Epidermal FABP Prevents Chemical-Induced Skin Tumorigenesis by Regulation of TPA-Induced IFN/p53/SOX2 Pathway in Keratinocytes

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Skin lipids (e.g., fatty acids) are essential for normal skin functions. Epidermal FABP (E-FABP) is the predominant FABP expressed in skin epidermis. However, the role of E-FABP in skin homeostasis and pathology remains largely unknown. Herein, we utilized the 7,12-dimethylbenz(a)anthracene and 12-O-tetradecanoylphorbol-13-acetate induced skin tumorigenesis model to assess the role of E-FABP in chemical-induced skin tumorigenesis. Compared to their wild-type littermates, mice deficient in E-FABP, but not adipose FABP, developed more skin tumors with higher incidence. 12-O-tetradecanoylphorbol-13-acetate functioning as a tumor promoter induced E-FABP expression and initiated extensive flaring inflammation in skin. Interestingly, 12-O-tetradecanoylphorbol-13-acetate-induced production of IFN-β and IFN-λ in the skin tissue was dependent on E-FABP expression. Further protein and gene expression arrays demonstrated that E-FABP was critical in enhancing IFN-induced p53 responses and in suppressing SOX2 expression in keratinocytes. Thus, E-FABP expression in skin suppresses chemical-induced skin tumorigenesis through regulation of IFN/p53/SOX2 pathway. Collectively, our data suggest an unknown function of E-FABP in prevention of skin tumor development, and offer E-FABP as a therapeutic target for improving skin innate immunity in chemical-induced skin tumor prevention.

INTRODUCTION

As the largest organ in mammals, skin consists of epidermis, dermis, and subcutaneous tissue and serves as a physical and immunologic barrier to the external environment. Keratinocytes are the primary cell type in the epidermis, undergoing continuous cycles of homeostatic proliferation and differentiation to maintain skin integrity and to protect against various environmental insults. Skin squamous cell carcinoma is the second most common skin cancer. Most of the increasing incidence is due to UV-induced squamous cell carcinoma, especially in light-skinned individuals with chronic cutaneous inflammation (Leiter et al., 2014; Lund et al., 2016). Despite recent studies revealing different cellular and molecular mechanisms, such as up-regulation of SOX2 in cancer stem cells, in the control of initiation and progression of primary skin squamous cell carcinoma (Boumahdi et al., 2014; Lapouge et al., 2012), how normal keratinocytes maintain their homeostasis and keep their functionality to prevent tumorigenesis during the process of continuous skin differentiation remains unknown.

Epidermal FABP (E-FABP, also known as mal1 or FABP5) was first cloned from psoriatic skin tissue due to its high up-regulation in psoriatic keratinocytes (Madsen et al., 1992). It belongs to the family of FABPs because it binds long-chain fatty acids and other hydrophobic ligands in the cytosol. Although FABPs are believed to play important roles in metabolic and inflammatory pathways through coordinating lipid transport and metabolism inside cells (Hotamisligil and Bernlohr, 2015; Storch and McDermott, 2009), the exact role of E-FABP in keratinocytes remains largely unknown. Several recent studies report that E-FABP expression in keratinocytes contributes to the water permeability barrier of the skin and promotes keratinocyte differentiation by enhancing fatty acid-mediated keratin 1 expression (Owada et al., 2002; Ogawa et al., 2011). Our group, and others, have demonstrated that E-FABP is also expressed in immune cells and plays a role in regulating T-cell differentiation and macrophage pro-inflammatory functions (Li et al., 2009; Moore et al., 2015; Zhang et al., 2014, 2015). Given the emerging role of E-FABP in

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Abbreviations: A-FABP, adipose FABP; DMBA, 7,12-dimethylbenz(a)anthracene; E-FABP, epidermal FABP; TPA, 12-O-tetradecanoylphorbol 13-acetate; WT, wild-type

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both keratinocytes and immune cells, we reasoned that E-FABP expression in the skin tissue might represent a previously unknown molecular mechanism in maintaining normal skin homeostasis and surveillance, thus playing a critical role in the prevention of environmentally induced tumorigenesis in skin.

7,12-Dimethylbenz(a)anthracene (DMBA)/12-O-tetradecanoylphorbol 13-acetate (TPA), also known as PMA in cellular studies) model is commonly used to chemically induce multistage skin tumorigenesis due to its easy performance (Abel et al., 2009; Kemp, 2005). The use of this two-step tumorigenesis model has identified many host-derived factors associated with skin tumor development. For example, p53 expression in stratified epithelia protects against DMBA/TPA-induced skin tumor growth and malignancy (Page et al., 2016). Constitutive expression of type I IFNs is critical in skin tumor prevention (Chen et al., 2009). Herein, we utilized the DMBA/TPA-induced skin tumorigenesis mouse model to determine whether and how host expression of E-FABP played a critical role in skin tumor development. We provide evidence to show that E-FABP expression in keratinocytes prevents skin tumor development by enhancing p53-mediated SOX2 down-regulation in keratinocytes.

RESULTS

Loss of E-FABP promotes DMBA/TPA-induced skin tumorigenesis

To determine the role of E-FABP in skin tumor development, wild-type (WT) and E-FABP<sup>e<sup>e</sup></sup> littermates were subjected to the DMBA/TPA protocol to chemically induce skin tumorigenesis. We did not observe any noticeable differences of the skin appearance after DMBA application between WT and E-FABP<sup>e<sup>e</sup></sup> mice. However, E-FABP<sup>e<sup>e</sup></sup> mice appeared to be more sensitive than WT mice in response to the TPA treatment with development of red skin in some TPA-treated areas, suggesting an early difference of TPA-induced inflammatory responses between WT and E-FABP<sup>e<sup>e</sup></sup> mice. Histological analysis showed an obvious keratinocyte proliferation with comparable infiltration of inflammatory cells in both WT and E-FABP<sup>e<sup>e</sup></sup> mice. Interestingly, keratinocytes in E-FABP<sup>e<sup>e</sup></sup> mice exhibited atypical dysplasia throughout the epidermis as compared to WT mice (Figure 1a and 1b). About 10 days after TPA treatment was initiated, E-FABP<sup>e<sup>e</sup></sup> mice began to develop small skin papillomas, while it took about 6 weeks before similar tumors were observed in WT mice (Figure 1c). With additional TPA applications, skin tumor numbers/mouse and incidence were significantly higher in E-FABP<sup>e<sup>e</sup></sup> mice than in WT mice.
(Figure 1c and 1d). Moreover, tumor burden as calculated by tumor volume/size in E-FABP−/− mice was greater than in WT mice (Figure 1e and 1f). As skin tissue also expresses adipose FABP (A-FABP), another FABP family member (Zhang et al., 2015), we also compared skin tumor development in WT and A-FABP−/− mice using the same DMBA/TPA protocol. There was no obvious impact of the DMBA/TPA regime in A-FABP−/− mice on skin tumor initiation and development as compared to their WT littermates (Supplementary Figure S1a and S1b online). These results indicate that expression of E-FABP, but not A-FABP, in mouse skin tissue is essential in suppressing chemical-induced skin tumorigenesis.

**TPA treatment induces E-FABP−independent and dependent inflammatory responses in skin**

Given the observed differences in skin responses to TPA treatment between WT and E-FABP−/− mice during tumor development, we first analyzed TPA-induced skin inflammatory responses in these mice. TPA treatment of WT and E-FABP−/− mice for 5 hours induced an early response of tumor-associated cytokine expression in skin tissues, such as IL6, IL1α, IL1β, TNF-α, IL10, and TGFβ1, but E-FABP deficiency had no impact on the production of these cytokines (Supplementary Figure S2a through S2f online). After 24 hours of TPA treatment, these acute inflammatory responses, including most cytokines and chemokines (CXCL10 and CXCL11), dissipated rapidly without significant differences between WT and E-FABP−/− mice (Figure 2a through 2f, Supplementary Figure S2g through S2j). However, we noticed that type I IFN-β and type III IFN-λ, but not type II IFN-γ, were significantly up-regulated in the skin of WT mice as compared to E-FABP−/− mice (Figure 2g and 2h, Supplementary Figure S2j). Consistently, elevated levels of IFN-β and IFN-λ in the skin of WT mice were confirmed by

![Figure 2. TPA treatment induces E-FABP−independent and dependent skin inflammation.](image-url)
confocal microscopy (Figure 2i and 2j). These results suggest that TPA induces both E-FABP-independent and E-FABP-dependent inflammatory responses during the promotion of tumor development and the diminished expression of IFN-β and IFN-λ may contribute to the exacerbated tumor development in E-FABP−/− mice.

**E-FABP is critical in PMA-induced production of IFN-β and IFN-λ in keratinocytes**

We noticed that TPA treatment significantly induced skin thickness. As E-FABP is the predominant FABP in skin (Zhang et al., 2015), we speculated that TPA treatment might impact E-FABP expression. To this end, TPA-treated mouse dorsal skin tissues were collected at different time points for analysis of FABP expression. We found that both mRNA and protein levels of E-FABP were significantly increased in response to TPA treatment (Figure 3a and 3b). In contrast, A-FABP expression levels in skin were much lower and did not change in response to TPA treatment (Figure 3c), suggesting a critical role of E-FABP, but not A-FABP, in TPA-induced skin pathogenesis. To confirm the essential role of E-FABP in IFN expression, we isolated keratinocytes from epidermis of WT and E-FABP−/− mice and measured FABP and IFN expression in the presence or absence of PMA stimulation. Similar to in vivo TPA treatment, E-FABP, but not A-FABP, was significantly up-regulated by PMA stimulation in vitro (Figure 3d and 3e). Strikingly, ablation of E-FABP expression in keratinocytes diminished the expression of IFN-β and IFN-λ in primary keratinocytes under both PMA-treated and untreated conditions (Figure 3f and 3g). To further verify the causal effect of E-FABP in IFN production, we knocked down E-FABP expression in the MPEK keratinocyte cell line (derived from the same C57/B6 genetic background) using small interfering RNA (Figure 3h) and demonstrated that E-FABP silencing significantly blocked PMA-induced IFN-l production in keratinocytes (Figure 3i). Of note, IFN-β levels were too low to be detected under the same condition. Altogether, our data indicate that E-FABP expression in keratinocytes is essential for IFN-β and IFN-λ production under TPA-induced conditions, thereby influencing chemically induced skin tumorigenesis.

**E-FABP deficiency suppresses p53 expression in keratinocytes**

To determine how E-FABP expression affected skin tumorigenesis in vivo, we focused on both extrinsic immune surveillance effects and tumor cell intrinsic factors. We first analyzed immune cell phenotypes in tumor-bearing WT and E-FABP−/− mice. As shown in Supplementary Table S1 (online), E-FABP deficiency did not show any apparent
phenotypic changes of immune cell populations in peripheral immune organs. When we further analyzed IFN-γ production in T cells, we did not find any significant alterations in both CD4+ and CD8+ T cells between WT and E-FABPe/e mice (Figure 4a through 4d). It appeared that adaptive T-cell responses did not apparently contribute to the observed tumor differences between WT and E-FABPe/e mice. To investigate whether E-FABP deficiency affected intrinsic tumor signals, we collected tumor samples from WT and E-FABPe/e mice and performed tumor signaling antibody arrays. Among the 250 cancer signaling proteins, we found 9 proteins, including the tumor-suppressive p53 protein, with >2-fold up-regulation in tumors from WT mice as compared to E-FABPe/e mice (Figure 4e, Supplementary Table S2 online). These data suggest that E-FABP deficiency may lead to the differential expression of these cancer signaling proteins, thus contributing to the accelerated tumorigenesis in E-FABPe/e mice.

Figure 4. E-FABP deficiency is associated with reduced p53 expression in keratinocytes. Draining lymph nodes (LNs) and spleen were collected from DMBA/TPA-treated tumor bearing mice and analyzed for (a) IFN-γ and IL17 production by gating on CD4+ T cells and (c) IFN-γ production in CD8+ T cells by flow intracellular staining. Average percentage of IFN-γ positive cells in CD4+ and CD8+ cells was shown in b and d, respectively (n = 4/group). (e) Cancer signaling proteins with relative fold >2 between WT and E-FABP−/− skin tumors were shown by protein array analysis. (f-i) Quantitative polymerase chain reaction (qPCR) analysis of relative expression of (f) β-catenin, (g) CDC25c, (h) Chk2, (i) C-Jun, (j) Myc, (k) p38, (l) and p53, in skin of naive WT and E-FABP−/− mice. (m) qPCR analysis of relative p53 levels in primary keratinocytes from WT and E-FABP−/− mice after PMA treatment for 24 hours (n = 3/group, *P < 0.05). (n) Western blotting analysis of p53 expression in skin cell lysates after anti-p53 immunoprecipitation. The supernatants after p53-immunoprecipitation were directly analyzed for the expression of E-FABP and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). DMBA, 7,12-dimethylbenz(a)anthracene; E-FABP, epidermal FABP; TPA, 12-O-tetradecanoylphororbol 13-acetate; WT, wild-type.
To assess whether E-FABP deficiency in keratinocytes per se or whether secondary changes due to tumor development were responsible for the differential cancer signaling proteins, we compared transcriptional levels of these proteins in skin tissues of naïve WT and E-FABP<sup>−/−</sup> mice. Interestingly, among the 7 proteins with detectable transcripts, including β-catenin, CDC25c, Chk2, c-Jun, Myc, p38, and p53, we only found that relative levels of p53 mRNA were significantly reduced when E-FABP was genetically deleted (Figure 4f through 4l). The reduced mRNA and protein levels of p53 in E-FABP<sup>−/−</sup> mice were confirmed in PMA-stimulated primary keratinocytes (Figure 4m and 4n). In contrast, E-FABP deficiency did not decrease the expression of other proteins in keratinocytes after PMA stimulation (Supplementary Figure S3 online). It is very likely that E-FABP deficiency predisposes mice to skin tumorigenesis due to the reduced expression of p53 in keratinocytes.

**IFN-β and IFN-λ increase p53 expression in keratinocytes and inhibit their proliferation**

It has been previously demonstrated that type I IFNs induced p53 gene transcription and protein expression for tumor suppression and antiviral defense (Takaoka et al., 2003). Given the observations mentioned, that E-FABP<sup>−/−</sup> keratinocytes exhibited impaired IFN-β/IFN-λ production and reduced p53 expression, we wondered whether IFN-β and IFN-λ directly induced activation of p53 and inhibited proliferation of keratinocytes. IFN-β and IFN-λ function through IFNAR1 and IFNLR1, respectively. We first measured the expression of IFNAR and IFNLR of WT and E-FABP<sup>−/−</sup> mice and found that E-FABP deficiency did not affect IFN receptor expression in the skin tissue (Figure 5a). Upon treatment with IFN-β and IFN-λ, MPEK keratinocytes exhibited strong IFN-induced responses, including up-regulation of IRF-7, STAT1, and Viperin (Figure 5b through 5d), which were consistent with anti-tumor signaling induced by IFN-β/λ (Romieu-Moureze et al., 2006). Most importantly, treatment with IFN-β or IFN-λ was able to induce p53 up-regulation in MPEK keratinocytes (Figure 5e). Consistent with other studies showing p53 as a brake on cell proliferation (Crochemore et al., 2002), we assessed keratinocyte proliferation by measuring Ki-67 expression with flow cytometric staining and showed that IFN treatment significantly inhibited MPEK keratinocyte proliferation as compared to untreated controls (Figure 5f and 5g). Notably, IFN-β and IFN-λ treatment did not induce apparent cell death of MPEK keratinocytes (Figure 5h and 5i). When primary keratinocytes from WT and E-FABP<sup>−/−</sup> mice were treated with IFN-β and IFN-λ, respectively, they exhibited similar responses as MPEK cells, suggesting that E-FABP deficiency does not appear to affect IFN-induced responses in primary keratinocytes (Supplementary Figure S4a through S4c). Taken together, our data suggest that E-FABP expression in keratinocytes promotes the production of IFN-β and IFN-λ, which up-regulate tumor suppressor p53, therefore, suppressing skin tumor development.

**E-FABP deficiency is associated with up-regulation of SOX2**

It is well known that p53 suppresses tumor development by targeting different genes and pathways involved in cell growth arrest, senescence, death, and angiogenesis (Wang and Sun, 2010). To further dissect the molecular mechanisms by which E-FABP expression provided a protective role against skin tumorigenesis, we performed gene microarray analysis using tumors from WT and E-FABP<sup>−/−</sup> mice. Of note, genes encoding cancer stemness markers (eg, SOX2) were among the most up-regulated genes in the E-FABP<sup>−/−</sup> tumors (Figure 6a, Supplementary Table S3 online). Using quantitative real-time polymerase chain reaction, we confirmed that SOX2 expression was significantly up-regulated in the E-FABP<sup>−/−</sup> keratinocytes (Figure 6b through 6d). With confocal analysis, we found that E-FABP was highly expressed in the epidermis of WT mice. Interestingly, when E-FABP was absent in the epidermis, SOX2 expression was up-regulated at the basolateral surface close to the dermis (Figure 6e). Moreover, we observed consistent SOX2 up-regulation in E-FABP<sup>−/−</sup> skin as compared to WT skin under either untreated or TPA-treated conditions (Figure 6f through 6h), further corroborating that E-FABP deficiency is associated with elevated levels of SOX2 expression in keratinocytes. Given the observations that SOX2 controls tumor initiation in squamous cell carcinoma and that the p53 pathway inhibits the expression of SOX2 (Boumahdi et al., 2014; Wang and Sun, 2010; Wang et al., 2016), our data reveal a previously unreported mechanism wherein E-FABP regulates IFN/p53/SOX2 axis in keratinocytes for prevention of skin tumorigenesis.

**DISCUSSION**

Although E-FABP is highly expressed in skin epidermis, the biological functions of E-FABP in skin remain largely unexplored. Using congenic E-FABP<sup>−/−</sup> and WT mice, we demonstrate that E-FABP expression is critical in suppression of DMBA/TPA-induced skin tumorigenesis. Mechanistically, E-FABP expression in skin of WT mice promotes IFN-β and IFN-λ production and responses, accompanied by up-regulated p53 expression and decreased SOX2 expression as compared to E-FABP<sup>−/−</sup> mice. Thus, E-FABP represents a previously unknown molecular mechanism by which the host maintains skin homeostasis and prevents environmental factor-induced skin tumor development.

The FABP family consists of nine members that bind hydrophobic lipid ligands (eg, endogenous or exogenous long-chain fatty acids and their derivatives) and coordinate their transportation, metabolism and functions (Hertzel et al., 2006; Hotamisligil and Bernlohr, 2015). Due to the tightly regulated patterns of tissue distribution, FABPs are named according to the tissue where they are predominantly regulated. For example, E-FABP and A-FABP are highly expressed in epidermis and adipose tissue, respectively. Considering their fatty acid binding characteristics, FABPs are believed to play important roles in cells responsible for lipid uptake, storage, and those that use lipids as a major energy source, such as macrophages, adipocytes, and memory T cells (Makowski et al., 2005; Nieman et al., 2011; Pan et al., 2017). Skin protects the host against environmental insults by providing an effective physical barrier, among which lipid synthesis, composition, and transportation are critical in maintaining normal skin integrity and function. E-FABP is the predominant FABP member expressed in keratinocytes (Zhang et al., 2015). However, the role of E-FABP...
in keratinocytes, especially in squamous cell carcinoma development, is unclear. Here, we provide evidence showing that E-FABP expression in skin is essential for inhibition of chemically induced skin tumorigenesis. Notably, A-FABP is also expressed in skin, although to a lesser extent, A-FABP expression does not appear to play a significant role in skin tumorigenesis.

Lipids in keratinocytes are composed of free fatty acids, cholesterol, and ceramides, among which polyunsaturated fatty acids are essential for skin structural integrity and normal skin barrier function (Hansen et al., 1958; Pappas, 2009). As E-FABP binds different dietary fatty acids with a high affinity for polyunsaturated fatty acids (Lee et al., 2015), keratinocytes deficient in E-FABP exhibit reduced incorporation of polyunsaturated fatty acids, including linoleic acids (Ogawa et al., 2011). We also noticed that skin epidermis was thinner in E-FABP−/− mice when compared to WT mice. It is worth noting that studies from our group and others have shown that polyunsaturated fatty acids taken up by macrophages or other types of cells promote the formation of lipid droplets, which function as a platform for the production of type I IFNs (Hinson and Cresswell, 2009; Mei et al., 2011; Zhang et al., 2014, 2017), thus, it was not surprising that we observed reduced production of both IFN-β and IFN-λ in E-FABP−/− keratinocytes. However, the restrictive expression pattern of

![Image of graphs and tables](https://www.jidonline.org/1931)
IFN-λ receptors on epithelial cells suggests its unique role in skin defense.

As immune sentinels, keratinocytes can discriminate pathogens, sense danger signals, and induce immune responses (Nestle et al., 2009). For example, keratinocytes can abundantly produce IFN-β in psoriasis and during wound healing (Zhang et al., 2016), which is consistent with our data showing TPA/PMA stimulation up-regulated IFN-β

Figure 6. SOX2 expression is up-regulated in skin of E-FABP deficiency mice. (a) Heatmap of differentially expressed genes with fold >3 in tumors from WT and E-FABP−/− mice. (b–d) Real-time polymerase chain reaction analysis of expression of (b) Gpx2, (c) OCT3, and (d) SOX2 in primary keratinocytes from WT and E-FABP−/− mice (n = 3/group, **P < 0.01 as compared to WT mice). (e) Confocal microscopic analysis of expression of E-FABP (green color) and SOX2 (red color) in keratinocytes of skin epidermis from WT and E-FABP deficiency mice (scale bar = 10 μm). (f–h) Western blotting analysis of SOX2 and E-FABP expression in skin of (f) untreated naïve mice, (g) mice treated with TPA for 1 day, and (h) mice treated with TPA for 3 days. E-FABP, epidermal FABP; TPA, 12-O-tetradecanoylphorbol 13-acetate; WT, wild-type.
production in primary keratinocytes. However, we noticed that resting/unstimulated keratinocytes do not produce detectable levels of IFN-β. In contrast, IFN-λ is constitutively produced by resting keratinocytes and can be up-regulated during external stimuli, indicating a unique role of IFN-λ in maintaining skin homeostasis. Given the observations that IFN-β induces p53 gene transcription and protein production for anti-tumor and anti-viral defense (Takaoka et al., 2003), we speculated that IFN-λ might exert similar functions in inducing the p53 response. Indeed, we demonstrate that IFN-λ treatment of keratinocytes up-regulates p53 protein, which explains why naive E-FABP−/− mice exhibit reduced expression of p53 as compared to naive WT mice. Moreover, E-FABP has been shown to promote keratinocyte differentiation through inducing expression of keratin 1 and involucrin (Dallaglio et al., 2013; Ogawa et al., 2011), our studies suggest that E-FABP is able to function via IFN-λ/p53 pathway to maintain normal cycling of keratinocytes under physiologic conditions and to prevent keratinocyte oncogenic transformation due to environmental insults.

Given the numerous targets of p53-regulating genes for tumor suppression, we analyzed the differentially expressed genes between WT and E-FABP−/− tumors to identify the potential targets mediating E-FABP/p53 protective effects. Interestingly, SOX2 was the most up-regulated gene expressed in E-FABP−/− tumors. As a transcription factor, SOX2 has been shown to be essential in maintenance of self-renewal and pluripotency of stem cells (Rizzino, 2009). Accumulated evidence also demonstrates that SOX2 is a key up-regulated oncogene promoting squamous cell carcinoma in skin, lung, colon, and breast (Boumahdi et al., 2014; Hussenet et al., 2010; Piva et al., 2014; Tani et al., 2007). More interestingly, recent studies report that the p53-dependent pathway directly controls a critical checkpoint of SOX2-mediated glial cell reprogramming and mesenchymal-to-epithelial transition (Brosh et al., 2013; Wang et al., 2016). In addition, we also observed that E-FABP deficiency is associated with other cancer stemness markers, such as Nanog, in the skin tissue before and after PMA treatment (data not shown). Integration of our observations and documented research suggests that E-FABP enhancement of p53 pathway inhibits SOX2 expression in keratinocytes, thus reducing chemical-induced skin tumorigenesis.

In summary, our studies demonstrate a previously unknown function of E-FABP in skin tumor prevention through regulating IFN/p53/SOX2 pathway, indicating that E-FABP expression in skin is critical in host against chemical-induced tumorigenesis. Thus, targeting E-FABP and related signaling molecules may provide strategies for skin cancer prevention and immunotherapy.

MATERIALS AND METHODS

Animals
Mice deficient for E-FABP or A-FABP were generated as described previously (Hotamisligil et al., 1996; Maeda et al., 2003). For the skin tumorigenesis model, 6- to 8-week-old male WT, E-FABP−/−, or A-FABP−/− mice (C57BL/6 background) were used for application of DMBA and TPA. Due to the carcinogenic properties, DMBA and TPA were applied strictly following the protocol approved by the Institutional Animal Care and Use Committee. See detailed DMBA/TAP-induced skin tumors and skin cell preparation in the Supplementary Materials (online).

Culture and treatment of keratinocytes
The mouse keratinocyte cell line, MPEK, was purchased from ZenBio (Research Triangle Park, NC). Cells were cultured and propagated in CnT-07 medium (ZenBio). These cells were treated with different concentrations of PMA, recombinant mouse IFN-β (100U) (BioLegend, San Diego, CA; cat#: 581302) or recombinant mouse IFN-λ3 (R&D System, Minneapolis, MN; cat#: 1789-ML-025/CF), respectively, in vitro for 7 or 14 hours. For primary keratinocytes isolated from WT or knockout mice, R5 (RPMI-1640 supplemented with 5% fetal bovine serum and 20 μg/ml gentamicin) was used for short-term culture. Complete minimum essential medium or CnT-07 medium was used for overnight culture.

Flow cytometry and confocal analyses
Flow cytometry and confocal analyses were performed as described previously (Rao et al., 2015). See detailed antibody and clone information in the Supplementary Materials.

Quantitative real-time polymerase chain reaction and gene expression microarray
For real-time quantitative polymerase chain reaction and microarray analyses, total RNA from cells was extracted using RNeasy Mini Kit (Qiagen, Valencia, CA). The cDNAs were synthesized using QuantiTect Reverse Transcription Kit (Qiagen). Quantitative polymerase chain reaction was performed with SYBR Green PCR Master Mix using ABI 7500 Real-Time PCR Systems or StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). Relative mRNA levels were determined using HPRT1 as a reference gene. Primer sequences are listed in Supplementary Table S4 (online). Gene expression microarray was performed at the Gene Expression Core at Mayo Clinic, Rochester, MN. The assigned Gene Expression Omnibus accession number was GSE109583.

Western blotting and protein array
Western blotting was performed as described previously (Zhang et al., 2014). Anti-p53 antibody (FL-393 and sc-98; Santa Cruz Biotechnology, Santa Cruz, CA), anti-SOX2 antibody (D-9, cat#sc-398254; Santa Cruz Biotechnology), anti-E-FABP antibody (cat#AF1476; R&D Systems), and anti-β-actin antibody (2F1-1, cat#643802; BioLegend), were used for overnight incubation (binding). For analysis of p53 expression in primary keratinocytes, skin lysates were immunoprecipitated with anti-p53 antibody (FL-393) before p53 immunoblotting (sc-98). Skin tumors from WT and E-FABP−/− mice (n = 3/group) were analyzed using the array for tumor signaling protein and phosphorylation (Full Moon BioSystems, Sunnyvale, CA).

E-FABP knockdown with small interfering RNAs
Duplex small interfering RNAs targeting the coding region of E-FABP were ordered from Integrated DNA Technologies (Coralville, IA). For E-FABP knockdown in the MPEK keratinocyte cell line, cells were transfected with 20 nM siRNA using Oligofectamine (Life Technologies, Carlsbad, CA) when 50% cells confluency was reached. Transfected cells were then treated 24 hours later.

Statistical analysis
Unpaired t test with Welch’s correction or Mann-Whitney test was used for data analyses. A p-value of <0.05 was considered significant.

CONFLICTS OF INTEREST
The authors state no conflict of interest.
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SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.02.041.

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