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Wnt signaling and the transcription factor Foxn1 contribute to cutaneous wound repair in mice

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ABSTRACT

Aim: The transcription factor Foxn1 is a regulator of scar-ended cutaneous wound healing in mice. However, the link between Foxn1 and Wnt signaling has not been explored in the context of cutaneous repair. Here, we investigate the effects of β-catenin-dependent and -independent Wnt signaling represented by Wnt10a and Wnt11, respectively, in healing of full-thickness cutaneous wounds in C57BL/6 mice.

Material and Methods: Quantitative polymerase chain reaction, western blot, and immunostaining were performed to assess the spatial and temporal distribution of Wnt10a, Wnt11, and β-catenin in skin during wound healing. A co-culture system consisting of keratinocytes transfected with an adenoviral vector carrying Foxn1-GFP and dermal fibroblasts (DFs) was employed to determine the influence of epidermal signals on the capacity of DFs to produce extracellular matrix (ECM) proteins *in vitro*. The levels of types I and III collagen in conditioned media from DFs cultures were examined via enzyme-linked immunosorbent assay.

Results: The expression of Wnt10a, Wnt11, and β-catenin increased at post-wounding days 14 and 21 when tissue remodeling occurred. Foxn1::Egfp transgenic mice experiments demonstrated that Wnts were abundant in the epidermis adjacent to the wound margin and to a lesser extent in the dermis. The Wnt10a signal colocalized with Foxn1-eGFP in the epithelial tongue and neo-epidermis during the initial stage of wound healing. Foxn1 overexpression in keratinocytes affected DFs function related to collagen synthesis.

Conclusions: Wnt ligands contribute to cutaneous wound repair, predominantly by engagement in ECM maturation. The data indicates a possible relationship between Foxn1 and Wnts in post-traumatic skin tissue.

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Skin; wound healing; scarring; Wnt signaling; Foxn1; extracellular matrix (ECM)

Introduction

The mammalian response to cutaneous wounds is characterized by rapid repair that is required to restore skin integrity[1]. Although each step of wound healing is tightly regulated for optimal skin recovery, the process involves excessive extracellular matrix (ECM) deposition and results in scar tissue formation. As the final stage of the regular mammalian tissue repair, scarring is inseparably accompanied by fibrosis that disrupts the physiological skin tissue architecture and impairs its function[1,2].

During regular wound healing, ECM synthesis begins approximately 2–3 days after injury and continues for an additional 2–4 weeks[3]. The early provisional matrix is formed primarily by collagen, elastin, proteoglycans, glycosaminoglycans, and hyaluronic acid, and its development eventually restores proper skin function. The maturation of granulation tissue marks the final, remodeling phase that reaches a steady state 21 days after wounding. However, this phase occurs over a prolonged period of time and might last for years[4,5]. Although multiple ECM components are implicated in post-trauma tissue fibrosis, collagen comprises the main structural component in fibrotic post-wounded skin[6,7]. In the context of wound repair, collagen I and III are the two dominant types[6,7].

Wnt signaling plays an important role in tissue homeostasis, and its fluctuation contributes to fibrogenesis in multiple organs[8–11]. Wnt ligands are secreted glycoproteins that bind to their receptors and induce a cascade of intracellular signaling events involving either the nuclear β-catenin accumulation leading to gene transcription (β-catenin-dependent) or effects in target tissue via a β-catenin-independent mechanism[12]. There is extensive literature suggesting that the cellular response to cutaneous injury and subsequent scar formation principally involves β-catenin-dependent Wnt signaling[9,13,14]. However, the role of Wnt signaling in cutaneous wound healing remains controversial; recent reports indicate that Wnt activity might be associated

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with hair follicle regeneration rather than tissue fibrosis[15].

Communication between the epidermis and underlying dermis is necessary for tissue homeostasis and involves the secretion of multiple factors[16]. We previously showed that the profiles of synthesized transcripts (e.g., Wnt10a, Wnt11, and β-catenin) and proteins that may be implicated in the molecular cross-layer dialogue differ between Foxn1-deficient (nude) and Foxn1-active (wild-type control) mice[17,18]. Foxn1 transcription factor deficiency corresponds with the unique ability of mammalian skin to regenerate when injury occurs[19–21]. In contrast, the regular (active) Foxn1 phenotype is manifested in terms of repair and skin scarring during the trauma of wounding[22].

The present study aimed to establish the role of β-catenin-dependent and -independent Wnt signaling represented by Wnt10a and Wnt11, respectively, in physiological (scar-forming), Foxn1-controlled wound healing. We established a full-thickness cutaneous wound model in C57BL/6 (B6) mice to assess the spatial and temporal distributions of the relevant members of the Wnt signaling cascade during the course of healing. Based on our previous reports[22,23], we also utilized Foxn1::Egfp transgenic mice to investigate the possible association of Wnt ligand with Foxn1 peak expression during wound healing. We also performed *in vitro* experiments on mouse primary keratinocytes transfected with Foxn1-GFP-expressing adenovirus (Ad-Foxn1) and co-cultured with dermal fibroblasts (DFs) to explore the effect of Foxn1 on ECM component production.

Methods

Animals and cutaneous wound model

The study was performed using newborn and adult (2–9 months) B6 wild-type and adult Foxn1::Egfp transgenic mice. Foxn1::Egfp mice possess an enhanced green fluorescent protein transgene that is driven by the Foxn1 regulatory sequence in the skin[22,23] (a kind gift from Professor Thomas Boehm, Max Planck Institute of Immunobiology and Epigenetics, Germany). All animals were bred and housed in a temperature- and humidity-controlled room (22 ± 2°C and 35–65%, respectively) with a 12-h light/12-h dark cycle at the Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland.

Full-thickness excisional wounds were created according to the procedure described previously[22,23]. Briefly, following anesthesia, four full-thickness wounds were made on the dorsal skin of mice using a 4-mm-diameter biopsy punch. Biopsy specimens were collected using an 8-mm biopsy punch on post-wounding days 1, 5, 7, 14, 21, and 36 (n = 4–7 animals per time point). Skin samples were frozen in liquid nitrogen for RNA and protein isolation or fixed for histological procedures. All experimental animal procedures were approved by the Ethics Committee of the University of Warmia and Mazury (Olsztyn, Poland), No. 28/2012.

Cell isolation and culture

Skin tissues samples were digested with 6 U/ml dispase I (Life Technologies) overnight at 4°C to separate the epidermis and dermis. The next day, separated epidermal tissue was digested for 3 min in 0.05% trypsin-EDTA solution and then filtered through 70-μm strainers for keratinocyte isolation. Keratinocytes were collected by a series of three trypsin digestions and filtrations, then centrifuged at 300 × *g* for 9 min at room temperature. The pelleted cells were suspended and maintained in keratinocyte seeding medium consisted of Dulbecco's modified Eagle's medium (DMEM/F-12; Sigma-Aldrich) containing 10% fetal bovine serum (FBS; Life Technologies), 10 μg/ml gentamicin, 0.25 μg/ml amphotericin B (Cascade Biologics), and 120 μM β-mercaptoethanol (Sigma-Aldrich). For DFs isolation from adult (2–-9 months) B6 mice, entire skin samples (without epidermis/dermis separation) were minced with scissors and enzymatically digested with 0.1% collagenase type I (Sigma-Aldrich) in phosphate-buffered saline with 1% penicillin/streptomycin for 75 min at 37°C. The DFs suspension was then filtered through 100-μm strainers and collected by centrifugation (264 × *g*, 5 min). Pelleted cells were suspended in DMEM/F12 supplemented with 15% FBS, with 1% penicillin/streptomycin. Primary keratinocytes (p = 0) and DFs (p = 0 or 1) were used for further experiments.

Keratinocyte transfection with adenoviruses

Keratinocytes isolated from newborn mice were cultured in inserts in CnT Basal Medium (CELLnTEC) and transfected with Foxn1-GFP-expressing (Ad-Foxn1) or control (Ad-GFP) adenoviruses. For co-cultures, keratinocytes cultured in inserts and DFs grown on the bottoms of 6-well plates were set up together 24 h after keratinocyte transfection and cultured for a further 24 or 48 h[17].

Collection of keratinocyte and DF-conditioned media

To study the effect of Foxn1 overexpression, keratinocyte conditioned media (KCM) from adenovirus-transfected keratinocytes and conditioned media from

co-cultured DFs (DF-CM) were harvested, centrifuged (300 × g, 15 min), and stored frozen at-20°C.

RNA isolation and real-time PCR

Total RNA was isolated from skin samples and cell cultures using TRIzol® Reagent (Thermo Fisher Scientific). cDNA was synthesized using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems by Thermo Fisher Scientific). Endogenous mRNA expression levels of *Hprt1* (chosen as a housekeeping gene), *Wnt10a, Wnt11, Ctnnb1, Col1a1, Col3a1, Tgfβ1, Tgfβ3, αSma* and *Tnc* (Table 1) were measured using TaqMan® Gene Expression Assays (Applied Biosystems by Thermo Fisher Scientific)[24]. Reactions were performed using an ABI ViiA™7 sequence detection system (Applied Biosystems by Life Technologies) with the following conditions: 10 min at 95°C, 45 cycles of 15 s at 95°C, and 1 min at 60°C. All results were normalized on the basis of *Hprt1* content and analyzed using the PCR Miner algorithm[25].

Protein isolation and western blot analysis

Frozen skin samples were homogenized in radioimmunoprecipitation assay buffer (RIPA) containing a protease inhibitor cocktail (Sigma-Aldrich). Thirty-five micrograms of proteins per sample were separated on 12% sodium dodecyl sulfate polyacrylamide gels and blotted onto polyvinyl difluoride membranes. Membranes were incubated separately with primary antibodies, including anti-Wnt10a (1:500, Abcam), anti-Wnt11 (1:500, Santa Cruz Biotechnology), anti-β-catenin (1:500, Cell Signaling Technology), and anti-Actb (1:1000, AbCam). After incubation with fluorescent anti-mouse (Cy5.5, 1:10,000, Rockland Immunochemicals Inc.) and anti-rabbit (IRDye 800, 1:5000, Rockland Immunochemicals Inc.) secondary antibodies, bands were visualized using the ChemiDoc™ Imaging System (Bio-Rad Laboratories, Inc.). Densitometric protein analysis was performed using Image Lab™ software, version 6.0 (Bio-Rad Laboratories, Inc.).

Immunofluorescence and immunohistochemistry

Immunohistochemical procedures were performed on formalin-fixed skin specimens that were embedded in paraffin, sectioned at 5 μm, and processed using the following primary antibodies: anti-Wnta10a (1:100, Abcam) and anti-Wnt11 (1:50, Santa Cruz Biotechnology). Antibody binding was detected using the ABC complex (Vector Laboratories, Inc.). Peroxidase activity was revealed using 3.3-diaminobenzidine (Sigma-Aldrich) as a substrate. Slides were counterstained with hematoxylin. Immunofluorescence assays were performed on paraformaldehyde-fixed and cryo-sectioned skin samples, using anti-GFP (1:400, Abcam), anti-Wnt10a (1:100, Abcam), and anti-Wnt11 (1:100, Santa Cruz Biotechnology) primary antibodies. Secondary antibodies labeled with Alexa Fluor 488 or Alexa Fluor 594 (Life Technologies) were used, and nuclei were counterstained with ProLong® Gold antifade reagent with DAPI (Life Technologies).

Tissue sections and stained cell cultures were visualized using an Olympus microscope (BX43), photographed with an Olympus digital camera (XC50), and analyzed with Olympus CellSens software. Confocal images were scanned and digitalized using an Olympus F10i-LIV laser scanning microscope integrated with Olympus CellSens Dimension Desktop 2.2 software.

Analysis of collagen types I and III

The concentrations of collagen types I and III in DF-CM were determined using Col1a1 and Col3a1 enzyme immunoassay kits (Wuhan EIAab Science Co., Ltd) according to the manufacturer's protocol.

Statistical analysis

Statistical analyses were performed using the GraphPad PRISM, version 6.02 software (GraphPad Software Inc.).

Table 1. Primer-probe sets used in real-time PCR.

Gene symbol	Gene full name	TaqMan® primer and probe sets ID
Wnt10a	*Wingless-type MMTV integration site family, member 10A*	Mm00437325_m1
Wnt11	*Wingless-type MMTV integration site family, member 11*	Mm00437327_g1
Ctnnb1	*Catenin, β1*	Mm00483039_m1
Col1a1	*Collagen, type I, alpha 1*	Mm00801666_g1
Col3a1	*Collagen, type III, alpha 1*	Mm01254476_m1
Tgfβ1	*Transforming growth factor, beta 1*	Mm01178820_m1
Tgfβ3	*Transforming growth factor, beta 3*	Mm00436960_m1
aSma (Acta2)	*Actin, alpha 2, smooth muscle, aorta*	Mm00725412_s1
Tnc	*Tenascin C*	Mm00495662_m1
Hprt1	*Hypoxanthine guanine phosphoribosyl transferase*	Mm01545399_m1

The significance of differences between groups was assessed using two-way analysis of variance (ANOVA), one-way ANOVA followed by Tukey's multiple comparisons, and paired Student's t-tests. Data are expressed as mean ± standard error of the mean. For all tests, $p < 0.05$ was considered statistically significant.

Results

Expression profiles of Wnt pathway members during wound healing

Given the large body of literature indicating that Wnt signaling exerts pro-fibrotic effects during healing[8,10,11], we investigated the expression profiles of the Wnt pathway ligands Wnt10a, Wnt11, and β-catenin during cutaneous wound repair in adult B6 mice (Figure 1).

Stable mRNA expression of Wnt members was observed from day 0 (control, uninjured skin) through days 1, 5, and 7 after injury, followed by a sharp, transient increase on post-wounding days 14 and 21, when tissue remodeling takes place (Figure 1(a–c), $p < 0.0001$). The analysis of Wnt10a and β-catenin protein levels showed results similar to the mRNA patterns of expression with strong correspondence between β-catenin mRNA (Figure 1(c)) and β-catenin protein (Figure 1(f,i), $p < 0.05$).

Localization of Wnt pathway representatives at the injury site

To investigate the spatial and temporal distribution of Wnts in injured skin, we performed immunohistochemical staining for Wnt10a and Wnt11 (Figure 2). At post-wounding day 2, Wnt10a (Figure 2(a–c)) and Wnt11 (Figure 2(g,h)) proteins localized to the epidermis adjacent to the wound margin, at the edges of the wound bed (Figure 2(a, b,g,h), arrows) and in the dermal layer (Figure 2(c,f), arrowheads). The strongest Wnt10a signal was detected at day 21 in all layers of stratified epidermis (Figure 2(e), arrows), and it also was observed in the dermal skin compartment (Figure 2(f), arrowheads)

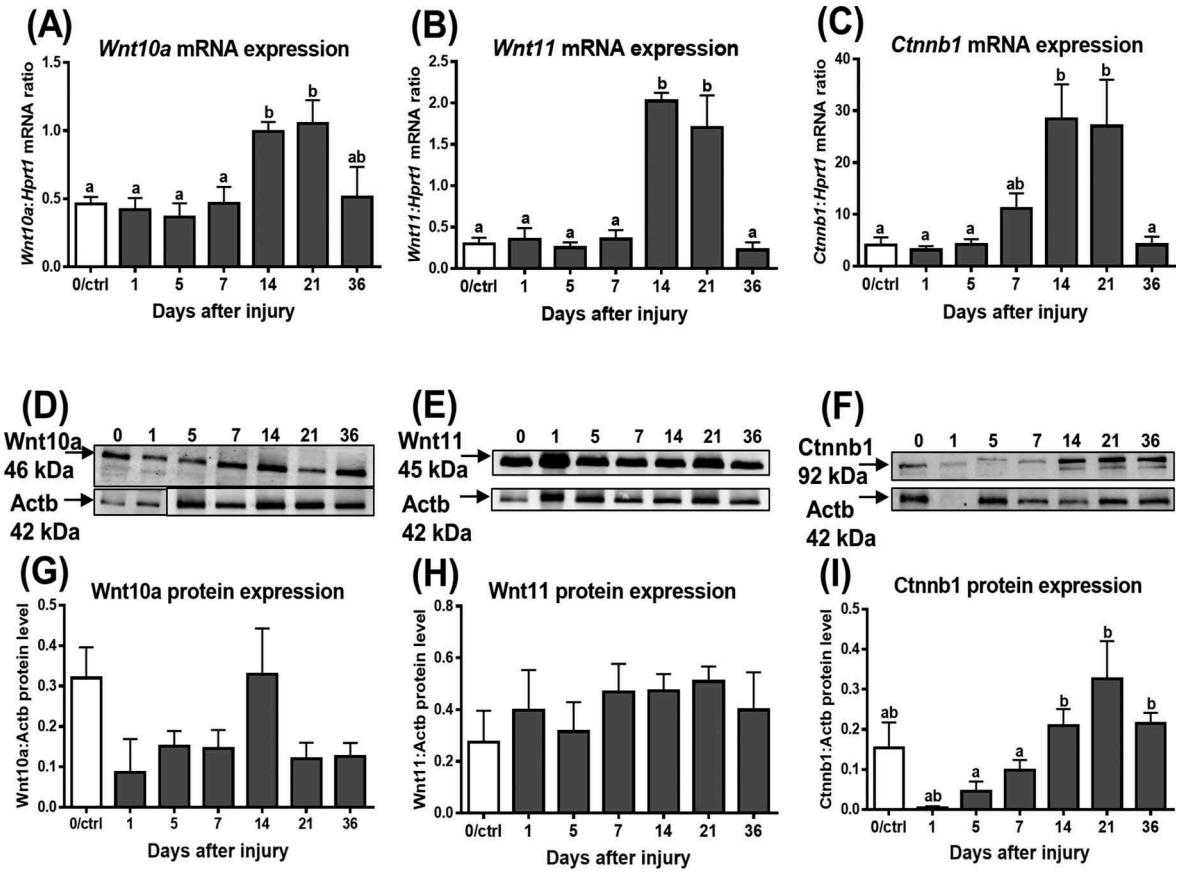
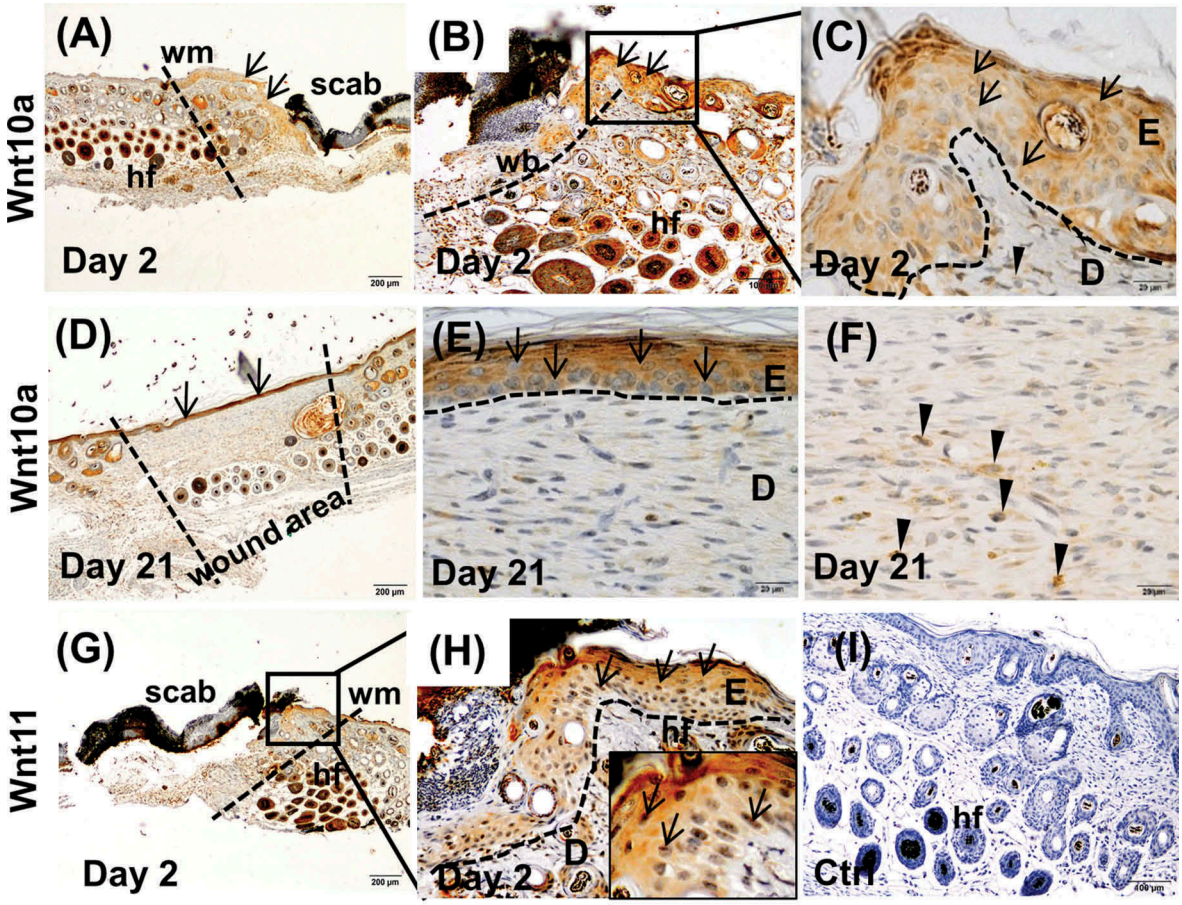


Figure 1. Expression of β-catenin-dependent and -independent Wnt pathway representatives during skin wound healing. Full-thickness 4-mm-diameter excisional wounds were created in the dorsal skin of B6 mice. Quantitative analysis of *Wnt10a* (a), *Wnt11* (b), and *β-catenin* (c) mRNA levels showed low and stable expression from day 0 up to post-wounding day 7, with sharp increases at days 14 and 21. Protein levels of Wnt10a (d, g), Wnt11 (e, h), and β-catenin (f, i) correlate with their transcript expression profiles. Data are expressed as mean ± standard error of the mean (n = 4), $^*P < 0.05$.



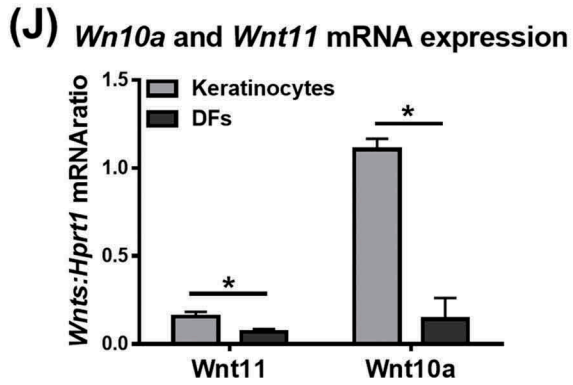


Figure 2. Wnt10a and Wnt11 expression in the skin (a–i) and cultured keratinocytes and DFs (2j). Immunohistochemical detection of Wnt10a and Wnt11 revealed their presence at the wound edges and in the leading epithelial tongue under the scab (a–c, g, and h). At post-wounding day 21, Wnt10a signal was greatly elevated in the neo-epidermis (d, e) and showed moderate expression in the dermis (f). In control sections, where primary antibodies were substituted with non-specific-IgGs, no positive signal was detected (i). Quantitative mRNA analysis showed higher *Wnt10a* and *Wnt11* expression in keratinocytes than in DFs (j). Arrows indicate Wnts signal in all layers of stratified epithelium. Arrowheads highlight Wnts presence in dermis. Dotted lines depict wound area (a, d, g) or it separates epidermis from underlying dermis (c, e, h). Abbreviations: D, dermis; DFs, dermal fibroblast; E, epidermis; hf, hair follicle; wm, wound margin. Scale bars: 200 μm (a, d, g), 100 μm (b, i), 50 μm (h), and 20 μm (c, e, f). Data are expressed as mean ± standard error of the mean (n = 4–5), *$P < 0.05$.

Moreover, strong expression of both ligands was observed in the hair follicles (Figure 2(a,b,d,g)).

Wnt10a and *Wnt11* mRNA analysis confirmed their expression in cultured keratinocytes and DFs (Figure 2(j)). However both Wnt signaling ligands were more highly expressed in keratinocytes than in DFs (both $p < 0.05$; Figure 2(j)). These results support the immunohistochemical results of Wnt10a

and Wnt11 protein in skin sections, showing that the epidermis is a principal source of relevant Wnt ligands.

Colocalization of Wnts and Foxn1 during wound healing

The expression profiles of Wnt10a and Wnt11 overlap with that of Foxn1 during the course of cutaneous wound healing, as we demonstrated previously[22,23]. To reconcile the finding of similar expression patterns of Foxn1 and Wnt members in injured skin, we performed double immunofluorescence for Wnt10a and Wnt11 on skin tissue sections collected from Foxn1::Egfp transgenic mice (Figure 3). Foxn1 and Wnt10a signals colocalized in the epidermis at injury sites (Figure 3(a–e), arrows). Robust signals from both proteins were specifically observed at the epidermal edge of the wound bed and in a leading epithelial tongue that invades the tissue under the scab and further accumulates in the neo-epidermis (Figure 3(a–d), arrows). Furthermore, Foxn1 and Wnt11 were expressed throughout the newly formed thick epidermis at post-wounding day 7 (Figure 3(f), arrows).

Effect of epidermal Foxn1 overexpression on DFs characteristics in vitro

The epidermis greatly contributes to post-trauma healing by engaging in re-epithelialization and activating epithelial-mesenchymal transition (EMT); both processes are mediated by Foxn1[22]. We assumed that Foxn1, the expression of which is restricted to the epidermis, might influence underlying connective tissue changes during cutaneous wound healing, particularly during the proliferative and remodeling phases when intensive ECM synthesis occurs.

For this purpose, we established a co-culture system for Ad-Foxn1-transfected keratinocytes with DFs (Figure 4). Foxn1 overexpression in keratinocytes significantly downregulated DFs mRNA levels of *Col3a* ($p<0.05$), *Tgfβ1*, and *αSma* (both $p < 0.01$; Figure 4(a)). Consistent with this, a substantial decrease in collagen type III was detected in DF-CM collected after 48 h of co-culture with Ad-Foxn1-transfected keratinocytes compared with DF-CM from Ad-GFP-transfected keratinocytes, control co-cultures ($p < 0.05$; Figure 4(c)). No differences in collagen type I were observed (Figure 4(b)).

Discussion

The present study examined the spatial and temporal distribution of Wnt glycoproteins throughout skin wound healing in B6 mice. The expression patterns of β-catenin-dependent and β-catenin-independent Wnt pathway representatives were identical and showed specific increases during the early remodeling phase (days 14–21). Wnt10a and Wnt11 were expressed in the epidermis and to a lesser extent in the dermis shortly after injury, however, their levels were greatly elevated at post-wounding day 21 when ECM remodeling occurs. Furthermore, given our previous reports indicating that the epidermal transcription factor Foxn1 is a novel regulator of skin wound healing in mice[22,23], we explored whether its overexpression in keratinocytes affected connective tissue function related to ECM synthesis. Notably, the level of collagen type III, a pro-regenerative indicator, decreased with Foxn1 overexpression (Figure 4).

There is considerable evidence that Wnt signaling is important in skin morphogenesis and homeostasis[26,27]. However, the role of this pathway in cutaneous wound healing remains controversial. Bastakoty et al. demonstrated that inhibition of the β-catenin-dependent Wnt pathway with topical application of small molecules (XAV 939 or pyrvinium) reduces fibrosis and promotes regeneration in full-thickness excisional wounds of the dorsal skin and ear injuries in C57BL/6J mice[13]. In contrast, using $Axin2^{LacZ/LacZ}$ mice that lack both copies of the negative Wnt regulator Axin2, Whyte et al. found that amplification of Wnt signaling enhanced healing of cartilage injury[28].

Our present findings show that during regular skin wound healing recognized as repair with scarring, the β-catenin-dependent and -independent Wnt pathway representatives (Wnt10a and Wnt11, respectively) robustly appear at post-wounding days 14 and 21, and this sharp increase corresponds with the early remodeling phase (Figure 1). These findings are consistent with *Wnt5*a and *Wnt11* expression in excisional wounds in mice, where both β-catenin-dependent and β-catenin-independent pathways contribute to scar tissue development during regular wound healing[29]. These findings indicate that involvement of the Wnt pathway in ECM dynamics favors scar tissue formation. However, the lack of Wnt "reporter" mice prevents clear demonstration of Wnt responsiveness to cutaneous wound healing[28,30]. This could be clarified by utilizing an experimental mouse model of dermal fibrosis including those induced by bleomycin as described by Akhmetshina et al.[31]. Although we studied adult (2–9 months old) mice, there is an increasing evidence of age-related changes in all phases of wound healing[23,32]. For this reason, Wnt function should be assessed at different ages

Wnt proteins play roles in cell-to-cell communication and thus act as morphogens in a broad range of tissues[33–35]. Following secretion, Wnts are distributed

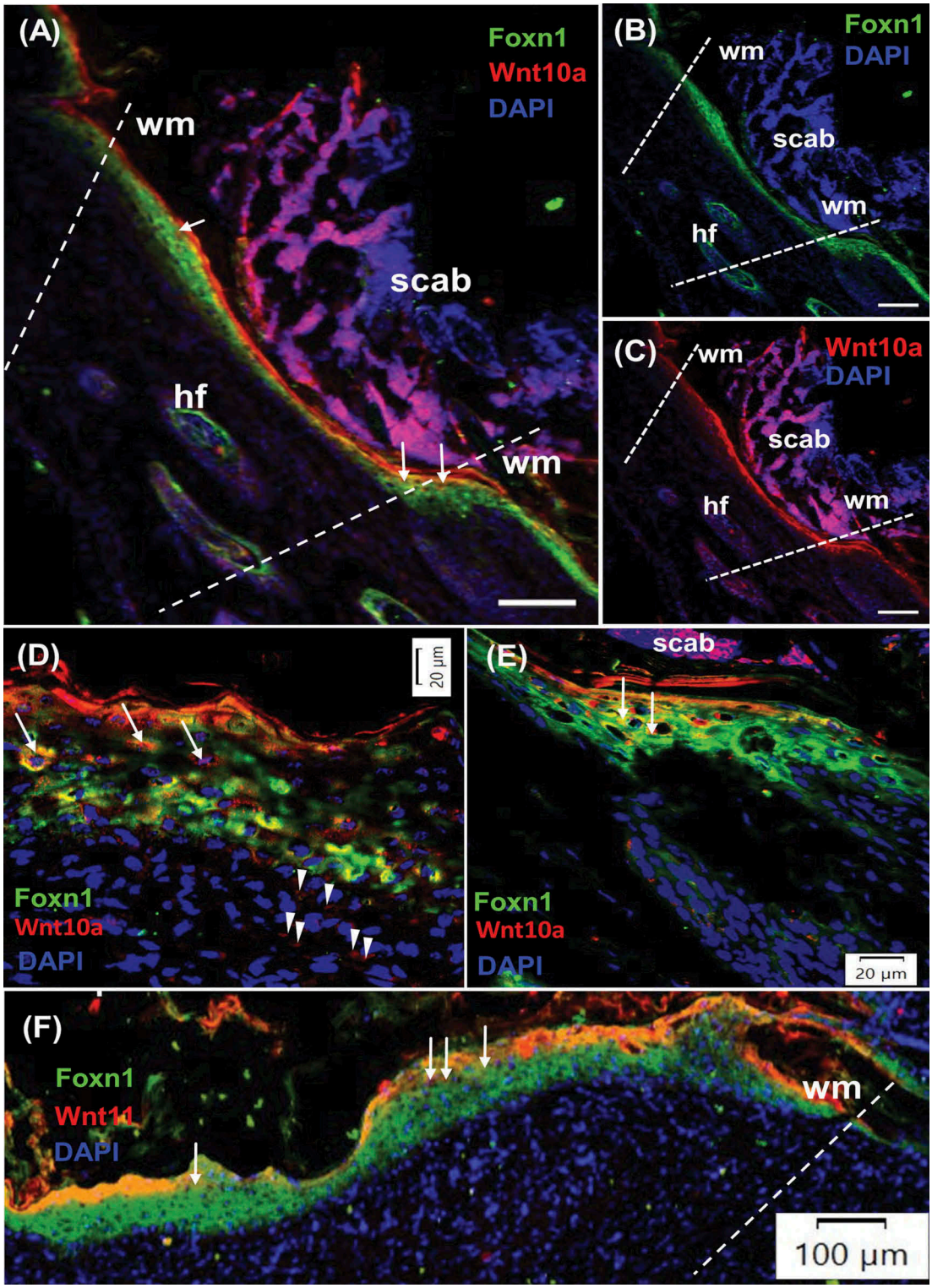


Figure 3. Foxn1 and Wnts post-wound skin localization. Confocal microscopy with double staining for Foxn1 and Wnt10a demonstrated colocalization (a, d, e) of Foxn1 (b) and Wnt10a (c) signals at the epidermal edge of the wound and in a leading epithelial tongue under the scab at post-wounding day 4 (a). Both proteins were expressed in hair follicles (a–c). The presence of Wnt10a in the dermis (d). Foxn1 colocalized in the epidermis with Wnt11 at post-wounding day 7 (g). Colocalization of specific signals indicated by arrows; arrowheads depict only dermal Wnt10a expression. Nuclei were counterstained with DAPI. Abbreviations: hf, hair follicle; wm, wound margin. Scale bars: 100 µm (a–c, g) and 20 µm (d–f).

to reach sensory cells that respond to the signal by modulating target gene expression[36]. The present results show that Wnt10a and Wn11 expression in the skin during wound healing is predominantly

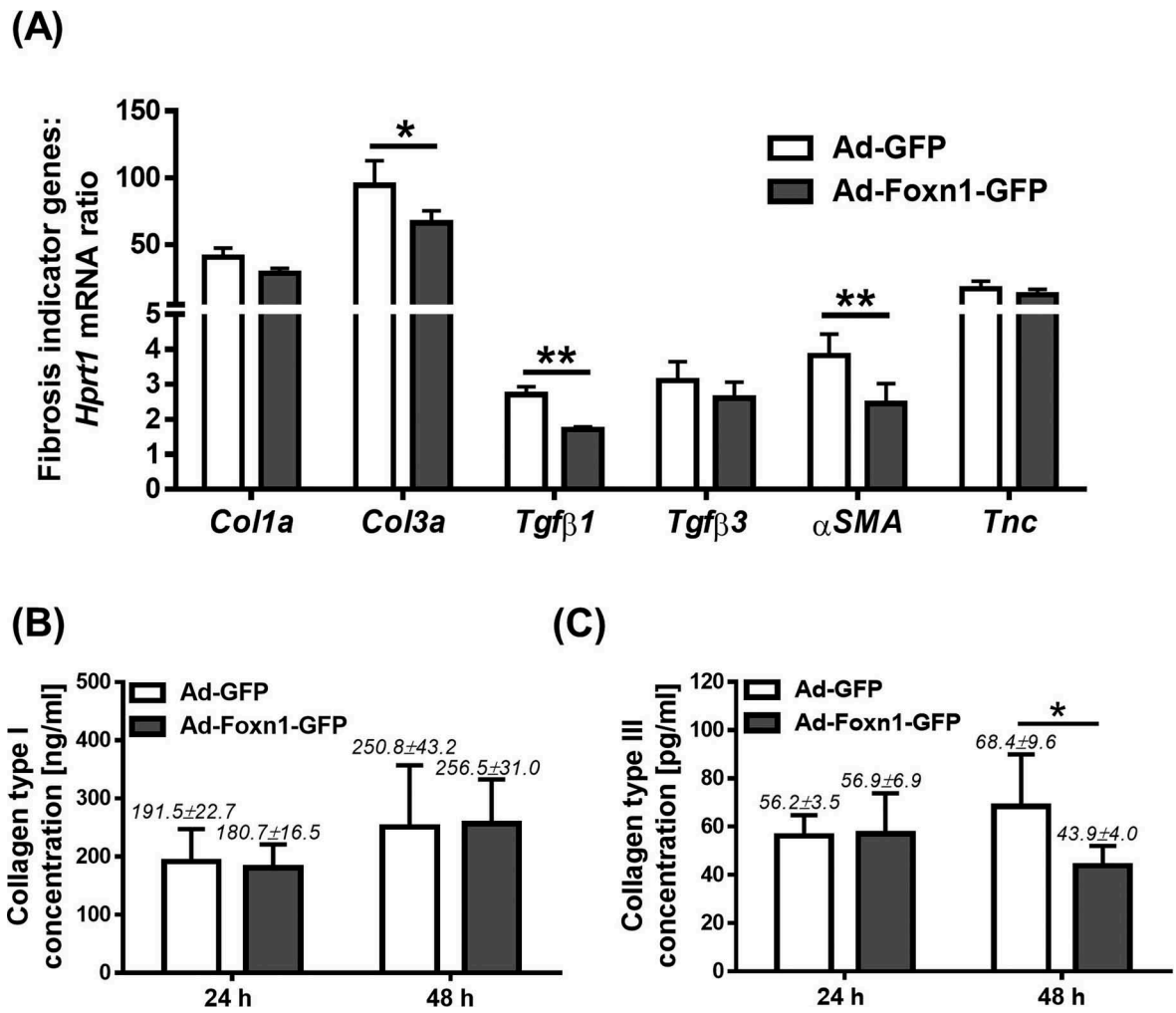


Figure 4. Foxn1 overexpression in primary keratinocytes influenced the ability of DFs to produce ECM components. Primary keratinocytes transfected with Ad-Foxn1-GFP or Ad-GFP (control) were co-cultured with DFs to mimic the *in vivo* epidermal–dermal relationship. Foxn1 overexpression downregulated pro-regenerative *Col3a1* mRNA and pro-fibrotic *Tgfβ1* and *αSma* transcripts (a). At the protein level, Foxn1 had no effect on the expression of collagen type I (b) but exerted an inhibitory effect on collagen type III secretion by DFs (c). *$P < 0.05$, **$P < 0.01$. Abbreviations: DFs, dermal fibroblast; ECM, extracellular matrix.

attributable to the interfollicular epidermis and hair follicles (Figures 2 and 3). This observation is supported by the significant upregulation of *Wnt10a* and *Wnt11* mRNA levels in primary keratinocytes compared to DFs. It suggests that the epidermis serves as a major source of Wnt ligands after the trauma of injury. Considering that Wnt proteins are able to travel to distant sites up to several dozen cell diameters away from their source of production[37], we may consider that Wnts synthesized in the epidermis influence deeper compartments of the skin (e.g., dermis), where they modulate DFs behavior. This concept is supported by Donati et al., who demonstrated that β-catenin-dependent Wnt pathway activation in keratinocytes leads to the secretion of an array of growth factors (i.e., Igf2 and Bmp2) that promote intradermal adipogenesis[16]. There is also evidence that Wnt ligands might use a short-distance mode of action, and this alternative pathway occurs mostly between neighboring cells[33].

The current study also indicates that Wnt10a, Wn11, and β-catenin expression patterns (Figure 1) overlap with the *Foxn1* mRNA profile during excisional wound healing in mice[23]. To the best of our knowledge, this is the first evidence of a spatial and temporal link between Foxn1 and Wnts in the context of cutaneous wound healing. Foxn1 expression in intact skin is restricted to the epidermis, where it is responsible for initiating terminal keratinocyte differentiation[38,39]. However, apart from being involved in re-epithelization during wound healing, Foxn1 also participates in EMT[22]. It has been postulated that Foxn1 activity after skin injury is associated with scar formation[22]; these conditions are

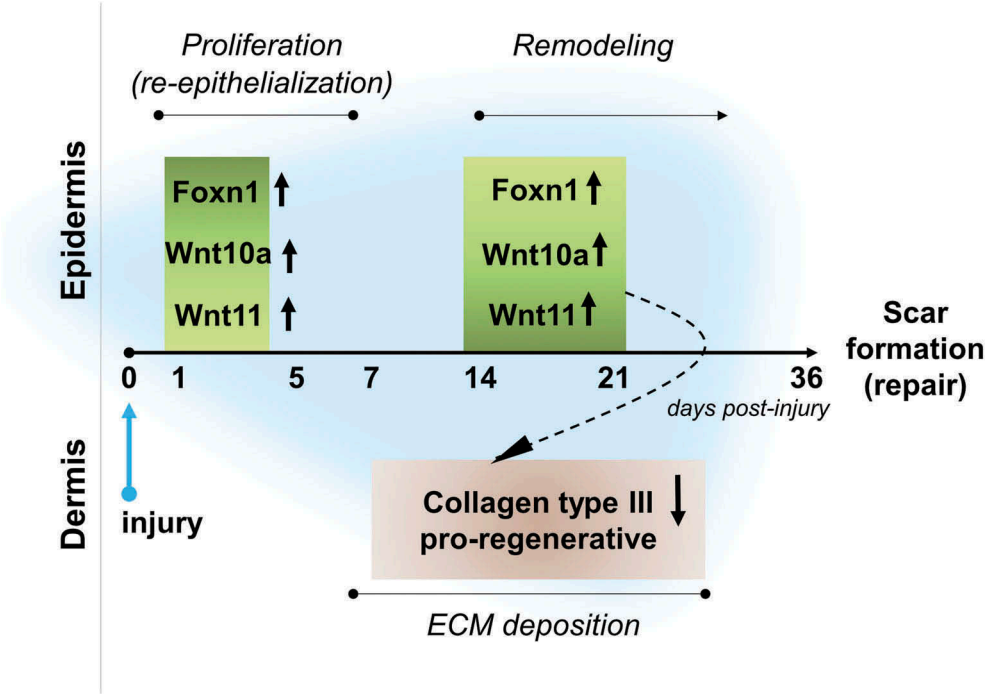


Figure 5. Schematic illustration of a possible relationship between Wnt10a, Wnt11, and Foxn1 during reparative skin wound healing in mice. Following injury, Wnt10a and Wnt11 are specifically expressed in the epidermis as early as days 2–4 (see Figures 2 and 3), indicating proliferation and re-epithelialization. A sharp and transient increase in Wnt signaling members occurs on post-wounding days 14 and 21 during the remodeling phase (see Figures 1–3). This characteristic profile of Wnt10a and Wnt11 coincides with Foxn1 expression during cutaneous wound healing[23]. Epidermal Foxn1 is involved in re-epithelization and EMT after the trauma of injury[22] and might also affect DFs by reducing pro-regenerative collagen type III secretion and thus facilitating scar formation healing.

also attributed to Wnt signaling at the injury site. Using an *in vitro* transwell system, we showed that Foxn1 overexpression in keratinocytes affects DFs (Figure 4) leading to reduced deposition of collagen type III (a marker of skin's ability to regenerate). Indeed, human fetuses that heal wounds via regeneration demonstrate a higher ratio of collagen type III and I than wounded skin in adults[40,41]. Moreover, Col3+/—mice show increased wound contraction and myofibroblast density[42]. Even more surprisingly, Foxn1 expression downregulates *Tgfβ1* and *αSma* mRNA levels in cultured DFs, suggesting that its pro-fibrotic effect might be mediated by downregulation of pro-regenerative collagen III rather than its effects on pro-fibrotic marker synthesis.

In conclusion, our study demonstrates the contribution of Wnt ligands to regular reparative skin wound healing. Importantly, we showed a correlation between Foxn1 and Wnt expression within the first days of injury *in vivo* (Figure 5). Furthermore, we found that Foxn1 overexpression modulates the capacity of DFs to produce ECM components *in vitro* (Figure 5). Whether there is the mechanistic link between Wnt signaling and Foxn1 in the skin as shown for the thymus[43] requires further study.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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