I κ B Kinase Alpha and Cancer

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I κ B kinase alpha (*Ikk- α*) gene mutations and IKK- α downregulation have been detected in various human squamous cell carcinomas (SCCs), which are malignancies derived from squamous epithelial cells. These squamous epithelial cells distribute to many organs in the body; however, the epidermis is the only organ mainly composed of stratified squamous epithelial cells, called keratinocytes. SCC is the second most common type of skin cancer. Reducing IKK- α expression promotes tumor initiation, and its loss greatly enhances tumor progression from benign papillomas to malignant carcinomas during chemical skin carcinogenesis in mice. Thus, IKK- α has emerged as a tumor suppressor for SCCs. Furthermore, inducible deletion of IKK- α in the keratinocytes of adult mice causes spontaneous skin papillomas and carcinomas, indicating that IKK- α deletion functions as a tumor initiator as well as a tumor promoter. This article discusses IKK- α biological activities and associated molecular events in skin tumor development, which may provide insight into the diagnosis, treatment, and prevention of human squamous cell carcinomas (SCCs) in the future.

Ikk- α Is a Target of Skin Carcinogenesis

REDUCTION OF I κ B kinase alpha (IKK- α) expression promotes tumor initiation, and its loss enhances tumor progression in chemical skin carcinogenesis (Park and others 2007). On the other hand, transgenic mice overexpressing IKK- α in the epidermal keratinocytes (Lori.IKK- α and K5.IKK- α) develop normally and remain healthy throughout their adult lives (Liu and others 2006, 2011). Elevated IKK- α levels repress skin tumor progression and metastasis. These biological activities indicate that retaining IKK- α levels is important for preventing skin tumor development.

Human *Ikk- α* is located at 10q24.31, close to *Pten* (10q23). It has been reported that alterations in more than 1 gene within the 10q22-10q26 region may be involved in human cancer development; however, *Pten* mutations are not found frequently in human and mouse SCCs (Petersen and others 1998; Kubo and others 1999; Mao and others 2004). Down-regulated IKK- α expression has been reported in various human SCCs (Liu and others 2006; Greenman and others 2007; Descargues and others 2008; Marinari and others 2008; Kwak and others 2011). The 5'-flanking regulatory elements of the *Ikk- α* gene contain cytosine-phosphate-guanine (CpG) islands that can be methylated, resulting in reduced IKK- α expression in human SCCs (Maeda and others 2007). Various *Ikk- α* mutations have been reported in human cancers (Liu and others 2006; Greenman and others 2007). In addition, *Ikk- α* mutations have been identified in skin papillomas and carcinomas derived from wild-type and *Ikk- α ^{+/-}* mice induced by the chemical carcinogens 7,12-dimethylbenz(a)anthracene

(DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA) (Park and others 2007). Interestingly, we found that DMBA/DMBA (complete skin carcinogenesis)-induced skin carcinomas contain more *Ikk- α* mutations and deletions than DMBA/TPA (2-stage skin carcinogenesis)-induced carcinomas in wild-type mice (our data not shown). We have observed more transition mutations (T $\rightarrow$ C and A $\rightarrow$ G) than transversion mutations in the *Ikk- α* gene in human and mouse skin SCCs (Liu and others 2006; Park and others 2007). We detected some "hot spot" mutations of *Ikk- α* in human and mouse SCCs. Some mutations cause amino acid substitutions or generate a stop codon, which may change the protein conformation or delete the protein. Deletions and insertions can cause frame-shift mutations, resulting in IKK- α deletion. We isolated several full-length IKK- α cDNAs from DMBA/DMBA-induced carcinomas and tested the activities of 3 IKK- α clones in regulating the expression of targets of IKK- α , cell cycle, and keratinocyte proliferation and differentiation (Zhu and others 2007; Liu and others 2008). The degree to which IKK- α activity is impaired is proportional to the number of mutations present in the IKK- α cDNA. Thus, various mutations of *Ikk- α* impair the normal functions of IKK- α and may contribute to skin tumor development.

In chemical skin carcinogenesis, DMBA induces activating *H-ras* mutations at the V61 and G12/13 codons at an early stage of tumor development and repeated treatment with TPA expands H-Ras-targeted cells (Balmain and Pragnell 1983). We found that although *Ikk- α ^{+/-}* mice develop 2 times more skin tumors than wild-type mice, the percentages of

H-ras mutations among wild-type and *Ikk- α ^{+/-}* papillomas and carcinomas are similar (Park and others 2007). We found no increase in *H-ras* mutations in *Ikk- α ^{+/-}* normal skin compared with wild-type normal skin 2 weeks after DMBA treatment. These results suggest that IKK- α reduction provides a growth advantage and cooperates with H-Ras in promoting skin tumor development. In addition, we do not observe *Ikk- α* mutations in the normal skin of wild-type and *Ikk- α ^{+/-}* mice treated with DMBA. Thus, DMBA may not directly target the *Ikk- α* gene at the tumor initiation stage. Since IKK- α deletion in keratinocytes can cause spontaneous skin papillomas and carcinomas in mice (Liu and others 2008), the mutations that cause IKK- α loss may contribute to the conversion of papillomas into carcinomas. Interestingly, skin tumors contained mutations in the *Ikk- α* gene, but not in those genes adjacent to the *Ikk- α* locus. It is not known why the *Ikk- α* gene predisposes to mutagenesis during carcinogenesis. It is also important to investigate whether some mutations in the *Ikk- α* gene can turn IKK- α function from a tumor suppressor to an oncogene.

Ultraviolet B (UVB) light is a common cause of human skin cancer and it is a complete carcinogen in skin carcinogenesis in mice (Xia and others 2010). UVB irradiation induces DNA damage and targets *p53*. Human cutaneous SCCs contain *p53* mutations (Jonason and others 1996). We found that IKK- α reduction increases UVB signature *p53* mutations and reduces apoptotic cells in the skin of UVB-treated mice (Xia and others 2010). *Ikk- α ^{+/-}* mice develop many more skin tumors than wild-type mice in UVB skin carcinogenesis. Thus, the mutagenesis of the *p53* tumor suppressor gene can be enhanced in IKK- α -deficient cells.

Overall, *Ikk- α* is a target of mutagens during skin carcinogenesis. These mutations may change the protein conformation to make IKK- α unstable or generate a stop codon that blocks IKK- α expression, both of which cause reduced IKK- α . Reduced IKK- α can promote the oncogenic H-Ras pathway in chemical skin carcinogenesis and increase *p53* mutations in UVB skin carcinogenesis (Fig. 1).

FIG. 1. The *Ikk- α* gene is a target of skin carcinogenesis.

IKK- α Levels Are Critical for Skin Tumor Development

Tumor development is antagonized by increased expression of many tumor suppressors in neoplasms, while mutations that downregulate the expression of these genes can promote tumor progression (Bartkova and others 2005). IKK- α levels are increased in response to UVB or TPA (Li and Karin 1998; Park and others 2007) (our data not shown). IKK- α expression is upregulated in mouse skin and skin papillomas induced by DMBA/TPA (Park and others 2007). We found that the induced IKK- α levels are lower in the skin and papillomas of *Ikk- α ^{+/-}* mice than in the skin and papillomas of wild-type mice. As a result, tumor latency is significantly shorter and tumor numbers are higher in *Ikk- α ^{+/-}* mice than in wild-type mice. Furthermore, we have demonstrated that a higher IKK- α level is more effective in repressing IKK- α loss-induced epidermal hyperproliferation and increasing terminal differentiation than a low IKK- α level in the skin of mice by using 2 transgenic mouse lines expressing different levels of transgenic IKK- α in the basal layer of the epidermis (Liu and others 2011). IKK- α represses epidermal growth factor receptor (EGFR), extracellular signal-regulated kinase (ERK), activator protein 1 (AP-1), and signal transducer and activator of transcription 3 (Stat3) activities, whereas IKK- α elevates the expression of Max dimer protein 1 (Mad1) and ovo-like 1 (Ovol1), c-Myc antagonists, and keratinocyte terminal differentiation in a dose-dependent manner in the skin of mice (Satterwhite and others 2001; Nair and others 2006; Descargues and others 2008). Taken together, these results indicate that maintaining an adequate dose of IKK- α is important for protecting the skin from various harmful stimulations and carcinogen attacks.

On the other hand, we have observed increased IKK- α levels in some cases of human SCCs (our unpublished data). The accumulated IKK- α is primarily contained within the cytoplasm of the human SCCs, while the majority of IKK- α is usually found in the nucleus of normal epidermal keratinocytes (Descargues and others 2008). In addition, we have shown that reintroduced IKK- α only rescues primary cultured *Ikk- α ^{-/-}* keratinocytes, but not transformed *Ikk- α ^{-/-}* keratinocytes (Liu and others 2009), indicating that the IKK- α -associated pathways are interrupted in transformed keratinocytes. Therefore, it is possible that the tumor cells no longer respond to IKK- α -mediated inhibitory signaling. It is also possible that the tumor cells carry an accumulation of mutated IKK- α which lacks the normal function of IKK- α . These questions remain to be addressed.

Molecular Events in IKK- α -Involved Skin Tumorigenesis

IKK- α (Chuk) is an 85-kD protein that contains a serine and threonine kinase domain, a leucine zipper, and a helix-loop-helix motif (Connelly and Marcu 1995; Mock and others 1995; DiDonato and others 1997). These domains are used to phosphorylate proteins, form homodimers and heterodimers with the homologous IKK- β protein, and interact with proteins. Although it has been shown that IKK- α forms complexes with the chromatin of many genes (Anest and others 2003; Yamamoto and others 2003; Zhu and others 2007; Liu and others 2008), it is not known whether IKK- α can directly bind to DNA to regulate gene expression. *Ikk- α ^{-/-}* mice

exhibit distinct phenotypes in different organs, suggesting that in these different types of cells, IKK- α functions differently. The most severe phenotype in *Ikk- α* knockout mice is the malformation of the epidermis, which displays striking hyperplasia and a lack of terminally differentiated skin-cornified layers, leading to the death of mutants soon after birth (Hu and others 1999; Li and others 1999; Takeda and others 1999).

IKK- α is 1 subunit of the IKK complex composed of IKK- α , IKK- β , and IKK- γ (Ghosh and Karin 2002). Nuclear factor (NF)- κ B inhibitor I κ B- α binds to NF- κ B proteins, masking the nuclear translocation signal within NF- κ B proteins, thereby blocking NF- κ B transcriptional activity. IKK- α and IKK- β , 2 highly conserved protein kinases, can phosphorylate I κ B α . This phosphorylation induces I κ B- α degradation, thereby freeing and activating the classical NF- κ B pathway (Ghosh and Karin 2002). Surprisingly, the activity of IKK- α in embryonic development and in regulating keratinocyte proliferation and differentiation does not require its kinase activity (Cao and others 2001; Hu and others 2001; Sil and others 2004). These findings indicate that a separate activity of the IKK- α homodimers is required for embryonic development and skin biology.

IKK- α loss acts as a tumor initiator in inducing spontaneous skin tumors in mice as well as in promoting skin tumor progression (Park and others 2007; Liu and others 2008). Such significant biological activity possessed by IKK- α suggests that a single gene mutation may be able to target multiple genes and pathways, which are involved in regulating cell growth control, cell death, gene expression, and genomic stability. Revealing these molecular events and identifying targets will provide insight into the prevention and therapeutic treatment of human SCCs.

EGFR/AP-1/Stat3 pathways

Inactivation of EGFR or reintroduction of IKK- α can prevent IKK- α deletion-induced epidermal hyperplasia and skin tumor development, indicating a cross-talk between IKK- α and EGFR (Liu and others 2008). Inhibitors for EGFR, ERK, A disintegrin and metalloprotease (Adam), RasN17 (dominant negative form), or reintroduced IKK- α can repress EGFR, Ras, and ERK activities, as well as levels of EGFR ligands in IKK- α -null keratinocytes and mouse skins. Thus, IKK- α antagonizes an EGFR-led autocrine pathway composed of EGFR, Ras, ERK, and EGFR ligands (including the ligands' activators). The majority of immuno-stained IKK- α is observed in the nuclei of normal epidermal cells in mice and humans (Marinari and others 2008; Liu and others 2011). We have shown that IKK- α forms complexes with the 5'-flanking regulatory regions of the genes *Egf*, *HB-Egf*, *amphiregulin*, and *Adam12,17,19* that encode proteins which can activate growth factors and cytokines by cleaving the precursors of these factors (Huovila and others 2005; Liu and others 2008). The binding of IKK- α to these genes is found to repress their expression levels, whereas, in the absence of IKK- α in keratinocytes, the expression levels of these genes are elevated. Thus, nuclear IKK- α functions to suppress the EGFR/Ras/ERK pathway to maintain skin homeostasis and prevent skin tumorigenesis.

In addition, we have reported increased AP-1 and Stat3 activities in IKK- α -null skin (Liu and others 2011). Reintroduced IKK- α or inactivated EGFR also represses AP-1

and Stat3 activities (Zenz and others 2003, 2008; Sano and others 2008). It is known that excessive AP-1 and Stat3 activities promote skin tumor development (Zenz and others 2003; Chan and others 2008; Sano and others 2008). Thus, IKK- α cross-talks with EGFR, AP-1, and Stat3 pathways in a loop. Targeting AP-1 or Stat3 signaling may also prevent skin tumor development in IKK- α deficient mice. Although EGFR regulates Stat3 activity, whether IKK- α directly regulates the Stat3 pathway remains to be investigated.

Mad1/Ovol1/Smad/TGF- β pathway

The transforming growth factor β (TGF- β)/Smad2/3 pathway is important for skin tumor development and skin homeostasis (Li and others 2006). IKK- α is found to interact with Smad2/3 transcriptional factors, which are required for TGF- β -induced expression of Mad1 and Ovol1, differentiation inducers, and c-Myc antagonists (Descargues and others 2008; Marinari and others 2008). IKK- α loss downregulates Mad1 and Ovol1 expression in human SCCs and mouse skin, whereas elevated IKK- α expression increases Mad1 and Ovol1 levels in keratinocytes and mouse skin (Descargues and others 2008; Liu and others 2011). Thus, IKK- α inhibits c-Myc activity via the TGF- β /Smad-mediated signaling pathway.

Cyclin D1 and p63

Overexpressed cyclin D1, a G1/S cell cycle regulator, promotes skin tumor development and has been observed in many different types of human tumors (Robles and others 1998; Yamamoto and others 2002). IKK- α loss has been reported to elevate cyclin D1 levels and lead to accumulation of nuclear cyclin D1 (Kwak and others 2011). Conversely, IKK- α was reported to repress cyclin D1 in response to treatment of UVB carcinogen (Song and others 2010). Furthermore, IKK- α and p63 knockout mice exhibit opposite phenotypes in the formation of the epidermis in mice (Hu and others 1999; Li and others 1999; Mills and others 1999; Takeda and others 1999; Yang and others 1999). *p63*^{-/-} mice develop a thin epidermal layer, but a strikingly thick epidermis is found in *Ikk- α* ^{-/-} mice. Cross-talking between the 2 genetic pathways has been demonstrated in the process of epidermal formation (Candi and others 2006; Koster and others 2007). Overexpressed p63 in the epidermis promotes skin tumor development in mice (Koster and others 2006). Thus, IKK- α loss deregulates cyclin D1 and p63, both of which are tumor promoters.

Cell cycle checkpoint

Genomic instability, such as gene mutations, deletions, aneuploid chromosomes, and amplified centrosomes, which can cause irreversible DNA damage, is the hallmark of skin malignant conversion from benign papillomas to malignant carcinomas (Aldaz and others 1988; Wang and others 1998). *Ikk- α* ^{+/-} mice develop 11 times more malignant carcinomas than wild-type mice, and most *Ikk- α* ^{+/-} carcinomas and half of *Ikk- α* ^{+/-} papillomas lose the wild-type *Ikk- α* allele in chemical carcinogenesis models (Park and others 2007), indicating that IKK- α loss promotes tumor progression. The G2/M cell cycle checkpoint controls cell progression. Cells need time to repair DNA errors after DNA damage occurs. The G2/M checkpoint arrests the cell cycle so that cells can do

this job. The defect in the G2/M checkpoint allows cells to carry DNA damage to daughter cells, thereby increasing genomic instability and promoting tumor progression. We have found that the G2/M cell cycle phase is reduced in primary cultured *Ikk- α ^{-/-}* keratinocytes in response to DNA damage (Zhu and others 2007). We have identified 14-3-3 σ , a G2/M cell cycle checkpoint protein (Chan and others 2000), as a target of IKK- α in G2/M cell cycle regulation. Chromatin consists of DNA wound around nucleosomes cores formed from histones (H). H2A-H2B and H3-H4 dimers each form individual core particles. Modifications on histones alter chromatin conformation, which positively or negatively regulate gene expression. Methylation on CpG islands is another regulatory mechanism for repressing gene transcription. The 14-3-3 σ gene contains 30 CpG islands. The methylation of 14-3-3 σ CpG islands is highly associated with many human epithelial cancers (Ferguson and others 2000). We found that 14-3-3 σ is highly methylated and downregulated in primary *Ikk- α ^{-/-}* keratinocytes compared with wild-type keratinocytes (Zhu and others 2007). H3-K9 trimethylation is associated with negative regulation of gene expression (Fischle and others 2003). We have shown that IKK- α interacts with H3, which forms a complex with the 14-3-3 σ locus. In the absence of IKK- α , the 14-3-3 σ gene is found to form a complex with trimethylated H3-K9, and its expression is downregulated. Reintroduced IKK- α reverses the methylation status of the 14-3-3 σ CpG islands and upregulates 14-3-3 σ expression (Zhu and others 2007). In addition, reintroduced IKK- α or 14-3-3 σ rescued the defect in the G2/M cell cycle checkpoint in response to DNA damage. Our result suggests that binding of IKK- α to H3 blocks the ability of trimethyltransferase Suv39h1 to access H3 (Rice and others 2003; Stewart and others 2005), thereby preventing H3-K9 methylation and, consequently, preventing 14-3-3 σ CpG methylation. Although 14-3-3 σ is expressed in the epidermis and papillomas, its expression is downregulated in poorly differentiated carcinomas lacking IKK- α (Dellambra and others 2000; Zhu and others 2007). Aged mice expressing reduced and mutated 14-3-3 σ develop spontaneous skin SCCs (Herron and others 2005). Thus, this IKK- α -mediated cell cycle regulation mechanism via 14-3-3 σ is important for skin tumor development.

Although IKK- α has been reported to phosphorylate serine S10 of H3 (Anest and others 2003; Yamamoto and others 2003), we found increased phosphorylated S10-H3 in the hyperplastic epidermis lacking IKK- α (Liu and others 2011). CpG islands are involved in regulating the expression of many genes. In particular, epigenetic regulatory mechanisms are important for embryonic development or maintaining stem cell properties. Thus, whether IKK- α utilizes this mechanism to regulate the expression of many genes in these biological events remains to be revealed.

The Effect of IKK- α Deficiency-Mediated Inflammation on Skin Carcinogenesis

Epidermal keratinocytes are capable of producing numerous cytokines, chemokines, and growth factors in response to various stimulations. These factors influence organ homeostasis and microenvironment. Excessive responses can contribute to the pathogenesis. As just mentioned, IKK- α deletion elevates EGFR, Stat3, and AP-1 activities (Liu and others 2011). Elevating one of these

pathways not only causes epithelial hyperplasia, but also induces inflammation (epithelial cell-derived inflammation), which promotes skin tumor development (Zenz and others 2003; Sano and others 2008; Liu and others 2011). We have shown elevated macrophages in the skin of *Ikk- α ^{fl/fl}/K5.IKK- α* mice, where EGFR, AP-1, and Stat3 activities and tumor necrosis factor (TNF)- α , interleukin (IL)-1, and IL-6 levels are dramatically elevated (Liu and others 2011). Reintroducing IKK- α or reducing EGFR levels abolishes infiltrating inflammatory cells, reduces cytokines, and inhibits epidermal cell hyperproliferation. Thus, epidermal cell-derived inflammation integrates with epidermal cell proliferation status.

The basal epidermal cells express keratin 5 (K5), and keratin 15 (K15) is expressed in a small population of hair follicular keratinocytes (Morris and others 2004). We have found that IKK- α deletion induced by K5.CreER or K15.CreRP1 induces spontaneous skin papillomas and carcinomas in mice (Liu and others 2008); however, we have observed more infiltrating leukocytes in *Ikk- α ^{fl/fl}/K5.CreER* skin than in *Ikk- α ^{fl/fl}/K15.CreRP1* skin. Indeed, the *Ikk- α ^{fl/fl}/K5.CreER* epidermis is thicker than the *Ikk- α ^{fl/fl}/K15.CreRP1* epidermis. We do not know whether K15- and K5-positive keratinocytes differ in their capacity to produce inducers of inflammation or there are more mutant K5 keratinocytes that can produce more inflammatory cytokines in the skin. Collectively, the epidermal cells lacking IKK- α are a resource for inducing microenvironmental inflammation.

NF- κ B is a group of transcriptional factors (RelA, RelB, c-Rel, p100/p52 [p100 is the precursor for p52], and p105/p50 [p105 is the precursor for p50]) that regulate the expression of many cytokines and chemokines and protect cells from apoptosis (Ghosh and Karin 2002). NF- κ B activity is frequently elevated in many human cancers (Van Waes and others 2007). IKK- α is one of the subunits of the IKK complex (Ghosh and Karin 2002). IKK- β and IKK- γ mainly lead to classical NF- κ B pathway through inducing I κ B- α degradation and freeing NF- κ B dimers (p65/p50) in the cytoplasmic compartment. The released NF- κ B dimers translocate to the nucleus, where they function as transcriptional factors. Furthermore, IKK- α homodimers can phosphorylate p100. This phosphorylation induces p100 processing to generate p52. RelB:p52 dimers translocate to the nucleus, leading to non-classical NF- κ B signaling activation. The function of IKK- α in embryonic development is independent of its kinase activity (Hu and others 2001; Sil and others 2004). None of the mice lacking any components of NF- κ B share epidermal phenotypes with *Ikk- α ^{-/-}* mice. On the other hand, K5.IKK- α and Lori.IKK- α transgenic mice develop normally (Liu and others 2006, 2011). We detected slightly decreased p100 and elevated p52 levels but no increased classical NF- κ B and IKK kinase activity in the skin of K5.IKK- α mice compared with wild-type skin, suggesting that slight variations in p100/p52 levels are not sufficient to cause skin lesions. In addition, we have shown elevated IL-1, TNF- α , and IL-6 expression and elevated NF- κ B DNA binding and IKK kinase activities in *Ikk- α ^{-/-}* keratinocytes compared with wild-type keratinocytes after TNF- α stimulation (Hu and others 2001). Interestingly, we also detected increased IKK kinase activity and reduced I κ B- α levels in skin carcinomas lacking IKK- α compared with papillomas and normal skin (Park and others 2007). Although it is assumed that IKK- β , a stronger kinase than IKK- α for I κ B α , may replace IKK- α in the IKK complex

FIG. 2. IKK- α loss deregulates multiple pathways in the skin. *Arrows:* promoting effect; *arrows with lines:* inhibiting effect; *lines with ?:* remaining to be determined. IKK- α , I κ B kinase alpha.

in the absence of IKK- α , resulting in increased IKK kinase activity, the precise mechanism for this event remains unclear. The importance of IKK kinase activity in skin carcinogenesis remains to be determined. In UVB skin carcinogenesis, we have detected higher levels of TNF- α , IL-1, IL-6, and monocyte chemoattractant protein-1 in *Ikk- α ^{+/-}* skin than in wild-type skin (Xia and others 2010). The medium from *Ikk- α ^{+/-}* keratinocyte culture is more active in promoting macrophage migration than wild-type keratinocyte culture medium, suggesting that keratinocytes lacking IKK- α can produce more macrophage inducers. In addition, we observed significantly reduced cell death in *Ikk- α ^{+/-}* skin compared with wild-type skin in UVB-treated mice during UVB skin carcinogenesis (Xia and others 2010). Thus, increased classical NF- κ B activity may promote inflammation and antiapoptotic activity to facilitate UVB carcinogenesis.

Moreover, IKK- α plays an important role in the development of lymphocytes and lymphoid organs (Kaisho and others 2001; Senftleben and others 2001; Kinoshita and others 2006; Lomada and others 2007). Normal immune responses are important for protecting the human body and preventing carcinogenesis. Either low or high immune responses may promote inflammation and tumorigenesis. We can use these skin tumor models to investigate whether and how mutant IKK- α -mediated immune defects affect tumorigenesis in mice and further develop immune therapies to treat cancer.

Conclusion

IKK- α loss activates or inactivates several important pathways that promote skin tumor development from tumor initiation to progression (Fig. 2). SCC is 1 major type of human epithelial cancer. Mouse cancer models, which mimic human diseases, can be used to study the pathogenesis, diagnosis, therapeutic treatment, and prevention of SCCs. Although we have developed the skin SCC mouse model, other types of SCC models have not yet been established. It is unclear whether the development of other types of SCCs requires IKK- α deficiency and additional alterations. Furthermore, we still do not know whether IKK- α deletion targets stage-specific squamous epithelial cells to initiate tumors, or it turns on oncogenes or represses tumor suppressors, which turns squamous epithelial cells at any stage into "cancer-initiating cells or cancer stem cells." In conclu-

sion, identifying driving forces for tumor development can provide alternative therapeutic avenues.

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Author Disclosure Statement

No competing financial interests exist.

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