The Role of Fibroblast Growth Factor-Binding Protein 1 in Skin Carcinogenesis and Inflammation

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Fibroblast growth factor-binding protein 1 (FGFBP1) is a secreted chaperone that mobilizes paracrine-acting FGFs, stored in the extracellular matrix, and presents them to their cognate receptors. FGFBP1 enhances FGF signaling including angiogenesis during cancer progression and is upregulated in various cancers. Here we evaluated the contribution of endogenous FGFBP1 to a wide range of organ functions as well as to skin pathologies using Fgfbp1-knockout mice. Relative to wild-type littermates, knockout mice showed no gross pathologies. Still, in knockout mice a significant thickening of the epidermis associated with a decreased transepidermal water loss and increased proinflammatory gene expression in the skin was detected. Also, skin carcinogen challenge by 7,12-dimethylbenz[a]anthracene/12-O-tetradecanoyl-phorbol-13-acetate resulted in delayed and reduced papillomatosis in knockout mice. This was paralleled by delayed healing of skin wounds and reduced angiogenic sprouting in subcutaneous matrigel plugs. Heterozygous green fluorescent protein (GFP)—knock-in mice revealed rapid induction of gene expression during papilloma induction and during wound healing. Examination of wild-type skin grafted onto Fgfbp1 GFP—knock-in reporter hosts and bone marrow transplants from the GFP-reporter model into wild-type hosts revealed that circulating Fgfbp1-expressing cells migrate into healing wounds. We conclude that tissue-resident and circulating Fgfbp1-expressing cells modulate skin carcinogenesis and inflammation.

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INTRODUCTION

The fibroblast growth factor-binding protein (FGFBP) family consists of three human and two murine (FGFBP1, 3) members, which are secreted chaperone proteins that bind to FGFs and enhance their biological activity (Tassi and Wellstein, 2006). As the best characterized member, FGFBP1 has been shown to bind to FGF1, 2, 7, 10, and 22 in a reversible manner through its C-terminal domain (Tassi et al., 2011). Paracrine FGFs (e.g., FGF1 and FGF2) are immobilized in the extracellular matrix and are released to bind to their cognate FGF receptors. In this context, FGFBP1 works as a modulator that chaperones the FGFs from their location in the extracellular matrix to target cells expressing FGF receptors.

FGFBP1 is expressed in epithelial cells in skin, stomach, eye, ileum, and colon (Aigner et al., 2002; Kurtz et al., 1997), and was found to act as an angiogenic switch molecule in cancer (Czubayko et al., 1997) and expressed in squamous cell carcinoma (SCC) (Czubayko et al., 1994) and pancreatic and colon cancer (Henke et al., 2006). Also, FGFBP1 is upregulated during a two-step chemical skin carcinogenesis challenge with 7,12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13- acetate (TPA) (Kurtz et al., 2004). We have previously investigated the role of FGFBP1

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Abbreviations: DMBA, 7,12-dimethylbenz[a]anthracene; FGFBP, fibroblast growth factor-binding protein; GFP, green fluorescent protein; KO, knockout; SCC, squamous cell skin cancer; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; WT, wild-type

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in a transgenic mouse model and found that conditionally expressed FGFBP1 accelerated angiogenesis in subcutaneously implanted matrigel plugs, enhanced wound healing, and reduced ischemic hindlimb injury (Tassi et al., 2011). Furthermore, FGFBP1 and FGFBP2 play a critical role during chicken development: knockdown of either caused embryonic lethality in part through vascular leakage (Gibby et al., 2009).

To investigate the function of endogenous FGFBP1, we generated a knockout (KO) and a knock-in green fluorescent protein (GFP)—reporter mouse model, to investigate the role of FGFBP1 in organ functions, skin homeostasis and repair, and during challenge by chemical carcinogenesis.

RESULTS

Generation of Fgfbp1-KO mice

The complete *Fgfbp1* open-reading frame contained in exon 2 on chromosome 5 was replaced by a floxed *Fgfbp1* gene and a neo-GFP cassette, resulting in Fgfbp1$^{+/loxP\text{-}neo\text{-}gfp}$ mice, which were then crossed with mice expressing cre (Figure 1a). The resulting mouse line expressed GFP in lieu of Fgfbp1 (Fgfbp1$^{+/gfp}$) and served to monitor activity of the *Fgfbp1* promoter. To generate Fgfbp1-KO mice, the Fgfbp1$^{loxP\text{-}neo\text{-}gfp}$ mice were crossed with mice expressing the recombinase Flpase and then with cre-expressing mice (Figure 1b—f). The mouse strain in this study is primarily C57Bl/6N and to a lower degree SV129N.

The epidermis of KO mice is thicker and has a reduced permeability

Because endogenous FGFBP1 is predominantly expressed in skin (Aigner et al., 2002), we initially focused on the analysis of the skin phenotype in the KO animals. The epidermis of adult (2—3 months old) but not juvenile KO mice (3 weeks old) showed a twofold increased thickness ($P < 0.05$; Figure 2a; Supplementary Figure S1a online). In contrast, the thicknesses of dermis, fat, and panniculus muscle were not significantly different between wild-type (WT) and KO (Figure 2b). The increased thickness in KO mice coincided with an increased proliferation index in basal keratinocytes (Figure 2c and e). Also, approximately 50% of the epidermis in KO mice showed multiple nucleated keratinocyte strata in contrast with a mostly single basal layer in WT (Figure 2d). Commensurate with epidermal thickening in KO mice, transepidermal water loss was decreased (Figure 2f). Also, the global expression analysis of skin RNA indicates significantly altered pathways related to barrier function in KO versus WT mice (Supplementary Figure S2a online) although staining for Claudin1 and Filaggrin showed no differences in the epidermis between WT and KO (Figure 2e). The analysis of RNA from the epidermis and from the dermis showed a higher expression of the macrophage marker F4/80 and Ffgr1c in the epidermis of KO mice (Figure 2g). In KO mice, both Fgfr1b and c are expressed at similar levels in the dermis and epidermis, whereas a 10-fold higher expression of the c-isoform was seen in the dermis versus the epidermis of WT animals (Figure 2g). Fgf7 and Fgfr2 were not differentially expressed in the epidermis or dermis (Supplementary Figure S1b).

FGFBP1 upregulation in patients' psoriatic lesions and SCC

Epidermal thickening observed in the Fgfbp1-KO model has been described in inflammatory pathologies of the skin such as psoriasis (Stern, 1997), and FGFR2 and FGF7 were found elevated in psoriatic skin (Guban et al., 2016). To assess FGFBP1 gene expression, we analyzed two previously published gene expression studies (Nair et al., 2009; Reischl et al., 2007) of paired samples of normal skin and psoriatic skin without and with lesions. FGFBP1 is significantly upregulated in lesions but not in unaffected skin (Supplementary Figure S3a and b online). The analysis of another study (Nindl et al., 2006) revealed that FGFBP1 is upregulated in actinic keratosis and invasive SCC relative to normal skin (Supplementary Figure S3c).

Skin epidermis of KO mice shows elevated proinflammatory gene expression

A psoriatic phenotype can be mimicked in mice by topical application of Aldara, a proinflammatory agent (Walter et al., 2013) (Figure 3a). Aldara contains imiquimod and activates the innate immune system via toll-like receptor-7 on neutrophils, macrophages, and dendritic cells and indirectly induces proliferation of keratinocytes (van der Fits et al., 2009). As a readout for activation by Aldara, we monitored myeloperoxidase activity, which increased during Aldara treatment in both WT and KO skin (Figure 3b). In response to Aldara, the expression of Fgfbp1 increased in WT mice (2.5-fold; Figure 3c) as did GFP activity in heterozygous GFP-reporter mice (Fgfbp1$^{+/gfp}$; 6-fold; Figure 3d and e). Aldara treatment also induced a striking 4.5-fold epidermal thickening (Figure 3f and g) and expression of the inflammatory genes Il6 and Il17a as well as the epithelial marker Krt16 (Figure 3h; Supplementary Figure S4a online). The expression of these genes was significantly elevated at baseline in the skin of KO mice, indicating a skin phenotype with activated immune response and thus increased epithelial proliferation (Figures 2b, d and 3f, g). It has been shown that the skin barrier function is highly dependent on FGF receptor expression (Yang et al., 2010). Fgfr1, 2, 3, 4 expressions, however, were not altered significantly in total skin (Supplementary Figure S5 online). These data suggest that the loss of Fgfbp1 induces a baseline increase in proinflammatory gene expression in KO skin comparable to the Aldara treatment effect in WT.

DMBA/TPA-induced skin papilloma formation is reduced and delayed in KO mice

Fgfbp1 expression is increased in mouse skin during carcinogen-induced papilloma formation (Kurtz et al., 2004) suggesting a potential role during carcinogenesis. Six-week-old GFP-reporter mice (Fgfbp1$^{+/gfp}$) treated topically with DMBA/TPA (Figure 4a) showed macroscopically visible GFP activity in papillomas (Figure 4b), GFP protein expression in the more differentiated outer keratinocyte layers of the epidermis (Figure 4c, magnified in Supplementary Figure S4b), and >2-fold GFP mRNA (Figure 4d). In WT skin, Fgfbp1 expression was induced similarly by 2.8-fold (Figure 4k). Skin biopsies taken from carcinogen-treated WT and KO mice showed a similar increase in hyperplastic keratinocyte layers as early as 12 days after the first treatment and maximal thickness at 57 days (Figure 4e). However, the appearance of skin papillomas was significantly ($P < 0.01$) delayed in KO mice: the first lesions in WT mice were found after 1.5 months (day 44) and only a month later (day 73) in