

Opposed expression of IKK α : loss in keratinizing carcinomas and gain in non-keratinizing carcinomas

Desheng Xiao¹, Jiantao Jia², Ying Shi^{2,3,4}, Chunyan Fu¹, Ling Chen^{2,3,4}, Yiqun Jiang^{2,3,4}, Li Zhou¹, Shuang Liu⁵, Yongguang Tao^{2,3,4}

¹Department of Pathology, Xiangya Hospital, Central South University, Changsha, Hunan, 410078, China

²Cancer Research Institute, Central South University, Changsha, Hunan, 410078, China

³Key Laboratory of Carcinogenesis and Cancer Invasion, Ministry of Education, Hunan, 410078, China

⁴Key Laboratory of Carcinogenesis, National Health and Family Planning Commission, Hunan, 410078, China

⁵Center for Medicine Research, Xiangya Hospital, Central South University, Changsha, Hunan, 410008, China

Correspondence to:

Yongguang Tao, e-mail: taoyong@csu.edu.cn

Shuang Liu, e-mail: liushuang1999@yahoo.com

Keywords: IKK α , keratinizing cancer, non-keratinizing cancer

Received: March 17, 2015

Accepted: July 24, 2015

Published: August 06, 2015

ABSTRACT

The functional role of IKK α *in vivo* is pretty complicated, largely due to its diverse functions through cell autonomous and non-autonomous manners. In addition, most of the studies on IKK α were derived from animal models, whether these findings hold true in human tumors remain unclear. Here we examined the expression of IKK α in nasopharyngeal carcinoma, which includes non-keratinizing carcinoma and keratinizing squamous cell carcinoma, and lung squamous cell carcinoma with keratinization and non-keratinization. We demonstrated that IKK α expression was almost negative in keratinizing cancer and higher expression of IKK α was found in non-keratinizing cancer, and that IKK α expression correlated with cellular differentiation of tumors in non-keratinizing nasopharyngeal carcinoma. These findings demonstrate that IKK α is diversely expressed in keratinizing and non-keratinizing carcinomas in the same type of cancer.

INTRODUCTION

Nuclear factor (NF)- κ B activation leads to a protumorigenic inflammatory microenvironment of various tumors [1]. The NF- κ B pathway is tightly stimulated by the I κ B-kinase (IKK) complex, which consists of two catalytic subunits, IKK α and IKK β , and a regulatory subunit, IKK γ [2]. Whereas, in most malignancies, the classical IKK β /IKK γ -dependent NF- κ B activation controls key functions for tumor initiation, promotion and progression in tumors [3].

The noncanonical NF- κ B pathway is correlated with IKK α , the role of IKK α in noncanonical NF- κ B pathway is more complex [4, 5]. Depending on the type of malignancy, IKK α can provide both tumor-promoting and tumor-suppressive mechanisms that are in most instances cell autonomous. Inhibition of IKK α prolongs survival and suppresses occurrence of metastatic diseases in models of mammary, prostate cancer and colorectal cancer [6–10].

IKK α controls expression of the inhibitor of metastasis maspin in breast and prostate cancer [7, 8] and is required for ErbB2-induced mammary tumorigenesis. In the latter case, NIK-dependent IKK α activation promotes expansion of tumor-initiating cells by directly phosphorylating the cyclin-dependent kinase inhibitor p27 [9].

In contrast, IKK α acts as a tumor suppressor in models of skin or lung squamous cell carcinoma (SCC), loss of IKK α enhances susceptibility to carcinogen-induced SCC in the skin and leads to the development of spontaneous lung SCC in mice [11, 12]. Importantly, during development of lung SCC, IKK α kinase inactivation culminates in the recruitment of tumor-promoting inflammatory macrophages and depletion of macrophages prevents SCC formation [12]. This is in clear contrast to the findings that IKK α promotes intestinal tumorigenesis by limiting recruitment of M1-like polarized myeloid cells [10], yet the reason for this diverse role of IKK α in macrophage activation profile

in these two different tumor entities remains currently unclear. However, it is possible that spontaneous lung SCC initiated by inactivation of IKK α belongs to keratinizing carcinoma according to the results [12]. Therefore, we assume that the expression level of IKK α might involve in the distinct subtype of in keratinizing and non-keratinizing carcinomas.

RESULTS AND DISCUSSION

To address the hypothesis that IKK α might involve in the distinct subtype of in keratinizing and non-keratinizing cancer, we used a subtype cancer that contains keratinizing and non-keratinizing cancers. Nasopharyngeal carcinoma (NPC), a prevalent cancer in southern China, is a common epithelial carcinoma in that is related with Epstein-Barr virus (EBV). NPC is classified into two major histological subtypes: non-keratinizing carcinoma (either differentiated or undifferentiated) and keratinizing squamous cell carcinoma [13, 14]. Poor differentiation is a hallmark of solid tumors and correlates with loss of control in tumor growth and poor prognosis of patients. Here we used immunohistochemistry analysis with IKK α primary antibody to detect the expression level of IKK α in three subtypes of the primary human NPC biopsies (keratinizing NPC, non-keratinizing with undifferentiated NPC and non-keratinizing with differentiated NPC). A total of five non-cancer nasopharyngeal and 43 NPC samples were evaluated. High IKK α staining was displayed in differentiated NPC specimens (77.78%), but only in a minority (22.22%) of undifferentiated NPC samples. As expected, weak IKK α staining was displayed in non-keratinizing NPC samples. Data in Figure 1A showed that IKK α was significantly lower in non-keratinizing NPC compared with the normal nasopharyngeal epithelium, while IKK α increased in non-keratinizing NPC and the highest of IKK α was expressed in differentiated NPC. Furthermore, the median score of IKK α expression in undifferentiated NPC specimens was markedly lower compared with the differentiated NPC, moreover, the score of IKK α expression in keratinizing NPC was significantly lower as compared with non-keratinizing NPC and non-cancer tissues (Figure 1B).

Next, to further confirm IKK α level in NPC biopsies, we used *in situ* hybridization (ISH) to analyze the mRNA level of IKK α . A total of 39 NPC samples were evaluated with ISH. Data in Figure 1C showed that IKK α was significantly absence in non-keratinizing, while IKK α increased in non-keratinizing NPC and the highest of IKK α was expressed in differentiated NPC. The median score of IKK α expression in undifferentiated NPC specimens was slightly lower compared with the differentiated NPC, moreover, the score of IKK α expression in keratinizing NPC was significantly lower as compared with non-keratinizing NPC (Figure 1D).

Our findings are consistent with recent findings show that IKK α is highly expressed in differentiated NPC and functions as an inhibitor of tumor growth, and that the expression of IKK α is positively linked with the survival of NPC patients and IKK α may be used as a novel target for differentiation therapy in NPC [15–18]. These findings demonstrated that IKK α is diversely expressed in keratinizing and non-keratinizing NPC.

Lung cancer is generally classified into a number of types including small cell carcinoma, squamous cell carcinoma (SCC), adenocarcinoma (ADC) and large cell carcinoma. SCC has been classified only by the degree of keratinization as a parameter of differentiation [19]. To further address the role of IKK α in keratinizing and non-keratinizing cancer, we chose five cases of normal lung, 20 case of SCC with keratinization and 42 cases of SCC with non-keratinization for this study. As expected in Figure 2A, weak IKK α staining was displayed in keratinizing SCC and strong IKK α staining was displayed in non-keratinizing SCC samples, moreover, the score of IKK α expression in keratinizing lung SCC was significantly lower as compared with non-keratinizing lung and non-cancer tissues (Figure 2B). Next, we used *in situ* hybridization (ISH) to analyze the mRNA level of IKK α in lung cancer. A total of 32 keratinizing and non-keratinizing lung cancer samples were evaluated with ISH. Data in Figure 2C showed that IKK α was significantly absence in non-keratinizing lung cancer, while IKK α increased in non-keratinizing lung SCC. Moreover, the score of IKK α mRNA expression in keratinizing lung SCC was significantly lower as compared with non-keratinizing lung SCC (Figure 1D). It indicated that loss of IKK α showed in keratinizing lung SCC and IKK α gained in non-keratinizing lung SCC.

To further address the role of IKK α in lung cancers, an *in silico* meta-analysis of IKK α expression profiles with Kaplan-Meier plotter (<https://kmplot.com>) performed on a cohort of lung cancers showed that higher expression of IKK α gene linked with overall survival in all lung cancer (1926 cases, Figure 3A) and adenocarcinoma (719 cases, Figure 3B), but not in lung SCC (525 cases, Figure 3C). Moreover, the higher expression of IKK α gene was correlated with first progression in all lung cancer (982 cases, Figure 3D) and adenocarcinoma (461 cases, Figure 3E). However, the higher expression of IKK α reversely was correlated with first progression in human lung SCC (141 cases, Figure 3F). It hints that IKK α may just function as initiator for cancer progression in lung SCC while IKK α only functions as a tumor suppressor in lung adenocarcinoma. Clearly, the analysis is contrast to the findings that IKK α reduction is associated with the development of spontaneous lung SCC in mice, which are associated with IKK α downregulation and inflammation [12].

Interestingly, IKK α comprises a nuclear localization signal and therefore also confers important nuclear

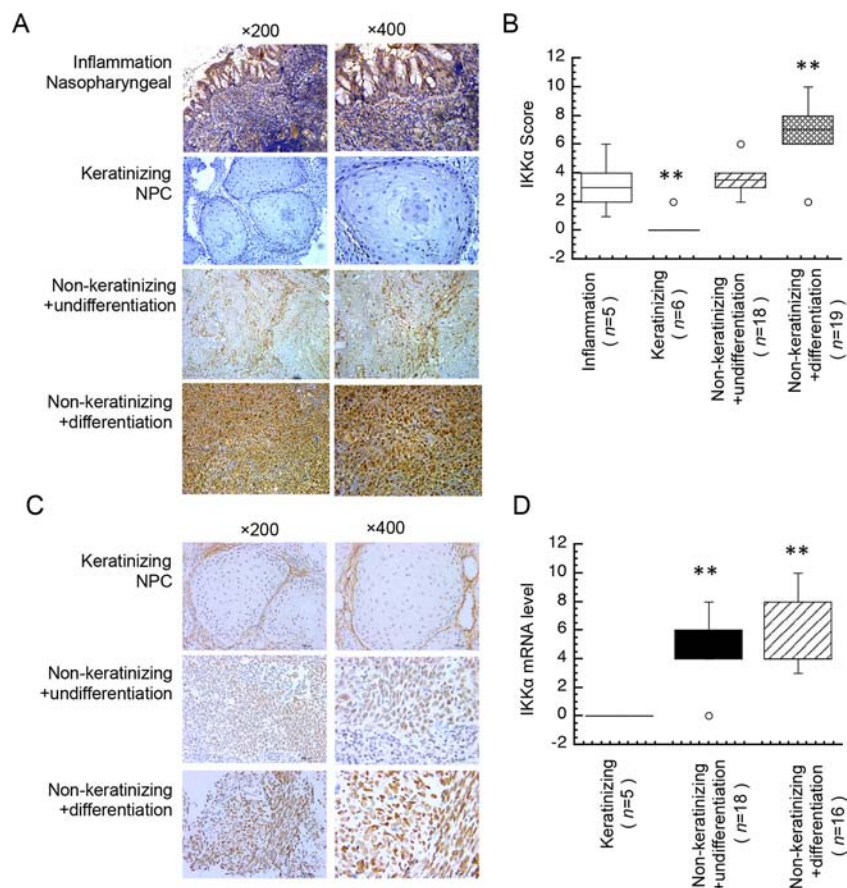


Figure 1: Different expression level of IKK α in NPC tissues. **A.** Immunohistochemical analysis was used to examine the protein level of IKK α in an inflamed nasopharyngeal and tissues from NPC patients. **B.** Expression protein level of IKK α in inflamed nasopharyngeal and subtypes of NPC tissues as indicated. **C.** The mRNA level of IKK α was analyzed by ISH in NPC tissues. **D.** Expression mRNA level of IKK α in subtypes of NPC tissues as indicated. *n*, number of analyzed samples, ** *p* < 0.01.

functions. In the nucleus, IKK α forms an intact complex with CREB binding protein (CBP) and contributes to NF κ B promoted gene expression through phosphorylation of histone H3 [20–22]. Additional tumor-promoting nuclear functions of IKK α include cell cycle regulation and chromosomal accessibility by phosphorylation of histone H3, Aurora B kinase, or the nuclear corepressor SMRT, which triggers its nuclear export with HDAC3 and its degradation [23, 24]. Phenotypic plasticity and functional heterogeneity are important features of tumors arising in various organs [25, 26]. Interestingly, depletion of Liver kinase B1 (Lkb1), a tumor suppressor encodes an evolutionarily conserved serine/threonine kinase, may initiate the transdifferentiation of lung adenocarcinoma in mice to SCC [27]. Whether IKK α shows the similar roles in the transdifferentiation remains further identification.

The epidermis is a squamous epithelium where keratinocytes follow a unique programme of terminal differentiation and cell death that leads to the formation of the cornified layer, the outermost skin barrier [28]. Keratinization is observed in the skin, tongue and external half of the lips where keratins produce the formation of

keratinization and cornification in skin modifications [29]. The somatic conditional depletion of IKK α using keratin 5 or keratin 14 promoters leads to tumor progression [12, 30]. However, in skin carcinogenesis assays, transgenic mice carrying active Ha-ras (K5-IKK α -Tg.AC mice) also develop invasive tumors, instead of the benign papillomas arising in wild type-Tg-AC mice also bearing an active Ha-ras [31], indicating a tumor promoter role of IKK α in skin cancer, similarly to what occurs in other neoplasias, including hepatocarcinomas [32], breast [33], prostate [34] and colorectal cancer [35]. Keratinized or non-keratinized epithelia is based historically on the notion that only the epidermis of skin modifications such as horns, claws and hooves is cornified, that non-modified epidermis is a keratinized stratified epithelium, and that all other stratified and non-stratified epithelia are non-keratinized epithelia [29]. Several factors such as keratins, filaggrin, loricrin, transglutaminases play critical roles in keratinization and keratinizing disorders in keratinocytes [36]. Recently, TMEM45A gene belongs to the large family of gene coding uncharacterized predicted transmembrane (TMEM) proteins and demonstrates

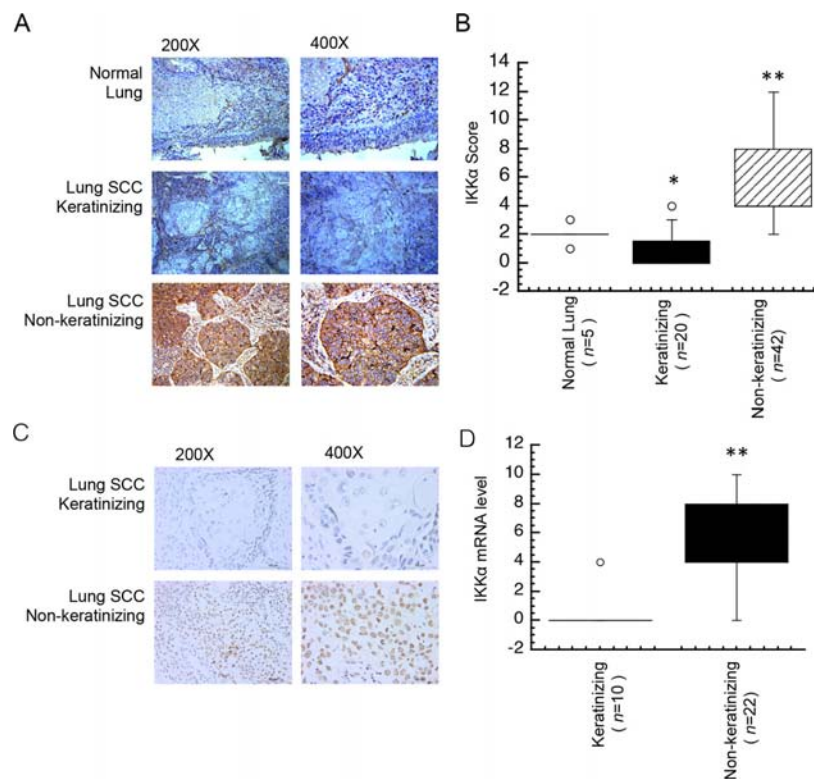


Figure 2: Different expression level of IKK α in lung SCC tissues. A. Immunohistochemical analysis was used to examine the protein level of IKK α in a normal lung and tissues from NPC patients. B. Expression protein level of IKK α in lung and subtypes of lung SCC tissues as indicated. C. The mRNA level of IKK α was analyzed by ISH in lung cancer tissues as indicated. D. Expression mRNA level of IKK α in subtypes of lung cancer tissues as indicated. *n*, number of analyzed samples, * *p* < 0.05, ** *p* < 0.01.

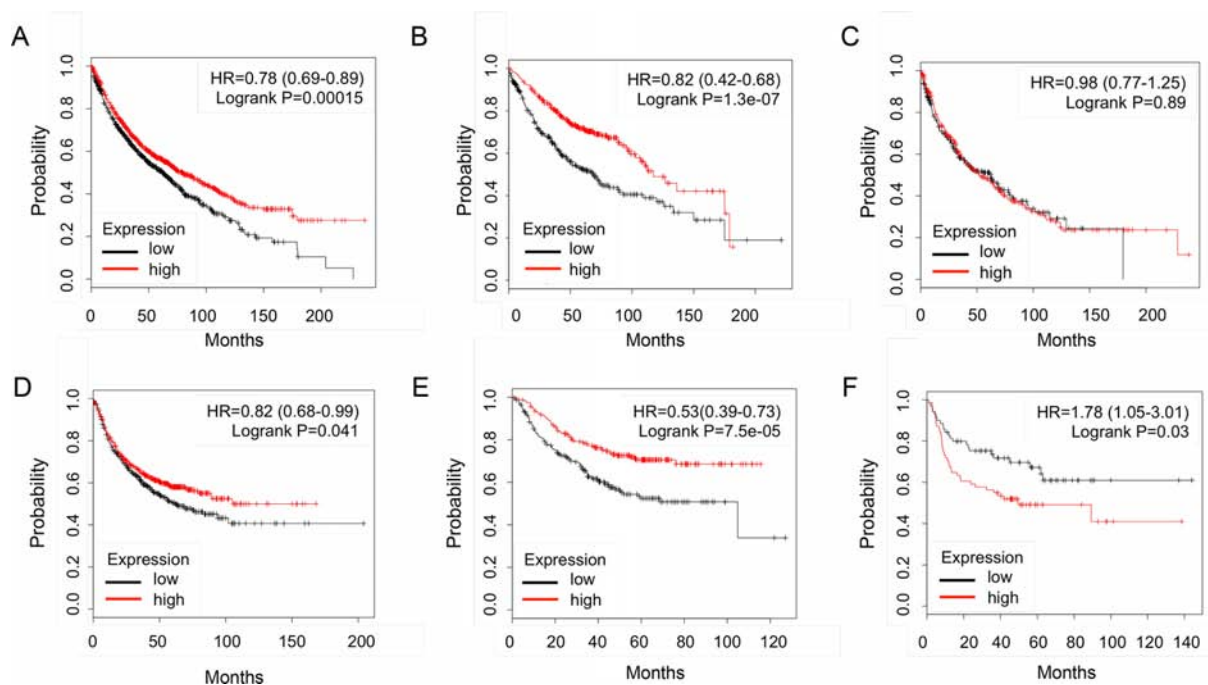


Figure 3: An in silico meta-analysis of IKK α expression profiles with Kaplan-Meier plotter in lung cancers. Overall survival analyzed in all lung cancer A. adenocarcinoma B. and SCC C. First progression analyzed in all lung cancer D. adenocarcinoma E. and SCC F.

that TMEM45A is strongly linked with epidermal keratinization [37]. It is not sure whether these factors contribute epidermal differentiation is a multi-step process regulated by specific pro-differentiation. The role of these factors in transdifferentiation of keratinizing and non-keratinizing SCCs from epidermis remains poorly known. Moreover, it is unclear that which factors and how initiate the transition between keratinizing and non-keratinizing cancer from epithelial cells. Whether and how IKK α as well as nuclear IKK α involves in the transition of NPC between keratinizing and non-keratinizing remains further identification.

Taken together, we found that IKK α expression was not expressed in keratinizing cancer but in non-keratinizing cancer, and that IKK α expression was linked with cellular differentiation in non-keratinizing NPC. These findings hint that IKK α is diversely expressed in keratinizing and non-keratinizing carcinomas in the same type of cancer.

MATERIALS AND METHODS

Tissue samples and clinical information

The ethical committee of our hospital approved the study. Archival materials with a diagnosis of keratinizing and non-keratinizing nasopharyngeal carcinoma, keratinizing and non-keratinizing lung squamous cell carcinoma were retrieved from the files of Xiangya Pathologic Anatomy Service. A total of seventy cases (5 of normal nasopharynx, 43 of NPC, 5 normal lung, and 62 of lung SCC) fit criteria for inclusion study. The histologic sections of all cases were re-reviewed and the diagnoses confirmed by the pathologist. Clinical information was extracted from the medical records. All patient data were de-identified.

Immunohistochemical analysis and ISH of biopsies

Five-micrometer-thick sections were obtained for immunohistochemical studies, which were performed on formalin-fixed, paraffin-embedded tissues using standard peroxidase immunohistochemistry techniques, heat-induced epitope retrieval buffer and primary antibodies against IKK α (Cat # IHC-00401, Bethyl). Appropriate positive and negative controls were included. All stained slides were initially reviewed and scored by the first author and re-viewed by three pathologists in a blinded fashion to ensure consistency of interpretation.

ISH was performed using the biotin labeled probe from ISH kit (Life technologies), according to the instruction of the manufacturers. The probe sequence was following:

5'-CAATGTGTTCTAGATGGAGTTAGAGGCTGTG
ATAGCTATATGGTT-3'.

IKK α staining was considered positively by ascertaining cytoplasmic and nuclear expression. The determination result was obtained from semi-quantitative classification according to 10 more visual fields ($\times 200$). The slides were first scored as 0 (negative), 1 (buff), 2 (pale brown), and 3 (tan). Positive expression of IKK α were scored as 0 (negative), 1+ (<10% of positively-staining tumor cells), 2+ (11–50% of positively-staining tumor cells), 3+ (50–75% of positively-staining tumor cells), and 4+ (>75% of positively-staining tumor cells). Both the scores by multiply were regarded as the determination result.

Statistical analysis and an *in silico* meta-analysis

The statistical association of expression of IKK α was analyzed using the SPSS 10.0 software. A two-tailed *P* value of less than 0.05 and 0.01 was considered to be statistically significant and much significant respectively. An *in silico* meta-analysis of IKK α expression profiles with Kaplan-Meier plotter from the webstie (<https://kmpplot.com>).

ACKNOWLEDGMENTS

This work was supported by the National Basic Research Program of China [2011CB504300(Y.T.); 2015CB553903(Y.T.)]; the Hunan Natural Science Foundation of China [12JJ1013(Y.T.); 2015JJ2173(L.S.)]; and the National Natural Science Foundation of China [81171881 and 81372427(Y.T.), 81271763(S.L.), 81302354(Y.S.)]; and the Hunan Provincial Innovation Foundation For Postgraduate [71380100002 (Y.J.)].

CONFLICTS OF INTEREST

The authors declare no conflict of interest. This manuscript has been read and approved by all the authors, and not submitted or under consider for publication elsewhere.

REFERENCES

1. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. *Cell*. 2008; 132:344–362.
2. Perkins ND. Integrating cell-signalling pathways with NF-kappaB and IKK function. *Nature reviews Molecular cell biology*. 2007; 8:49–62.
3. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nature reviews Immunology*. 2005; 5:749–759.
4. Senftleben U, Cao Y, Xiao G, Greten FR, Krahn G, Bonizzi G, Chen Y, Hu Y, Fong A, Sun SC, Karin M. Activation by IKK α of a second, evolutionary

- conserved, NF-kappa B signaling pathway. *Science*. 2001; 293:1495–1499.
5. Vallabhapurapu S, Karin M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annual review of immunology*. 2009; 27:693–733.
 6. Cao Y, Luo JL, Karin M. IkappaB kinase alpha kinase activity is required for self-renewal of ErbB2/Her2-transformed mammary tumor-initiating cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:15852–15857.
 7. Luo JL, Tan W, Ricono JM, Korchynskiy O, Zhang M, Gonias SL, Cheresch DA, Karin M. Nuclear cytokine-activated IKKalpha controls prostate cancer metastasis by repressing Masp1. *Nature*. 2007; 446:690–694.
 8. Tan W, Zhang W, Strasner A, Grivennikov S, Cheng JQ, Hoffman RM, Karin M. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. *Nature*. 2011; 470:548–553.
 9. Zhang W, Tan W, Wu X, Poustovoitov M, Strasner A, Li W, Borchering N, Ghassemian M, Karin M. A NIK-IKKalpha module expands ErbB2-induced tumor-initiating cells by stimulating nuclear export of p27/Kip1. *Cancer cell*. 2013; 23:647–659.
 10. Goktuna SI, Canli O, Bollrath J, Fingerle AA, Horst D, Diamanti MA, Pallangyo C, Bennecke M, Nebelsiek T, Mankan AK, Lang R, Artis D, Hu Y, Patzelt T, Ruland J, Kirchner T, et al. IKKalpha promotes intestinal tumorigenesis by limiting recruitment of M1-like polarized myeloid cells. *Cell reports*. 2014; 7:1914–1925.
 11. Liu B, Xia X, Zhu F, Park E, Carbajal S, Kiguchi K, DiGiovanni J, Fischer SM, Hu Y. IKKalpha is required to maintain skin homeostasis and prevent skin cancer. *Cancer cell*. 2008; 14:212–225.
 12. Xiao Z, Jiang Q, Willette-Brown J, Xi S, Zhu F, Burkett S, Back T, Song NY, Datla M, Sun Z, Goldszmid R, Lin F, Cohoon T, Pike K, Wu X, Schrumph DS, et al. The pivotal role of IKKalpha in the development of spontaneous lung squamous cell carcinomas. *Cancer cell*. 2013; 23:527–540.
 13. Lieberman PM. *Virology*. Epstein-Barr virus turns 50. *Science*. 2014; 343:1323–1325.
 14. Mesri EA, Feitelson MA, Munger K. Human viral oncogenesis: a cancer hallmarks analysis. *Cell host & microbe*. 2014; 15:266–282.
 15. Deng L, Li Y, Ai P, Xie Y, Zhu H, Chen N. Increase in IkappaB kinase alpha expression suppresses the tumor progression and improves the prognosis for nasopharyngeal carcinoma. *Mol Carcinog*. 2015; 54:156–65.
 16. Valentine R, Dawson CW, Hu C, Shah KM, Owen TJ, Date KL, Maia SP, Shao J, Arrand JR, Young LS, O'Neil JD. Epstein-Barr virus-encoded EBNA1 inhibits the canonical NF-kappaB pathway in carcinoma cells by inhibiting IKK phosphorylation. *Molecular cancer*. 2010; 9:1.
 17. Xie Y, Li Y, Peng X, Henderson F Jr., Deng L, Chen N. Ikappa B kinase alpha involvement in the development of nasopharyngeal carcinoma through a NF-kappaB-independent and ERK-dependent pathway. *Oral oncology*. 2013; 49:1113–1120.
 18. Yan M, Zhang Y, He B, Xiang J, Wang ZF, Zheng FM, Xu J, Chen MY, Zhu YL, Wen HJ, Wan XB, Yue CF, Yang N, Zhang W, Zhang JL, Wang J, et al. IKKalpha restoration via EZH2 suppression induces nasopharyngeal carcinoma differentiation. *Nature communications*. 2014; 5:3661.
 19. Fisseler-Eckhoff A. [Malignant lung tumors—histomorphological classification, immunohistological techniques and prognostic factors] *Kongressband / Deutsche Gesellschaft für Chirurgie Deutsche Gesellschaft für Chirurgie Kongressl*. 2001; 118:590–595.
 20. Anest V, Hanson JL, Cogswell PC, Steinbrecher KA, Strahl BD, Baldwin AS. A nucleosomal function for IkappaB kinase-alpha in NF-kappaB-dependent gene expression. *Nature*. 2003; 423:659–663.
 21. Yamamoto Y, Verma UN, Prajapati S, Kwak YT, Gaynor RB. Histone H3 phosphorylation by IKK-alpha is critical for cytokine-induced gene expression. *Nature*. 2003; 423:655–659.
 22. Huang WC, Ju TK, Hung MC, Chen CC. Phosphorylation of CBP by IKKalpha promotes cell growth by switching the binding preference of CBP from p53 to NF-kappaB. *Molecular cell*. 2007; 26:75–87.
 23. Chariot A. The NF-kappaB-independent functions of IKK subunits in immunity and cancer. *Trends in cell biology*. 2009; 19:404–413.
 24. Hinz M, Scheidereit C. The IkappaB kinase complex in NF-kappaB regulation and beyond. *EMBO reports*. 2014; 15:46–61.
 25. Tata PR, Mou H, Pardo-Saganta A, Zhao R, Prabhu M, Law BM, Vinarsky V, Cho JL, Breton S, Sahay A, Medoff BD, Rajagopal J. Dedifferentiation of committed epithelial cells into stem cells *in vivo*. *Nature*. 2013; 503:218–223.
 26. Visvader JE. Cells of origin in cancer. *Nature*. 2011; 469:314–322.
 27. Han X, Li F, Fang Z, Gao Y, Li F, Fang R, Yao S, Sun Y, Li L, Zhang W, Ma H, Xiao Q, Ge G, Fang J, Wang H, Zhang L, et al. Transdifferentiation of lung adenocarcinoma in mice with Lkb1 deficiency to squamous cell carcinoma. *Nature communications*. 2014; 5:3261.
 28. Eckhart L, Lippens S, Tschachler E, Declercq W. Cell death by cornification. *Biochim Biophys Acta*. 2013; 1833:3471–3480.
 29. Bragulla HH, Homberger DG. Structure and functions of keratin proteins in simple, stratified, keratinized and cornified epithelia. *Journal of anatomy*. 2009; 214:516–559.
 30. Liu B, Willette-Brown J, Liu S, Chen X, Fischer SM, 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 and differentiation*. 2011; 18:1854–1864.

31. Alameda JP, Moreno-Maldonado R, Fernandez-Acenero MJ, Navarro M, Page A, Jorcano JL, Bravo A, Ramirez A, Casanova ML. Increased IKKalpha expression in the basal layer of the epidermis of transgenic mice enhances the malignant potential of skin tumors. *PLoS One*. 2011; 6:e21984.
32. Jiang R, Xia Y, Li J, Deng L, Zhao L, Shi J, Wang X, Sun B. High expression levels of IKKalpha and IKKbeta are necessary for the malignant properties of liver cancer. *Int J Cancer*. 2010; 126:1263–1274.
33. Merkhofer EC, Cogswell P, Baldwin AS. Her2 activates NF-kappaB and induces invasion through the canonical pathway involving IKKalpha. *Oncogene*. 2010; 29:1238–1248.
34. Mahato R, Qin B, Cheng K. Blocking IKKalpha expression inhibits prostate cancer invasiveness. *Pharmaceutical research*. 2011; 28:1357–1369.
35. Fernandez-Majada V, Aguilera C, Villanueva A, Vilardell F, Robert-Moreno A, Aytes A, Real FX, Capella G, Mayo MW, Espinosa L, Bigas A. Nuclear IKK activity leads to dysregulated notch-dependent gene expression in colorectal cancer. *Proc Natl Acad Sci U S A*. 2007; 104:276–281.
36. Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. *Nature reviews Molecular cell biology*. 2005; 6:328–340.
37. Hayez A, Malaisse J, Roegiers E, Reynier M, Renard C, Haftek M, Geenen V, Serre G, Simon M, de Rouvroit CL, Michiels C, Poumay Y. High TMEM45A expression is correlated to epidermal keratinization. *Experimental dermatology*. 2014; 23:339–344.