against clinical isolate sequences using RAST server. (C) Relative gene expression of fumC and other TCA cycle enzymes by clinical *S. aureus* strains grown to exponential phase in LB compared to wild type (WT) USA300 LAC. Each data point is the mean value ± SEM. *p<0.01 and ****p<0.0001 by Mann-Whitney test comparing AD isolate and USA300.

**Figure 2. Clinical strains harbor metabolic mutations.** (A) Diagram of glycolysis and TCA cycle. (B) Heatmap of clinical isolates in TCA cycle, glycolytic, and small colony variant (SCV) genes. (C) Fumarate levels measured in cell media after 5-hour infection in HEKn cells and keratinocytes (HEKn) and those stimulated with clinical AD strains and WT USA300 LAC, and (B) WT USA300 JE2 and WT WT LAC. Each data point is the mean value ± SEM *p<0.05 and ****p<0.0001 by one- (C) or two-way ANOVA (A, B).

**Figure 3. *S. aureus* metabolism is linked to IL-1β production.** (A) A Seahorse extracellular flux analyzer monitored metabolic activity via oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of uninfected primary keratinocytes (HEKn). (B) Fumarate accumulation, which have been shown to be linked to glycolysis and epigenetic changes resulting in trained immunity. (C) Fumarate levels measured in cell media after 5-hour infection in HEKn cells and keratinocytes (HEKn) and those stimulated with clinical AD strains and WT USA300 LAC, and (B) WT USA300 JE2 and WT WT LAC. Each data point is the mean value ± SEM *p<0.01 and ****p<0.0001 by one- (C) or two-way ANOVA (A, B, E).

### CONCLUSIONS

- Chronic colonizing *S. aureus* isolates from the skin of atopic dermatitis patients represent a diverse population that are associated with varying levels of clinical disease.
- *S. aureus* isolates have adapted to the skin environment by metabolic changes, including *fumC* upregulation.

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