Tie2-dependent knockout of HIF-1 impairs burn wound vascularization and homing of bone marrow-derived angiogenic cells

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Aims

Hypoxia-inducible factor 1 (HIF-1) is a heterodimer composed of HIF-1α and HIF-1β subunits. HIF-1 is known to promote tissue vascularization by activating the transcription of genes encoding angiogenic factors, which bind to receptors on endothelial cells (ECs) and bone marrow-derived angiogenic cells (BMDACs). In this study, we analysed whether HIF-1 activity in the responding ECs and BMDACs is also required for cutaneous vascularization during burn wound healing.

Methods and results

We generated mice with floxed alleles at the Hif1a or Arnt locus encoding HIF-1α and HIF-1β, respectively. Expression of Cre recombinase was driven by the Tie2 gene promoter, which is expressed in ECs and bone marrow cells. Tie2Cre+ and Tie2Cre− mice were subjected to burn wounds of reproducible diameter and depth. Deficiency of HIF-1α or HIF-1β in Tie2-lineage cells resulted in delayed wound closure, reduced vascularization, decreased cutaneous blood flow, impaired BMDAC mobilization, and decreased BMDAC homing to burn wounds.

Conclusion

HIF-1 activity in Tie2-lineage cells is required for the mobilization and homing of BMDACs to cutaneous burn wounds and for the vascularization of burn wound tissue.

Keywords

Hypoxia • Wound healing • Conditional knockout • Angiogenesis

1. Introduction

Burn injuries are a major health problem affecting 1 million Americans each year.1 Vascularization is an important component of wound healing and occurs by angiogenesis, the sprouting of new branches from preexisting vessels, and vasculogenesis, the recruitment of nonresident cells that participate in de novo blood vessel formation. Hypoxia induces the expression of angiogenic factors such as vascular endothelial growth factor (VEGF), stromal cell-derived factor 1 (SDF-1), and placental growth factor (PLGF).2,3 Transcriptional activation of the genes encoding VEGF and SDF-1 is mediated by hypoxia-inducible factor 1 (HIF-1),1,3,5 which functions as a master regulator of vascular responses to hypoxia/ischaemia.3 HIF-1 is a heterodimer composed of a constitutively expressed HIF-1β subunit [also known as the aryl hydrocarbon receptor nuclear translocator (ARNT)] and an O2-regulated HIF-1α subunit.6 HIF-2, which also mediates vascular responses to hypoxia, is a heterodimer composed of HIF-1β and an O2-regulated HIF-2α subunit.7 Compared with excisional wounds, burn wounds are associated with decreased HIF-1α levels.8 HIF-1α haploinsufficiency in mice that are heterozygous for a germline null (knockout) allele at the Hif1α locus results in deficient burn wound vascularization with impaired expression of HIF-1α and SDF-1.9 Ageing also impairs HIF-1α expression and burn wound vascularization.10

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Both local and systemic responses to wounding contribute to the reparative vascularization process. Endothelial cells (ECs) in preexisting vessels are activated to initiate angiogenesis in the injured tissue. In addition, the HIF-1-dependent release of angiogenic cytokines from the wound elicits the mobilization and homing of bone marrow-derived angiogenic cells (BMDACs), which can participate in vasculogenesis and stimulate angiogenesis through paracrine mechanisms. BMDACs express progenitor cell markers (e.g., Sca1+) and cell surface receptors that determine the angiogenic cytokines to which they can respond (e.g. CXCR4+ BMDACs respond to SDF-1). Recent evidence indicates that most BMDACs are pro-angiogenic myeloid cells. The mobilization of Sca1+/CXCR4+ BMDACs after burn wounding is impaired in HIF-1α haploinsufficient mice. Cutaneous electroporation of a plasmid vector encoding a constitutively active form of HIF-1α induces BMDAC mobilization and promotes excisional wound healing in diabetic mice.

In the present study, we used a mouse burn model to evaluate the effect of targeted HIF-1α or HIF-1β deficiency in Tie2-lineage ECs and BMDACs on wound vascularization. Conditional knockout of the Hif1α or Arnt gene in these cells to eliminate HIF-1 or both HIF-1 and HIF-2 activities, respectively, resulted in a failure of BMDAC mobilization and homing to burn wounds, which contributed to impaired wound vascularization.

2. Methods

2.1 Generation of conditional Hif1α and Arnt knockout mice

Mice with Tie2Cre-dependent knockout at the Hif1α or Arnt locus were generated by crossing animals containing lacZ sites flanking exon 2 of Hif1α or Arnt (Jackson Laboratory, Bar Harbor, ME, USA). lacZ sites were originally bred into the Hif1α or Arnt loci. Mice obtained by analysis of DNA from tail biopsies by PCR. Primers used to distinguish floxed from non-floxed alleles were: 5′-GGAG-3′ (floxed) and 5′-GTGT-3′ (non-floxed) for Hif1α or Arnt (Jackson Laboratory).

2.2 Burn wounding protocol

All animal protocols were approved by The Johns Hopkins University Animal Care and Use Committee and conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Three- to four-month-old mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection. The depth of anesthesia was confirmed by lack of toe pinch response. We utilized a burn wounding protocol that was developed in rats and adapted for mice.

Two burns of 1.2 cm diameter each were produced on the dorsum, representing 10% of total body surface area. Contact time of 4 s was chosen to produce a standardized full-thickness burn. Fluid resuscitation was performed by intraperitoneal injection of normal saline (0.4 mL/kg) within 1 h after burning. Buprenorphine (0.1 mg/kg) was administered by subcutaneous injection for analgesia during the first 24 h.

2.3 Wound area measurement

Wound area was determined immediately after burn wounding and on days 3, 7, 14, and 21. The wound perimeter was demarcated on clear acetate paper, digitized at 600 dpi using a flatbed scanner, and converted to surface area units (cm²). Wound areas were measured by two independent investigators in a blinded fashion and mean values were reported.

2.4 Laser Doppler perfusion imaging

Blood flow in wound areas was determined with a laser Doppler perfusion imaging device (Moor Instruments, Devon, UK), which utilizes a 633 nm He–Ne laser to measure subcutaneous blood flow. Serial measurements were obtained immediately after burn wounding and on days 3, 7, and 14. Scans were performed at a fixed distance of 70 cm. A region of interest encompassing the whole burned region was selected and the mean blood flow was calculated using LDISOF software (Lisca, Sweden). To calculate peak blood flow, the data points were modelled to a Gaussian function according to the following equation:

\[ Q(t) = A \times \exp\left(-\frac{(t - t_0)^2}{(2\sigma)^2}\right) \]

where Q(t) is the blood flow at time t, A the peak blood flow, t0 the time at peak flow, and σ the standard deviation of the peak blood flow.

2.5 Flow cytometry

Circulating Sca1+/CXCR4+ and Tie2+/CXCR4+ cells were quantified using flow cytometry. Non-burned mice and mice 3-day post-burn were anaesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). The depth of anaesthesia was confirmed by toe pinch and blood samples were collected by cardiac puncture. Red blood cells were lysed with ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was incubated with anti-CXCR4, anti-Sca1, or anti-Tie2 antibodies (eBiosciences) followed by ammonium chloride and the mononuclear fraction was inc...
cervical dislocation under ketamine/xylazine anaesthesia 8 h after BMDAC injection and normal (non-burned) skin and burn wound were collected, incubated in tissue lysis buffer [5 mM EDTA, 0.2% SDS, 200 mM NaCl, 100 mM Tris–HCl (pH 7.5), and 100 g/mL Proteinase K] for 18 h at 55°C. Genomic DNA was isolated by phenol–chloroform extraction and isopropanol precipitation. BMDAC homing was analysed by qPCR using primers for Sry (Y-chromosome) and Nme1 (autosomal) gene sequences. Homing was calculated as the efficiency-corrected26 Sry/Nme1 signal ratio from burn wounds compared with normal skin, according to the following expression: 27

\[
R = \frac{E_{C_q \text{Syr}(B)}}{E_{C_q \text{Nme1}(B)}} \times \frac{E_{C_q \text{Nme1}(N)}}{E_{C_q \text{Syr}(N)}},
\]

where \( R \) is the relative homing to burned when compared with non-burned skin in each mouse; \( E_{C_q \text{Syr}} \) and \( E_{C_q \text{Nme1}} \) are the efficiencies for Sry and Nme1 primer pairs, respectively; and \( C_q \) is the threshold cycle value for Sry or Nme1 in the burned (B) or non-burned (N) samples.

### 2.8 Statistical analysis

Data are shown as mean ± SEM. Differences between two groups were assessed with Student’s t-test. Flow and wound closure curves were analysed with two-way ANOVA with Bonferroni post hoc comparisons. Ratios and percentages were normalized with a log_{10} conversion previous to parametric test analysis. The parameters of Gaussian functions were adjusted to experimental data using non-linear regression resulting in high correlation coefficients (\( r > 0.95; P < 0.05 \)).

### 3. Results

#### 3.1 Efficiency of Tie2-dependent knockout of Hif1a and Amt genes

To quantify knockout efficiency in Hif1a^{f/f}-Tie2Cre^{+} and Amt^{f/f}-Tie2Cre^{+} mice, we isolated genomic DNA from bone marrow, peripheral blood mononuclear cells, and spleen, which are tissues that contain large numbers of bone marrow-derived cells, and from the lungs, in which ECs comprise approximately half of the total cell number. qPCR assays were performed using primers designed to detect the floxed exon (Hif1a exon 2 or Amt exon 6) and primers that detect the next downstream intact exon. The ratio of qPCR signal from targeted (floxed):downstream (non-floxed) exon was determined. After this normalization procedure, we measured knockout efficiency in tissues from Tie2Cre^{+} mice relative to the signal from Tie2Cre^{−} tissues. A reduction in amplification of the floxed exon of >50% was observed in both knockout strains (i.e. Hif1a^{f/f} and Amt^{f/f}) in all four tissues analysed (\( P < 0.05 \) by Student’s t-test, \( n = 3–6 \); Figure 1). Deletion of the Hif1a exon 2 was more efficient in the three tissues containing bone marrow-derived cells, whereas deletion of Amt exon 6 was more efficient in EC-rich lung tissue. In contrast, no significant deletion was observed in skin, reflecting the minor contribution of ECs to total cell number in this tissue.

#### 3.2 Delayed wound closure and blood flow in Tie2Cre^{+} knockout mice

Complete wound closure was achieved by day 21 of follow-up in all mice (Figure 2A). However, non-linear regression analysis of burn wound closure, using serial wound area data obtained by digital planimetry, revealed that the rate of closure was significantly decreased in Hif1a^{f/f}-Tie2Cre^{+} and Amt^{f/f}-Tie2Cre^{+} mice when compared with their Tie2Cre^{−} littermates. The time to 50% wound closure was 6.66 ± 0.04 days in Hif1a^{f/f}-Tie2Cre^{+} mice compared with 5.43 ± 0.02 days in Hif1a^{f/f}-Tie2Cre^{−} mice (\( P < 0.01; n = 12–14 \)) and 10.75 ± 0.98 days in Amt^{f/f}-Tie2Cre^{+} mice compared with 8.27 ± 0.56 days in Amt^{f/f}-Tie2Cre^{−} mice (\( P < 0.05; n = 8–20 \)). A significant effect on wound healing of genetic background differences between Hif1a^{f/f} and Amt^{f/f}-Tie2Cre^{+} mice, which was independent of Tie2Cre status, was also observed.

Laser Doppler perfusion imaging of burn wounds in these mice showed that blood flow transiently increased during burn wound healing as previously described,17 resulting in an inverted U-shaped curve (Figure 2B, top panels). Statistical analysis with two-way ANOVA demonstrated a significant overall difference between Tie2Cre^{−} and Tie2Cre^{+} genotypes with respect to blood flow kinetics in both knockout strains (\( P < 0.05; n = 8–20 \)). Peak blood flow was significantly decreased in the burn wounds of Hif1a^{f/f}-Tie2Cre^{+} (202 ± 14 PU) when compared with Hif1a^{f/f}-Tie2Cre^{−} (245 ± 14 PU) mice and in the burn wounds of Amt^{f/f}-Tie2Cre^{+} (338 ± 21 PU) when compared with Amt^{f/f}-Tie2Cre^{−} (416 ± 15 PU) mice (Figure 2B, bottom panels; \( n = 8–20; P < 0.05 \) by Student’s t-test).
3.3 Arnt knockout impairs vascularization after burn wounding

To determine whether the persistent differences in wound blood flow reflected differences in vascularization, we performed immunohistochemistry of burn wound tissues, which were harvested from Arntf/f-Tie2Cre+ and Arntf/f-Tie2Cre− mice on day 21. Blood vessels were detected in the dermis of the wound by immunohistochemical staining with anti-CD31, which is expressed by vascular ECs, and αSMA, which is expressed by vascular pericytes and smooth muscle cells (Figure 3A). Blinded quantification of the number of CD31+ and αSMA+ cells revealed a significant reduction in vascular density within burn wounds of Arntf/Tie2Cre+ mice when compared with their Arntf/Tie2Cre− littermates (Figure 3B).

3.4 Hif1α knockout impairs vascularization after burn wounding

Analysis of day 21 wounds by immunohistochemistry revealed a significant reduction in the number of CD31+ blood vessels in Hif1af/f-Tie2Cre+ mice when compared with Hif1af/f-Tie2Cre− littermates (Figure 4). The mean luminal area of αSMA+ blood vessels in the wounds on day 21 was also significantly reduced.

3.5 Decreased BMDAC mobilization after burn wounding in knockout mice

We previously demonstrated that BMDACs are mobilized into peripheral blood after burn wounding.9,10 We analysed the levels of circulating Sca1+/CXCR4+ and CXCR4+/Tie2+ BMDACs on day 3 after burn wounding. Compared with non-burned control mice, burn wounding induced a 3.7-fold increase in circulating Sca1+/CXCR4+ BMDACs in both Hif1af/Tie2Cre− and Arntf/Tie2Cre− mice (Figure 5A; P < 0.05.
compared with non-burned controls and burned Cre\(^+\) mice; \(n = 3\)–9). A 3.9- and 2.6-fold increase in circulating Tie2\(^+\)/CXCR4\(^+\) BMDACs was observed in Hif1a\(^{−/−}\)-Tie2Cre\(^−\) and Arntf\(^{−/−}\)-Tie2Cre\(^−\) mice, respectively (Figure 5B; \(P < 0.05\) compared with non-burned controls and burned Cre\(^+\) mice; \(n = 3\)–8). In contrast, there was no increase in Sca1\(^+\)/CXCR4\(^+\) or Tie2\(^+\)/CXCR4\(^+\) BMDACs on day 3 after burn wounding in Hif1a\(^{−/−}\)-Tie2Cre\(^−\) or Arntf\(^{−/−}\)-Tie2Cre\(^−\) mice (Figure 5A and B). These data indicate that Tie2-dependent knockout of HIF-1\(\alpha\) or HIF-1\(\beta\) eliminates BMDAC mobilization in response to burn wounding.

### 3.6 HIF-1 knockout impairs the homing of cultured BMDACs to burn wounds

We next analysed whether the homing of BMDACs to burn wounds requires HIF-1 activity in the burn wound tissue, BMDACs, or both. BMDACs obtained from male donors were injected intravenously into female recipients 48 h after burn wounding. Eight hours later, samples of burn wound and normal (non-burned) skin were isolated and qPCR was performed using primers specific for Sry, which is a gene located on the Y chromosome and therefore a marker of BMDACs from the donor male mouse. The ratio of Sry signal in burned:non-burned skin is a quantitative measure of BMDAC homing to the burn wound.\(^{25}\)

To determine whether HIF-1 activity in Tie2-lineage cells within the burn wound is required for BMDAC homing, we injected BMDACs from Tie2Cre\(^−\) donors into burned recipients that were either Tie2Cre\(^−\) or Tie2Cre\(^+\). When Hif1a\(^{−/−}\)-Tie2Cre\(^−\) BMDACs were injected into Hif1a\(^{−/−}\)-Tie2Cre\(^−\) recipients, the mean ratio of Sry signal in burned:non-burned skin was 7.9 ± 1.7, indicating efficient homing of BMDACs to the wound, whereas in Hif1a\(^{−/−}\)-Tie2Cre\(^+\) recipients, the ratio was 0.6 ± 0.2, indicating a complete loss of BMDAC homing (Figure 6A, left panel). The difference between groups was statistically significant (\(P < 0.05\) by Student’s t-test; \(n = 4\)). Similar significant differences were observed when Arntf\(^{−/−}\)-Tie2Cre\(^−\) BMDACs were injected...
The purpose of this study was to assess the vascular response to burn wounding; (i) delayed wound closure and reduced wound blood flow during wound healing; (ii) impaired wound vascularization; (iii) inhibited mobilization of endogenous BMDACs; and (iv) impaired BMDAC homing to the wound. The effect of HIF-1α or HIF-1β loss-of-function was observed, despite large differences in the rate of wound healing and blood flow dynamics in the Hif1αff and Arntff strains, which are likely due to differences in genetic background. This work complements previous studies demonstrating that in aged, diabetic, or germ-line HIF-1α haploinsufficient mice, loss of HIF-1α expression results in failure to induce expression of genes encoding angiogenic growth factors, failure to mobilize BMDACs, and failure to vascularize and repair burn wounds. 9,10 excisional wounds, 17,28–30 ischaemic skin flaps, 5,31 and ischaemic muscle resulting from femoral artery ligation. 24,25,32,33

The current study focused on the effect of HIF-1α or HIF-1β deficiency that was restricted to Tie2-lineage cells. Tie2 is a tyrosine kinase receptor for angiopoietins that is expressed primarily by ECs. 21,34,35 Hypoxia induces Tie2 expression in ECs 36,37 and a subset of monocytes 38 and pericytes 39 through diverse cell-specific mechanisms. The presence of Tie2+ BMDACs in Amf1ff-Tie2Cre+ and Hif1αff-Tie2Cre+ mice indicates that HIF-1 (or HIF-2) is not absolutely required for Tie2 expression. However, expression of a dominant negative form of HIF-2α from the Flk1 promoter, which may be expressed earlier in embryogenesis than the Tie2 promoter, was associated with decreased Tie2 expression. 40 Tie2 is expressed in myeloid 41 and haematopoietic stem cells in the bone marrow 42,43 and circulating Tie2+ cells promote endothelial repair after vascular injury 44 or soft tissue ischaemia. 35 Consequently, we measured Tie2+ circulating BMDACs after burn wounding in Amf1ff-Tie2Cre−
and Hif1α<sup>−/−</sup>-Tie2Cre<sup>−/−</sup> mice. Tie2+/CXCR4<sup>−</sup> and Sca1+/CXCR4<sup>−</sup> BMDACs were mobilized on day 3 and these responses were impaired in both Arntf<sup>−</sup>-Tie2Cre<sup>−/−</sup> and Hif1α<sup>−/−</sup>-Tie2Cre<sup>−/−</sup> mice, indicating that Tie2-lineage BMDACs participate in the vascular response triggered by burn wounding. Studies of burn patients have demonstrated that circulating BMDACs are correlated with increased circulating BMDACs in ischaemic tissue through the expression of genes encoding β3-integrins.25

In the recipient, ECs as well as early responding bone marrow cells may play critical roles in the homing response. We have previously shown that HIF-1 controls the expression of hundreds of genes in vascular ECs.50 Previous studies have also demonstrated that in ECs, HIF-1 mediates autocrine signalling by fibroblast growth factor 2 and VEGF, which is required for tumor vascularization.51,52 Results from the current and previous studies underscore the master regulatory role that HIF-1 plays in vascularization, as it is essential for both the production of angiogenic factors and the response of ECs and BMDACs to these factors.

Our findings offer insight into the role of HIF-1α in the pathophysiology of burn wound healing and implicate BMDACs as a potential therapeutic in burn patients. Recent studies have demonstrated that increasing HIF-1α expression either by gene therapy or pharmacological induction promoted angiogenesis in diabetic wound models.17,28,29 HIF-1α gene therapy combined with BMDAC therapy in a limb ischaemia model in aged mice improved perfusion and limb salvage, whereas either therapy alone was ineffective.25,33 Studies are underway to investigate whether combined HIF-1α gene therapy and BMDAC therapy promotes the vascular/repairative response in this burn wound model.

**Conflict of interest:** none declared.

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