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MINIREVIEW

FOXN1 Transcription Factor in Epithelial Growth and Wound Healing

Anna I. Grabowska,[a] Tomasz Wilanowski[b]

Laboratory of Neuroplasticity, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland[a]; Laboratory of Signal Transduction, Department of Cell Biology, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warsaw, Poland[b]

ABSTRACT FOXN1 is a prodifferentiation transcription factor in the skin epithelium. Recently, it has also emerged as an important player in controlling the skin wound healing process, as it actively participates in reepithelialization and is thought to be responsible for scar formation. FOXN1 positivity is also a feature of pigmented keratinocytes, including nevi, and FOXN1 is an attribute of benign epithelial tumors. The lack of FOXN1 favors the skin regeneration process displayed by nude mice, pointing to FOXN1 as a switch between regeneration and reparative processes. The stem cell niche provides a functional source of cells after the loss of tissue following wounding. The involvement of prodifferentiation factors in the regulation of this pool of stem cells is suggested. However, the exact mechanism is still under question, and we speculate that the FOXN1 transcription factor is involved in this process. This review analyzes the pleiotropic effects of FOXN1 in the skin, its function in the tumorigenesis process, and its potential role in depletion of the stem cell niche after injury, as well as its suggested mechanistic role, acting in a cell-autonomous and a non-cell-autonomous manner during skin self-renewal.

KEYWORDS FOXN1, cancer, inflammatory response, regeneration, skin wound healing, stem cells

Accepted manuscript posted online 12 June 2017

Citation Grabowska AI, Wilanowski T. 2017. FOXN1 transcription factor in epithelial growth and wound healing. Mol Cell Biol 37:e00110-17. https://doi.org/10.1128/MCB.00110-17.

Address correspondence to Anna I. Grabowska, an.grabowska@nencki.gov.pl.

Skin serves as a barrier against the external environment. It consists of the epidermis, dermis, and subcutaneous adipose cells. The epidermis is a squamous, stratified epithelium composed of keratinocytes. The basal layer of keratinocytes is mitotically active and is a reservoir for epidermal stem cells (SCs). In normal, healthy skin, keratinocytes undergo a process of differentiation—they move upward, form outer layers, and then become flattened and are sloughed from the surface of the skin (1). Skin maintains a balance between proliferation and differentiation, and there are various factors, like transcription factors, microRNAs (miRNAs), etc., that affect this transitional change from multipotent proliferating basal keratinocytes to differentiating cells withdrawn from the cell cycle (2). Wounding perturbs the balance between growth and differentiation of epidermal cells. Signaling pathways and transcription factors, which govern epidermal stratification, also affect the self-renewal potential of damaged tissue (3, 4). Wound healing is a complicated process that is affected by the types of insults causing the injury (1) through immunological, endocrine, and neural system responses (5, 6). The reparative process does not result in complete recovery of a functional tissue in mammals, and as a consequence, a scar is formed (5, 7). An excessive connective tissue response instead of regular wound healing results in raised scarring, causing hypertrophic scars and keloids, which are detrimental from both esthetic and functional points of view (8). It is well established that human and rodent embryos up to the third trimester of gestation can heal skin injuries in a scarless way (9) in a process akin to regeneration. Regeneration is manifested by the capacity of cells to develop into a whole functional organism. The stem cell niche provides a functional

source of cells for self-renewal of intact tissues and after the loss of a portion of the cells following wounding (1, 10). In general, there are two main theories about how SC clones arise: by asymmetric division or neutral competition (11, 12). In the former model, a slow-cycling (DNA label-retaining) cell gives rise to one stem cell and one daughter cell that has limited division potential, as it is fated to differentiate; in the latter model, stem cells (committed progenitors) divide with probabilistic fate into cycling or differentiated cells (13, 14). However, it is suggested that until the homeostasis of the stem cell niche is maintained, it is of less importance which mechanism of division is followed by SCs (12); nevertheless, the involvement of prodifferentiation factors in the regulation of the pool of stem cells after wounding is suggested.

It was recently reported that nude (*Foxn1*-deficient) mice regenerate back skin injuries (15). Taking into consideration that the developmental transition point from scarless to scar-forming skin wound healing coincides with the initiation of *Foxn1* expression (16), this makes FOXN1 an attractive signaling molecule to investigate in reparative versus regenerative processes. Importantly, our recent transcriptomic analysis of *Foxn1*-positive and *Foxn1*-negative skin samples revealed huge differences in the gene expression profiles and in the numbers of signaling pathways affected (17). Additionally, the fact that FOXN1 governs the transition from basal to suprabasal layers makes FOXN1 a potential transcription factor affecting the stem cell niche. Thus, the aim of this review is to expound on the FOXN1 transcription factor as an important contributor to skin morphogenesis, self-renewal, and injury-induced signal response.

FOX TRANSCRIPTION FACTOR FAMILY

FOXN1 belongs to the forkhead box (FOX) transcription factor family, whose members are involved in the regulation of organogenesis, proliferation, maintenance of the stem cell niche, cell cycle control, metabolic homeostasis, cancer, and aging (18, 19). FOXN1, like other FOX proteins, possesses a conserved DNA binding domain (DBD), first described in the homeotic *Drosophila melanogaster* transcription factor Forkhead (FKH), previously named "winged helix." It has been shown that mutations of the associated gene cause a double-head phenotype in the fruit fly (20, 21). *Fox* genes have divergent functions and/or target tissues, and their expression is often temporally altered. FOXN1 is considered to be a transcriptional activator because it has a transcriptional activation domain adjacent to the C-terminal motif (22). FOXN1 may function as a "pioneer" factor, which means that it has access to nucleosome-bound DNA, allowing other transcription factors to act at those sites and enabling pleiotropic transcriptional activity (23). Only two other FOX transcription factors, both from the FOXO subfamily, were implicated as regulators of the skin wound healing process, FOXO1 (24) and FOXO3 (25).

NUDE PHENOTYPE

The lack of functional FOXN1 protein leads to a nude phenotype in mice and nude/severe combined immunodeficiency (nude/SCID) syndrome in humans, which is characterized by the lack of a thymus and, consequently, primary T-cell immunodeficiency, a lack of visible hair, and skin and nail abnormalities (16, 26, 27). More than 20 years ago, it was shown by positional cloning that a loss-of-function mutation in the *Foxn1* gene, originally named winged-helix nude (*Whn*) or HNF3/forkhead homolog-11 (*Hfh11*), is responsible for the loss of a functional DBD (28). The role of FOXN1 in the formation of the thymus and immunological system is studied extensively and has recently been reviewed in detail (23, 29, 30). Briefly, thymic epithelial cell lineages express the FOXN1 transcription factor, which is essential for T cell development (31, 32). *Foxn1* is expressed in endoderm-originating thymic tissue, with coexpression of characteristic epidermal markers (23). Interestingly, recent cell fate studies showed a reprogramming potential of FOXN1-positive keratinocytes, as when they were cocultured with fibroblasts *in vitro*, they supported the differentiation of hematopoietic stem cells (HSCs) into the T-cell lineage (33).

Foxn1 is expressed in the skin in the epidermis and hair follicles, and also, in the

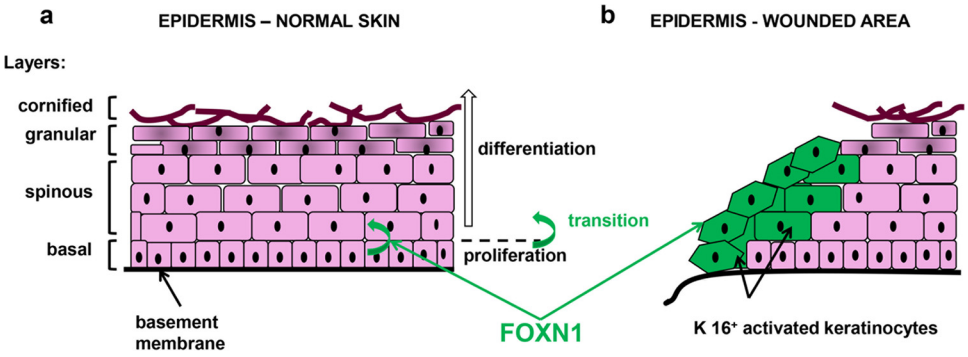

FIG 1 Schematic view of the role of FOXN1 in uninjured (a) and injured (b) skin epithelium. In normal skin, FOXN1 participates in the transition from the proliferative to the differentiation state, and in wounded areas, it is coexpressed with K16-positive ($K16^+$) activated keratinocytes.

submatrix region of the nails, oral cavity, tongue, and nasal placode (16, 34). In the skin, *Foxn1* expression has been found only in epithelial cells (35, 36).

ROLE OF FOXN1 IN THE SKIN

Foxn1 expression is triggered when cells transit from the proliferative (basal layer) to the differentiated state (suprabasal and remaining layers of keratinocytes) (16, 27). It is readily visible in murine hair follicles (HFs) as they undergo synchronized dynamic cycles of active growth (anagen), regression (catagen), and quiescence (telogen). The FOXN1 level peaks during the anagen stage, while it is absent in the telogen stage (36). Anagen is linked to the activation of multipotent SCs, which exit their niche and differentiate (2). In HFs, FOXN1-positive cells are submatrix cells that proliferate rapidly and presumably transit to differentiation (16). Within the interfollicular epidermis (IFE), the activity of FOXN1 was reported as being most prominent in the first suprabasal layer of keratinocytes, which correlates with their exit from the cell cycle and the initiation of differentiation; the vast majority of the proliferative basal cells of the IFE are FOXN1 negative (Fig. 1a) (16, 34). The generation of protective, terminally differentiated layers of the skin requires a constitutive complement source of cells from SCs dispersed in the basal layer (1). FOXN1-positive cells coexpress keratins 1 and 10 (K1 and K10) (early differentiation markers), indicating that *Foxn1* is a prodifferentiation gene.

Strictly connected with skin morphogenesis is the pigmentation process—an important functional defense system in which FOXN1 is a crucial player (37). A mechanistic role of FOXN1 involves the initiation of fibroblast growth factor 2 (FGF2) release, which stimulates melanocytes to transfer the pigment directly to FOXN1-positive cells (suprabasal layer) (36). FGF2, as keratinocyte's mitogen, promotes the proliferation of neighboring basal keratinocytes of IFE (36, 37); however, FOXN1-positive suprabasal cells with signs of early differentiation are not susceptible to autocrine promitotic signals (36). FOXN1 is responsible indirectly for the proliferation of basal keratinocytes and directly for their transition to differentiating suprabasal cells (27). Thus, it seems that FOXN1 governs the balance between proliferation and differentiation events.

FOXN1 was shown to undergo a posttranslational modification, palmitoylation, that enables its cellular stability and trafficking (38). Point mutation in palmitoyl acyltransferase (PAT) causes the loss of palmitoylation activity and results in disruption of hair shaft differentiation and hyperplasia of IFE, which are accompanied by a decreased level of FOXN1 together with the upregulation of its negative regulator, epidermal growth factor receptor (EGFR).

Skin wound healing consists of overlapping, coordinated phases, the first of which involves a hemostasis-inducing inflammatory response to avoid infection (39). Next, the proliferation and migration of different cells within the wound area lead to erasing of granulation tissue (5, 6). In the last, remodeling phase, all the signals that were rapidly evoked are ceased, and disorganized collagen fibers form scar tissue (6). FOXN1 was

reported by our team as a damage response signal during the first two phases of wound healing, and we consider FOXN1 responsible for scar formation (40). In our studies, the levels of FOXN1 expression have clearly been raised in all keratinocytes except basal ones at the wound margin and the postinjury epithelium. However, during reepithelialization, all keratinocytes in the leading tip were FOXN1 and keratin 16 (K16) positive, indicating an active hyperproliferative state (Fig. 1b). Elevated K16 expression is characteristic for postinjury skin; however, it was observed at high levels in uninjured skin of Murphy Roths Large (MRL) mice (another example of a mammal showing the phenomenon of regeneration), which are able to perfectly regenerate a digit after its amputation (41). Consistent with this, uninjured skin of nude mice also displayed increased K16 levels (17). The presence of activated keratinocytes in intact skin of MRL and nude mice was explained by Cheng and others and by us as a condition that favors a more effective response to injury (17, 41).

FOXN1 INVOLVEMENT IN SIGNALING PATHWAYS

The molecular mechanism of FOXN1 functioning in the skin is not well known. Despite many scientific efforts, only a few target genes have been identified, and the precise molecular mechanisms of their induction and regulation are poorly understood. The microarray data from comparisons of primary human epidermal keratinocytes and keratinocytes with induced human FOXN1 revealed increased expression of more than 30 genes (42). The greatest number of upregulated genes is classified into functional groups mostly connected with signaling, growth arrest, cytokines, metabolism, and extracellular matrix (ECM). One of the upregulated genes was K10, indicating the promotion of keratinocyte differentiation. Our recent microarray data encompassing transcriptome profiles of uninjured skin samples of adult wild-type controls, nude mice, and mouse fetuses at embryonic days 14 (E14) and 18 (E18) have shown a large number of differentially expressed genes (17), indicating broad pleiotropic effects of FOXN1. In the comparison of skin regeneration models (E14 embryos and nude adult mice [with *Foxn1* loss-of-function mutation]) versus reparative-skin-healing models (E18 embryos and wild-type adult mice [*Foxn1* positive]), approximately 2,000 genes were revealed by our team as differentially expressed (13). Downregulated genes in the regeneration model were associated with differentiation and upregulated genes with ECM remodeling, immune response, and interestingly, terminal differentiation, represented by involucrin and filaggrin. Genes exclusively enriched in nude mice encoded members of the Wnt family and matrix metalloproteinases (MMPs), in contrast to genes associated with the Notch and bone morphogenetic protein (BMP) signaling pathways, which were downregulated (17).

In HFs, the position of the FOXN1 transcription factor in the regulatory signaling networks is not clearly defined, as sometimes it is positioned downstream from Notch canonical signaling (43), whereas in other reports it is downstream from BMP (44, 45) and upstream from Notch, as a direct activator of the *Notch* gene by binding to its promoter (45). Nevertheless, it was shown that there is competition between BMP and Wnt signaling within the bulge stem cells, affecting the balance of stem cell activity. Inhibited BMP and raised Wnt signaling (observed also in the skin of nude mice) favors the activation of quiescent SCs, indicating that BMP acts as a switch for activation/inactivation of the bulge stem cell niche (46). Thus, the downregulation or loss of *Foxn1* could contribute to SC activation and the creation of a proregenerative environment in nude mice. The data connected with Wnt, Notch, and BMP pathways in the IFE are more limited. It was suggested that BMP changes the fate of proliferating stem cells by transiting amplifying cells toward differentiation, which is coupled with the overexpression of K10 and involucrin (47). It was shown that the dermal BMP-FGF axis, activated by Wnt of epidermal origin, is required for proper epidermal stratification, while simultaneously, BMP4 promotes epithelial growth in a non-cell-autonomous manner by activating basal keratinocytes through FGF7 and FGF10 signaling (4). Interestingly, it was reported that the basal layer of IFE was comprised in its majority by SCs (committed progenitors), which were both the source and the target for Wnt

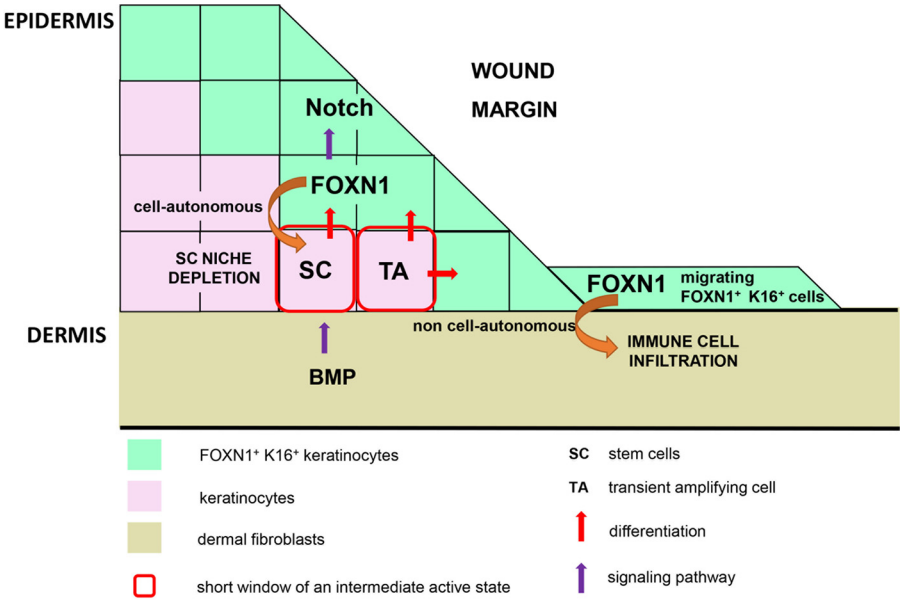

FIG 2 Proposed model of FOXN1 involvement in skin wound healing. FOXN1 activates the differentiation of epidermal stem cells/progenitor cells in a cell-autonomous way and determines scar formation in a non-cell-autonomous way through a directed immune response (i.e., chemokine and cytokine infiltration) into the dermal wound area.

signaling molecules, while Wnt inhibitors were secreted to the suprabasal, differentiated layers (14). Taking into consideration that FOXN1 is a prodifferentiation factor responsible for the transition from basal to suprabasal layers (16, 27) and that the lack of FOXN1 leads to significant Wnt signaling upregulation together with downregulation of Wnt inhibitors (17) one could speculate that the lack of FOXN1 favors the proliferation and activation of SCs within the IFE, which creates a proregenerative environment. So, conversely, FOXN1 could contribute to the depletion of the stem cell niche as one of the BMP downstream targets (Fig. 2). Similarly to FOXN1, Notch is a prodifferentiation factor (48), and recently it has been reported to act as an injury response signal in the inflammatory and proliferation phases of wound healing in suprabasal keratinocytes (49). This process mirrors the postwounding expression of *Foxn1* (40). This observation supports their common function in the BMP/Notch signaling pathway suggested in HFs (45). Interestingly, it was shown that epidermal Notch recruits a subset of inflammatory cells in the postwounding dermis in a non-cell-autonomous way (49). It was assumed that that immunological defect does not have a direct impact on the mechanism of wound healing in nude mice (50). However, earlier studies showed that T-cell deficiency in the skin of nude mice leads to enhanced breaking strength and collagen deposition, which were reduced to the values observed in wild-type controls when syngeneic T lymphocytes were reconstructed (51). Even though a lack of T cells does not directly affect skin wound healing, it is intriguing to consider whether FOXN1 has a non-cell-autonomous effect on the inflammatory response in the skin wound healing process.

NUDE MICE (*Foxn1* DEFICIENT) FROM THE SKIN POINT OF VIEW

The skin of nude mice has no visible hair, but it possesses a large number of hair follicles with bent hair shafts not able to rise above the surface of the skin (16). Interestingly, hair cycling was determined to be functional by the presence of cyclin D1 mRNA in nude mice (52). It is well known that these mice do not produce hair cortex (16) and form an abrogated inner root sheath (IRS) (27). There were reported differences in the distribution of SC/progenitor fractions in HFs of nude mice versus wild-type controls. The stem cell markers SOX2 (sex-determining region Y [SRY] box 2) and OCT4 (octamer-binding transcription factor 4) are downregulated, in contrast to the upregu-

lation of LHX2 (LIM homeobox protein 2), which is responsible for anagen progression, and the Wnt signaling target gene, β-catenin, which is uniformly expressed at high levels in the nude mice (52). Similarly, in spiny mice, which are another case of mammals able to regenerate their tissues, the readout of Wnt inductive signaling, LEF1, was present at high levels in both epidermal and dermal cells of forming hair placodes (53).

The skin of *Foxn1*-deficient mice is thicker and hyperplastic (16), exhibits distinctive biochemical and physical characteristics (50), and in general, seems to display immature embryonic features (17). It was reported that the spatiotemporal expression pattern of specific keratins in the IFE of nude mice is altered. K5 is expressed throughout the epidermis, colocalizing with K1 and spreading robustly in suprabasal cells, while filaggrin and involucrin accumulate more in the outer layers of the epidermis (34, 35). These characteristics indicate that suprabasal cells maintain basallike features and that the differentiation process is disrupted in those animals. It was also shown that FOXN1 induction results in increased mitogenic potential of basal keratinocytes, as evidenced by upregulation of the proliferation marker Ki67, while the remaining postmitotic layers are K6 positive, a sign of hyperproliferation of the skin (27). *In vitro* studies showed that mitogen-activated protein kinase (MAPK) is an upstream regulator of FOXN1, as inhibition of MAPK allows the expression of *Foxn1* and induces the onset of differentiation (35). Interestingly, nude mouse skin has a large population of OCT4-positive dermal cells (52). *In vitro* studies of primary dermal fibroblasts from nude mice revealed that they produce a higher content of both collagen I and III, although at the same time, they produce active matrix metalloproteinase 9 (MMP-9) and MMP-13, which are completely absent under the wild-type conditions (15). As the dermal part of the skin is responsible for scar formation, *Foxn1*-deficient mice exhibit a much higher stemness potential of the dermis, and the fibroblasts of nude mice produce large amounts of ECM components but, simultaneously, are able to turn over these components, which prevents ECM restoration.

It was shown that *Foxn1*-deficient mice are an example of a regeneration model in mammals (15, 50, 54). Ear holes made with a 4-mm punch were regrown with signs of regeneration reminiscent of rapid reepithelialization, blastemalike structure formation, and chondrogenesis (54). Interestingly, in the same studies, the differentiation potential of ear mesenchymal stem cells (EMSCs) was the same in nude and wild-type mice, pointing attention to factors providing the niche of stem cells for the regeneration process. Blastema formation was also shown in an ear wound healing model in spiny mice (53). There, the signs of regeneration were manifested as fast reepithelialization, activated Wnt signaling, and markers of blastema, i.e., the presence at the wound site of undifferentiated disorganized keratinocytes, a higher ratio of collagen III to collagen I, the accumulation of tanescin C, and the absence of alpha-smooth muscle actin (α-SMA). Incisional wounds made on the backs of nude mice revealed an exclusive regeneration response, while immunodeficient *Rag1*-deficient and SCID mice (lacking B and T cells, respectively) formed scars similarly to the control (C57BL/6J) mice (50). This may suggest that the immunological defect does not have a direct impact on the mechanism of healing and allows attention to focus on the role of FOXN1 in the skin reparative process. However, surprisingly, the most significant characteristic was a unique, bimodal pattern of upregulated MMP-9 and MMP-13 during the early and late phases of wound healing (15). MMP-9 expression was observed in the epidermis in the early phase of wound healing in both nude and wild-type mice but, interestingly, it was displayed at a late stage (36 days postinjury) only in the dermal fraction of nude mice. Thus, nude mice are able to actively remodel tissues after wounding.

SKIN CARCINOGENESIS IN NUDE MOUSE MODEL

Wound healing and carcinogenesis processes have much in common, as similar signaling pathways are involved in their initiation (55), ECM reorganization (56), and activation of SCs (57, 58). However, the key difference between wound healing and cancer is that the injury response is a temporary, self-limited process, contrary to the

constant changes resulting in invasion and malignancy observed in cancer (55, 57). Besides that, injured and cancer tissues have similar gene signatures. It was recently shown that SC lineages acquire plasticity that enables their activation, which is facilitated by SOX9 and KLF5 transcription factors in both phenomena: wound healing and tumorigenesis (58).

Nude mouse skin, which shows the distinctive features listed above, displays a reduced incidence of tumor formation in response to standard carcinogenesis protocols (59, 60). This is despite the fact that these mice cannot generate mature T cells and, consequently, are unable to mount many types of adaptive immune responses. This observation can be explained, at least in part, by a decrease in the proliferative potential of epithelial cells in the nude mice. FOXN1 is a key regulatory factor involved in maintaining the balance between keratinocyte growth and differentiation (27, 61). In particular, FOXN1 regulates the initiation of terminal differentiation of keratinocytes in the stepwise or temporal regulation of differentiation, as FOXN1 can ensure that the differentiation program is carried out in proper sequence (16, 35, 42). A disruption in the balance between keratinocyte proliferation and differentiation can result in various skin abnormalities, including skin cancer. In particular, in adult mice, the constant state of dynamic equilibrium between self-renewal, terminal differentiation, and barrier maintenance has been linked to perturbations in skin disorders, including skin tumors (62).

SKIN CANCER IN HUMAN PATIENTS

FOXN1 is highly expressed in seborrheic keratoses (SKs), which are common, benign epithelial tumors. However, it is almost undetectable in cutaneous squamous cell carcinomas (SCCs). As is the case in the nude mouse model, the involvement of FOXN1 in the early stages of keratinocyte differentiation is crucial. In this context, the expression of *FOXN1* is positively regulated by the fibroblast growth factor receptor 3 (FGFR3) signaling pathway and negatively regulated by epidermal growth factor receptor–extracellular signal-regulated kinase (EGFR/ERK) signaling. Furthermore, knockdown of *FOXN1* expression in primary human keratinocytes cooperates with oncogenic RAS in the induction of SCC-like tumors, whereas increased *FOXN1* expression triggers SCC cells to shift to a benign SK-like tumor phenotype. Thus, FOXN1 can serve as a determinant of benign versus malignant keratinocyte tumor development (63). Interestingly, in mice, a similar dependence was observed in the case of *Notch1*: the loss of *Notch1* facilitated carcinogenesis by creating a woundlike microenvironment (64). FOXN1 plays an additional function in SKs, promoting melanocyte migration, which provides a possible explanation for another commonly observed feature of SKs, their increased pigmentation. This is also a characteristic of epidermal nevi, suggesting that even in this case, FOXN1 expression is increased (63).

Other examples of human skin tumors include dermatofibromas (DFs), which are common benign fibrohistiocytic tumors, and dermatofibrosarcoma protuberans (DFSPs), slowly growing dermal neoplasms of intermediate malignancy. Overexpression of *FOXN1* was observed in the epidermal regions of DFs, suggesting that these regions are similar to SKs both in terms of histological features and the activation of FOXN1. On the other hand, *FOXN1* expression was negative to weakly positive in the epidermal regions of DFSPs (65). Thus, DFs and DFSPs, akin to SKs and SCCs, provide another example where *FOXN1* expression allows a determination between benign and malignant tumor development. In addition, like SKs, the epidermal regions of DFs often display hyperpigmentation. This, again, may be caused by *FOXN1* overexpression in epidermal keratinocytes, which promotes melanogenic stimulation in adjacent epidermal melanocytes (65).

INFLAMMATION

A perturbed balance between keratinocyte proliferation and differentiation does not have to be the only mechanism of FOXN1 involvement in skin cancer. Recent studies have found a link between inflammation and many chronic diseases, including cancer

(66). For example, mouse strains with subacute skin barrier defects are more prone to chemically induced skin tumor development (64). In the latter study, Demehri et al. suggested that the underlying mechanism is general and is dependent on the development of a tumor-promoting chronic inflammatory microenvironment in the skin. Thus, conversely, the distinctive process of inflammation in the nude mice may act as a tumor-suppressive environment and may explain the lower incidence of skin tumor formation in these mice.

The reduced level of proscarring cytokines in the nude mice has another consequence, scarless wound healing. This ability is manifested by a lack of scar formation, low levels of collagen, high and sustained levels of hyaluronic acid, low levels of proscarring cytokines, and a low level of inflammation, all features consistent with scar-free healing in mammalian fetuses (50). These data, together with the T-cell deficiency in nude mice and similar levels of macrophage content in postwounding skin tissues in nude and wild-type mice, are consistent with observations that wounds to the skin of mammalian fetuses and gingival tissue show a reduced inflammation phase during the healing process, a condition that has been linked to scarless healing (15).

EMT

The epithelial-to-mesenchymal transition (EMT) is one of the characteristic cellular-reprogramming processes affecting both cancer and wound healing (55, 67). However, cells which undergo tumorigenic transformation exhibit cancer stem cell properties, while in turn, cells within the wound bed exhibit a transient migratory mesenchymal phenotype, which is a sign of EMT (55). In addition to its role in the regulation of keratinocyte differentiation and inflammation, FOXN1 can be involved in skin carcinogenesis and wound healing through yet another process, EMT. Our recent data implicate FOXN1 in the EMT process, resulting in the formation of scar tissue (40). The expression of *Foxn1* during mouse wound healing was found to colocalize with various EMT markers, such as Snail1 and MMP-9. Additionally, quantitative measurements of E-cadherin and N-cadherin surface markers within FOXN1-positive cells revealed a population of cells with a transient E-cadherin$^+$/N-cadherin$^+$ phenotype in postinjury tissue, which could indicate an active EMT process during skin wound healing. In light of previous findings showing that *Foxn1*-deficient (nude) mice exhibit scar-free skin healing, it was proposed that FOXN1 activity or inactivity acts as a major switch between reparative (scar-forming) and regenerative (scar-free) healing (68).

PROSPECTS

It is evident from the experimental data that the role of FOXN1 transcription factor in the skin is complex. Currently it is known that FOXN1 is essential for the development and maintenance of skin epithelia, as well as for scar formation. It has a pleiotropic effect on the skin: it seems to control skin morphogenesis at multiple levels, as it directly promotes keratinocyte differentiation and indirectly stimulates epidermal growth through FGF2. FOXN1 also affects the process of pigmentation, is differentially expressed in benign and malignant tumors, and can be an active contributor to the skin wound healing process. It is likely that FOXN1 governs the homeostasis of the SC niche as a negative regulator, as the lack of FOXN1 creates a proregenerative environment that potentially supports the expansion of the SC niche and its activation. It is also possible that FOXN1 can act in a non-cell-autonomous way by affecting immune cell subsets and, consequently, contributes to scar formation. Future studies will be necessary to determine the exact direct molecular mechanisms of FOXN1 functions in the skin.

ACKNOWLEDGMENTS

We thank Malgorzata Kossut for her helpful suggestions and critical reading of the manuscript.

This article was funded by statutory funds of Nencki Institute of Experimental Biology of Polish Academy of Sciences.

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