





Review

Context-Dependent Role of IKK β in Cancer

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Received: 31 October 2017; Accepted: 1 December 2017; Published: 8 December 2017

Abstract: Inhibitor of nuclear factor kappa-B kinase subunit beta (IKK β) is a kinase principally known as a positive regulator of the ubiquitous transcription factor family Nuclear Factor-kappa B (NF- κ B). In addition, IKK β also phosphorylates a number of other proteins that regulate many cellular processes, from cell cycle to metabolism and differentiation. As a consequence, IKK β affects cell physiology in a variety of ways and may promote or hamper tumoral transformation depending on hitherto unknown circumstances. In this article, we give an overview of the NF- κ B-dependent and -independent functions of IKK β . We also summarize the current knowledge about the relationship of IKK β with cellular transformation and cancer, obtained mainly through the study of animal models with cell type-specific modifications in IKK β expression or activity. Finally, we describe the most relevant data about IKK β implication in cancer obtained from the analysis of the human tumoral samples gathered in The Cancer Genome Atlas (TCGA) and the Catalogue of Somatic Mutations in Cancer (COSMIC).

Keywords: IKK β ; cancer; animal model; transgenic mice; oncogene; tumor suppressor gene

1. Introduction

Cancer is the generic name given to several dozens of different diseases with the common characteristic of lacking control of cell proliferation, leading to the production of an excessive number of cells that can affect the normal function of organs and tissues. This process is usually the consequence of one or more activating mutations in oncogenes that promote cellular proliferation and/or inactivating mutations in tumor suppressor genes. The number of genes implicated in cancer is expanding constantly as we learn more about gene function; nowadays, it is estimated that more than 1% of human genes are implicated in cancer (COSMIC v82) [1,2]. Some of these genes have the intriguing property of showing both tumor protective and tumor accelerating activities, depending on the specific mutational effect or the cellular context. One such gene is *IKBKB*, which encodes a Ser/Thr kinase named Inhibitor of nuclear factor kappa-B kinase subunit beta, which is usually abbreviated in the literature as IKK2, IKKb or IKK β . IKK β is a protein able to phosphorylate a number of substrates not completely known yet (for a recent review, see [3]). In the following sections, we present our current knowledge of the relationship of this multifaceted protein with tumoral transformation in different cell types.

2. IKK β and the Regulation of NF- κ B Pathway

IKK β was first identified as part, along with the kinase IKK α and the regulatory subunit NF- κ B essential modulator (NEMO)/IKK γ , of the IKK complex, a master regulator of the ubiquitous family of dimeric transcription factors NF- κ B (for a review, see [4]). As they form a dimer, IKK α and IKK β share common functions, mainly as NF- κ B regulators but both of them also have additional individual functions not shared with the other IKK subunit [3]. NF- κ B is crucial for the proper functioning of cells, as it regulates cell survival, proliferation, apoptosis and other essential processes; besides, NF- κ B orchestrates both inflammation and immune responses [5,6]. Not surprisingly, considering the variety and importance of the processes regulated by NF- κ B, its constitutive activity is frequently associated to cancer development [7,8]. On the other side, the inhibition of NF- κ B activation decreases chemically induced lung carcinogenesis, demonstrating the contribution of NF- κ B to carcinogen-induced inflammation and consequent tumor formation [9].

I κ B (inhibitor of κ B) is a family of inhibitory proteins of the NF- κ B pathway that sequester NF- κ B dimers in the cytoplasm. I κ B members contain an ankyrin repeats domain, functionally implicated in the interaction with NF- κ B proteins. The IKK complex is responsible for phosphorylation at specific Ser residues of members of the I κ B family (for a review, see [10,11]). In general, as a result of this phosphorylation, cytoplasmic I κ B proteins are polyubiquitinated and subsequently degraded by the proteasome, thus releasing NF- κ B dimers and allowing their translocation into the nucleus where they can regulate gene expression. I κ B α itself is a target of NF- κ B, thus establishing an autoregulatory feedback loop [10,11]. The case of the I κ B protein p105 (or NF κ B1) is different, as it serves as both a NF- κ B subunit precursor and an I κ B protein. p105 is phosphorylated by IKK β and consequently processed to an active component of the NF- κ B family, p50 [12,13].

The control of NF- κ B activity by IKK β also expands to other proteins different from I κ B proteins (Figure 1). This is the case of the NF- κ B subunit p65, whose transcriptional factor activity is increased upon phosphorylation by IKK β [14]. Furthermore, IKK β phosphorylates and as a result increases the stability of Tumor necrosis factor alpha-induced protein 3 (TNFAIP3, also named A20), a negative regulator of NF- κ B in inflammation and immunity [15] which in turn prevents IKK β activation [16]. NEMO, the regulatory subunit of the IKK complex, is phosphorylated by IKK β in Ser residues at positions 43, 68 and 85; NEMO-Ser⁶⁸ phosphorylation leads to modification of the activity of the IKK complex, which possibly represents a form of fine-tuning of NF- κ B activity [17]. B-cell lymphoma/leukemia 10 (BCL10), a protein whose mutation is implicated in certain types of lymphoma, is phosphorylated at multiple sites by IKK β and consequently degraded. BCL10 promotes NF- κ B activation after receptor stimulation in several cell types, including lymphocytes. Therefore, IKK β -mediated BCL10 phosphorylation represents a negative feedback loop for termination of NF- κ B signaling in T lymphocytes [18].

In summary, IKK β is fundamental in regulating NF- κ B activity by phosphorylation of proteins with a leading role in the regulation of NF- κ B activity, such as p65, I κ B members and NEMO and also other NF- κ B family members (A20, BCL10 or p105). By this important function, changes in the expression or activity of IKK β can turn out in changes in the expression of many other proteins with different cellular outcomes. But, as discussed in the following section, this is not the only way that IKK β exploits for modifying cellular proliferation and physiology.

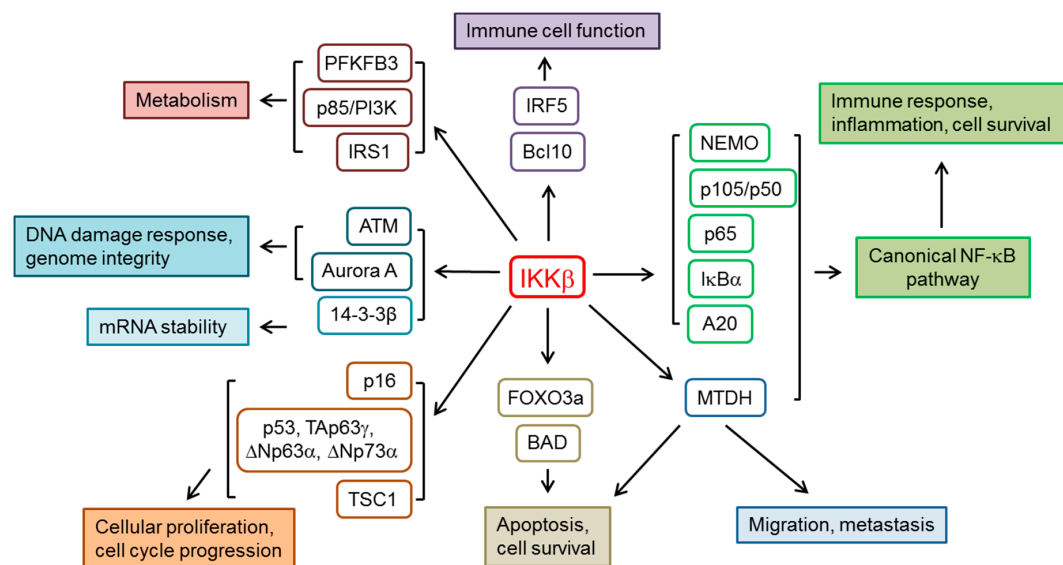


Figure 1. Main substrates of IKK β and the affected biological functions. mRNA: messenger RNA. PFKFB3: 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase isoform 3; p85/PI3K: p85 regulatory subunit of phosphoinositide 3-kinase; IRS1: Insulin receptor substrate 1; ATM: ataxia-telangiectasia mutated kinase; Aurora A: Aurora A kinase; TSC1: tuberous sclerosis 1; IRF5: Interferon regulatory factor 5; FOXO3a: Forkhead box O3a; MTDH: Metadherin.

3. Other IKK β Substrates and Cellular Functions

As previously mentioned, IKK β also phosphorylates a number of molecules that leads to changes in fundamental signaling and developmental pathways such as mechanistic target of rapamycin (mTOR), insulin and Wnt signaling. IKK β also affects cellular metabolism, autophagy, cellular responses to DNA damage and immune responses, as discussed in the following paragraphs. Not surprisingly, changes in IKK β activity can affect the majority of the biological capabilities considered as “hallmarks of cancer” [19]. In this section, we briefly review the most relevant non-NF- κ B substrates of IKK β , grouping them functionally.

3.1. Cellular Proliferation and Cell Cycle Progression

Especially important from the point of view of cancer is the activity of IKK β over tumor suppressor proteins. p53 is one of such substrates, being phosphorylated by IKK β at Ser 366, in the C-terminal regulatory domain. This phosphorylation marks p53 for ubiquitination and posterior degradation [20] and represents one of the reciprocal negative regulation steps that exist between p53 and NF- κ B pathway [21]. IKK β also phosphorylates other members of the p53 superfamily, as TAp63 γ , hindering its interaction with the transcriptional co-activator p300 and thus inhibiting TAp63 γ transcriptional activity [22]. In addition, IKK β phosphorylates and negatively regulates Δ Np63 α in response to extrinsic stimuli, as tumor necrosis factor alpha (TNF α) or chemotherapeutic agents; consequently, cells become susceptible to cell death in response to cellular stress or DNA damage [23]. Finally, IKK β phosphorylates and stabilizes Δ Np73 α in keratinocytes, thus antagonizing p53 activity; this function could be important in cellular transformation and in certain forms of cancer [24].

It is noteworthy that IKK β can also act over p16, one of the tumor suppressor proteins encoded by the Cyclin Dependent Kinase Inhibitor 2A (CDKN2A) gene. In studies performed in WI-38 human lung fibroblasts and other cell lines, it was demonstrated that IKK β binds to and phosphorylates p16 at Ser 8. This modification inactivates p16, preventing the inhibitory function of p16 over Cyclin Dependent Kinase 4 (CDK4), thus allowing progression to the phase of the cell cycle in which DNA is replicated (S phase) [25].

TSC1 is a tumor suppressor gene whose mutation leads to the tuberous sclerosis complex syndrome, a genetic disorder that causes benign tumors in skin, brain and other organs. *TSC1* is part of a protein complex that negatively regulates the activity of the mTOR pathway. It has been demonstrated in breast cancer cell lines that IKK β is a *TSC1* kinase whose activity leads to *TSC1* suppression, mTOR activation and enhanced angiogenesis, favoring tumor development. Interestingly, these IKK β -mediated alterations are also found in clinical samples of breast cancer [26].

Taken together, these data indicate that IKK β activity could favor tumor formation by means of its negative regulation of proteins with a fundamental role in cell cycle regulation and tumor suppression, as p53 family members, p16 and *TSC1*.

3.2. Immune Cell Function

The immune system is a key element to avoid the apparition and progression of tumoral lesions. IKK β affects the immune system by multiple ways and correct IKK β functioning is fundamental for the establishment of a fully functional immune system, a process probably implicating both NF- κ B-dependent and -independent functions of IKK β . Thus T cell-specific IKK β activity is needed for the formation of regulatory and memory T cells [27] and it has been recently published that genetic deletion or pharmacologic inhibition of IKK β hampers Treg-mediated suppression of activated T cells, so improving the antitumor response of these cells [28]. IKK β also directly regulates IRF5 (Interferon Regulatory Factor 5), a transcription factor important in the innate immune response to virus infection. IRF5 phosphorylation by IKK β causes its dimerization and nuclear translocation, where IRF5 can activate the transcription of target genes, as interferon beta (IFN β) and other inflammatory cytokines [29,30].

3.3. Metabolism

There are several links between IKK β and metabolism. IRS1, a member of the insulin receptor substrate family, is an adaptor protein that transmits signaling from insulin and IGF1 receptors to the PI3K/AKT (Protein Kinase B) and mitogen-activated protein kinase (MAPK) pathways, among others. IRS1 mutations are associated to insulin resistance and type II diabetes. Phosphorylation of IRS1 by IKK β can contribute to impairment of insulin signaling pathways and to the insulin resistance mediated by inflammatory processes [31].

IKK β also influences cellular metabolism by regulating the inhibition of PI3K/AKT pathway in response to starvation. This effect is mediated by phosphorylation of the p85 regulatory subunit of PI3K in response to nutrient deprivation. Interestingly, these results have been observed both in cultured cells and in a variety of tissues in response to metabolic restriction [32]. Another mechanism of modulation of cellular energy metabolism by IKK β comes from the regulation of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase isoform 3 (PFKFB3), a major driver of aerobic glycolysis. IKK β phosphorylates PFKFB3, leading to its subsequent inhibition in the low glutamine environments that can take place as tumors grow. This mechanism is considered an adaptation to metabolic stress, increasing the ability of cancer cells to survive at low glutamine concentration [33].

3.4. DNA Damage, Genome Integrity and mRNA Stability

Currently, we know a number of additional IKK β substrates that take part in fundamental cellular processes and whose deregulation is related to some forms of cancer (for a review, see [3,34,35]). Among them, we can cite ATM, a protein kinase implicated in the response to DNA damage that is mutated in the disorder ataxia-telangiectasia. In case of DNA damage, IKK β is activated, translocates into the nucleus and phosphorylates ATM, promoting DNA repair [36].

Also important from the point of view of cancer is the role that IKK β plays in the regulation of Aurora A kinase, an important regulator of cell cycle progression and maintenance of spindle bipolarity. Reduction of IKK β activity leads to an increase in the amount of Aurora A and concomitantly to spindle defects, aneuploidy and cellular transformation [37]. Recently, Shen et al. have described that

decreased phosphorylation of Aurora A by IKK β leads to cell division and developmental defects in a zebrafish model of embryos lacking IKK β [38].

Finally, phosphorylation of 14-3-3 β by IKK β results in modulation of posttranscriptional regulation of gene expression by affecting messenger RNA (mRNA) stability [39] and could therefore lead to changes in multiple cellular functions related to cancer.

3.5. Apoptosis, Cell Survival and Cell Migration

FOXO3a is a transcription factor that controls cell cycle progression and induces apoptosis. In some tumors, as a consequence of AKT activation, FOXO3a is excluded from the nucleus, thus being unable to regulate the transcription of its target genes and contributing to tumor development. Hu et al. have found that several breast tumors have nuclear exclusion of FOXO3a not in response to AKT activation but associated to expression of IKK β : so, IKK β inactivates and directs FOXO3a to degradation, thus inducing tumorigenesis in breast cells [40].

Interestingly, the study of the TNF α -induced phosphoproteome in the MCF-7 breast cancer cell line has revealed MTDH as other IKK β substrate [41]. MTDH is a regulator of multiple pathways, including Wnt and NF- κ B (through I κ B α degradation). MTDH acts as an oncogene and promotes cell proliferation and migration, metastasis and angiogenesis; in addition, its overexpression correlates with bad prognosis in several cancer types, including melanomas, breast tumors, hepatocellular carcinomas and esophageal squamous cell carcinomas (SCCs). MTDH phosphorylation by IKK β is important for TNF- α -mediated gene expression and NF- κ B regulation [41]. In other contexts, IKK β can also inhibit TNF α -induced apoptosis by phosphorylation-mediated inhibition of the proapoptotic protein BAD [42].

4. Mouse Models for the Study of IKK β Role in Cancer

Experiments performed almost 20 years ago revealed that IKK β functions cannot be assumed by IKK α or other proteins, as *Ikkkb* knock-out mouse embryos die at mid-gestation due to massive apoptosis in the hepatocytes [43–45], a phenotype similar to that observed in knock-out mice lacking p65 or other NF- κ B subunits. The lethal phenotype of IKK β -null embryos is partially rescued by simultaneous inactivation of the receptor 1 of tumor necrosis factor (TNFR1), indicating that the hepatic apoptosis observed in IKK β -null mice is dependent on TNF α toxicity [45]. Curiously, the consequences of a lack of IKK β in humans seem to be less harmful as homozygous deletion of the *IKBKB* gene is not embryonic lethal—at least in some patients—but leads to a lack of Treg and $\gamma\delta$ T cells and to severe immunodeficiency [46].

In the last years, animal models with tissue-specific modified IKK β expression have shed light on the divergent functions of IKK β in tumoral transformation of different organs and cell types (lung, melanocytes, pancreas, liver, intestine, skin and oral stratified epithelia), as we describe in this section (summarized in Table 1).

4.1. Lung Cancer

In lung, IKK β activity in alveolar epithelial cells contributes to tumoral transformation and cancer development, as its genetic inactivation impairs tumor proliferation induced by concomitant Kras^{G12D} and p53 knock-down and increases tumor latency [47]. In addition, IKK β chemical inhibition in mouse models of lung cancer also reduces tumor cell proliferation and tumor growth [47,48] and slows tumor progression [48]. Furthermore, Zaynagetdinov et al. showed that transgenic mice with inducible expression of active IKK β in airway epithelial cells, following exposure to chemical carcinogens, exhibit enhanced lung tumorigenesis. Increased tumor formation was preceded by increased proliferation of airway epithelial cells and enhanced influx of regulatory T lymphocytes [49].

Interestingly, IKK β activity in myeloid cells also affects tumorigenesis, as IKK β ablation in this cell population decreases the pulmonary inflammatory process induced by tobacco smoke and abrogates lung tumorigenesis [50].

Table 1. Summary of mouse models relevant for the study of the role of IKK β in cancer.

Type of Tumor/IKK β Modification	Phenotypic Effect	Proposed Mechanism	References
Lung Cancer			
Lentiviral-mediated <i>Ikkbb</i> deletion in a lung model of adenocarcinoma expressing <i>Kras</i> ^{G12D} and shp53.	Attenuated tumor proliferation and significantly prolonged mouse survival.	Down-regulation of the NF- κ B target TIMP-1 and ERK pathway; reduced cell proliferation.	[47]
Melanoma			
Dox-induced <i>Ikkbb</i> deletion in melanocytes expressing HRas ^{G12V} in INK4A/ARF-null background.	Inhibition of melanoma tumor development.	p53-dependent cell cycle arrest and apoptosis.	[51]
<i>Ikkbb</i> deletion in myeloid cells in a mouse model injected with BRAF ^{V600E} /PTEN ^{-/-} melanoma cells.	Growth of cutaneous and lung melanoma tumors.	Myeloid IKK β promotes antitumor immunity by modulating the chemokine CCL11 and the innate immune response.	[52]
Pancreatic Cancer			
Cre-mediated <i>Ikkbb</i> deletion in a model expressing <i>Kras</i> ^{G12D} in the pancreas.	Reduced progression of pancreatic neoplasia.	Downregulation of inflammatory cytokines and chemokines; downregulation of Notch signaling; PPARG inhibition.	[53]
Cre-mediated <i>Ikkbb</i> deletion in a model expressing <i>Kras</i> ^{G12D} in the pancreas (also in INK4A/ARF null background).	Reduced formation of pancreatic neoplasia and of pancreatic ductal adenocarcinomas.	Inhibition of inflammation and NF κ B activation.	[54]
Liver Cancer			
Cre-mediated <i>Ikkbb</i> deletion in hepatocytes.	Enhanced DEN-induced hepatocarcinogenesis.	Increased cell death and compensatory proliferation mediated by increased ROS production and JNK1 activation.	[55,56]
<i>Ikkbb</i> deletion in initiated hepatocytes transplanted onto mice expressing PLAU in hepatocytes.	Enhanced formation of hepatocellular carcinomas.	Enhanced ROS production and STAT3 activation.	[57]
Cre-mediated <i>Ikkbb</i> deletion in hepatocytes and hematopoietic-derived Kupffer cells.	Decreased DEN-induced hepatocarcinogenesis and reduced hepatocyte regeneration.	Diminished induction of hepatic mitogens (IL-6, TNF α and HGF).	[55]
Intestinal Cancer			
Cre-mediated <i>Ikkbb</i> deletion in intestinal epithelial cells.	Decreased tumor incidence in a colitis-associated cancer model.	Enhanced p53-independent apoptosis and defective Bcl-xL induction in tumor promotion.	[58]
Cre-mediated <i>Ikkbb</i> deletion in myeloid cells.	Decreased tumor incidence and size in a colitis-associated cancer model.	Reduced expression of proinflammatory mediators without effect on apoptosis.	[58]

Table 1. Cont.

Type of Tumor/IKK β Modification	Phenotypic Effect	Proposed Mechanism	References
Expression of constitutively active IKK β in intestinal epithelial cells.	Spontaneous tumorigenesis and enhanced carcinogenesis induced by APC mutation or chemical treatments.	Activation of Wnt signaling and production of a pro-inflammatory intestinal microenvironment.	[59]
<i>Ikkkb</i> deletion in mesenchymal cells mediated by a constitutive ColVI-Cre transgene.	Protection against inflammation-induced intestinal carcinogenesis.	IKK β in mesenchymal cell causes an increase in IL-6 production and STAT3 activation.	[60]
<i>Ikkkb</i> deletion in mesenchymal cells mediated by an inducible Col1a2Cre-ER transgene.	Stimulated intestinal proliferation, increased angiogenesis and promotion of colonic tumor growth.	IKK β down-regulates TGF β signaling and HGF secretion.	[61]
Non-Melanoma Skin Cancer			
Constitutive K14-Cre mediated <i>Ikkkb</i> deletion in epidermal keratinocytes.	Severe inflammatory skin disease leading to death before postnatal day 10.	Unbalanced immune skin homeostasis, mediated by TNF α .	[62]
Inducible K14-CreER mediated <i>Ikkkb</i> deletion in epidermal keratinocytes.	Skin inflammation, hair follicle disruption and epidermal pseudoepitheliomatous hyperplasia but not tumor formation.	<i>Ikkkb</i> deletion leads to STAT3 and ERK1/2 activation.	[63]
Unexpected <i>Ikkkb</i> deletion in skin keratinocytes mediated by a GFAP-Cre transgene.	Skin hyperplasia, inflammation and development of SCCs in part of the mice.	Increased TNF α expression in lesions.	[64]
Unexpected <i>Ikkkb</i> deletion in skin keratinocytes mediated by a OX40-Cre transgene.	Hyperplasia and inflammatory skin lesions.	Increased TNF α expression in lesions and T lymphocyte activation.	[65]
IKK β overexpression in epidermal keratinocytes by a K5-IKK β transgene.	Resistance to tumor development in chemically-induced NMSC models.	Tumor-protective function of IKK β is mediated by tumor suppressor proteins p16 and/or p19.	[66]
Oral and Esophageal Cancer			
IKK β overexpression in oral epithelial keratinocytes by a K5-IKK β transgene.	Spontaneous oral tumoral lesions and increased malignancy after oral chemical carcinogenesis.	Enhanced oral inflammation with infiltration of granulocytes, macrophages and B lymphocytes.	[67]
Expression of constitutively active IKK β in esophageal epithelia.	Esophagitis and increased angiogenesis in esophageal stroma.	Increased production and secretion of GM-CSF and TNF.	[68]

ROS: reactive oxygen species; SCC: squamous cell carcinoma; NMSC: non-melanoma skin cancer.

4.2. Melanoma

Mice with melanocyte-specific *Ikkkb* deletion showed less melanoma incidence than *Ikkkb* wild type mice in a melanoma model that lacks p16 and p19 and expresses a mutant form of H-Ras. This melanoma-promoting activity of IKK β is mediated, at least partially, by preventing cell cycle arrest and through modification of the expression of regulators of cell cycle, as CDK2, CDK4 and Aurora kinases A and B [51]. These authors also studied the effect of IKK β in myeloid cells during melanoma tumorigenesis in a series of experiments injecting intravenously allogenic *Braf*^{V600E}/*Pten*^{-/-} or syngeneic B16F0 melanoma cells into recipient mice lacking IKK β in myeloid cells. At difference to the effect described above for lung cancer, IKK β in myeloid cells is essential for the establishment of an efficient antitumorigenic immune response: myeloid cells lacking IKK β are less able to phagocytize and digest melanoma cells than myeloid cells expressing wild type IKK β [52]. These results reveal an interesting aspect of the role of IKK β in cancer: IKK β actually has both tumor promoting and tumor suppressing activities over melanoma development, depending on the targeted cell type (melanocytes and myeloid cells, respectively).

4.3. Pancreatic Cancer

Constitutive IKK β activity promotes leukocyte infiltration and induces acute pancreatitis [69,70]. Pancreas-specific genetic deletion of *Ikkkb* in a *Kras*^{G12D} mouse model of pancreatic ductal adenocarcinoma considerably delayed carcinogenesis and led to lower grade pancreatic lesions. These effects depend partially on modifications of IL-1 α induction and of NF- κ B activity on the one hand and on downregulation of Notch signaling and of the expression of the Notch-target genes *Hes1* and *Hey1*, on the other hand [53,54].

4.4. Liver Cancer

In hepatocellular carcinoma (HCC) studies, a somewhat more complex situation has been described: in an experimental model of hepatic carcinogenesis induced by diethylnitrosamine (DEN), a lack of IKK β in hepatocytes results in an increase in reactive oxygen species (ROS) production and in the incidence of HCCs—both in number and size—indicating an antitumoral role for IKK β in hepatocytes. By contrast, deletion of IKK β in both hepatocytes and Kupffer cells (liver resident macrophages which produce mitogens in response to liver damage) led to the development of fewer HCCs than mice expressing normal amounts of IKK β , indicating a IKK β protumoral role in Kupffer cells [55]. The effect of hepatocyte IKK β over HCC is mediated by negative regulation of signal transducer and activator of transcription 3 (STAT3) signaling [57] and by c-Jun N-terminal kinases (JNK) pathway [56].

4.5. Intestinal Cancer

Regarding intestinal cancer, different animal models have been studied. *Ikkkb* deletion in intestinal epithelial cells by a Villin-Cre transgene resulted in lower colon cancer incidence in a carcinogen-induced model, by enhanced apoptosis during tumor promotion [58]. *Ikkkb* deletion in myeloid cells by a Cre transgene directed by regulatory elements from the Lysozyme 2 gene had also an antitumoral effect but mediated by a decrease in expression of proinflammatory mediators [58]. The expression of a constitutively active form of IKK β in intestinal epithelial cells of transgenic mice induced spontaneous intestinal tumor formation, as well as enhanced tumorigenesis in models of carcinogen- or mutation-induced colorectal cancer [59]. These effects were accompanied of increased production of cytokines and chemokines and of increased β -catenin activation and Wnt signaling in the intestinal inflammatory microenvironment [59]. The mechanism through which IKK β activation in intestinal epithelial cells accelerates intestinal tumor promotion is the upregulation of inducible nitric oxide synthase (iNOS), which induces DNA damage [71].

Recently, two reports about the role of IKK β in mesenchymal cells over intestinal tumorigenesis have been published [60,61]. These articles are a good example of how very different outcomes can be obtained in response to subtle differences in the induced IKK β alterations, illustrating the complexity of IKK β functions. Constitutive *Ikkbb* deletion in mesenchymal cells by means of a ColVI-Cre transgene led to protection against inflammation-induced intestinal carcinogenesis [60], indicating an intestinal tumor-promoting role for IKK β in mesenchymal cells. When mesenchymal *Ikkbb* inactivation was accomplished by an inducible Col1a2Cre-ER transgene, intestinal tumor growth is promoted upon carcinogenic treatments [61]. As the authors of these reports discuss, these apparently opposite results could be due to differences in the mesenchymal populations targeted in both experiments, being the Col1a2Cre-ER transgene expressed in more cells than the ColVI-Cre transgene. Other possible explanation relies on the temporal differences in *Ikkbb* inactivation in both models. In addition, IKK β could have distinct functions in different fibroblast populations.

4.6. Non-Melanoma Skin Cancer, Oral and Esophageal Cancer

Mice with *Ikkbb* inactivation in skin keratinocytes suffer from a TNF α -mediated inflammatory skin disease that is rescued in the absence of TNFR1 [62]; these results highlight the importance of keratinocyte IKK β in the maintenance of a correct balance of inflammatory cells in the skin. Interestingly, *Ikkbb* deletion in skin resulted in epidermal hyperplasia and activation of STAT3 and ERK1/2 pathways but not in tumor formation [63]. Other animal models with floxed *Ikkbb* genes aimed to study IKK β functions in T cells and astrocytes led unexpectedly to cre-mediated *Ikkbb* deletion in skin keratinocytes. These mice showed a hyperplastic skin phenotype associated to inflammation that in some cases led to SCC development [64,65].

The expression of a constitutively active form of IKK β in epithelial cells of murine esophagus leads to esophagitis and increased angiogenesis in the esophageal stroma. These phenotypes were accompanied by increased esophageal infiltration of immune cells and increased levels of GM-CSF and TNF [68]. Unfortunately, the reduced life span of these mice makes difficult to perform experiments for the study of the contribution of IKK β to esophageal carcinogenesis.

We have studied mice overexpressing human IKK β in stratified epithelia under the transcriptional control of Keratin K5-derived regulatory sequences; this model is also illustrative of the varying effects of IKK β over different cell types. K5-IKK β transgenic mice are prone to the appearance of oral epithelia lesions in palate and forestomach, associated to a higher presence of infiltrating cells. These spontaneous lesions were frequently benign (from hyperplastic dysplasia to carcinoma in situ) but SCCs were also observed in some animals [67]. When K5-IKK β is accompanied by a v-Ha-Ras transgene (Tg.AC mice [72]) and subjected to an experimental oral cancer treatment, control Tg.AC mice developed benign oral lesions, whereas the majority of double transgenic K5-IKK β /Tg.AC mice developed multiple foci of highly dysplastic invasive SCCs in the oral epithelia and in the non-glandular stomach. In short, overexpression of IKK β in murine oral epithelia leads to an increase both in spontaneous tumorigenesis and in the malignancy observed after chemical induction of oral tumors, indicating an oncogenic role for IKK β in oral cancer [67].

However, when we probed this transgenic line for the role of IKK β in non-melanoma skin cancer, a completely opposing outcome was obtained, as mice overexpressing IKK β in keratinocytes were refractory to development of skin tumors under carcinogenesis protocols, indicating a tumor suppressor role for IKK β in skin [66]. K5-IKK β keratinocytes express an increased amount of several tumor suppressor proteins (p53, p16 and p19). In order to check if the antitumoral effect of IKK β in skin is related to any of these tumor suppressor proteins, we performed carcinogenesis experiments in K5-IKK β mice that simultaneously lack p53 in skin or p16 and p19 in every cell. These experiments showed that the skin tumor protective function of IKK β is independent of p53 but dependent on the proteins coded by the *Ink4a/Arf* locus (p16 and p19), as the number of tumors generated in K5-IKK β mice in the absence of p16 and p19 were similar to the number generated in control mice; interestingly, in *Ink4a/Arf* null background, K5-IKK β mice developed a significantly higher amount of

undifferentiated and spindle SCCs (the most malignant types of SCCs), indicating that the skin tumor suppressive function of IKK β turns into an oncogenic role in absence of p16 and/or p19. In order to clarify which *Ink4a/Arf*-coded tumor suppressor protein mediates these IKK β skin suppressive cancer functions, it will be needed to generate K5-IKK β mice lacking p16 or p19 individually and to study their sensitivity to skin cancer.

5. Lessons from Human Tumoral Samples

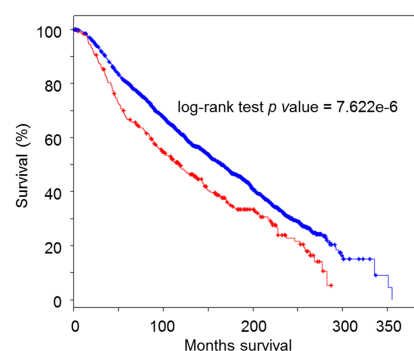
In the last years, a huge amount of genomic and expression data from numerous human tumoral samples have been generated. These data show association in some cases between tumor development and genetic or expression alterations in *IKBKB*.

For example, IKK β expression seems to be a risk factor in ovarian cancer and higher expression of IKK β in human ovarian cancer samples is associated to lower patient survival. In agreement with these data, pharmacological inhibition of IKK β or downregulation by RNA interference in ovarian cancer cell lines led to a decrease in some characteristics related to tumoral aggressiveness (as anchorage-independent growth or invasion through basement membrane) [73].

IKBKB was recognized as a cancer gene by COSMIC based on the finding of *IKBKB* activating mutations in Lys¹⁷⁵ in around 8% of splenic marginal zone lymphomas of B cells; this mutation renders a constitutively active IKK β protein [74]. A relatively high number of mutations in *IKBKB* have been found in skin basal cell carcinomas, although the interpretation of these data is difficult due to the high mutation rate found in this particular tumor type [75]. In spite of this, in general, *IKBKB* is rarely mutated and COSMIC finds only five tumor types with mutations and always at low proportions. Lys¹⁷⁵ Glu is the most frequently found mutation, although apparently there are no hotspots. In The Cancer Genome Atlas (TCGA [76–79]) there are also very few mutations affecting the *IKBKB* gene.

Gene fusions involving *IKBKB* have also been found, although this process does not seem to be a common mechanism of activation of the gene, as at the moment only a few have been found in breast [80] and prostate tumors [81].

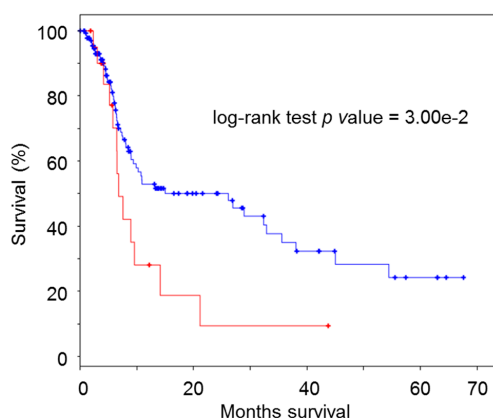
Homozygous deletions are also rare, being found in TCGA mainly in prostate (5% of tumors) and liver tumors (around 4%). No particular association with any tumor variable or mutation has been found. It is intriguing that some tumors show loss of one single *IKBKB* allele, in special liver and prostate tumors (40% of the tumors) and head and neck SCCs (35%), which seems to indicate a tumor suppressive role of IKK β in these cancer types. Interestingly, heterozygous loss or low expression of *IKBKB* is associated to mutations in *TP53* and to impaired overall survival in breast tumors (Figure 2A) and in Head and Neck Squamous Cell Carcinomas (HNSCCs) (Figure 2B).



	Total cases	No. cases deceased	Median months survival
Cases with alterations in <i>IKBKB</i>	308	205	117.9
Cases without alterations in <i>IKBKB</i>	1672	938	164.6

(A)

Figure 2. Cont.



	Total cases	No. cases deceased	Median months survival
Cases with low <i>IKBKB</i> expression	21	13	13.6
Cases with normal or high <i>IKBKB</i> expression	138	56	52.3

(B)

Figure 2. (A) Survival (months), in the TCGA Breast METABRIC cohort, separating tumors with homozygous or heterozygous deletion of *IKBKB* (red) from tumors without loss of *IKBKB* (blue). Tumor data and statistical and graphical tools are from cBioportal [78,79]; (B) Survival (months) in the TCGA Head and Neck Squamous Cell Carcinomas (HNSCC, Nature 2015 cohort), separating tumors with low *IKBKB* expression (fold change < -1.5) (red) from all the rest (blue). Tumor data and statistical and graphical tools are from cBioportal [78,79].

The analysis of the data included in cBioportal indicates that overexpression of *IKBKB* and copy-number amplifications are common, being *IKBKB* amplified at high level in around 25% of neuroendocrine prostate tumors. The same holds true in 10% of breast tumors and in around 5% of several other types of tumors in the uterus, ovary, esophagus, lung and bladder. In these tumor types, the possible existence of correlations between *IKK β* expression and any biological or clinical variable is not known but in concordance with the previously mentioned association between low expression of *IKBKB* and mutations in *TP53* in breast cancer, overexpression of *IKBKB* in breast cancer correlates negatively with *TP53* mutation but positively with ER-positive status ($p < 0.0001$, Fisher's exact test, both for data in the METABRIC and TCGA (provisional) breast cancer cohorts).

Overall, these data add to the growing body of *IKK β* protumorigenic and antitumorigenic evidence obtained using genetically modified animal models, indicating that the effect of *IKK β* over tumoral transformation is cell-type specific, favoring carcinogenesis in many cell types but not in others. The knowledge of the biological determinants that define the pro- or anti-tumorigenic role of *IKK β* is a challenge that remains open. Consequently, further work is needed, both in animal models and with human-derived tumoral samples, to unravel the cell-type specificity and complex activities of *IKK β* in cancer.

Acknowledgments: Financial support: This research was supported partially by funds from Fondo Europeo de Desarrollo Regional (FEDER) and by grants from the Fondo de Investigaciones Sanitarias (FIS) from the Spanish government (PI14/01403; CB16/12/00228; PI17/00578). We would like to apologize to our colleagues whose work has not been cited here.

Conflicts of Interest: The authors declare no conflict of interest.

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