# IκB kinase α functions as a tumor suppressor in epithelial-derived tumors through an NF-κB-independent pathway (Review)

YUXIN XIE[1], KEQI XIE[2], QIHENG GOU[3] and NIANYONG CHEN[1]

[1]Department of Radiation Oncology, Cancer Center and State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, Sichuan 610041; [2]Department of Anesthesiology, Mianyang Central Hospital, Mianyang, Sichuan 621000; [3]State Key Laboratory of Biotherapy/Collaborative Innovation Center of Biotherapy, West China Hospital, Sichuan University, Chengdu, Sichuan 610041, P.R. China

Received June 5, 2015; Accepted July 6, 2015

DOI: 10.3892/or.2015.4229

**Abstract.** Recent studies have shown that IκB kinase α (*IKKα*) plays an important role in human skin cancer and acts as a major regulator for keratinocyte terminal differentiation and proliferation. *IKKα* deficiency or mutation is associated with human tumor development; thus, overexpression of *IKKα* could prevent tumor progression. However, findings suggest that *IKKα* is equally essential for many other epithelial-derived tumors. In the present study, we discussed the role of *IKKα* as a tumor suppressor in *IKKα*-mediated epithelial-derived tumors and its activation pathway, which is different from the traditional NF-κB pathway. The present study provides theoretical basis for understanding the molecular mechanisms involved in *IKKα*-related tumors.

**Contents**



*Correspondence to:* Professor Nianyong Chen, Department of Radiation Oncology, Cancer Center and State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, 37 Guoxuexiang, Wuhou, Chengdu, Sichuan 610041, P.R. China
E-mail: nchenyy@yahoo.com

*Abbreviations: IKKα*, IκB kinase α; NF-κB, nuclear factor-κB; TSGs, tumor-suppressor genes; HLH, helix-loop-helix; SCC, squamous cell carcinoma; ERK, extracellular signal-regulated kinase; MCP-1, monocyte chemoattractant protein-1; EGFR, epidermal growth factor receptor; TGF-β, transforming growth factor β; EMT, epithelial-mesenchymal transition; VEGF, vascular endothelial growth factor; CDK1, cyclin-dependent kinase 1; PFS, progression-free-survival; MSI, microsatellite instability; SSRs, simple sequence repeats; MMR, mismatch repair; NPC, nasopharyngeal carcinoma; MMP-9, matrix metalloproteinase-9; EBNA1, Epstein-Barr nuclear antigen 1; AIB1/SRC-3, amplified in breast 1/steroid receptor coactivator-3; RANKL, RANK ligand

*Key words: IKKα*, tumor suppressor, epithelial-derived tumor, signaling pathway, NF-κB

## 1. Introduction

Inactivation of tumor suppressor genes (TSGs), whose structural abnormalities and functional disorders lead to changes in their regulatory mechanism, plays an important role in the development and progression of human tumors. The role of TSGs involved in normal differentiation of tissues and inhibition of carcinoma has gained increasing attention (1,2). Numerous studies have shown that the mutations and/or deletions of *IKKα* frequently occur in human epithelial-derived carcinogenesis, while *IKKα* stable expression appears in corresponding normal tissues (3-6).

## 2. Structure and traditional functions of *IKKα*

*IKKα* is a 85-kDa polypeptide containing an amino-terminal serine-threonine kinase catalytic domain and carboxyl-proximal helix-loop-helix (HLH) and leucine zipper-like (LZip) amphipathic α-helical domains (7,8). *IKKα* and *IKKβ*, which share similar substrate specificities as catalytic subunits, form a specific IκB kinase complex with the regulatory subunit *IKKγ*/NEMO, thus contributing to kinase activity regulation in the cytoplasm (9).

A series of surrounding signals, such as aberrant cytokine production, integrins, growth factors and cytokine receptors activate the *IKK* complex for the phosphorylation and ubiquitylation of IκBs, resulting in subsequent proteasomal degradation of p105 to p50. This is the canonical NF-κB pathway (Fig. 1A), which leads to the nuclear entry of NF-κB1/RelA (p50/p65) (10,11). *IKKα* also forms *IKKα*/*IKKα* homodimers to stimulate an alternative NF-κB pathway by transformation of NF-κB2/p100/RELB into p52/RELB heterodimers, which translocate into the nucleus and exert

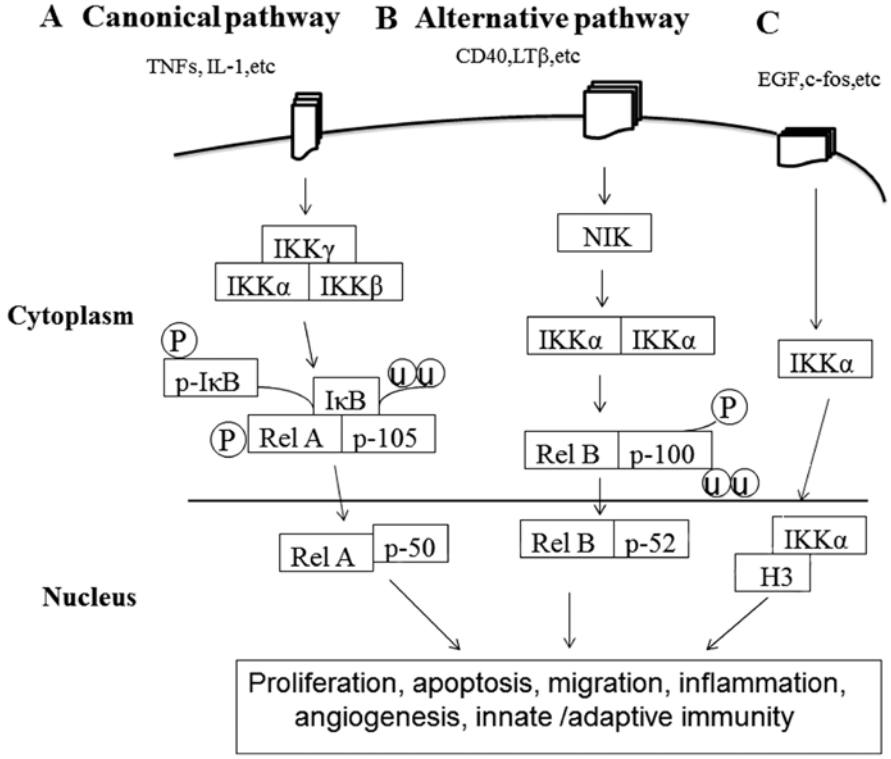


Figure 1. A proposed schematic diagram for activation of pathways involved in the function of IKKα in cancer pathogenesis. (A) Agents activating the canonical NF-κB pathway include aberrant cytokine production, integrins, such as tumor necrosis factor (TNF)-α, IL-1 and corresponding cytokine receptors. Stimuli-induced activation of the multiprotein IKK complex results in the phosphorylation of NH2-terminal serine residues in IκBs and rapid separation from p105/RELA through a ubiquitin-26 S proteasome pathway. Subsequently, proteasome degradation of p105 to p50 and phosphorylation of RelA (p65) is stimulated, leading to nuclear translocation of the NF-κB1/RelA (p50/p65) complex, which activates gene expression regulating proliferation, apoptosis, migration, inflammation, angiogenesis and innate immunity. (B) The alternative pathway is triggered by other aberrant TNF cytokines and lymphotoxin β (LTβ). Stimuli-induced activation of kinase NIK phosphorylates and activates IKKα/IKKα homodimers, leading to degradation and phosphorylation of NF-κB2/p100 precursor protein to p52 and nuclear translocation of the p52/RelB complex. The alternative pathway is the major provider of adaptive immunity in mammals. (C) IKKα may also translocate to the nucleus and modulate global levels of histone H3 phosphorylation on Ser10 in response to mitogenic EGF stimuli.

transcriptional control (12) (Fig. 1B). Furthermore, *IKKα* may directly translocate to the nucleus and bind to histone 3 (H3) (13) (Fig. 1C). Ultimately, these processes contribute to gene expression and regulation of proliferation, apoptosis, migration, tumorigenesis, inflammation, angiogenesis and innate immunity (Fig. 1).

### 3. Role of *IKKα* in normal epithelial and skin squamous cell carcinoma (SCC)

*IKKα functions as a major regulator in keratinocyte proliferation and differentiation.* Calcium ($Ca^{2+}$) is essential for the induction and maintenance of the terminal differentiation status in the epidermis (14,15). However, primary cultured *IKKα*$^{-/-}$ keratinocytes fail to undergo terminal differentiation, which is also not induced by $Ca^{2+}$. Conversely, reintroduction of wild-type (WT) or kinase-inactivated *IKKα* induces terminal differentiation of keratinocytes, while this does not occur when *IKKβ* is reintroduced, even though it exhibits a similar structure and function with *IKKα*. Furthermore, compared to *IKKβ* in the cytoplasm exclusively, *IKKα* exists both in the cytoplasm and nucleus (4,16). *IKKα* shows nuclear localization in the normal epidermis of humans and mice (3,17). In the nucleus, *IKKα* does not exert kinase activity and has a special role different from that of *IKKβ*. Moreover, mutant *IKKα* is not only incapable of moving to the nucleus, but it also fails to induce terminal differentiation of *IKKα*$^{-/-}$ keratinocytes (17). These findings indicate that nuclear *IKKα* regulates keratinocyte proliferation and $Ca^{2+}$-dependent differentiation. However, how $Ca^{2+}$ is involved in the process of *IKKα*-induced keratinocyte terminal differentiation remains to be determined.

*Function of IKKα as a tumor suppressor in skin SCC.* Studies have demonstrated that *IKKα* mutations are present in human SCC and that a marked reduction in *IKKα* expression occurs in poorly differentiated human and mouse cutaneous SCCs (18). In studies of chemical carcinogen-induced (19) or UVB-induced (20-22) skin carcinogenesis, the findings have shown that lack of *IKKα* expression promotes the development of skin papillomas and carcinomas in *IKKα*$^{+/-}$ mice. In chemical carcinogen-induced skin carcinogenesis, reduced levels of *IKKα* promote the oncogenic H-Ras pathway and enhance the mitogenic activity, extracellular signal-regulated kinase (ERK) activity and overexpression of growth factors (19); and in addition, influence UVB-related p53 mutations, upregulate the expression of monocyte chemoattractant protein-1 (MCP-1/CCL2), TNF-α, IL-1 and elevate macrophage migration, which are crucial for accelerating UVB skin carcinogenesis (21,23). In contrast, increased IKKα expression antagonizes mitogenesis

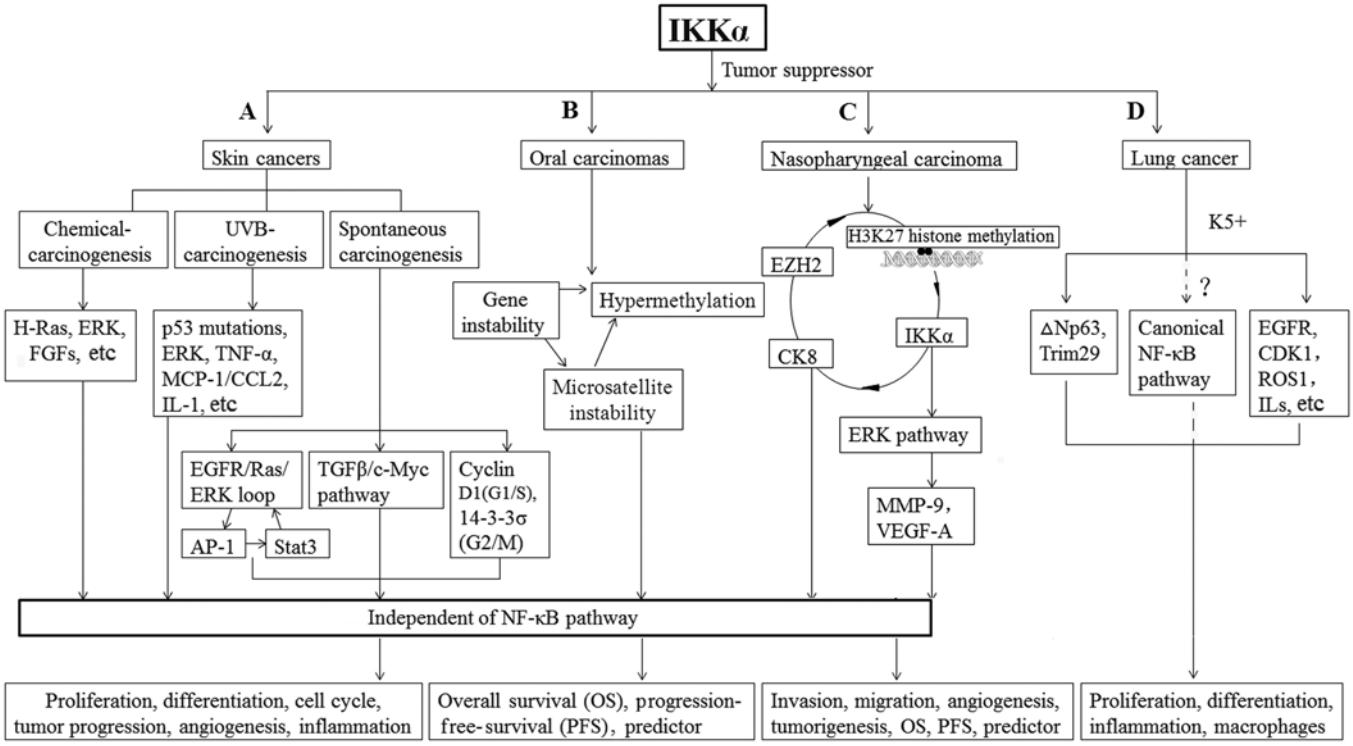


Figure 2. Mechanism of IKKα as a tumor suppressor in epithelium-derived carcinoma development from tumor initiation to progression. (A) In chemical carcinogen-induced skin cancer, reintroduction of IKKα or kinase-inactive IKKα represses DMBA-induced H-Ras mutations or TPA-induced excessive ERK activity or excessive expression of EGF and FGFs. Reduction in IKKα expression promotes expansion of cells carrying UVB-induced-p53 mutations and induces the recruitment of macrophages and inflammation by upregulating expression of cytokines and MCP-1/CCL2. In spontaneous carcinogenesis, IKKα suppresses the transcription of EGFR ligands and their ligand activators, resulting in inhibition of EGFR and ERK activities. IKKα cross-talks with EGFR, AP-1 and Stat3 pathways in a loop. Moreover, IKKα inhibits the TGF-β/Smad2/3/c-Myc pathway and contributes to the regulation of cyclin D1 in the G1/S and 14-3-3σ in the G2/M phase to maintain a normal balance in the cell cycle. (B) In oral carcinoma, the genetic instability of IKKα and hypermethylation of its CpG islands causes some promoter hypermethylation and epigenetic inactivation of gene transcription. (C) Reduced EZH2 inhibits H3K27 histone methylation on the IKKα promoter and relieves IKKα transcriptional repression. Overexpression of IKKα reduces CK13 and increase CK8 expression to induce epithelial differentiation. Through another manner, IKKα suppresses NPC through the ERK pathway by regulating MMP-9. IKKα is independent of the NF-κB pathway in the above process. (D) In spontaneous lung SCCs, kinase-dead IKKα knock-in suppresses the expression of p63, Trim29, K5 and EGFR/ERK activity and CDK1, ROS1, IL-1 levels to keep balance between cell proliferation and differentiation and prevents an excessive inflammatory microenvironment. However, the increased canonical NF-κB pathway contributes to IKKα-related lung SCC development and this requires further elucidation.

and angiogenesis, thus antagonizing the development of malignant carcinomas and metastases (Fig. 2A).

Overall, *IKKα* plays an important role in skin cancer, and the maintenance of an adequate level of IKKα is essential for protecting the skin from various harmful stimulations and carcinogen attacks. It is necessary to investigate whether certain mutations in *IKKα* convert its function from a tumor suppressor to an oncogene.

*Mechanism of IKKα in IKKα-mediated skin tumorigenesis*

*The EGFR/ERK/EGF/HB-EGF/ADAM signaling pathway.* Inactivation of epidermal growth factor receptor (EGFR) or reintroduction of *IKKα* prevents *IKKα*-induced epidermal hyperplasia and skin cancer development, indicating a cross-talk between *IKKα* and EGFR (3,19). *IKKα* deletion causes elevation in EGFR and ERK activity, EGF and HB-EGF levels and the downstream expression of ADAM sheddases (such as, ADAM9, 10, 12, 17 and 19), which could cleave EGF and HB-EGF precursors to generate their active soluble forms (24). These molecules are activated in the EGFR autocrine loop. The EGFR/Ras pathway is important for keratinocyte differentiation, regulation of proliferation and skin tumor development. This autocrine loop in *IKKα*-lacking cells could ultimately result in excessive proliferation and dedifferentiation of the epidermis. Conversely, reintroduction of *IKKα* or inactivation of EGFR and/or EGFR inhibitors, blocks *IKKα* loss-induced epidermal hyperproliferation and skin tumors in mice. Furthermore, *IKKα* represses the EGFR/Ras/ERK loop via the suppression of the transcription of genes that encode EGFR ligands (3,25).

In addition, studies have shown that reintroduced *IKKα* or inactivated EGFR also represses AP-1 and Stat3, whose excessive activity accelerates skin tumor development (26-28). However, the cross-talk between *IKKα* and the EGFR, AP-1, and Stat3 pathways in a loop remains to be investigated (25). Collectively, *IKKα* contributes to an EGFR-mediated loop and represses cell proliferation, resulting in induction of terminal differentiation in skin keratinocytes (Fig. 2A).

*The TGF-β/Smad2/3/c-Myc pathway.* The transforming growth factor β (TGF-β)/Smad2/3 pathway is also important for skin homeostasis and skin tumor development (Fig. 2A). Upon treatment with TGF-β, *IKKα* forms a complex with Smad2 or Smad3 on the promoters of Mad genes (16,29). Studies have suggested that loss of *IKKα* downregulates Mad1, Mad2, Mad3, Mad4 and Ovol1 expression and increases

c-Myc activity, which is associated with cell differentiation in keratinocytes. Excessive c-Myc activity inhibits the prevention of cell cycle exit and cell apoptosis, thereby destroying regular cell differentiation. Moreover, a reduced level of Max/Mad dimers induces the competition with c-Myc, which can form dimers with Max as well, thus abnormally enhancing c-Myc/Max dimer-induced cell proliferation (30,31).

Although TGF-β acts as a tumor suppressor at the early stage of tumor development and affects cell proliferation, differentiation and inflammation (32), the levels of TGF-β are high in SCC. Accumulating evidence has revealed that TGF-β plays a bidirectional role in cancer progression (33,34). Studies suggest that TGF-β works as a tumor promoter by modifying tumor promoter-induced cell proliferation and inflammation, stimulating angiogenesis and promoting epithelial-mesenchymal transition (EMT) (35). In skin cancer, TGF-β fails to exert its tumor suppressor activity in *IKKα*-null or *IKKα*-mutated tumor cells, owing to the fact that *IKKα* is a major target of mutagenesis in tumorigenesis. Thus, *IKKα* is crucial for *TGF-β*-induced tumor suppressor activity during skin tumorigenesis.

*The role of IKKα in the cell cycle*

*IKKα and cyclin D1.* Several studies suggest that *IKKα* regulates the turnover and subcellular distribution of cyclin D1 by inducing its phosphorylation of cyclin D1 (36) (Fig. 2A). Cyclin D1 is an important regulator of cell-cycle initiation and G1/S transition. Cyclin D1 binds to Cdk4 and Cdk6 to form a pRB kinase, which phosphorylates and deactivates the tumor-suppressor protein pRB. Upon phosphorylation, pRB loses its repressive activity for the E2F transcription factor, which activates the transcription of several genes required for cell-cycle initiation and G1/S transition. Furthermore, overexpression of cyclin D1 plays a critical role in tumor development through stimulating various growth factors, such as anchorage-independent growth and vascular endothelial growth factor (VEGF) (37).

*IKKα* is critical for the phosphorylation of cyclin D1 at the T286 residue, which is indispensable for the transport of the protein out of the nucleus during the S phase of the cell cycle and for its degradation (38). *IKKα*$^{-/-}$cells have cyclin D1 in the nucleus and therefore, abnormally activate CDK4/6 kinase activity and pRb phosphorylation, leading to the deregulation of the G1/S checkpoint. This process may be enhanced by the presence of cyclin D1 mutations (39); at the same the T286 residue remains in the nucleus and promote tumorigenesis.

*IKKα and 14-3-3σ.* 14-3-3σ, as a G2/M cell cycle checkpoint, regulates the cell cycle and allows cells to repair genetic errors, thus preventing mutagenesis and genomic instability (40). It is specifically and highly expressed in keratinocytes and other epithelial cells. A large number of studies have shown that *14-3-3σ* is silenced in many human epithelial cancer cells. Studies show that the level of 14-3-3σ is low in *IKKα*$^{-/-}$ cells but is restored after the reintroduction of *IKKα* (Fig. 2A). Furthermore, the protein is a downstream target of *IKKα* in the pathway of *IKKα*-mediated cell cycle regulation and functions in response to DNA damage (41,42).

Moreover, 14-3-3σ is also a target of *IKKα* for maintaining genomic stability. *14-3-3σ* contains many CpG islands. Its hypermethylation and loss of final *σ* expression are the most consistent molecular alterations in malignancies. Studies have confirmed that *14-3-3σ* CpG islands are hypermethylated in *IKKα*$^{-/-}$ keratinocytes, which is associated with trimethyl-H3-K9, a protein essential to DNA methylation. An entire kinase domain (300 aa) deletion of *IKKα* does not permit *IKKα* to bind to the N-terminal tail of H3, which fails to prevent trimethylation of H3-K9 at the *14-3-3σ* and fails to induce the expression of 14-3-3σ. Conversely, binding of *IKKα* to H3 probably prevents H3-K9 trimethylation, thereby shielding the *14-3-3σ* locus from hypermethylation and maintaining genomic stability. Thus, *IKKα* plays an important role in epigenetic regulatory mechanisms of *14-3-3σ* for cancer prevention (41). However, further studies may be necessary to unveil whether *IKKα* utilizes this mechanism to regulate a series of genes, providing the foundation for exploiting its hypermethylation as a marker for the early detection of malignancies.

Therefore, *IKKα*-mediated cell cycle regulation mechanism via cyclinD1 or 14-3-3σ is important for prevention of skin tumors.

## 4. The role of *IKKα* in lung SCC tumorigenesis

Studies have revealed a decrease in *IKKα* RNA expression level in human lung cancer cell lines (4). Recent findings have demonstrated the pivotal role of *IKKα* in the development of spontaneous lung SCC. Studies have established a kinase-dead *IKKα* knock-in (*IKKα*$^{K44A/K44A}$, *IKKα*$^{KA/KA}$) mouse model, in which the lysine (K) at amino acid 44, the ATP-binding site, was replaced with alanine (A), to develop spontaneous lung SCCs to determine the role of *IKKα* in normal bronchial epithelium and its related carcinomas. The mice did not display any obvious abnormalities at the time of birth, indicating that *IKKα* kinase inactivation does not affect mouse embryonic development. However, spontaneous lung tumors, whose weight was timely increased, appeared in *IKKα*$^{KA/KA}$ mice from 4 to 10 months of age and the animals began to die after 6-10 months. In that case, *IKKα* levels in the lungs of *IKKα*$^{KA/KA}$ newborns were low and markedly decreased at 4 months of age. Therefore, reduction in *IKKα* levels contributed to the development of spontaneous lung tumors. Furthermore, atypical squamous hyperplasia development in the fore-stomach, esophagus and skin was also investigated in the *IKKα-deficient* mice. However, reintroduction of the *IKKα* transgene prevented the development of the tumors (43). These results demonstrated that high levels of *IKKα* prevent lung tumor development.

Some studies suggest that lung SCCs are derived from keratin 5-positive (K5$^{+}$) basal cells of the pseudostratified bronchial epithelium. K5$^{+}$ epithelial cells lacking *IKKα* may be targets for SCC development (4,44). *IKKα*$^{KA/KA}$ mice expressed high levels of K5, as well as transcription factor p63, tripartite motif-containing 29 (Trim29) proteins, and Ki67, which was similar to the expression profile in human lung SCCs (43).

*p63*, a member of the tumor suppressor *p53* family, is required for the formation of the epidermis, other stratified epithelia and epithelial appendages (45). The N-terminal-truncated form of p63 (ΔNp63) is predominately expressed in the epidermis and is overexpressed in various epithelial cancers, where it exerts oncogenic activities (46). In addition,

*Trim29* overexpression has been studied in human lung, bladder, colon, ovarian, endometrial and gastric cancers. In these cell types, Trim29 promotes cell proliferation and inhibits *p53* activity (47,48). These findings highlighted that increased epithelial cell-specific *p63* and *Trim29* may contribute to lung SCC development. Compared to WT mice, ΔNp63 and Trim29 protein levels were increased in the lungs of $IKK\alpha^{KA/KA}$ mice and further increased in the lung affected by SCCs. Chromatin immunoprecipitation (ChIP) assay was used to explain how *IKKα* regulates the expression of both Trim29 and ΔNp63. The results showed that nuclear *IKKα* bound to the promoter regions of *Trim29* and *p63*, which was associated with high levels of H3 lysine 27 trimethylation (H3K27me3), a negative transcription modifier and low levels of H3 lysine 4 trimethylation (H3K4me3), a positive transcription modifier, thus leading to the control of their expression. This process occurred both in human and mouse lung epithelial cells (43,49,50). In consequence, nuclear *IKKα* regulates the expression of both Trim29 and ΔNp63 at the transcription level by modifying the chromatin structure of *Trim29* and *p63* in an epigenetic manner to prevent tumor development (Fig. 2D).

In addition, the levels of insulin growth factor 1 (IGF-1), cyclin-dependent kinase 1 (CDK1) and the activities of EGFR, ERK and p38 were elevated in the lungs of $IKK\alpha^{KA/KA}$ mice and were considerably increased in SCC compared to WT lungs, which is similarly to human. Excessive inflammatory cells and the microenvironment, cytokines and chemokines were also present in the lungs of $IKK\alpha^{KA/KA}$ mice. Increased expression levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1b, IL-6, chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-X-C motif) ligand 5, CCL11 and CCL8 were observed in the lungs of $IKK\alpha^{KA/KA}$ mice as well. Excessive amounts of macrophages also caused increased inflammation, epithelial cell proliferation, DNA damage and activity of many pathways that may contribute to lung carcinogenesis in $IKK\alpha^{KA/KA}$ mice (43,51) (Fig. 2D).

Unlike the malfunction of the nuclear factor κB (NF-κB) pathway in skin cancer (52), increased activity of the canonical NF-κB pathway was found to contribute to lung SCC development in $IKK\alpha^{KA/KA}$ mice. Whether the NF-κB pathway can be used as a therapeutic target to prevent and treat lung SCCs remains to be elucidated (Fig. 2D).

## 5. The novel role of *IKKα* in head and neck cancer

*IKKα and oral carcinomas*. Since *IKKα* is expressed in normal oral keratinocytes but its expression is frequently decreased in carcinoma cells, further studies have been conducted to investigate the role of *IKKα* in oral carcinoma tissues and analyze its prognostic significance in comparison with clinicopathological parameters and patient survival. Results indicated that *IKKα* levels decreased in 44.9% of carcinoma patients and the expression of the protein was closely associated with progression-free survival (PFS), independent of other risk factors. Therefore, *IKKα* was considered to be a significant independent predictor of mortality due to carcinomas (53).

*In vitro*, *IKKα* is located in the nucleus and is upregulated upon differentiation in oral carcinomas. Furthermore, *IKKα* shows genetic instability in its locus and hypermethylation of its CpG islands (53). Studies have also shown that the genetic instability is frequently associated with promoter hypermethylation, resulting in epigenetic inactivation of gene transcription (54). Otherwise, there is a close correlation between the promoter hypermethylation and microsatellite instability (MSI). MSI, also referred to as simple sequence repeats (SSRs), is originally associated with DNA mismatch repair (MMR), which plays a prominent role in the correction of errors made during DNA replication, genetic recombination and in the repair of small deletions and loops in DNA. Moreover, it closely associates with gene silencing through promoter hypermethylation. Thus, MSI may be important in *IKKα*-related oral tumorigenesis (55,56). In fact, most of the MSI carcinomas were methylated in a specific site critical for *IKKα* expression, which is important for the activity of *IKKα* as a tumor-suppressor gene in oral carcinoma regulation (Fig. 2B). However, since in some carcinomas, the methylation status did not correlate with immunoreactivity, other mechanisms regulating the gene expression remain to be investigated (53,57). Similar to skin cancer, in oral carcinoma, *IKKα* in the nucleus suppresses malignancy by acting on cell differentiation independent of canonical NF-κB activation in oral carcinoma.

*IKKα and nasopharyngeal carcinoma*. Nasopharyngeal carcinoma (NPC) is one of the common epithelium-derived carcinomas in the head and neck. The effect of *IKKα* as the tumor suppressor in NPC cells or the survival of NPC patients has recently been investigated.

*IKKα* is upregulated upon differentiation in NPC cell lines *in vitro*. Differential expression of *IKKα* exists in NPC cells, which is negatively correlated with invasiveness, migration and angiogenesis, but not with proliferation. Lack of *IKKα* could lead to increased cancer invasion, migration and angiogenesis; on the contrary, reintroduction of *IKKα* abrogated these biological behaviors (58). Moreover, *IKKα* inhibited tumorigenesis in mice inoculated with *IKKα*-transfected NPC cells *in vivo*. These processes were found to be independent of *IKKα* kinase activity and the conventional effect of *IKKα* on NF-κB pathways (58,65).

Furthermore, this study (58) showed that 26.8% of a total of 157 NPC patients exhibited low expression or deletion of *IKKα*. Although the *IKKα* expression levels had no correlation with tumor stage, recurrence, distant metastasis, age and gender, the survival (OS or DFS) of NPC patients was significantly associated with *IKKα* expression. Similar to T stage, lymph node metastasis, locoregional recurrence and distant metastasis, *IKKα* expression significantly influenced clinical prognosis. Its high expression could serve as an independent favorable predictor for NPC patients.

Similar to oral carcinoma cell lines, the CpG islands of the *IKKα* gene were heavily methylated in NPC cell lines and clinical specimens. Studies have shown that increased *IKKα* expression could induce differentiation and prevent NPC development by reducing enhancer of zeste homologue 2 (EZH2)-related H3K27 histone methylation of the *IKKα* promoter (59). Importantly, overexpression of *IKKα* results in fusiform morphological change, reduction in CK13 and increase in involucrin and CK8 without activating the NF-κB pathway, leading to differentiation of NPC cells (59-61).

These findings suggest an important role of *IKKα* for NPC differentiation in an epigenetic mechanism.

Furthermore, what is the downstream signaling pathway or mechanism that regulates this newly recognized suppressive effect of *IKKα* on NPC? Findings have shown that *IKKα* inhibits NPC development through inactivation of ERK1/2 and its phosphorylation (pERK1/2) in the ERK pathway, but it is independent of EGFR, although it usually acts as the upstream protein in the ERK pathway, thus resulting in aberrant activation of the ERK signaling pathway (65). This finding is consistent with the malfunction of *IKKα* in the proliferation of NPC cell lines (62). Furthermore, matrix metalloproteinase-9 (MMP-9), but not MMP-2, is an essential downstream molecule in the IKKα-related ERK pathway for repressing NPC progression (Fig. 2C).

EBV is ubiquitously associated with the carcinogenesis of NPC (63). In addition, research has shown that Epstein-Barr nuclear antigen 1 (EBNA1) inhibits the canonical NF-κB pathway and contributes to the pathogenesis of NPC, by inhibiting *IKKα*/*IKKβ* phosphorylation (64). Thus, the correlation between *IKKα*, EBNA1 and NF-κB in NPC was investigated, although the expression of EBNA1 was high and undifferentiated expressed in all EBV-positive NPC cell lines. Studies strongly suggest that *IKKα* plays a crucial role as a tumor suppressor in NPC and is not related to tumor-associated protein EBNA1 (65).

## 6. A different role of *IKKα* in hormone-related breast and prostate cancer

As stated above, *IKKα* plays a pivotal role as a tumor suppressor in epithelial-derived tumors, particularly in SCCs; however, it has been determined that *IKKα* also functions as a tumor promoter in hormone-related breast (66) and prostate cancers (67). One study showed that it provides an important linking effect between RANK signaling and cyclin D1 expression in the development of the mammary gland (68). It was also demonstrated that the C57BL/6 female mouse expresses a mutant $IKK\alpha^{AA}$ knock-in allele in which alanine replaces serine to inactivate *IKKα* kinase activity, impairing the proliferation of mammary epithelial cells and thus leading to a severe lactation defect. However, this defect is completely reversed by the reintroduction of a mammary specific *cyclin D1* in the $IKK\alpha^{AA/AA}$ mammary epithelium. Furthermore, it has been found that *IKKα* kinase activity is involved in cyclin D1 expression and this process is activated by RANK ligand (RANKL), an efficient NF-κB activator (68).

In breast cancer, *IKKα* phosphorylates ERα and the nuclear hormone receptor co-activator AIB1/SRC-3 (amplified in breast 1/steroid receptor coactivator-3) and thus activates the transcription of estrogen-responsive genes, including *cyclin D1* and *c-myc*, to enhance proliferation of ER(+) breast cancer cells (66). In addition, another finding showed that the epidermal growth factor receptor (Her-2) activated *IKKα* to induce tumor invasion through the NF-κB canonical pathway in $HER^{2+}/ER^{-}$ breast cancer cells (69).

Except for breast cancer, a study demonstrated that nuclear *IKKα* activation by the RANK ligand inhibits maspin expression and leads to metastatic progression of mouse and human prostate cancers (67). Therefore, *IKKα* functions as an oncogenic factor, in conjunction with the hormone-related genes, to contribute to the development of breast and prostate cancers through NF-κB-dependent or -independent pathways.

## 7. Conclusions

As a novel and potential suppressor, *IKKα* activates or inactivates comprehensive pathways that prevent the development of a variety of epithelium-derived carcinomas from tumor initiation to progression (Fig. 2). Except for the cytoplasmic effect of *IKKα* on the NF-κB pathway, the role of nuclear *IKKα* is much more essential to epithelium-derived carcinomas. Although an increasing amount of research has explored the function and mechanism of *IKKα* in such carcinomas, the cross-talk among them has not yet been established. It is still unclear whether *IKKα* deletion targets stage-specific squamous epithelial cells to initiate tumors or it turns on oncogenes or represses tumor suppressors and whether *IKKα* plays a role in stem cell maintenance, renewal or division. On the other hand, *IKKα* is also suggested as a tumor promoter. Therefore, whether *IKKα* functions differently in different types of tumors warrants further investigation. Collectively, *IKKα* is a major regulator to participate in the downstream gene cascade, leading to repression of cell hyperproliferation, angiogenesis, epithelial-mesenchymal transition and malignant progression. Furthermore, *IKKα* may be a potential therapeutic strategy for preventing carcinoma.

## Acknowledgements

The present study was supported by a grant from the National Natural Science Foundation of China (no. 81072217) (to C.N.).

## References

1. Berger AH, Knudson AG and Pandolfi PP: A continuum model for tumour suppression. Nature 476: 163-169, 2011.
2. Stovall DB, Cao P and Sui G: SOX7: From a developmental regulator to an emerging tumor suppressor. Histol Histopathol 29: 439-445, 2014.
3. Liu B, Xia X, Zhu F, Park E, Carbajal S, Kiguchi K, DiGiovanni J, Fischer SM and Hu Y: IKKalpha is required to maintain skin homeostasis and prevent skin cancer. Cancer Cell 14: 212-225, 2008.
4. Kwak YT, Radaideh SM, Ding L, Li R, Frenkel E, Story MD, Girard L, Minna J and Verma UN: Cells lacking IKKalpha show nuclear cyclin D1 overexpression and a neoplastic phenotype: Role of IKKalpha as a tumor suppressor. Mol Cancer Res 9: 341-349, 2011.
5. Marinari B, Ballaro C, Koster MI, Giustizieri ML, Moretti F, Crosti F, Papoutsaki M, Karin M, Alema S, Chimenti S, *et al*: IKKalpha is a p63 transcriptional target involved in the pathogenesis of ectodermal dysplasias. J Invest Dermatol 129: 60-69, 2009.
6. Marinari B, Moretti F, Botti E, Giustizieri ML, Descargues P, Giunta A, Stolfi C, Ballaro C, Papoutsaki M, Alemà S, *et al*: The tumor suppressor activity of IKKalpha in stratified epithelia is exerted in part via the TGF-beta antiproliferative pathway. Proc Natl Acad Sci USA 105: 17091-17096, 2008.
7. McKenzie FR, Connelly MA, Balzarano D, Muller JR, Geleziunas R and Marcu KB: Functional isoforms of IkappaB kinase alpha (IKKalpha) lacking leucine zipper and helix-loop-helix domains reveal that IKKalpha and IKKbeta have different activation requirements. Mol Cell Biol 20: 2635-2649, 2000.
8. Connelly MA and Marcu KB: CHUK, a new member of the helix-loop-helix and leucine zipper families of interacting proteins, contains a serine-threonine kinase catalytic domain. Cell Mol Biol Res 41: 537-549, 1995.

9. Ghosh S and Karin M: Missing pieces in the NF-kappaB puzzle. Cell 109 (Suppl): S81-S96, 2002.
10. Sakurai H, Suzuki S, Kawasaki N, Nakano H, Okazaki T, Chino A, Doi T and Saiki I: Tumor necrosis factor-alpha-induced IKK phosphorylation of NF-kappaB p65 on serine 536 is mediated through the TRAF2, TRAF5, and TAK1 signaling pathway. J Biol Chem 278: 36916-36923, 2003.
11. Van Waes C, Yu M, Nottingham L and Karin M: Inhibitor-kappaB kinase in tumor promotion and suppression during progression of squamous cell carcinoma. Clin Cancer Res 13: 4956-4959, 2007.
12. Van Waes C: Nuclear factor-kappaB in development, prevention, and therapy of cancer. Clin Cancer Res 13: 1076-1082, 2007.
13. Anest V, Cogswell PC and Baldwin AS Jr: IkappaB kinase alpha and p65/RelA contribute to optimal epidermal growth factor-induced c-fos gene expression independent of IkappaBalpha degradation. J Biol Chem 279: 31183-31189, 2004.
14. Elias PM, Ahn SK, Denda M, Brown BE, Crumrine D, Kimutai LK, Kömüves L, Lee SH and Feingold KR: Modulations in epidermal calcium regulate the expression of differentiation-specific markers. J Invest Dermatol 119: 1128-1136, 2002.
15. Liu B, Zhu F, Xia X, Park E and Hu Y: A tale of terminal differentiation: IKKalpha, the master keratinocyte regulator. Cell Cycle 8: 527-531, 2009.
16. Liu B, Park E, Zhu F, Bustos T, Liu J, Shen J, Fischer SM and Hu Y: A critical role for I kappaB kinase alpha in the development of human and mouse squamous cell carcinomas. Proc Natl Acad Sci USA 103: 17202-17207, 2006.
17. Sil AK, Maeda S, Sano Y, Roop DR and Karin M: IkappaB kinase-alpha acts in the epidermis to control skeletal and craniofacial morphogenesis. Nature 428: 660-664, 2004.
18. Park E, Liu B, Xia X, Zhu F, Jami WB and Hu Y: Role of IKKalpha in skin squamous cell carcinomas. Future Oncol 7: 123-134, 2011.
19. Park E, Zhu F, Liu B, Xia X, Shen J, Bustos T, Fischer SM and Hu Y: Reduction in IkappaB kinase alpha expression promotes the development of skin papillomas and carcinomas. Cancer Res 67: 9158-9168, 2007.
20. Zhu F, Park E, Liu B, Xia X, Fischer SM and Hu Y: Critical role of IkappaB kinase alpha in embryonic skin development and skin carcinogenesis. Histol Histopathol 24: 265-271, 2009.
21. Xia X, Park E, Liu B, Willette-Brown J, Gong W, Wang J, Mitchell D, Fischer SM and Hu Y: Reduction of IKKalpha expression promotes chronic ultraviolet B exposure-induced skin inflammation and carcinogenesis. Am J Pathol 176: 2500-2508, 2010.
22. Peinado C, Kang X, Hardamon C, Arora S, Mah S, Zhang H, Ngolab J and Bui JD: The nuclear factor-kappaB pathway down-regulates expression of the NKG2D ligand H60a in vitro: Implications for use of nuclear factor-kappaB inhibitors in cancer therapy. Immunology 139: 265-274, 2013.
23. Fujimoto H, Sangai T, Ishii G, Ikehara A, Nagashima T, Miyazaki M and Ochiai A: Stromal MCP-1 in mammary tumors induces tumor-associated macrophage infiltration and contributes to tumor progression. Int J Cancer 125: 1276-1284, 2009.
24. Huovila AP, Turner AJ, Pelto-Huikko M, Karkkainen I and Ortiz RM: Shedding light on ADAM metalloproteinases. Trends Biochemical Sci 30: 413-422, 2005.
25. Liu S, Chen Z, Zhu F and Hu Y: IkappaB kinase alpha and cancer. J Interferon Cytokine Res 32: 152-158, 2012.
26. Liu B, Willette-Brown J, Liu S, Chen X, Fischer SM and Hu Y: IKKalpha represses a network of inflammation and proliferation pathways and elevates c-Myc antagonists and differentiation in a dose-dependent manner in the skin. Cell Death Differ 18: 1854-1864, 2011.
27. Zenz R, Eferl R, Scheinecker C, Redlich K, Smolen J, Schonthaler HB, Kenner L, Tschachler E and Wagner EF: Activator protein 1 (Fos/Jun) functions in inflammatory bone and skin disease. Arthritis Res Ther 10: 201, 2008.
28. Sano S, Chan KS and DiGiovanni J: Impact of Stat3 activation upon skin biology: A dichotomy of its role between homeostasis and diseases. J Dermatol Sci 50: 1-14, 2008.
29. Descargues P, Sil AK, Sano Y, Korchynskyi O, Han G, Owens P, Wang XJ and Karin M: IKKalpha is a critical coregulator of a Smad4-independent TGFbeta-Smad2/3 signaling pathway that controls keratinocyte differentiation. Proc Natl Acad Sci USA 105: 2487-2492, 2008.
30. Gandarillas A: The mysterious human epidermal cell cycle, or an oncogene-induced differentiation checkpoint. Cell Cycle 11: 4507-4516, 2012.
31. Pulverer B, Sommer A, McArthur GA, Eisenman RN and Luscher B: Analysis of Myc/Max/Mad network members in adipogenesis: Inhibition of the proliferative burst and differentiation by ectopically expressed Mad1. J Cell Physiol 183: 399-410, 2000.
32. Drabsch Y and ten Dijke P: TGF-β signalling and its role in cancer progression and metastasis. Cancer Metastasis Rev 31: 553-568, 2012.
33. Mordasky Markell L, Perez-Lorenzo R, Masiuk KE, Kennett MJ and Glick AB: Use of a TGFbeta type I receptor inhibitor in mouse skin carcinogenesis reveals a dual role for TGFbeta signaling in tumor promotion and progression. Carcinogenesis 31: 2127-2135, 2010.
34. Ikushima H and Miyazono K: TGFbeta signalling: A complex web in cancer progression. Nat Rev Cancer 10: 415-424, 2010.
35. Ravindran A, Mohammed J, Gunderson AJ, Cui X and Glick AB: Tumor-promoting role of TGFbeta1 signaling in ultraviolet B-induced skin carcinogenesis is associated with cutaneous inflammation and lymph node migration of dermal dendritic cells. Carcinogenesis 35: 959-966, 2014.
36. Kwak YT, Li R, Becerra CR, Tripathy D, Frenkel EP and Verma UN: IkappaB kinase alpha regulates subcellular distribution and turnover of cyclin D1 by phosphorylation. J Biol Chem 280: 33945-33952, 2005.
37. Tashiro E, Tsuchiya A and Imoto M: Functions of cyclin D1 as an oncogene and regulation of cyclin D1 expression. Cancer Sci 98: 629-635, 2007.
38. Diehl JA, Cheng M, Roussel MF and Sherr CJ: Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. Genes Dev 12: 3499-3511, 1998.
39. Alt JR, Cleveland JL, Hannink M and Diehl JA: Phosphorylation-dependent regulation of cyclin D1 nuclear export and cyclin D1-dependent cellular transformation. Genes Dev 14: 3102-3114, 2000.
40. Chan TA, Hwang PM, Hermeking H, Kinzler KW and Vogelstein B: Cooperative effects of genes controlling the G(2)/M checkpoint. Genes Dev 14: 1584-1588, 2000.
41. Zhu F, Xia X, Liu B, Shen J, Hu Y, Person M and Hu Y: IKKalpha shields 14-3-3sigma, a G(2)/M cell cycle checkpoint gene, from hypermethylation, preventing its silencing. Mol Cell 27: 214-227, 2007.
42. Dellambra E, Golisano O, Bondanza S, Siviero E, Lacal P, Molinari M, D'Atri S and De Luca M: Downregulation of 14-3-3sigma prevents clonal evolution and leads to immortalization of primary human keratinocytes. J Cell Biol 149: 1117-1130, 2000.
43. Xiao Z, Jiang Q, Willette-Brown J, Xi S, Zhu F, Burkett S, Back T, Song NY, Datla M and Sun Z: The pivotal role of IKKalpha in the development of spontaneous lung squamous cell carcinomas. Cancer Cell 23: 527-540, 2013.
44. Hackett NR, Shaykhiev R, Walters MS, Wang R, Zwick RK, Ferris B, Witover B, Salit J and Crystal RG: The human airway epithelial basal cell transcriptome. PloS One 6: e18378, 2011.
45. Ye S, Lee KB, Park MH, Lee JS and Kim SM: p63 regulates growth of esophageal squamous carcinoma cells via the Akt signaling pathway. Int J Oncol 44: 2153-2159, 2014.
46. Koster MI, Dai D, Marinari B, Sano Y, Costanzo A, Karin M and Roop DR: p63 induces key target genes required for epidermal morphogenesis. Proc Natl Acad Sci USA 104: 3255-3260, 2007.
47. Cambiaghi V, Giuliani V, Lombardi S, Marinelli C, Toffalorio F and Pelicci PG: TRIM proteins in cancer. Adv Exp Med Biol 770: 77-91, 2012.
48. Sho T, Tsukiyama T, Sato T, Kondo T, Cheng J, Saku T, Asaka M and Hatakeyama S: TRIM29 negatively regulates p53 via inhibition of Tip60. Biochim Biophys Acta 1813: 1245-1253, 2011.
49. Hayashi A, Yamauchi N, Shibahara J, Kimura H, Morikawa T, Ishikawa S, Nagae G, Nishi A, Sakamoto Y and Kokudo N: Concurrent activation of acetylation and tri-methylation of H3K27 in a subset of hepatocellular carcinoma with aggressive behavior. PloS One 9: e91330, 2014.
50. Tie F, Banerjee R, Saiakhova AR, Howard B, Monteith KE, Scacheri PC, Cosgrove MS and Harte PJ: Trithorax monomethylates histone H3K4 and interacts directly with CBP to promote H3K27 acetylation and antagonize Polycomb silencing. Development 141: 1129-1139, 2014.
51. Ring BZ, Seitz RS, Beck RA, Shasteen WJ, Soltermann A, Arbogast S, Robert F, Schreeder MT and Ross DT: A novel five-antibody immunohistochemical test for subclassification of lung carcinoma. Mod Pathol 22: 1032-1043, 2009.

52. Hu Y, Baud V, Oga T, Kim KI, Yoshida K and Karin M: IKKalpha controls formation of the epidermis independently of NF-kappaB. Nature 410: 710-714, 2001.
53. Maeda G, Chiba T, Kawashiri S, Satoh T and Imai K: Epigenetic inactivation of IkappaB Kinase-alpha in oral carcinomas and tumor progression. Clin Cancer Res 13: 5041-5047, 2007.
54. Choi JD and Lee JS: Interplay between epigenetics and genetics in cancer. Genomics Inform 11: 164-173, 2013.
55. Ahuja N, Mohan AL, Li Q, Stolker JM, Herman JG, Hamilton SR, Baylin SB and Issa JP: Association between CpG island methylation and microsatellite instability in colorectal cancer. Cancer Res 57: 3370-3374, 1997.
56. Bairwa NK, Saha A, Gochhait S, Pal R, Gupta V and Bamezai RN: Microsatellite instability: an indirect assay to detect defects in the cellular mismatch repair machinery. Methods Mol Biol 1105: 497-509, 2014.
57. Gu L, Zhu N, Findley HW, Woods WG and Zhou M: Identification and characterization of the IKKalpha promoter: Positive and negative regulation by ETS-1 and p53, respectively. J Biol Chem 279: 52141-52149, 2004.
58. Deng L, Li Y, Ai P, Xie Y, Zhu H and Chen N: Increase in IkappaB kinase alpha expression suppresses the tumor progression and improves the prognosis for nasopharyngeal carcinoma. Mol Carcinog 54: 156-165, 2015.
59. Yan M, Zhang Y, He B, Xiang J, Wang ZF, Zheng FM, Xu J, Chen MY, Zhu YL, Wen HJ, *et al*: IKKalpha restoration via EZH2 suppression induces nasopharyngeal carcinoma differentiation. Nat Commun 5: 3661, 2014.
60. van Dorst EB, van Muijen GN, Litvinov SV and Fleuren GJ: The limited difference between keratin patterns of squamous cell carcinomas and adenocarcinomas is explicable by both cell lineage and state of differentiation of tumour cells. J Clin Pathol 51: 679-684, 1998.
61. Huang WG, Cheng AL, Chen ZC, Peng F, Zhang PF, Li MY, Li F, Li JL, Li C, Yi H, *et al*: Targeted proteomic analysis of 14-3-3sigma in nasopharyngeal carcinoma. Int J Biochem Cell Biol 42: 137-147, 2010.
62. Sullu Y, Demirag GG, Yildirim A, Karagoz F and Kandemir B: Matrix metalloproteinase-2 (MMP-2) and MMP-9 expression in invasive ductal carcinoma of the breast. Pathol Res Pract 207: 747-753, 2011.
63. Busson P, Ooka T and Corbex M: Nasopharyngeal carcinomas and Epstein-Barr virus: From epidemiology and detection to therapy. Med Sci (Paris) 20: 453-457, 2004 (In French).
64. Valentine R, Dawson CW, Hu C, Shah KM, Owen TJ, Date KL, Maia SP, Shao J, Arrand JR and Young LS: Epstein-Barr virus-encoded EBNA1 inhibits the canonical NF-kappaB pathway in carcinoma cells by inhibiting IKK phosphorylation. Mol Cancer 9: 1, 2010.
65. Xie Y, Li Y, Peng X, Henderson F Jr, Deng L and Chen N: Ikappa B kinase alpha involvement in the development of nasopharyngeal carcinoma through a NF-kappaB-independent and ERK-dependent pathway. Oral Oncol 49: 1113-1120, 2013.
66. Park KJ, Krishnan V, O'Malley BW, Yamamoto Y and Gaynor RB: Formation of an IKKalpha-dependent transcription complex is required for estrogen receptor-mediated gene activation. Mol Cell 18: 71-82, 2005.
67. Luo JL, Tan W, Ricono JM, Korchynskyi O, Zhang M, Gonias SL, Cheresh DA and Karin M: Nuclear cytokine-activated IKKalpha controls prostate cancer metastasis by repressing Maspin. Nature 446: 690-694, 2007.
68. Cao Y, Bonizzi G, Seagroves TN, Greten FR, Johnson R, Schmidt EV and Karin M: IKKalpha provides an essential link between RANK signaling and cyclin D1 expression during mammary gland development. Cell 107: 763-775, 2001.
69. Merkhofer EC, Cogswell P and Baldwin AS: Her2 activates NF-kappaB and induces invasion through the canonical pathway involving IKKalpha. Oncogene 29: 1238-1248, 2010.