TRPV1-associated sensory-immune signature distinguishes nonhealing diabetic foot ulcers

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We acknowledge the following funding sources: R01DK136241 and NIH Bench-to-Bedside, an award made possible by the NIH Office of Clinical Research.

Chronic wounds affect 8.2 million Medicare beneficiaries, costing $28.1–96.8 billion annually[1]. Pruritus is often prevalent in chronic ulcers and may decrease with healing.[2-4] Although nonhealing involves inflammatory dysregulation,[5,6] the relationship between sensory–immune signaling and DFU healing outcomes remains unclear. We investigated whether itch- and nociception-associated transcriptional programs distinguish healing from nonhealing DFUs using integrated bulk and single-cell transcriptomics.

Bulk RNA-seq datasets (GSE134431: DFU Healer n=7, Nonhealer n=6; GSE97615: acute wounds n=8) were integrated with controls (n=12; total n=33); batch correction was confirmed by PCA (Fig. 1A). Gene-set enrichment evaluated sensory, itch, neuropeptide, axonal, histamine, and mast-cell pathways. Bulk RNA-seq interrogated genes spanning nociceptor/TRP-neurotrophic (TRPV1, TRPA1, NTRK1, SCN10A, SCN11A, PIRT, NGF), histaminergic (HRH1, HRH2, HRH4, HDC, TPSAB1), pruritus cytokine (IL31, IL31RA, IL33, IL4, IL13,

TSLP), and substance P/NK1R (TAC1, TACR1). Single-cell RNA-seq localized findings by cell type.

PCA of batch-corrected bulk RNA-seq data demonstrated condition-associated separation, with acute wounds clustering distinctly from DFUs and controls (Fig. 1A). Among 24 candidate sensory–immune genes, TRPV1 was the only gene significantly upregulated in DFU Nonhealers compared with healers (Fig. 1B, left). Relative to diabetic foot skin (DFS), DFU healers exhibited significantly reduced MRGPRX2 and increased POSTN, whereas Nonhealers demonstrated increased F2RL1/PAR2 expression (Fig. 1B, middle–right). F2R/PAR1 was not significantly altered, and MRGPRX2, POSTN, F2RL1/PAR2, and F2R/PAR1 did not significantly distinguish healing outcomes (adjusted $P \geq 0.30$). Single-cell RNA-seq revealed distinct cell-type-specific patterns of the candidate genes from bulk transcriptomic analysis. Single-cell analysis revealed cell-type-specific detection of sensory–immune and stress-response genes across clinical groups (Fig. 1C). Compared with healers, nonhealers exhibited increased TRPV1 detection in arterial smooth muscle cells, HRH1 in macrophages, and IL33 in keratinocytes and vascular endothelial cells. Substance P signaling components showed distinct cellular distributions, with TAC1 primarily detected in fibroblasts and TACR1 in vascular endothelial cells across clinical groups. Stress-response genes RELA and CDKN1A showed widespread detection across epithelial, stromal, and immune populations, with increased CDKN1A detection in Nonhealer keratinocytes and arterial smooth muscle cells.

Nonhealing DFUs exhibit a distinct sensory–immune transcriptional signature characterized by selective TRPV1 upregulation, the only candidate gene significantly distinguishing healers from Nonhealers. Altered MRGPRX2, POSTN, and F2RL1/PAR2 expression relative to diabetic foot skin further highlights broader pruritogenic and inflammatory

pathway alterations in DFUs. Single-cell analyses revealed cell-type-specific differences in TRPV1, HRH1, and IL33 detection across vascular, immune, and epithelial compartments, alongside distinct distributions of substance P signaling components and widespread stress-response gene detection. Together, these findings identify TRPV1-associated sensory–immune signaling as a previously underexplored feature of nonhealing DFUs and support further investigation of TRPV1 as a candidate biomarker and potential therapeutic target. Future studies integrating clinical itch assessments and functional validation may clarify the contribution of these pathways to impaired wound healing.

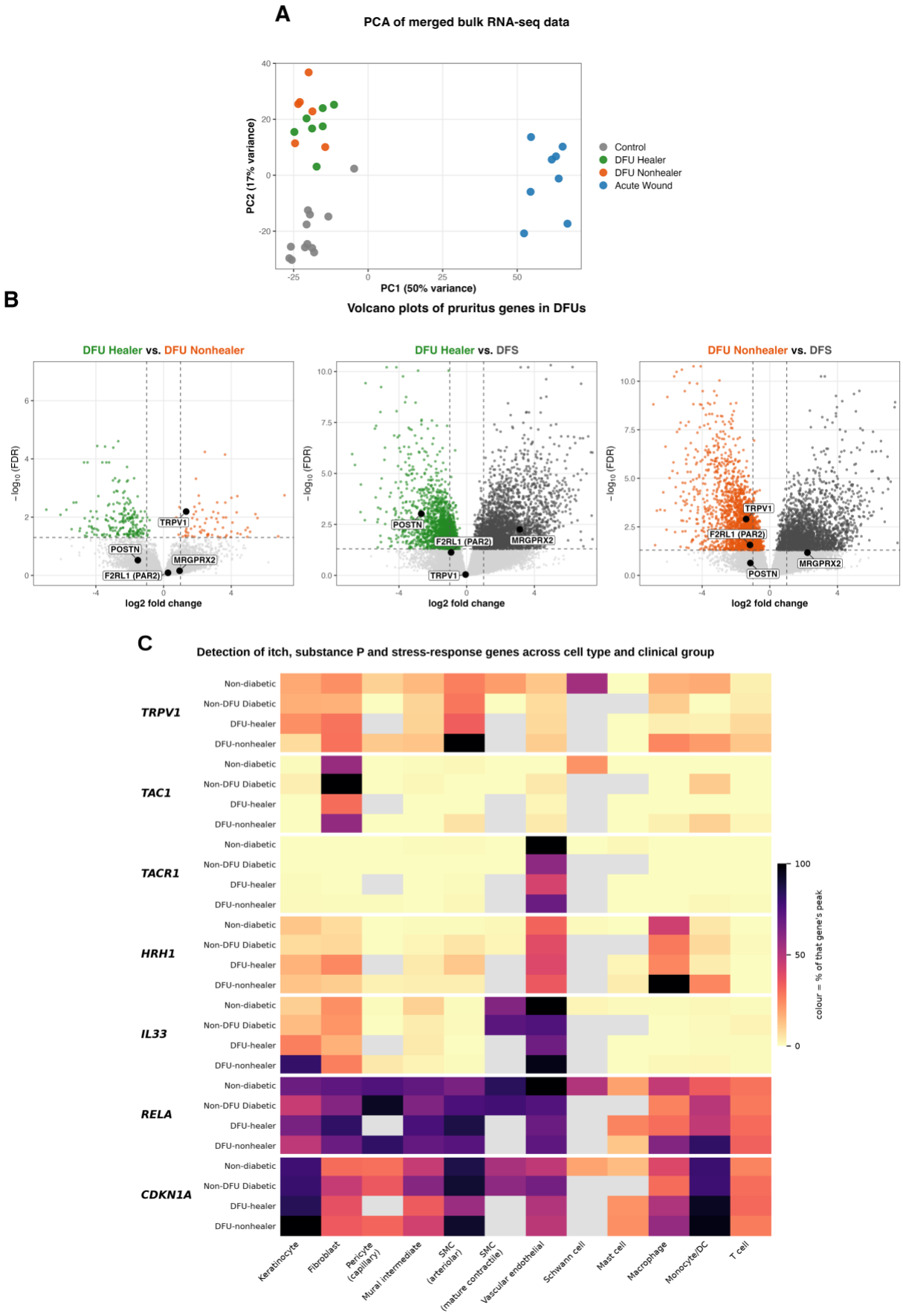

A
PCA of merged bulk RNA-seq data
PC2 (17% variance)
PC1 (50% variance)
Control
DFU Healer
DFU Nonhealer
Acute Wound
B
Volcano plots of pruritus genes in DFUs
DFU Healer vs. DFU Nonhealer
DFU Healer vs. DFS
DFU Nonhealer vs. DFS
−log10 (FDR)
log2 fold change
TRPV1
POSTN
MRGPRX2
F2RL1 (PAR2)
C
Detection of itch, substance P and stress-response genes across cell type and clinical group
TRPV1
TAC1
TACR1
HRH1
IL33
RELA
CDKN1A
Non-diabetic
Non-DFU Diabetic
DFU-healer
DFU-nonhealer
colour = % of that gene's peak
Keratinocyte
Fibroblast
Pericyte (capillary)
Mural intermediate
SMC (arteriolar)
SMC (mature contractile)
Vascular endothelial
Schwann cell
Mast cell
Macrophage
Monocyte/DC
T cell

Figure 1. Sensory–immune gene expression in diabetic foot ulcers (DFUs).

(A) PCA of merged bulk RNA-seq data (GSE134431 and GSE97615) after ComBat-seq batch correction (n=33: control 12, DFU healer 7, DFU nonhealer 6, acute wound 8). Points are samples, colored by condition.

(B) Volcano plots of differential expression for DFU healer vs. nonhealer (left; merged dataset, DESeq2 with dataset as a covariate), DFU healer vs. diabetic foot skin (DFS; middle) and DFU nonhealer vs. DFS (right; both GSE134431 only; DFS n=8, healer n=7, nonhealer n=6). Significance used the Wald test with Benjamini–Hochberg FDR correction across all genes in each comparison. Dashed vertical lines mark a 2-fold change (log2 fold change = ±1), and the dashed horizontal line marks FDR = 0.05. Genes with FDR ≥ 0.05 are grey; colored points are significant genes, colored by the group in which they are higher (green, healer; orange, nonhealer; dark grey, DFS). Black points label TRPV1, MRGPRX2, F2RL1 (PAR2) and POSTN. Axes are truncated, so a few extreme genes fall outside the plotting area.

(C) Heatmap showing the percentage of cells with detectable transcripts of *TRPV1*, *TAC1*, *TACR1*, *HRH1*, *IL33*, *RELA*, and *CDKN1A* across cell types and clinical groups in the single-cell RNA-seq dataset (GSE165816). Values represent the percentage of cells in which each gene was detected. Color is scaled independently for each gene relative to its maximum detection percentage across all cell type/group combinations (maximum detection: *TRPV1* = 4.5%, *TAC1* = 3.6%, *TACR1* = 23%, *HRH1* = 61.9%, *IL33* = 44.7%, *RELA* = 28%, *CDKN1A* = 68%). Grey cells indicate cell type/group combinations containing fewer than 50 cells.

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