Macrophage-specific inhibition of the histone demethylase JMJD3 decreases STING and pathologic inflammation in diabetic wound repair

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Macrophage plasticity is critical for normal tissue repair following injury. In pathologic states such as diabetes, macrophage plasticity is impaired, and macrophages remain in a persistent proinflammatory state; however, the reasons for this are unknown. Here, using single-cell RNA sequencing of human diabetic wounds, we identified increased JMJD3 in diabetic wound macrophages, resulting in increased inflammatory gene expression. Mechanistically, we report that in wound healing, JMJD3 directs early macrophage-mediated inflammation via JAK1,3/STAT3 signaling. However, in the diabetic state, we found that IL-6, a cytokine increased in diabetic wound tissue at later time points post-injury, regulates JMJD3 expression in diabetic wound macrophages via the JAK1,3/STAT3 pathway and that this late increase in JMJD3 induces NfκB-mediated inflammatory gene transcription in wound macrophages via an H3K27me3 mechanism. Interestingly, RNA sequencing of wound macrophages isolated from mice with JMJD3-deficient myeloid cells (Jmjd3fl/flLyz2Cre) identified that the STING gene (Tmem173) is regulated by JMJD3 in wound macrophages. STING limits inflammatory cytokine production by wound macrophages during healing. However, in diabetic mice, its role changes to limit wound repair and enhance inflammation. This finding is important since STING is associated with chronic inflammation, and we found STING to be elevated in humans and murine diabetic wound macrophages at late time points. Finally, we demonstrate that macrophage-specific, nanoparticle inhibition of JMJD3 in diabetic wounds significantly improves diabetic wound repair by decreasing inflammatory cytokines and STING. Taken together, this work highlights the central role of JMJD3 in tissue repair and identifies cell-specific targeting as a viable therapeutic strategy for nonhealing diabetic wounds.

Keywords: wound healing; epigenetics; diabetes; JMJD3; STING

Cellular & Molecular Immunology (2022) 19:1251–1262; https://doi.org/10.1038/s41423-022-00919-5

INTRODUCTION

Tissue repair after injury is a highly orchestrated process occurring in overlapping stages of coagulation, inflammation, proliferation, and remodeling [1, 2]. The inflammatory phase is further divided into an early phase, wherein macrophages (Mφs) promote inflammation and pathogen destruction, and a late phase, where Mφs promote tissue repair. We and others have identified that in the setting of type 2 diabetes (T2D), the inflammatory phase is significantly prolonged, which retards progression through the remaining stages of repair. In diabetes, during the inflammatory phase, inflammatory Mφs are initially decreased, resulting in impaired early inflammation, followed by a late, robust Mφ transition to an inflammatory phenotype that persists and does not allow wound healing to progress along its normal course [3–5]. The reasons for this dysregulated inflammation are complex; however, we previously identified epigenetic mechanisms that regulate gene expression in wound Mφs during normal tissue repair and are altered in pathologic states. Despite an increased understanding of the role of epigenetic alterations in wound repair, the precise enzymes involved and their kinetic regulation during normal and diabetic wound repair remain incompletely understood.

We recently identified that the histone demethylase Jumonji domain-containing protein D3 (JMJD3) controls inflammation in vascular disease [6]. Specifically, JMJD3 demethylates the lysine
27 site on histone 3 (H3K27), resulting in the opening of chromatin that renders promoter sites accessible for transcription factor binding [6–8], thus effectively increasing gene expression. Demethylation by JMJD3 at the promoter sites reverses the repressive effect of H3K27 trimethylation (H3K27me3) and results in gene activation and active transcription. We previously found that JMJD3 is increased in the presence of the fatty acid palmitate, which is known to be elevated in obesity-induced diabetes and in diabetic bone marrow progenitor cells [9]. Nevertheless, there remains a dearth of information on JMJD3 in normal and diabetic wound Mφs and its role in inflammation and tissue repair. Given that JMJD3 has been shown to regulate inflammation in other tissues and be increased in diabetic bone marrow, we examined the regulation and role of JMJD3 in wound Mφs during normal and diabetic tissue repair.

Herein, using human wound single-cell RNA sequencing (scRNAseq) and primary wound Mφs isolated from diabetic and control murine wounds, we show that Jmdj3 in Mφs is necessary for inducing early inflammation in normal wound repair but is pathologically elevated in diabetic Mφs at later stages of repair. Mechanistically, we found that IL-6, a cytokine present at high levels in diabetic peripheral blood and wound tissues, drives the Janus kinase 1,3/signal transducer and activator of transcription 3 (JAK1,3/STAT3) pathway to increase Jmdj3 expression in diabetic wound Mφs and that abrogation of this pathway leads to decreased Mφ-mediated inflammation in diabetic wounds. Interestingly, wound Mφs from our mice with JMJD3-deficient myeloid cells (Jmdj3fl/flLy2Cre+) analyzed by bulk RNA-seq showed that Jmdj3 regulates the STING gene (Tmem173), which, in addition to NFκB-mediated inflammation, contributes to chronic inflammation in diabetes. Finally, we demonstrated that local, Mφ-specific inhibition of JMJD3 using nanoparticles dramatically improves diabetic wound repair by decreasing NFκB and STING-mediated inflammation. Together, these results suggest a central role for JMJD3 in normal and diabetic wound repair and identify JMJD3 as a viable therapeutic target for nonhealing diabetic wounds.

RESULTS

JMJD3 is increased early in wound Mφs, increases NFκB inflammatory cytokine gene expression via H3K27me3 and is regulated by JAK1,3/STAT3 in wound Mφs

Epigenetic-based histone modifications have been shown by our group and others to regulate Mφs phenotype and function during wound repair [10–12]. JMJD3 plays a vital role in inflammation in other vascular tissues, and given the importance of Mφ-activated inflammation in wound repair, we investigated JMJD3 in wound Mφs following injury. First, to establish the normal kinetics of JMJD3 following injury, we sorted wound Mφs (CD3/CD19/NK1.1/Ly6G/CD11b+) daily for 10 days following wounding (6 mm punch biopsy) and analyzed Jmdj3 expression. We identified a significant increase in Jmdj3 on Day 3 post-injury, followed by a rapid decrease over the course of repair (Fig. 1A). By Day 5 (following the acute inflammatory phase), Jmdj3 expression in wound Mφs had returned to baseline levels. Next, to examine the relevance of JMJD3 in wound Mφs during repair, we generated a myeloid-specific, JMJD3-deficient mouse strain using the Cre recombinase Lox-P system [13] (Jmdj3fl/Ly2Cre+). Confirmation of reduced Jmdj3 specifically in wound Mφs isolated from the Jmdj3fl/Ly2Cre+ mice was obtained (Supplementary Fig. 1A).

Given that we and others have shown that NFκB-mediated inflammation is important in wound Mφs and is often epigenetically regulated, we specifically examined the expression of NFκB-mediated genes previously identified to be important for wound repair (e.g., Il1b, Tnfa) in isolated wound Mφs from the Jmdj3fl/Ly2Cre+ mice and littermate controls at Day 3 post-wounding. As expected, we identified significant decreases in Il1b and Tnfa as well as Il2 and Il23 expression in the wound Mφs from the Jmdj3fl/Ly2Cre+ mice (Fig. 1B, Supplementary Fig. 1B). This phenomenon reduced Il1b and Tnfa expression in the Jmdj3fl/Ly2Cre+ wound Mφs and was also observed at the protein level by ELISAs (Fig. 1C). Since JMJD3 alters gene expression through demethylation of H3K27me3, we examined wound Mφs from the Jmdj3fl/Ly2Cre+ mice by chromatin immuno-precipitation (ChIP) for H3K27me3 at the NFκB binding sites of the Il1b and Tnfa gene promoters. We found a significant increase in the repressive H3K27me3 mark at these NFκB binding sites on the promoters of Il1b and Tnfa (Fig. 1D, Supplemental Fig. 1C).

Our group previously showed that Type 1 interferons (IFN-I), such as IFN-β, are increased in normal wound tissue early during the inflammatory phase of wound repair [4, 11]. Although IFN-β can activate numerous downstream pathways, one of the more commonly studied pathways involves the activation of Janus kinase (JAK) proteins, which leads to tyrosine phosphorylation that, in turn, activates signal transducers and activators of transcription (STAT) proteins and translocates dimerized, phosphorylated proteins to the nucleus [14–16]. Furthermore, IFN-β has been shown by our group and others to modulate epigenetic enzyme expression in wounds and other vascular tissues, where they function as transcriptional regulators [3, 12, 17]. Given the known early spike of IFN-β in wound tissue undergoing normal repair processes, we examined the effect of IFN-β stimulation on Jmdj3 in wound Mφs. Wound Mφs were sorted on Day 3 post-injury, stimulated ex vivo with IFN-β (100 U/mL; 8.5 ng/mL) for 6 h and analyzed for Jmdj3 expression. Jmdj3 expression was significantly increased in wound Mφs following IFN-β stimulation, and this effect was abrogated in wound Mφs sorted from the mice deficient in the IFN-β receptor (Ifnar−/−) (Fig. 1E). To examine the downstream effects of IFN-β stimulation on JMD3 and H3K27me3 regulation of inflammatory cytokines, we isolated wound Mφs ex vivo, treated them with IFN-β and examined them by ChIP analysis for H3K27me3 at NFκB binding sites on promoters of inflammatory genes important for wound repair (e.g., Il1b, Tnfa). We found that levels of H3K27me3 were significantly decreased at NFκB binding sites on Il1b and Tnfa gene promoters following IFN-β stimulation of wound Mφs (Fig. 1F). Next, to determine whether IFN-β regulates Jmdj3 expression via JAK/STAT signaling, we examined JAK1, JAK3 and STAT3 levels following IFN-β stimulation of wound Mφs ex vivo. We confirmed prior reports [18, 19] that IFN-β increases JAK1 and 3 and STAT3 phosphorylated protein levels, and this effect is negated in Ifnar−/− mice (Supplementary Fig. 2). To study the effect of JAK1/3 on Jmdj3 expression in wound Mφs, we used tofacitinib, a commercially available competitive inhibitor of JAK1/3, and treated isolated wound Mφs ex vivo with IFN-β and tofacitinib (100 μM) for 6 h. We found that inhibition of JAK1/3 led to significantly decreased levels of Jmdj3 in the wound Mφs (Fig. 1G), and when we examined the Il1b and Tnfa gene promoters by ChIP, we found that JAK1/3 blockade resulted in increased H3K27me3 at the NFκB binding sites of these genes, resulting in decreased gene activation (Fig. 1H). Next, we studied the effects of STAT3 on Jmdj3 expression in wound Mφs. We used the Cre/LoxP system to generate myeloid-depleted STAT3 mice (Stat3fl/Ly2Cre+) as previously described [13]. Wound Mφs (CD3/CD19/NK1.1/Ly6G/CD11b+) isolated on Day 3 from myeloid-cell STAT3-deficient mice were isolated by sorting and stimulated ex vivo with IFN-β for 6 h. These Mφs exhibited significantly decreased Jmdj3 expression (Fig. 1I), and ChIP analysis of NFκB-mediated inflammatory promoters showed that Il1b and Tnfa had increased H3K27me3 levels (Fig. 1J). Taken together, these data suggest that the increased IFN-β observed in early wound inflammation regulates early Jmdj3 expression in normal wound Mφs via the JAK1/STAT3/STAT3 pathway.

JMJD3 is increased in diabetic wound Mφs during the late inflammatory phase

Given that NFκB-mediated inflammatory cytokines have been shown to be elevated in diabetic wounds [3, 5, 20] and that JMJD3
JAK1,3 inhibition with tofacitinib (100 μM) was examined whether JMJD3 was elevated in diabetic wound Mφs, we isolate wound Mφs and find that JDMD3, along with IL-6, TNF-α, and IL-23, was significantly increased in Mφs from chronic wounds of T2D patients (Fig. 2A, Supplementary Fig. 3). Similarly, we generated a murine model of obesity and T2D by administering a high-fat diet chow (60% carbohydrates versus 12% in normal Chow) for 12-20 weeks. These mice with diet-induced obesity (DIO) exhibit insulin resistance and impaired glucose tolerance [21]. Wound Mφs (CD3−/CD19−/NK1.1−/Ly6G−/CD11b+) were isolated every 48 h post-wounding from the mice with DIO and demonstrated increased Jmd3 expression on Days 5–10 during the late stages of wound repair, when wound Mφs should have transitioned from a pro- to an anti-inflammatory phenotype (Fig. 2B). Furthermore, we noted a sustained elevation of the inflammatory genes Il6, Il1b, and Tnfα in diabetic wound Mφs, while Il1b was significantly reduced on Day 1 in diabetic wound Mφs and remained unchanged or decreased relative to that of nondiabetic wounds throughout the time course (Supplementary Fig. 4A–D), suggesting that early IFN-β production in wounds suppresses inflammatory cytokine production and is necessary for healthy wound repair.

Since we and others have previously shown that diabetic wounds fail to upregulate IFN-β post-injury and during wound repair [11, 22–24], we examined our previously published human wound bulk RNA-seq dataset for other potential cytokines/chemokines that are elevated in diabetic wound tissue and may regulate the Jak/Stat pathway and Jmd3 in diabetic wound Mφs [10]. As others have previously identified [25, 26], we found increased IL-6 in human T2D wounds (Fig. 2C). Mechanistically, IL-6 binds to the IL-6 receptor (IL-6R), which is common on Mφ cell surfaces, and this complex then couples with the transmembrane spanning IL-6R subunit-b gp-130. Binding with gp-130 facilitates homodimerization that leads to stimulation of the Jak/Stat3 pathway downstream [18, 27]. Importantly, IL-6 has been shown to specifically upregulate Stat3 compared to other Stat proteins [28–30]. To directly examine the effects of IL-6 on Jmd3 in diabetic wound Mφs, we isolated wound Mφs from the mice with DIO and treated them ex vivo with recombinant IL-6 (rIL-6; 20 nM).

regulates inflammatory cytokines in normal wound Mφs, we examined whether Jmd3 was elevated in diabetic wound Mφs. We determined the translational relevance by first examining human wound Mφs analyzed previously by our group [10] using single-cell RNA sequencing (scRNA-seq) from T2D and non-T2D patients and found that Jmd3, along with IL-6, TNF-α, IL-1β, and IL-23, was significantly increased in Mφs from chronic wounds of T2D patients (Fig. 2A, Supplementary Fig. 3). Similarly, we generated a murine model of obesity and T2D by administering a high-fat diet chow (60% carbohydrates versus 12% in normal Chow) for 12-20 weeks. These mice with diet-induced obesity (DIO) exhibit insulin resistance and impaired glucose tolerance [21]. Wound Mφs (CD3−/CD19−/NK1.1−/Ly6G−/CD11b+) were isolated every 48 h post-wounding from the mice with DIO and demonstrated increased Jmd3 expression on Days 5–10 during the late stages of wound repair, when wound Mφs should have transitioned from a pro- to an anti-inflammatory phenotype (Fig. 2B). Furthermore, we noted a sustained elevation of the inflammatory genes Il6, Il1b, and Tnfα in diabetic wound Mφs, while Il1b was significantly reduced on Day 1 in diabetic wound Mφs and remained unchanged or decreased relative to that of nondiabetic wounds throughout the time course (Supplementary Fig. 4A–D), suggesting that early IFN-β production in wounds suppresses inflammatory cytokine production and is necessary for healthy wound repair.

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We observed a significant increase in Jmd3 expression in DIO wound Mφs after treatment with rIL-6, and this effect was abrogated with an IL-6 receptor inhibitor (200 nM; LMT-28), a small molecule that competitively blocks phosphorylation of the IL-6 receptor beta subunit glycoprotein-130 [31] (Fig. 2D). To inhibit JAK1/3, we used tofacitinib (100 μM) in diabetic wound Mφs, and this effect was reversed after treatment with tofacitinib in the presence of rIL-6 (Fig. 2G). A concomitant increase in H3K27me3 at the Il1b and Tnfα promoters when examined by ChIP (Fig. 2H) was noted with tofacitinib treatment. We also examined the role of STAT3 by isolating wound Mφs from Stat3f/fLyz2Cre+ mice on a high-fat diet for 12-20 weeks (DIO) and treated them ex vivo with rIL-6. In these cells, we observed a decrease in Jmd3 expression (Fig. 2I) and an increase in H3K27me3 at the Il1b and Tnfα promoters when examined by ChIP (Fig. 2J). Together, these data suggest that in diabetic wound tissue, JMJD3 increases in the later stages of wound healing, is regulated by IL-6 through a JAK1/3/STAT3 pathway, and, as such, may be partially responsible for the persistent inflammatory phenotype seen in diabetic wound Mφs.
JMJD3 regulates STING in pathologic wound Mψs

To identify additional relevant genes that Jmjd3 may regulate in wound repair, we isolated wound Mψs (CD3/CD19/NK1.1/Ly6G/CD11b+) from myeloid-depleted JMJD3 mice (Jmjd3fl/Lyz2cre+) and littermate controls and performed bulk RNA sequencing analysis. We then examined this dataset for differentially regulated genes that were previously associated with inflammation and/or wound repair. This analysis revealed that in JMJD3-deficient mice, in addition to other inflammatory genes, Tmem173, a gene involved in the cytosolic GMP-AMP synthase - stimulator of interferon genes (cGAS-STING) inflammatory pathway, was significantly decreased (Fig. 3A). cGAS-STING has been shown to sustain chronic inflammation in pathologic conditions such as obesity-induced diabetes [32-35], myocardial infarction [17], and chronic inflammatory diseases [36-39]. Although the downstream effects of STING-mediated inflammation in cancer and other chronic inflammatory conditions have been extensively studied, the upstream regulation of Tmem173 gene expression is unknown.

To further investigate our findings from the RNA-seq data, we isolated wound Mψs from our Jmjd3fl/Lyz2cre+ mice and littermate controls and found decreased Tmem173 in the Jmjd3-deficient wound Mψs, consistent with our RNA-seq data (Fig. 3B). Furthermore, ChIP analysis at the GATA1 binding site on the Tmem173 gene promoter revealed a concomitant increase in the H3K27me3 mark at the Tmem173 promoter in wound Mψs (Fig. 3C). Additionally, when we examined scRNA-seq data from human T2D and non-T2D laboratory [4], we observed elevated Tmem173 in the human T2D wound Mψs (Fig. 3D). We studied the kinetics of Tmem173 expression in diabetes by isolating wound Mψs from the mice with DIO and their ND controls up to 10 days after wounding and analyzed them for Tmem173 expression. We observed that in DIO wound Mψs, Tmem173 increased late in the wound healing process (Fig. 3E) compared to that of the littermate controls. Notably, the slight increase observed in Tmem173 at Day 10 in the ND wound Mψs did not translate to the protein level (Supplementary Fig. 5A). Wound Mψs obtained on Day 5 post-wounding revealed that the mice with DIO had significantly increased phosphorylated STING protein levels (p-STING), as shown by immunofluorescence (Fig. 3F, G), and STING levels, as shown by Western blots (Supplementary Fig. 5B).

Diabetic STING knockout mice, Tmem173−/− (DIO STING−/−), were generated by subjecting Tmem173−/− mice to a high-fat diet for 12-20 weeks, and a wound healing curve was obtained against age-matched littermate controls. The Tmem173−/− mice with DIO exhibited improved wound healing at Days 4-7 (Fig. 3H), correlating with the days of greatest Tmem173 expression. Histologic examination of Day 5 wounds from the DIO STING−/− mice and the control mice revealed higher collagen deposition by trichrome staining and improved wound contraction (Fig. 3I). Next, we examined the role of STING in promoting downstream inflammation in wound Mψs and noted that in DIO Tmem173−/− wound Mψs isolated on Day 5 post-injury, there was a significant decrease in the inflammatory cytokines Il6, Il1b, and Tnfa, with a concomitant increase in the reparative gene mannose receptor complex 1 (Mrc1) (Fig. 3J-M). Examination of nondiabetic wound Mψs revealed that in the Tmem173−/− mice, there was a significant increase in the inflammatory cytokines Il1b, Il6, and Tnfa (Supplementary Fig. 6A-C) with nonsignificant changes in Mrc1, suggesting a context-specific, dichotomous role for STING in regulating Mψ-mediated inflammatory cytokines and wound repair (Supplementary Fig. 6D, E).

To explore this issue further, we examined downstream STING pathway signaling by western blotting for the levels of p-IFR3, p-TBK1 and p-NFkB (p65) in wound Mψs from the nondiabetic Tmem173−/− mice and their littermate controls. We noted that wound Mψs from the Tmem173−/− mice displayed decreased p-IFR3, p-TBK1 and p-NFkB levels (Supplementary Fig. 7A-D), suggesting that in the nondiabetic state, STING may regulate the early production of IFN-1 and signals primarily through a TBK1/IRF3 axis, contributing to normal wound repair. As expected, a wound healing curve of the Tmem173−/− mice showed impaired wound healing compared to that of the littermate controls, further suggesting that a baseline level of STING activity is necessary for normal wound repair (Supplementary Fig. 6E).

We then examined the downstream STING pathway in the setting of diabetes. In addition to the decreased inflammatory markers noted in DIO Tmem173−/− wound Mψs (Fig. 3J-M), Western blotting revealed that DIO Tmem173−/− wound Mψs, at baseline and with rIL-6 stimulation, exhibited increased p-IRF3, p-TBK1 and p-NFkB expression (Supplementary Fig. 7E-H) compared to the controls. These data suggest that in the diabetically treated, STING may have reduced effects on the TBK1/IRF3/IFN-1 pathway.

Subsequently, we explored the role of JMJD3 in the upstream regulation of Tmem173 expression in diabetic wound Mψs. We observed significantly decreased levels of the transcriptionally repressive H3K27me3 mark at the Tmem173 promoter in diabetic wound Mψs compared to the normal diet controls (Fig. 3N). Examination of other histone methylation and acetylation marks at the Tmem173 promoter did not show other transcriptionally activating modifications (Supplementary Fig. 5C, D). To simulate diabetic wound inflammatory cytokine expression conditions in our mouse model, we stimulated Day 5 wound Mψs from normal diet-fed mice ex vivo with rIL-6 (20 nM), and increased Tmem173 expression was observed (Fig. 3O). When these Mψs were examined by immunofluorescence, there was significantly increased phospho-STING staining that was abrogated in the Jmjd3fl/Lyz2cre+ wound Mψs (Fig. 3P, Q). We then investigated whether upstream blockade of JMJD3 production affects Tmem173 transcription. Wound Mψs were isolated at 5 days post-wounding and stimulated with rIL-6 agent for 6 h with and without the JAK1/3 inhibitor tofacitinib (100 μM). Quantitative PCR analysis revealed decreased Tmem173 expression (Fig. 3R) and a concomitant increase in the H3K27me3 mark at the Tmem173 promoter binding site when wound Mψs were treated with rIL-6 and tofacitinib (Fig. 3S). Furthermore, when wound Mψs from myeloid-depleted STAT3 mice (Stat3fl/Lyz2cre+) were isolated and treated ex vivo with rIL-6, they exhibited decreased Tmem173 expression (Fig. 3T) and increased H3K27me3 marks at the Tmem173 promoter binding site (Fig. 3U) compared to those of their littermate controls. Finally, we observed that myeloid depletion of Jmjd3 in diabetes results in increased production of the anti-inflammatory cytokine IL10 (Supplementary Fig. 8A), which results in elevated levels of repressive anti-inflammatory Ly6C+ CD11b+ Mψs critical for tissue repair (Supplementary Fig. 8B, analyzed by flow cytometry as previously described by our laboratory [4]). Taken together, these results suggest that JMJD3 regulates STING expression and thereby influences the Mψ-mediated inflammatory phenotype in wound healing.

Macrophage-specific JMJD3 inhibition improves tissue repair in diabetic wounds

Given the central role of JMJD3 activity in Mψ-mediated inflammation in tissue repair and its pathologic role in sustaining inflammation in diabetic wounds, we theorized that cell-specific targeting of JMJD3 would be a viable therapeutic target. To examine this, we synthesized Mψ-targeting nanoparticles [10, 40] by linking dextran-laden nanoparticles with a known JMJD3 covalent inhibitor, GSK-J1 (41) (Fig. 4A). Dextran is known to be efficiently taken up by tissue Mψs due to binding of the mannose receptor (CD206) and is used as a standard label for Mψs in animal models [40, 42-44]. First, we verified their Mψ specificity (Supplementary Fig. 9) and then performed subcutaneous injections (1 mg/kg) into the wounds of the mice with DIO daily following wounding for 7 days. Wound healing, compared to that of the placebo-treated mice and the DIO Jmjd3fl/Lyz2cre+ mice (phenotypic control), was monitored and analyzed by ImageJ.
software and histology. We observed that Mφ-specific nanoparticle inhibition of JMJ3 resulted in significantly improved wound healing in the GSK-J1 nanoparticle-treated group and was most pronounced in the later days of wound healing. This period is consistent with the days of highest Jmj3 and Tmem173 expression in diabetic wound Mφs. For comparison, the DIO Jmj3+/-Lyz2+/- mice exhibited improved wound healing similar to the nanoparticle-treated cohort. Histologically, wounds harvested on Day 5 of nanoparticle treatment had increased epithelialization compared to the placebo controls, signifying advancement through the normal stages of tissue repair in the treated wounds (Fig. 4B). To confirm that the observed effects were mediated by...
JMJD3 inhibition in Mφs, we isolated wound Mφs (CD3-/CD19-/NK1.1-/Ly6G-/CD11b+) from the nanoparticle-treated mice and their controls on Day 5 after wounding and analyzed them. These assays revealed significantly decreased Tmem173, Il1b and Tnfa expression in the wound Mφs from treated wounds compared to the placebo-treated wound Mφs by gene expression and protein levels (Fig. 4C; Supplementary Fig. 10). Taken together, these data suggest that Mφ-specific targeting of JMJD3 in diabetic wounds ameliorates persistent inflammation resulting from the NFκB and STING pathways and results in improved diabetic wound repair, making this a viable therapeutic strategy.

**DISCUSSION**

Nonhealing diabetic wounds are highly prevalent, with limited therapeutic options [45, 46]. Here, we showed that JMJD3 is critical for regulating Mφ-mediated inflammation in normal wound repair. We also identified that persistent, pathologic inflammation, as seen in T2D, is mediated by the expression of JMJD3 in Mφs past the initial inflammatory phase of wound repair. Uproregulation of JMJD3 in diabetic wound Mφs led to decreased repressive H3K27 trimethylation at NFκB binding sites at the promoters of inflammatory genes, resulting in increased inflammatory gene transcription. Herein, using human wound Mφs from chronic diabetic wounds and wound Mφs from DIO murine models, we show that JMJD3 is regulated by the JAK1,3/STAT3 pathway, whereas in diabetic wounds, IL-6 is the primary driver of JAK1,3/STAT3 signaling in Mφs (Fig. 5). Furthermore, RNA sequencing analysis of Mφs from myeloid cell-deficient JMJD3 mice showed that JMJD3 regulates Tmem173, which is known to perpetuate chronic inflammation in T2D [32, 36, 37]. We found that Tmem173 is elevated in diabetic wound Mφs and contributes to impaired wound healing by decreasing TBK1/IRF3/IFN-I signaling. Finally, we found that Mφ-specific nanoparticle inhibition of JMJD3 in diabetic wounds led to decreased inflammatory cytokine production and improved tissue repair. Together, this work highlights the central role of JMJD3 in normal and pathologic wound healing.

Initial investigations into the role of JMJD3 in wound healing have revealed that global JMJD3 inhibition leads to impaired keratinocyte migration and decreased epithelial maturation, resulting in poor wound healing. We, therefore, hypothesized that targeting JMJD3 specifically in Mφs could improve the outcome of diabetic wound repair. To test this hypothesis, we developed a novel Mφ-specific nanoparticle inhibition strategy. Using this approach, we were able to achieve effective targeting of JMJD3 in Mφs while avoiding off-target effects in other cell types. Our results demonstrate that Mφ-specific nanoparticle inhibition of JMJD3 in diabetic wounds leads to decreased inflammatory cytokine production and improved tissue repair. This suggests that targeting JMJD3 in Mφs is a promising therapeutic strategy for the treatment of diabetic wounds.
wound healing [47–49]. These studies examined JMJD3 in keratinocyte functions and identified that JMJD3 played a role in wound repair via Notch activation in keratinocytes. A more recent report examined the role of microRNA regulation of JMJD3 in human keratinocytes and fibroblasts during the early phase of wound healing [48]. This study showed that JMJD3 is necessary for keratinocyte function in early wound healing, which correlates with our findings on the importance of JMJD3 in Mϕs in the acute inflammatory phase of wound healing. Finally, a number of studies have examined the role of JMJD3 in regulating fibrosis, a key component of the tissue repair cascade, following myocardial infarction [50], in hepatocytes [51] and in diabetic renal tissue [52]. In these studies, JMJD3 was shown to facilitate fibrotic events in the organ beds, signifying the central role this enzyme plays in tissue repair processes. Despite these findings, the role of JMJD3 specifically in wound Mϕs and in diabetic wound repair was undefined. Furthermore, the upstream regulation of JMJD3, either in keratinocytes or other cells, in tissue repair has not been explored. We identified a key role for JMJD3 in Mϕs in regulating early inflammatory gene expression in normal wounds and that pathologic expression of JMJD3 in late wound Mϕs, as seen in diabetes, results in increased late inflammatory gene expression. In both normal and diabetic Mϕs, Jmdj3 expression was regulated by JAK1/3/STAT3, although the ligands regulating this signaling pathway differed. In normal wounds, our group found that IFN-β is elevated at early time points, and this does not occur in diabetic wounds [11]. In this work, we found that this early IFN-β drives Jmdj3 expression in normal wound Mϕs, but in diabetic wounds that have decreased IFN-β after injury, IL-6 acts to drive the JAK1/3/STAT3 signaling pathway.

STING is an endoplasmic reticulum protein that promotes persistent inflammation in malignancy and infection [53, 54]. A recent report detailed the role that STING plays in potentiating lipotoxicity-induced inflammation within pancreatic beta-cells and therefore facilitating the insulin resistance characteristic of diabetes in a murine model [55]. While this and other studies provide a direct link between STING, inflammation, and diabetes/obesity [34, 35], there remains limited information on the role of STING in promoting the chronic inflammatory state observed in diabetic wound repair. Furthermore, STING has not been examined in normal or pathologic wound repair. In particular, wounds, especially nonhealing diabetic wounds, are often a milieu of various pathogens in addition to cellular debris [5] that can serve as ligands for the STING inflammatory pathway. Our study attempts to fill this gap by examining STING in Mϕs in wound repair.

STING signaling can proceed by activation of TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3), resulting in the transcription of genes encoding type 1 interferons (IFN-I). [56] The TBK1/IRF3 signaling pathway often counteracts the NFκB pathway, which is responsible for increased proinflammatory cytokine production, such as that of IL-6 and TNF-α. [57, 58] By modulating TBK1/IRF3/IFN-I signaling, STING can act in a dichotomous manner to promote or inhibit inflammation depending on the disease context—a phenomenon that has been previously reported in oncology where chronic STING activation is linked to inflammation-induced carcinogenesis and metastasis via noncanonical NFκB signaling. [59–61]

Our studies indicate that in nondiabetic wound Mϕs, Ilnb is elevated by Day 1 and gradually diminishes over the wound healing course. This pattern correlates with Tmem173 expression kinetics in nondiabetic wounds and the increased TBK1/IRF3 signaling observed. Together, these findings confirm the importance of IFN-I in healthy wound healing, as previously reported [11, 62], and suggest that STING is partially responsible for the early increase in IFN-I in nondiabetic wounds, is important for suppressing late inflammatory cytokine production and promotes tissue repair in nondiabetic wounds. However, late and sustained expression of STING in diabetes does not lead to increased STING activation and its downstream IFN-I cytokines. Rather, sustained STING expression in diabetes is detrimental to wound healing due to decreased IL-1β production and increased IL-6, TNF-α, and IL-6 production, all of which act in concert to sustain a proinflammatory Mϕ phenotype. We further show that STING is modulated by JMJD3 in diabetic wound Mϕs and that blockade of STING in the diabetic setting can lead to decreased inflammation, an increased reparative Mϕ phenotype, and improved diabetic wound repair. Although this study provides valuable insight into the mechanisms behind dysregulated Mϕ inflammation in diabetic wounds, some limitations must be addressed. First, within our myeloid-specific JMJD3 murine model, we used the Lyz2Cre system. We acknowledge that there is no Cre-specific transgenic line perfect for blocking Mϕ expression of JMJD3...
for Msps given that there is overlap between monocytes/Msp, neutrophils, and dendritic cells due to their close lineage relationship. Second, we recognize that other epigenetic enzymes may regulate Msp function in diabetic wounds and that these enzymes do not work in isolation but rather contribute at different phases of wound repair to the cell phenotype [63–65]. Third, we realize that IL-6 is unlikely to be the only driver of JAK1,3/STAT3 signaling in wounds, although IL-6 elevation in diabetic wounds is well documented [66, 67], and we show that it is persistently elevated in diabetic wound Msps. Finally, we have not directly examined the interplay between Msps and other structural cells, such as smooth muscle cells or fibroblasts, that exist within the wound. These local interactions clearly impact Msp signaling and phenotype and likely influence the overall epigenetic program of these cells in wound repair. We acknowledge that studies examining these interactions are necessary to fully understand the factors responsible for impaired diabetic wound healing. We also acknowledge that JMD3-mediated modulation of Msp inflammatory activity may affect other cell types involved in the wound healing cascade and that this requires further exploration.

To conclude, our study provides important mechanistic information that JMD3 regulates inflammation in wound Msps by decreasing H3K27me3 on inflammatory genes. Our findings emphasize the importance of understanding the role of epigenetic enzymes in the wound healing cascade and that this requires further study.

**MATERIALS AND METHODS**

**Mice**

Mice were maintained in the University of Michigan Biomedical Sciences and Research Building in the Unit for Laboratory and Animal Medicine (ULAM), which is a pathogen-free animal facility. Mouse experiments were conducted with approval from our Institutional Animal Care and Use Committee (IACUC), and all regulatory and safety standards were strictly adhered to. Male C57BL/6 mice (RRID: IMSR_JAX:000664) were delivered at 6-7 weeks of age from the Jackson Laboratory (Bar Harbor, ME) and were maintained in breeding pairs in the ULAM facilities and were fed a normal chow diet (13.5% kcal fat; LabDiet). Jmd3fl/fl mice were created as previously reported by our laboratory. [12] Jmd3lox/− mice were then bred with B6.129P2-Lyz2tm1(Cre)Ifo/J (Lyz2Cre) mice from the Jackson Laboratory to obtain mice deficient in Jmd3 monocytes, Msps and granulocytes [66]. Floxed-cre mice were genotyped regularly after birth with custom primers. STING−/− mice were obtained from the Jackson Laboratory (MPYS5; RRID: IMSR_JAX:025805; B6(Cg)-Stingtm1.Cennj). For induction of a diabetic phenotype, male C57BL/6 mice were maintained on a standard high-fat diet (60% kcal saturated fat, 20% proteins, 20% carbohydrate; Research Diets, Inc.) for 12-18 weeks to yield the diet-induced obesity (DIO) model of type 2 diabetes mellitus [69, 70]. After the appropriate time period, HD-fed (DIO) mice developed obesity and insulin resistance with fasting blood sugars in the mid-200s and elevated insulin levels. All DIO/control animals underwent procedures at 20-32 weeks of age with IACUC approval. For these experiments, only male mice were used, as female mice do not develop DIO.

**Human wound isolation**

All experiments using human samples were approved by the IRB at the University of Michigan (IRB #: HUM00098915) and were conducted in accordance with the principles in the Declaration of Helsinki. Biopsies from human diabetic wounds (n = 4) versus normal skin samples (n = 38) were collected. The diabetic samples for scRNA-seq were from patients with an average age of 60 years, who all had diabetes (A1c > 7), hypertension, hyperlipidemia, and coronary artery disease. In the nondiabetic patient group, the average age was 70 years, with half the patients having hypertension, hyperlipidemia, and coronary artery disease. Wounds were obtained from the specimens using an 8 mm punch biopsy tool and processed for reverse transcription PCR (RT–PCR) as described for the murine wounds. RNA with RNA integrity number scores greater than 8 were used, and all values were compared to 28S/18S ratios and other housekeeping genes.

**Wound healing**

Mice were anesthetized, dorsal hair was removed with Veet (Reckitt Benckiser) and rinsed with sterile water, and two full-thickness back wounds were created by 6-mm punch biopsy with or without wound splinting. The initial wound surface area was recorded, and digital photographs were obtained daily using an 8 mp iPad camera as previously described [71]. Photographs contained an internal scale to allow for standard calibration. The wound area was quantified using ImageJ software (National Institute of Health, Bethesda, MD) and calculated as a percentage of initial wound area. In all pharmacologic dosing experiments, local injection with the drug or vehicle control was performed at four points along the wound edge [20].

**Wound cell digestion**

Wounds were harvested from the dorsum of mice postmortem following CO2 asphyxiation. Wounds were then minced finely with sharp scissors, and suspensions were placed in a LiberaseTM (50 mg/mL; Sigma-Aldrich, St Louis, MO, cat. no. S101020001) and DNase I (20 U/mL; Sigma-Aldrich, St Louis, MO, cat. no. 9003-98-9) solution at 37 °C for 30 min for enzymatic digestion. RPMI + FBS was then added to stop the reaction, and the wound cell suspensions were then gently plunged and filtered through a 100 μm filter to result in a single cell suspension. Cells were then magnetically sorted for CD3, CD19, NK1.1, Ly6G and CD11b+ cells and cultured ex vivo for RNA, cDNA, or protein studies.

**Magnetic-activated cell sorting (MAC) of murine wounds**

MAC sorting of wound samples was performed [72]. Briefly, wound cell isolates were incubated with fluorescein isothiocyanate (FITC)-labeled anti-mouse anti-CD3 (RRID: AB_312660), anti-CD19, and anti-Ly6G (BioLegend) followed by Anti-FITC MicroBeads (Miltenyi Biotec). The resulting flowthrough was then incubated with anti-CD11b MicroBeads to isolate the non-neutrophils, nonlymphocytes, and CD11b+ cells. Cells were saved in TRIzol (Invitrogen) for quantitative RT–PCR analyses.

**Immunofluorescence studies**

Isolated wound CD11b+ Msps were incubated on 1.5 mm cover slips under various stimuli by relevant cytokines for 1 h at 37 °C. A 4% paraformaldehyde solution in media was made, and the cells were allowed to fix for 30 min at room temperature. The media solution was aspirated and washed 3x with PBS, and the cover slips were blocked in blocking buffer (1x PBS, 5% fetal bovine serum, 0.3% Triton X-100) for 1 h at room temperature. The primary p-STING antibody (phospho-STING (Ser365) (D1C4T), Cell Signaling Technology) was prepared at a 1:400 concentration in Antibody Dilution Buffer (1x PBS, 1% bovine serum albumin, 0.3% Triton X-100) and applied to the fixed cells overnight at 4 °C. The primary antibody solution was aspirated, and the cells were washed 3 times with PBS and treated with FITC-conjugated anti-rabbit IgG (1:200 v/v; Thermo Fisher) secondary antibody diluted in Antibody Dilution Buffer for 2 h at RT in the dark. Cells were washed and mounted onto microscope slides with Prolong Gold Antifade Reagent with DAPI (Cell Signaling). Slides were allowed to incubate in the dark at RT for 24 h prior to visualization by confocal microscopy (Nikon A1 inverted confocal, 10x magnification). FITC immunofluorescence intensity was analyzed by ImageJ (NIH), and statistical significance was obtained using Student’s t test.

**RNA extraction**

Total RNA extraction was performed with TRIzol (Invitrogen) using the manufacturer’s directions. RNA was extracted using chloroform, isopropyl alcohol, and ethanol. Superscript III Reverse Transcriptase (Thermo Fisher Scientific) kits were used to synthesize cDNA from extracted RNA. cDNA primers for Jmd3, Ihb, and Tmem173 were purchased from Applied Biosciences. RT–PCR was conducted with 2x TaqMan Fast PCR mix and run on a 7500 Real-Time PCR system (Applied Biosciences), and data were then reviewed in a relative quantification analysis to the 18S ribosomal RNA. All samples were assayed in triplicate. Data were then compiled in Excel (Microsoft) and presented using Prism software (GraphPad v9).

**Chromatin immunoprecipitation assay**

Chromatin immunoprecipitation assays were performed as described previously [9]. Briefly, following ex vivo studies, wound Msps (CD3+CD19/CD11b-CD19-) were crosslinked in 1% formaldehyde for 10 min at RT, and pellets were stored at -80 °C until analysis. Cells were lysed for

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10 min on ice in SDS lysis buffer with a protease inhibitor cocktail (Sigma-Aldrich), syringe passed and sonicated to generate 100-300 base pair fragments. Five percent of the total chromatin volume was used as the input control. The rest of the chromatin was subsequently incubated with antibodies against trimethylated H3K4, trimethylated H3K9, trimethylated H3K27, or rabbit polyclonal IgG (Millipore Sigma) as a nonspecific antibody control. This step was followed by addition of Protein A Sepharose beads (Thermo Fisher Scientific) for 1 h at 4 °C. The pellet was washed, and bound DNA eluted 2x for 15 min at room temperature with 5 min at 65 °C at the end of the second elution. The combined eluates were reverse cross-linked for 5 h at 65 °C. Samples were stored at −20 °C, followed by protease K digestion for 1 h at 45 °C. DNA was purified using phenol/chloroform/isooamyl alcohol solution. The precipitated DNA was analyzed by quantitative real-time PCR on a TaqMan 7500 sequence detection system. The following primers were used to amplify DNA in samples: Il1b (forward) 5’ GCGAGGGTCGTTGGTGATG 3’ and (reverse) 5’ CAGTCTGATATGCCAAGGGTCG 3’, Tnfα (forward) 5’ TCTGTAGTCTGCCCAGATGTT 3’ and (reverse) 5’ TATGGGCTCTACACCTGCTG 3’, Il12 (forward) 5’ AGTGAATTCCGGAGGCCCCAC 3’ and (reverse) 5’ CTTTCCAGGACGTGTG TCTC 3’, Il22 (forward) 5’ GGCCTCCAAAGAGGAGAGAT 3’ and (reverse) 5’ CCACCTGTGTGTGAGA GAGATG 3’, Nos2a (forward) 5’ CCAACATGAGGCCCA CAC 3’ and (reverse) 5’ GCTTCCATAAAGAGGAGAGAT 3’ and (reverse) 5’ CACCTGTGTGTGAGA GAGATG 3’. 

Western blot
MAC sorted, CD11b+ wound Mϕ cells were subjected to cell lysis buffer and protease inhibitor cocktail. Protein suspensions were then standardized for protein concentrations using a Bradford protein assay (Bio-Rad). Equal amounts of protein were mixed with loading buffer and subjected to 4%–18% Tris-glycine gel electrophoresis under reducing conditions. Proteins were then wet-transferred at 100 V for 1 hour in Tris-glycine transfer buffer (Invitrogen) to nitrocellulose membranes and probed with primary antibodies (all Cell Signaling; p-JAK1 (D7N4Z), JAK1 (6G4), p-JAK3 (D44E3), JAK3 (D7812), p-STAT3 (D3A7), STAT3 (D22G), STING (D2P7F), IL-1β (3A6), β-actin (8H1D01), p-NFκB p65 (Ser536; 93H1), NFκB p65 (D14E12), p-TBK1 (DS252), TBK1 (EB183), p-IRF3 (D601M), IRF3 (D8389) and GAPDH (D1H11)) diluted to 1:500 v/v in 5% bovine serum albumin in Tris buffered saline with Tween buffer overnight at 4 °C with agitation. Nitrocellulose membranes were then washed and incubated with anti-rabbit IgG or anti-mouse IgG HRP-conjugated secondary antibody (Cell Signaling, Inc.) for 1 h at RT with shaking and visualized with timed chemiluminescence (Thermo Fisher Scientific). Densitometry was calculated using ImageJ (NIH), and statistical significance was obtained using unpaired Student’s t test.

Enzyme-linked immunosorbent assay (ELISA)
Wound Mϕs were MACs isolated and stimulated in culture for 4 h in RPMI. After stimulation, the cell-free supernatant was collected and analyzed by specific enzyme immunoassay kits for IL10, IFN-γ, IL-1β, and TNF-α (all ELISAs from Cayman Chemical) according to the manufacturer’s instructions.

Drug loading was determined to be 2.5 wt% via UV–Vis.

For mouse experiments, the dextran or dextran GSK-J1 conjugated solid was reconstituted in phosphate buffered saline (1 mg/kg) and injected subcutaneously at 4 points along the punch biopsy wounds daily. An 8-megapixel iPad camera with an internal scale was used to record wound size daily. Wound closure was then measured as a percentage of the initial wound area. Images were evaluated by 2 independent, blinded observers. Wound area was calculated using ImageJ software (NIH).

Histology
Whole wounds were excised from mice or humans using a 6.8-mm punch biopsy. Wound sections were fixed in 10% formalin overnight before embedding in paraffin. Sections (5 μm) were stained with Masson’s trichrome for evaluation of re-epithelialization, granulation and collagen deposition. Images were quantified on ImageScope software and ImageJ at 20X magnification. Percent re-epithelialization was calculated by measuring the distance traveled by epithelial tongues on both sides of the wound divided by the total distance [73].

Bulk RNA sequencing and scRNA-seq analyses
Generation of single-cell suspensions for scRNA-seq was performed in the following manner: Following informed consent from patients and in accordance with University of Michigan IRB Study # HUM00098915, skin was harvested via punch biopsy from diabetic and nondiabetic control wounds. Samples were incubated overnight in 0.4% Disperse (Life Technologies, Thermo Fisher Scientific) in HBSS (Gibco, Thermo Fisher Scientific) at 4 °C. The epidermal and dermal layers were separated. The epidermis was digested in 0.25% Trypsin-EDTA (Gibco, Thermo Fisher Scientific) with 10 units/mL DNase I (Thermo Fisher Scientific) for 1 h at 37 °C and subsequently quenched with PBS (Atlanta Biologicals) and stored at −80°C. The epidermis was re-suspended with a 100 μm mesh and a 4% collagenase II (Life Technologies, Thermo Fisher Scientific) and 0.2% Collagenase V (Millipore Sigma) in plain RPMI medium for 1.5 h at 37 °C, and strained through a 100 μm mesh. Epidermal and dermal cells were combined in a 1:1 ratio for scRNA-seq by the University of Michigan Advanced Genomics Core on the 10x Genomics Chromium System. Libraries were sequenced on the Illumina NovaSeq 6000 sequencer. NovaSeq was used as the sequencing platform to generate 151 bp paired-end reads. We conducted adapter trimming and quality control procedures as described previously [74]. The reads were then mapped using STAR [75] to build human GRCh37, and gene expression levels were quantified and normalized by HTSeq [76] and DESeq2 [77]. Negative binomial models in DESeq2 were used to conduct differential expression analysis. To increase the sample size of the control samples, we used skin biopsies obtained from our previous study [78]. For bulk RNA sequencing and scRNA-seq data accession, the numbers include GSE154556 and GSE154557 (Gene Expression Omnibus). For scRNA-seq data, data processing, including quality control, read alignment, and gene quantification, was conducted using 10X Genomics Cell Ranger software. Seurat was then used for normalization, data integration, and clustering analysis [79]. All clustered cells were mapped to corresponding cell types by matching cell cluster gene signatures with putative cell type–specific markers.

Statistical analysis
GraphPad Prism software (RRID:SCR_002798) version 9.2.0 was used to analyze the data. Data were analyzed for a normal distribution, and significant differences among multiple groups were obtained using Student’s t tests. All p values less than or equal to 0.05 were considered significant.

REFERENCES
1. Veltnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res. 2009;37:1528–1542. https://doi.org/10.1177/0300060510365213.
2. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: a cellular perspective. Physiol Rev. 2019;99:665–711. https://doi.org/10.1152/physrev.00006.2019.
3. Gallagher KA, Joshi A, Carson WH, Thaller M, Allen R, Makke S, et al. Epigenetic changes in bone marrow progenitor cells influence the inflammatory phenotype and alter wound healing in type 2 diabetes. Diabetes. 2015;64:1420–1430. https://doi.org/10.2337/db14-0872.
4. Kimball A, Schaller M, Joshi A, Davis FM, denDekker A, Koury M, et al. LysC(H) blood monocyte/macrophage drive chronic inflammation and impair wound healing in diabetes mellitus. Arterioscler Thromb Vasc Biol. 2018;38:1112–1114. https://doi.org/10.1161/ATVBAHA.118.310703.
5. Boniakowski AE, Kimball AS, Jacobs BN, Kunkel SL, Gallagher KA. Macrophage-mediated inflammation in normal and diabetic wound healing. J Immunol. 2017;199:17–24. https://doi.org/10.4049/jimmunol.1700223.
6. Davis FM, Tsoi LC, Melvin WJ, denDekker A, Wasikowski R, Joshi AD, et al. Inhibition of macrophage histone demethylase JMJD3 protects against abdominal aortic aneurysms. J Exp Med. 2021;212:1883–1893. https://doi.org/10.1084/jem.20201839.
7. Lagunas-Rangel FA. KDM6B (JMJD3) and its dual role in cancer. Biochimie. 2021;184:63–71. https://doi.org/10.1016/j.biochi.2021.02.005.
8. Ding Y, Yao Y, Gong X, Zhou Q, Chen J, Tian M, et al. JIMD: a critical epigenetic regulator in stem cell fate. Cell Commun Signal. 2021;19:72. https://doi.org/10.1186/s12964-021-00753-8
9. Davis DM, denDekker A, Joshi AD, Schaller MA, Bermick J, et al. The history of cytokine/gp130 signaling and its regulation. Mol Biol. 2005;12:545 https://doi.org/10.1038/nsmb941
10. Kimball AS, Davis FM, denDekker A, Joshi AD, Schaller MA, Bermick J, et al. The role of JAK/STAT signaling in normal and adaptive immunity in acute respiratory viral infection. PLoS Pathog. 2015;11: e1005338 https://doi.org/10.1371/journal.ppat.1005338
11. Kos CH. Cre/loxP system for generating tissue-specific knockout mouse models. Nutr Rev. 2004;62:243–246. https://doi.org/10.1036/nm04june243-246
12. Garbers C, Aparicio-Siegmund S, Rose-John S. The IL-6/gp130/STAT3 signaling axis: a highly regulated and multifaceted pathway. Biochimie. 2020;184:1389–1403 https://doi.org/10.1016/j.biochi.2020.06.015
13. akbari M, Hassan-Zadeh V. IL-6 signalling pathways and the development of type 2 diabetes. Diabetes. 2019;68:1099–1108. https://doi.org/10.2337/db18-0052
14. Xiao TS, Fitzgerald KA. The cGAS-STING pathway for DNA sensing. Mol Cell. 2013;51:135–139. https://doi.org/10.1016/j.molcel.2013.07.004
15. Dhanarwani R, Takahashi M, Sharma S. Cytosolic sensing of immuno-stimulatory DNA, the enemy within. Curr Opin Immunol. 2018;50:82–87. https://doi.org/10.1016/j.coi.2017.11.004
16. Kim Y, Li G, Tao J, Wu N, Kannadi MR, Bi Y, et al. Double knockout of Akt2 and AMPK accentuates high fat-diet induced cardiac abnormalities through a cGAS-STING-mediation mechanism. Biochem Biophys Res Commun. 2020;528:671 e255. https://doi.org/10.1016/j.bbrc.2020.05.115
17. King KR, Aguirre AD, Ye YX, Sun Y, Roh JD, Ng RP, et al. IRF3 and type I interferons involved in lipotoxic injury of pancreatic beta cells in type 2 diabetes. Mol Cell Endocrinol. 2020;515:https://doi.org/10.1016/j.mce.2020.110890
18. Kurauti MA, Costa-Júnior JM, Ferreira SM, Santos GJ, Sponton C, Carneiro EM, et al. Type 1 interferon induced epigenetic regulation of macrophages suppresses innate and adaptive immunity in acute respiratory viral infection. PLoS Pathog. 2015;11: e1005338 https://doi.org/10.1371/journal.ppat.1005338
19. Kröetz DN, Allen RM, Schaller MA, Cavallaro C, Ito T, Kunkel SL, et al. Type I interferon fuel a fatal response to myocardial infarction. Nat Med. 2017;23:1481–1487. https://doi.org/10.1038/nm.4428
20. Murray PJ. The JAK-STAT signaling pathway: input and output integration. J Immunol. 2007;178:2623–2629. https://doi.org/10.4049/jimmunol.178.5.2623
21. Schindler C, Levy DE, Decker T. JAK-STAT signaling: from interferons to cytokines. J Biol Chem. 2007;282:20059–20063. https://doi.org/10.1074/jbc.R700016200
22. Gallagher KA, Liu ZJ, Xiao M, Chen H, Goldstein LJ, Bueno DG, et al. Diabetes impairs monocyte recruitment and endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 α. J Clin Invest. 2007;117:1249–1259. https://doi.org/10.1172/JCI297910
23. Tschopp M, Heimann ML. Overview of rodent models for obesity research. Curr Protoc Neurosci. 2002;Chapter 9:Unit 9 10. https://doi.org/10.1002/0471142301.ns0910s17
24. Atzard F, Jiia J, Diedische M, Potier C, Potier L, Velho G, et al. Monocytopoiesis, monocyte morphological anomalies and hyperinflammation characterize severe COVID-19 in type 2 diabetes. EMBO Mol Med. 2020:12:10308 https://doi.org/10.15252/emmm.202013038
25. Melvin, WJ, Audo CO, Davis FM, Sharma SB, Joshi A, DenDekker A, et al. Coro- navirus induces diabetic macrophage-mediated inflammation via SETO. Proc Nat Acad Sci USA. 2018;115:https://doi.org/10.1073/pnas.2010711118 (2021).
26. Kumar NP, Siddharth N, Raaj D, Buranekha VW, Nutman TJ, Babu S. Type 2 diabetes mellitus is associated with altered CD8(+) T and natural killer cell function in inflammation and insulin resistance by suppressing the mtDNA release-activated cGAS-STING pathway. Biochim Biophys Res Commun. 2020;528:271 e255. https://doi.org/10.1016/j.bbrc.2020.05.115
27. Garbers C, Aparicio-Siegmund S, Rose-John S. The IL-6/gp130/STAT3 signaling axis: a highly regulated and multifaceted pathway. Biochimie. 2015;127:70–75. https://doi.org/10.1016/j.biochi.2015.02.008
28. Skinnmets C, Boulanger ML, Garcia KC. IL-6 signaling in diabetes: a complex network of crosstalk. Biochim Biophys Acta. 2019;1859:658–675 https://doi.org/10.1016/j.bbadis.2019.165855
29. Jiang Y, Bakhom SF. The cytosolic DNA-sensing cGAS-STING pathway in cancer. Cancer Discov. 2020;10:26–39. https://doi.org/10.1158/2159-8290.CD-19-0761
30. Hu HQ, Qiao JT, Liu FQ, Wang JB, Sha S, He Q, et al. The STING-IRF3 pathway is involved in lipidic injury of pancreatic beta cells in type 2 diabetes. Mol Cell Endocrinol. 2020;518:110890 https://doi.org/10.1016/j.mce.2020.110890
31. C.O. Audu et al.
56. Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature. 2009;461:788–792. https://doi.org/10.1038/nature08476

57. Motwani M, Pesiridis S, Fitzgerald KA. DNA sensing by the cGAS-STING pathway in health and disease. Nat Rev Genet. 2019;20:657–674. https://doi.org/10.1038/s41576-019-0151-1

58. Wu J, Sun L, Chen X, Du F, Shi H, Chen C, et al. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science. 2013;339:826–830. https://doi.org/10.1126/science.1229963

59. Hoong BYD, Gan YH, Liu H, Chen ES. cGAS-STING pathway in oncogenesis and cancer therapeutics. Oncotarget. 2020;21:2930–2955. https://doi.org/10.18632/oncotarget.27673

60. Bakhoun SF, Ngo B, Laughney AM, Cavallo JA, Murphy CJ, Ly P, et al. Chromosomal instability drives metastasis through a cytosolic DNA response. Nature. 2018;553:467–472. https://doi.org/10.1038/nature25432

61. Dunphy G, Flannery SM, Almíne JF, Connelly DJ, Paulus C, Jansson KL, et al. Non-canonical activation of the DNA sensing adaptor STING by ATM and IFI16 mediates NF-κB apoptosis signaling after nuclear DNA damage. Mol Cell. 2018;71:745–760 e745. https://doi.org/10.1016/j.molcel.2018.07.034

62. Wolf, SJ, Audu CO, Joshi A, denDekker A, Kimball AS, Evanoff H, et al. Sepsis. 2021;140:455–62. https://doi.org/10.1016/j.jid.2019.06.143

63. Gao Y, Xie Z, Ho C, Wang J, Li Q, Zhang Y, et al. LR1G promotes keratinocyte migration and wound repair through regulation of HIF-1α/stability. J Invest Dermatol. 2020;140:455–464 e458. https://doi.org/10.1016/j.jid.2019.06.143

64. Davis FM, Schaller MA, Dendekker A, Joshi AD, Kimball AS, SCt, Evanoff H, et al. Sepsis induces prolonged epigenetic modifications in bone marrow and peripheral macrophages impairing inflammation and wound healing. Arterioscler Thromb Vasc Biol. 2019;39:2353–2366. https://doi.org/10.1161/ATVBAHA.119.312754

65. Long M, Rojo de la Vega M, Wen Q, Bharara M, Jiang T, Zhang R, et al. An essential role of NRF2 in diabetic wound healing. Diabetes. 2016;65:780–793. https://doi.org/10.2337/db15-0564

66. Meeks KAC, Bentley AR, Gouveia MH, Chen G, Zhou J, Lei L, et al. Genome-wide analyses of multiple obesity-related cytokines and hormones informs biology of cardiometabolic traits. Genome Med. 2021;13:156 https://doi.org/10.1186/s13073-019-00971-2

67. Dmitriyeva M, Kozhakhmetova Z, Urazova S, Kozhakhmetov S, Turebayev D, Toleubayev M. Inflammatory biomarkers as predictors of infected diabetic foot ulcer. Curr Diabetes Rev. 2021;18:204 https://doi.org/10.2174/1573399817666210928144706

68. Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Forster I, et al. Enhanced TH1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. Immunity. 1999;10:39–49. https://doi.org/10.1016/S1074-7613(00)80005-9

69. Corredor J, Yan F, Shen CC, Tong W, John SK, Wilson G, et al. Tumor necrosis factor regulates intestinal epithelial cell migration by receptor-dependent mechanisms. Am J Physiol Cell Physiol. 2003;284:C93–961. https://doi.org/10.1152/ajpcell.00309.2002

70. Toye AA, Lippiat JD, Proks P, Shimomura K, Bentley L, Hugill A, et al. A genetic and physiological study of impaired glucose homeostasis control in C57BL/6J mice. Diabetologia. 2005;48:675–686. https://doi.org/10.1007/s00125-005-1680-z

71. Nathan C. Points of control in innate immune signaling. J Invest Dermatol. 2014;134:1828–1838. https://doi.org/10.1038/jid.2014.28

72. Mirza RE, Fang MM, Ennis WJ, Koh TJ. Blocking interleukin-1β induces a healing-associated wound macrophage phenotype and improves healing in type 2 Diabetes. Diabetes. 2013;62:2579–2587. https://doi.org/10.2337/db12-1450

73. Nishiyama T, Kii I, Kashima TG, Kikuchi Y, Ohazama A, Shimazaki M, et al. Delayed re-epithelialization in periorificial-deficient mice during cutaneous wound healing. PLoS One. 2011;e18410 https://doi.org/10.1371/journal.pone.0018410

74. Li B, Tsii LC, Swindell WR, Gudjonsson JE, Tejstav I, Johnston A, et al. Transcriptome analysis of porsias in a large case-control sample: RNA-seq provides insights into disease mechanisms. J Invest Dermatol. 2014;134:1828–1838. https://doi.org/10.1038/jid.2014.28

75. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zalecki C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics. 2013;29:15–21. https://doi.org/10.1093/bioinformatics/bts635

76. Anders S, Pyl PT, Huber W. HTSeq—a python framework to work with high-throughput sequencing data. Bioinformatics. 2015;31:166–169. https://doi.org/10.1093/bioinformatics/btu638

77. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550 https://doi.org/10.1186/s13059-015-0550-8

78. Tsio LC, Rodriguez E, Stolzl D, Wehkamp U, Sun J, Gerdes S, et al. Progression of acute-to-chronic atopic dermatitis is associated with quantitative rather than qualitative changes in cytokine responses. J Allergy Clin Immunol. 2020;145:1406–1415. https://doi.org/10.1016/j.jaci.2019.11.047

79. Butler A, Hoffman P, Smibert P, Papalexi E, Satija R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. Nat Biotechnol. 2018;36:411–420. https://doi.org/10.1038/nbt.4096

ACKNOWLEDGEMENTS

We wish to thank Robin Kunkel for aid with the graphics and illustration and Lisa Johnson for aid with the histology, staining and quantification.

AUTHOR CONTRIBUTIONS

Experimental conception and plan: COA, AMS, MAC, BL and KAG. Data acquisition: COA, WJM, XX, JYM, TMB, ADJ, SJW, ECB, KDM, HD, SBS, and JS. Data Interpretation: COA, WJM, XX, JYM, RW, LCT, FMD, ADJ, SJW, ECB, KDM, HD, SBS, JEG, ATO, BBM, AMS, SLK, BL, MAC and KAG. Manuscript Writing: COA and KAG. Manuscript Editing and Proofreading: All authors reviewed, edited and accepted the manuscript in its final submitted form.

FUNDING

This work was supported in part by National Institute of Health grants R01 – HL137919 (KAG), R01 – DK124290-01 (KAG, BBM), R01 – AR 079863 01 (KAG), R01 – HL156274-01A1 (KAG), and R01 – DK 127331 01 A1 (KAG), Doris Duke Foundation CSDA 2017079 (KAG), NIH F32-DK126471 (COA), the Vascular and Endovascular Surgical Society Resident Research Award (COA), the Society for Vascular Surgery Resident Research Award (COA) and the Coller Surgical Society Resident Research Award (COA).

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41423-022-00919-5.

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