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REVIEW ARTICLE

IKKs and tumor cell plasticity

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Nuclear factor κB (NF-κB) transcription factors are the central hubs of signaling pathways connecting proinflammatory signals to cell survival, proliferation and cytokine production. In cancers, NF-κB signaling influences many aspects of tumor development, from initiation to metastasis. These functions are mediated by tumor-induced plasticity that allows tumor cells to adapt and survive in changing conditions within the tumor microenvironment. Tumor cell plasticity is shaped by the inflammatory microenvironment in tumors. This review focuses on inhibitor of NF-κB kinases, the direct upstream elements of NF-κB regulation, specifically on their conventional and non-conventional functions in animal models of tumorigenesis from the recent literature.

Introduction

A relationship between inflammation and cancer has been proposed since Rudolph Virchow observed an increase in the number of infiltrating leukocytes in tumors in 1863 [1]. Since then, a large body of evidence has accumulated supporting such a link, and the underlying molecular mechanisms have gradually been uncovered. Today we know that a history of chronic inflammation can be attributed to more than 17% of malignancies worldwide [2]. Prolonged intake of non-steroidal anti-inflammatory drugs significantly lowers the risk of certain cancers, such as breast and colon cancer [3], suggesting that therapies targeting inflammatory processes rather than directly killing tumor cells may overcome complications due to resistance of tumors to current therapeutics.

Drug resistance is acquired from cellular heterogeneity within tumors, which in turn arises from tumor cell plasticity. The mechanisms leading to tumor cell

Abbreviations
ATM1, ataxia telangiectasia mutated serine/threonine kinase 1; BAFF, B-cell activating factor; CAC, colitis-associated colorectal cancer; CAF, cancer-associated fibroblast; CaP, prostate cancer; CRC, colorectal cancer; CYLD, cylindromatosis lysine 63 deubiquitinase; DEN, diethylnitrosamine; DMBA, 7,12-dimethylbenzanthracene; E2F1, E2F transcription factor 1; EGFR, epithelial growth factor receptor; EMT, epithelial–mesenchymal transition; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FOXA2, forkhead box protein A2; FOXO3a, forkhead box protein O3a; HCC, hepatocellular carcinoma; IFN, interferon; IKK, inhibitor of κB kinase; IL, interleukin; IκB, inhibitor of nuclear factor κB; JNK, c-Jun N-terminal kinase; LT, lymphotoxin; MAPK, mitogen-activated protein kinase; mTOR, mechanistic target of rapamycin; NEMO, NF-κB essential modulator; NF-κB, nuclear factor κB; NMSC, non-melanoma skin cancer; NPM, nucleophosmin; Rac1, Rac Family Small GTPase 1; RANK, receptor activator of nuclear factor κB; RIPK1, receptor-interacting serine/threonine-protein kinase 1; ROS, reactive oxygen species; SCC, squamous cell carcinoma; SMRT, silencing mediator of retinoic acid and thyroid hormone receptor; SOD, superoxide dismutase; STAT3, signal transducer and activator of transcription 3; TAX1BP1, Tax1 binding protein 1; TGFβ, tumor growth factor beta; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; TSC, tuberous sclerosis complex subunit; VEGF-A, vascular endothelial growth factor A; XBP1, X-box-binding protein 1.

plasticity include mutations, epithelial–mesenchymal transition (EMT), dedifferentiation and inflammation, which help cancer cells adapt to changes and threats within the tumor microenvironment. Among these mechanisms, inflammation plays a central role. As will be explained in detail, the inflammatory microenvironment provides all the conditions for cells to accumulate further mutations, to dedifferentiate to gain stem cell-like properties and to go through the EMT for invasion and formation of distant metastases. Therefore, we need a better understanding of inflammatory pathways and their regulators to be able to develop superior therapeutic options that target tumor cell plasticity and tumor development. This review particularly focuses on inhibitor of nuclear factor κB (IκB) kinases (IKKs) as the master regulators of inflammatory signaling mechanisms that shape tumor cell plasticity in various cancer models.

Tumor cell plasticity

Plasticity by definition means the ability to change or adapt to varying conditions. Cellular plasticity is required for tumor cells to adapt to an ever-changing tumor microenvironment for growth and survival amidst various environmental threats. Most of the tumors arising from a single cell have an unlimited proliferative ability yet differ in many other capabilities such as self-renewal, handling stress to avoid death, escaping immune surveillance and invading to form distant metastases. All these differences within tumors are the result of a wide-ranging cellular heterogeneity [4–6]. In addition to heterogeneity, the great level of complexity within tumors is achieved by elaborate interaction of various cell types in response to alteration of the tumor microenvironment [7]. Altogether, cellular plasticity-driven heterogeneity accounts for the development of resistance to chemotherapeutic reagents.

How is tumor cell plasticity achieved? There are basically four fundamental mechanisms, namely mutation, dedifferentiation/transdifferentiation, EMT and inflammation (Fig. 1). Together with epigenetic interactions, tumor cell fusion and exocytic vesicles, these shape the cellular plasticity of the tumors [8]. However, this review focuses only on the fundamental mechanisms that lead to plasticity during the initiation, progression and metastasis of tumors.

The first source of tumor cell plasticity is mutations. As seen in the process of evolution, heterogeneity – variation – must pre-exist in tumor cell populations before any change in the tumor microenvironment occurs. Mutations are key players in cellular heterogeneity within tumors [9]. Driver mutations, such as chromosomal or microsatellite instability and loss of DNA proofreading or repair machinery, increase tumor cell susceptibility to further mutations to escape cell death or to gain new features required for malignant transformation [10]. Besides, mutations within a solid tumor can give rise to different populations that take part in invasion, metastasis, drug resistance and tumor recurrence [11]. Mutations are not only a component of tumor cell plasticity, but also the basis of all the other mechanisms leading to it. Therefore, every fundamental feature of tumor cell plasticity is based on acquiring sufficient mutations to achieve heterogeneity within the tumors for the growth and expansion of cancer cells.

The second fundamental mechanism that drives tumor cell plasticity is dedifferentiation and/or transdifferentiation, respectively the ability of tumor cells to acquire stem cell-like characteristics supporting continuous proliferation of growing tumors and to shift back to differentiated states [8]. In a given tissue, stem cells are the only cell type that maintains tissue homeostasis following damage or renews the tissue by transforming into any other cell type [8,11,12]. Normally, differentiated cells lose this capability, but differentiated cells within tumors can dedifferentiate, adopting a stem cell-like phenotype [11], and therefore cancer stem cells can arise from the tissue stem cells or from differentiated quiescent cells to sustain tumor growth. This ability is essential to repopulate the cancer stem cell niche against any cellular stress leading to the loss of stem cells [13], thus making stemness and dedifferentiation major assets for tumor cell plasticity.

The third important mechanistic insight into tumor cell plasticity is related to EMT, which grants tumor cells the ability to invade deeper into the tissue or to form distant metastases [14]. When tumors grow to their natural boundaries limited by the surrounding tissues and extracellular matrix, it is a challenge for their metabolically active cells to maintain a nutrient supply sufficient for their high demands [15]. To do so, some tumor cells remodel the extracellular matrix to open up extra space for new tumor cells to invade by coordinating fibroblasts and leukocytes, while others induce angiogenesis to initiate a new blood supply together with endothelial cells, and others still intravasate into the blood circulation to find new places to grow without competition [14,15]. Since only mesenchymal-like cells can activate molecular pathways related to motility, almost all these processes require EMT for cells to gain motility to leave the tumor stroma and form new colonies [16]. However, the mesenchymal state is not as proficient as the epithelial state in terms of proliferative ability [17,18]. Epithelial

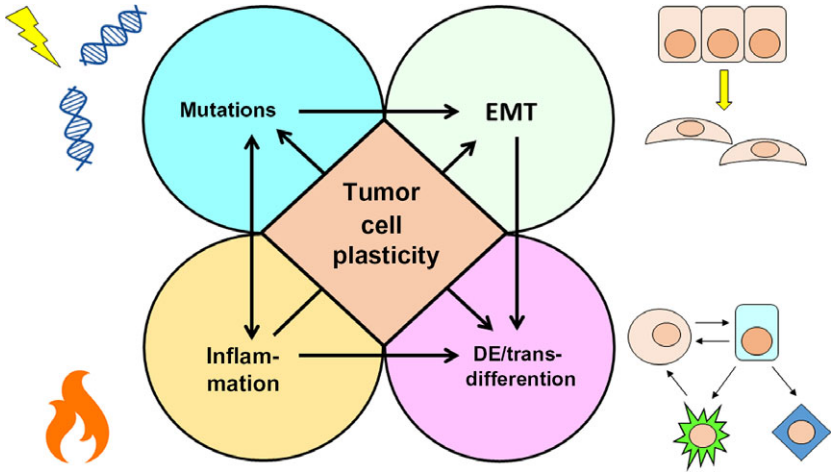

Fig. 1. Mechanisms of tumor cell plasticity. Mutations, EMT, dedifferentiation/transdifferentiation and inflammation are the main mechanisms that gives rise to tumor heterogeneity. Heterogeneity is important in enabling tumor cells to adapt to and survive environmental threats.

cells have a basal attachment for efficient proliferation and additionally paracrine growth signals become more effective when the cells are closely packed. Although mesenchymal cells lose these advantages, metastasizing mesenchymal cells still have the capacity to return to a highly proliferative epithelial state to form distant colonies [19–21]. Therefore, epithelial–mesenchymal plasticity is a two-way process helping the tumor cells reprogram their physiology according to the changing demands of the tumor microenvironment.

The last but not least important mechanism of tumor cell plasticity is the ability to create a chronic inflammatory environment within solid tumors. An inflammatory microenvironment can promote tumor growth by triggering the release of growth hormones from surrounding stromal or immune cells, leading to altered cytokine profiles within the tumor, and thus evasion of immunosurveillance. Chronic inflammation also induces a wound healing response, angiogenesis or EMT to enable tumor cells to invade and eventually escape to form metastases [22,23]. Recently, it has been found that a microenvironment that favors growth and increased mutational rates due to reactive oxygen species (ROS) stress and compensatory proliferation upon excessive cell death can initiate tumorigenesis as well [24]. Taken together, an inflammatory environment can be the driving force for tumor cell plasticity. Eventually, the success of tumor cells not only results from their intrinsic ability to alter their own physiology but also depends on how they transform the whole tumor microenvironment to promote tumor growth.

Inflammation as the driving force for tumor cell plasticity

Inflammation is the natural response, elicited through concerted activity of infiltrating leukocytes, cytokine secretion and tissue regeneration, when the host is faced with tissue injury or pathogens, in an attempt to heal a wound or eliminate a pathogen [25]. Normally, inflammation is resolved as soon as the threat to tissue integrity has been eliminated. However, incomplete elimination of a pathogen or failure to properly shut down the inflammatory pathways will result in chronic inflammation. This is one of the hallmarks of cancer, leading to continuous cytokine and protease secretion, and production of ROS and nitric oxides that eventually create an inflammatory microenvironment favoring tumor initiation [24,26]. This microenvironment has many cellular components, namely tumor cells, stromal cells, and innate and adaptive immune cells. It is the intricate signaling among these cell types that tilts the balance towards protumorigenic or antitumorigenic immunity [27].

Among all the molecular pathways that bridge inflammatory regulation of tumor microenvironment and cellular plasticity, nuclear factor κB (NF-κB) signaling represents a central hub due to its versatile roles in inflammation and tissue regeneration [28–31]. While chronic inflammation increases susceptibility to cancer development, NF-κB signaling can also contribute to tumor promotion in cancers not associated with pre-existing inflammation by creating an inflammatory tumor microenvironment [32]. The NF-κB family comprises proteins that regulate expression of genes

involved in inflammation, innate and adaptive immune responses, cell survival, and cancer [28,33–35]. Although both NF-κB (e.g. RelA, RelB, cRel, p50, p52) and its regulatory IκB subunits (e.g. IκBα, IκBβ, IκBε, Bcl3, p100, p105) are numerous, little has been revealed of the mutations that affect the functions of these molecules in different diseases [36]. Consequently, our knowledge about their regulation has come mainly from genetic models of upstream regulatory elements, such as IKKs, in cancers of hematopoietic origin [37].

A core element of NF-κB activation is the IKK complex built from two catalytic subunits, IKKα and IKKβ kinases, and a regulatory subunit, NF-κB essential modulator (NEMO)/IKKγ [29,31]. We know that NF-κB subunits are regulated by the IKK complex, which is in turn regulated by diverse upstream signaling mechanisms comprising growth, death, stress and pathogen recognition receptors. Activation of NF-κB transcription factors is highly dependent on both classical and alternative pathways that mediate degradation of IκB and IκB-related proteins [29,30]. The classical (or canonical) pathway utilizes IKKβ- and IKKγ-dependent IκBα degradation in response to proinflammatory stimuli (such as interleukin (IL)-1, tumor necrosis factor (TNF)-α and toll-like receptor ligands) releasing active NF-κB dimers into the nucleus, where they bind to DNA and mediate gene expression [38,39]. In contrast, the alternative (or non-canonical) pathway can be activated by ligands (such as B-cell activating factor (BAFF), lymphotoxin (LT) -$\alpha_1\beta_2$ and CD40L) acting on their specific receptors (BAFFR, LTβR and CD40, respectively) and depends exclusively on IKKα and its upstream kinase, NF-kB-inducing kinase [40–43]. Throughout the text we will use the term 'conventional' to refer to the NF-κB-dependent (comprising both classical and alternative pathways) functions of IKKs and 'non-conventional' for the NF-κB-independent functions or targets of IKKs. Still, it cannot be ruled out that IKKα/β activity on non-conventional targets or pathways may also be induced through similar upstream elements to those inducing conventional (classical or alternative) NF-κB signaling pathways [44]; nonetheless, IKKα/β activity on non-conventional targets may not require any of the downstream elements activated by NF-κB transcription factors [45,46]. To better illustrate conventional and non-conventional targets of IKKs in different cancers, Tables 1 and 2.

Despite the fact that canonical IKKs have structural and functional similarities, they have distinct roles, and diverse phenotypes have been reported for IKKα and IKKβ knockout mice [32]. Many studies have addressed the role of IKK subunits in tumor development because of their therapeutic potential, which is largely absent for NF-κB or IκB subunits. Although there is a potential use of specific peptides blocking the interaction of non-kinase subunits of signaling complexes, such as IKKγ in IKK complex, to terminate downstream signaling [47], the number of such studies is still limited compared with studies addressing catalytic subunits of IKK complexes [48–50]. Therefore, in the next section, we concentrate on IκB kinases as the key mediators of cytokine signaling that are able to orchestrate inflammation in the tumor microenvironment. The focus on both conventional and non-conventional functions, with specific examples of *in vivo* animal models of solid tumors, will explain how these molecules affect tumor cell plasticity by shaping the tumor microenvironment.

Specific roles of IKKs in tumor cell plasticity

Though the roles of canonical NF-κB signaling have long been well described in many cancers, specific functions of IKKβ or IKKα in tumor cell plasticity were poorly characterized until recently. Accumulating evidence suggests that in their non-conventional roles, these kinases possess a greater level of complexity in the way they regulate inflammation, proliferation and metastasis due to their ability to phosphorylate diverse substrates [51]. Table 1 summarizes conventional and non-conventional targets or signaling pathways controlled by IKKs and their effects on tumor cell plasticity in different cancers (also see Fig. 2). Detailed information on the non-conventional IKKα/β targets in different signaling pathways can be found in Table 2. In contrast to IKKβ, which spends most of its time in the cytoplasm chasing canonical and non-conventional targets, IKKα translocates into the nucleus where it regulates non-conventional substrates such as p65, histone H3, p27, forkhead box protein A2 (FOXA2) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) to direct various mechanisms in inflammation and cancer (Table 2 and Fig. 3) [32,51–53].

IKKs are involved in tumor-elicited inflammation through their indispensable roles in regulating inflammatory cells and their interactions with tumor cells within the tumor microenvironment. Specifically, IKKs regulate proinflammatory or anti-inflammatory signaling machinery through phosphorylation of numerous targets, such as NF-κB, protein inhibitor of activated STAT1 (PIAS), interferon regulatory factor 3/7 and Tax1 binding protein 1 (TAX1BP1). These targets in turn regulate downstream factors such as TNF, IL-1, IL-6, IL-18, interferon (IFN)-α/β, IFNγ, various other

Table 1. Conventional and non-conventional targets/signaling pathways controlled by IKKs and their effects in tumor cell plasticity in different cancers. Gsk3β, glycogen synthase kinase 3β; iNOS, inducible nitric oxide synthase; NMSC, non-melanoma skin cancer; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; SCC, squamous cell carcinoma.

Cancer type	IKK subunit	Role	Target molecules/ pathways	Effect in mechanisms regulating tumor cell plasticity	References
Colorectal	IKKβ	Oncogene	NF-κB, Wnt, Twist, Bcl-XL, IL-6, STAT3, iNOS, Rac1, NOTCH	Inflammation, stemness, dedifferentiation, metastasis, survival, proliferation	[59,62–65,70,77]
	IKKα	Oncogene	NF-κB, p65, IFNγ, TAX1BP1	Anti-inflammation, proliferation, tumor tolerance	[54,83,86]
Breast	IKKβ	Oncogene	FOXO3a, TSC1, GSK3β, NOTCH, IL-6, Snail, Twist, HER2	Inflammation, proliferation, survival, stemness	[68,87,88,90–92,94,97, 103,104]
	IKKα	Oncogene	Myc, mTOR, p27, HER2, Maspin1	Proliferation, survival, metastasis	[95,98,105–108]
Liver	IKKβ	Oncogene	NF-κB, IL-6, STAT3, JNK, SOD, XBP1, PFKFB3	Inflammation, proliferation, ER stress, survival	[56, 110–113, 116, 123]
	IKKα	Oncogene	FOXA2, NOTCH, ATG16 l1	Proliferation, inflammation, survival	[57,117]
Skin	IKKβ	Tumor suppressor	P16/CDKN2a	Stemness, proliferation	[148]
Melanoma, SCC, papilloma	IKKα	Tumor suppressor	ERK, p53, TGFβ, cyclin D1, Myc, NPM	Anti-inflammatory, anti-proliferative, stemness, dedifferentiation	[132,134,135,138–141]
NMSC	IKKα	Oncogene	EGFR, VEGF-A, Maspin1, MMP9	Proliferation, EMT, metastasis	[142,143,147]
Lung	IKKβ	Oncogene	NF-κB, IL-6, JNK	Proliferation	[150,152]
	IKKα	Tumor suppressor	NF-κB	Anti-inflammatory, Anti-proliferative, tumor surveillance	[153]
Prostate	IKKα	Oncogene	Maspin1, IKKβ, E2F1	Metastasis, proliferation, regeneration	[156–158]
Ovarian	IKKβ	Oncogene	NF-κB, IL-6, IL-11	Inflammation, proliferation, survival	[160,161]
Pancreas	IKKα	Tumor suppressor	NF-κB	Anti-inflammatory	[163]

cytokines and signal transducer and activator of transcription 3 (STAT3) [45,54,55], which also have pleiotropic effects on proliferation, oxidative stress, endoplasmic reticulum (ER) stress and anti-apoptotic machinery, challenging genomic integrity and facilitating mutations, another important mechanism in tumor cell plasticity.

Besides these inflammatory targets, IKKs can also directly or indirectly regulate many proliferative factors, such as (a) cyclin D1, epidermal growth factor receptor (EGFR), tumor growth factor β (TGFβ), c-Myc and FOXO3a, which in turn favor mutations due to increased proliferation [46]; (b) many components in survival pathways activated upon stress such as CYLD Lysine 63 Deubiquitinase, X-box-binding protein 1 (XBP1), superoxide dismutase (SOD), Rac Family Small GTPase 1 (Rac1), ATG16 l1, receptor-interacting serine/threonine-protein kinase 1 (RIPK1), extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) [45,56–59]; and (c) various controlling factors such as p53, Aurora A, ATM Serine/Threonine Kinase 1 or nucleophosmin (NPM) that orchestrate factors to preserve genomic integrity in other cancers [45,51]. IKKs also control factors such as SMRT, FOXA2, histone deacetylase 3, histone H3α, Smad2/3 or E2F transcription factor 1 (E2F1) participating in NOTCH, Wnt or TGFβ pathways [46,51]. The latter is important signaling machinery as highly dividing tumor cells require stemness and dedifferentiation/transdifferentiation to maintain the ability to regenerate or adapt to various stressful conditions within the tumor microenvironment. Last but not least, the specific functions of IKKs in tumor cell plasticity include their abundant functions in controlling EMT through factors such as Wnt, Maspin1, Twist and Snail [8,60]. Formation of tumor cell plasticity highly depends on the regulation of canonical and non-canonical targets by the tissue- and context-specific roles of

Table 2. Detailed information about non-conventional IKKα/β targets in different signaling pathways. The information in this table is compiled mostly from studies with animal tumor models. For more exhaustive information about non-conventional IKKα/β targets in various signaling machinery, not necessarily related to tumor cell plasticity, refer to previous reviews [45,46,51]. AIB1/SRC-3 alliases for NCOA3, nuclear receptor coactivator 3; ERα, esterogen receptor alpha; MMP9, matrix metalloprotease 9; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3.

Non-conventional IKK targets	Targeted by	NEMO-dependency	Catalytic activity	Cellular localization	Cell type	References
Histone H3α	IKKα	Yes	Yes	Nucleus	Fibroblasts, intestinal, breast	[40,53,86,97]
SMRT	IKKα	Yes	Yes	Nucleus	Intestinal	[52]
NPM	IKKα	No	Yes	Nucleus	Skin	[141]
Maspin1	IKKα	No	No (through p27)	Nucleus	Breast, skin, prostate	[108, 143, 156]
p27	IKKα	No	Yes	Nucleus	Breast (skin, prostate?)	[106]
Cyclin D1	IKKα	No	Yes (through histone H3α)	Nucleus	Breast	[175]
FOXA2	IKKα	No	Yes	Nucleus	Hepatocytes	[117]
E2F1	IKKα	No	Yes	Nucleus	Prostate	[158]
ATG16 l1	IKKα	Yes	Yes	Cytoplasm	Intestinal	[57]
TAX1BP1	IKKα	No	Yes	Cytoplasm	Fibroblasts	[83]
EGFR, VEGF-A, MMP9	IKKα	Unclear	Unclear	Cytoplasm	Skin	[147]
c-Myc, integrin-α6	IKKα	No	Yes	Nucleus	Skin	[147]
ERα, AIB1/SRC-3	IKKα	Unclear	Yes	Cytoplasmic	Breast	[97]
TSC1	IKKβ	Unclear	Yes	Cytoplasm	Breast, hepatocytes	[88,122]
XBP1	IKKβ	Yes	Yes	Cytoplasm	Hepatocytes, (adipocytes?)	[56]
PFKFB3	IKKβ	Yes	Yes	Cytoplasm	Breast, fibroblasts	[123]
FOXO3a	IKKβ	Unclear	Yes	Cytoplasm	Breast	[87]
RIPK1	IKKα/β	Yes	Yes	Cytoplasm	Hepatocytes	[115]
NOTCH	IKKα/β	No	No (through SMRT or FOXA2/NUMB)	Nucleus	Intestinal and breast (through SMRT), hepatocytes (through FOXA2)	[52,103]
IL-6	IKKα/β	Yes	No (through NOTCH)	Nucleus	Breast	[103]
STAT3	IKKα/β	Yes	No (through NOTCH)	Nucleus	Breast	[103]

the IKK kinases. To better illustrate this, we give several examples of the pivotal roles IKKs play in shaping tumor cell plasticity in what follows.

Among many studies associating NF-κB signaling with almost any cancer in the past two decades, there have been only a limited number providing mechanistic insights, and even fewer describing the roles of IKKs in animal models of tumorigenesis. Since we believe that the complex regulations and interactions within the tumor microenvironment can be best understood through the use of genetic disease models and transgenic animal models, here we select those studies, classified by individual cancers, to demonstrate how canonical or non-canonical IKK functions affect tumor cell plasticity. Of note, however, due to the genetic and physiological differences between these species, there is a possibility that not all the information derived from animal models can be translated into human disease. Recent literature has witnessed an increased interest in patient-derived xenografts or patient-derived organoid models to better approximate and model human diseases. Although these are powerful tools for rapid and reliable drug screening, toxicological assessment and combinatorial targeting, they fail to establish complex interactions within the tumor microenvironment. To resolve such problems, advances in cell-based co-cultures of multiple origin may be a good alternative. Developments in this area or in related cell-based techniques will eventually eliminate the requirement for animal use in cancer or any other disease research, yet the information derived from such studies about the specific functions of IKKs in various solid malignancies is very limited at the present.

Cancer specific functions of IKKs in tumor cell plasticity

Colorectal cancer

Constitutive NF-κB activation, a common theme in most cancers, is one of the leading reasons that tumors develop resistance against chemotherapy or radiation. NF-κB activity is crucial for the activation of anti-apoptotic signaling that protects tumor cells from

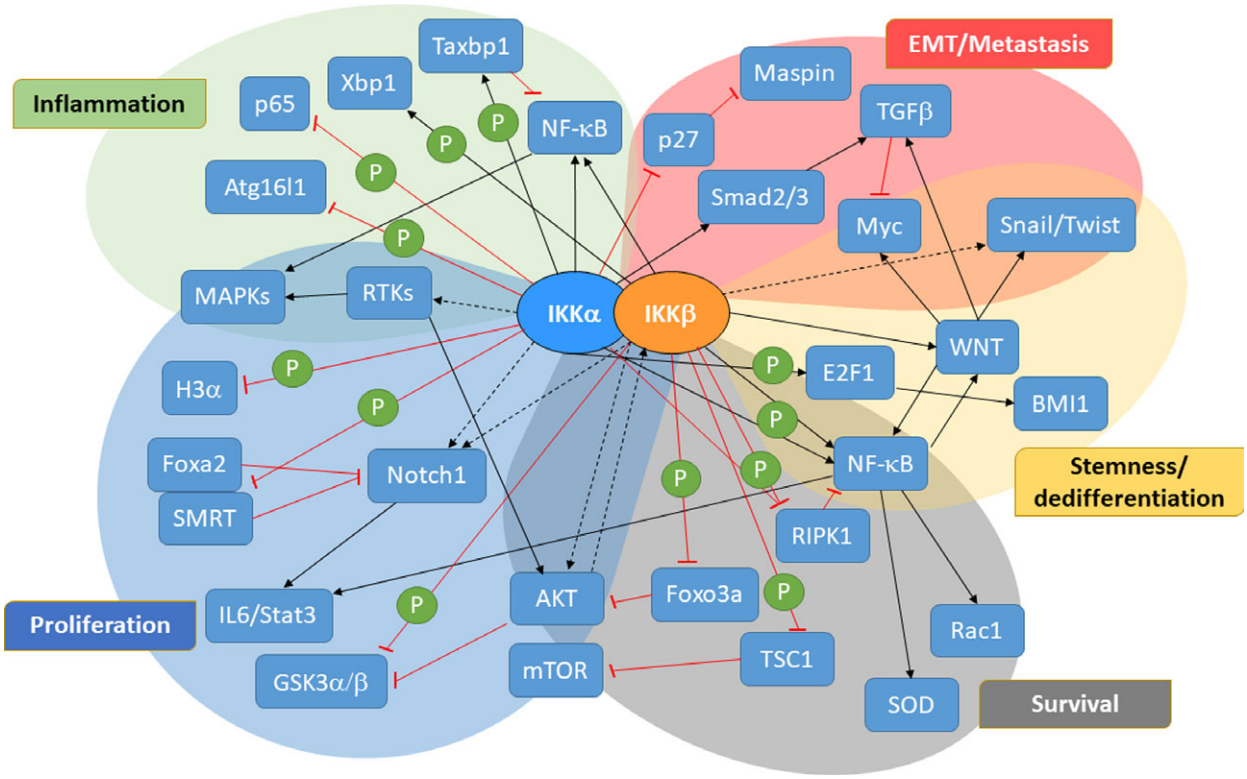

Fig. 2. Signaling pathways governed by IKKs in tumor development and tumor cell plasticity. A summary of the combination of signaling molecules and pathways targeted by IKKs in different cancers. It should be noted that not all pathways are active at the same time or work in the same way in each cancer. Most of these pathways are differentially regulated in different cancers and even in different subtypes. Tables 1 and 2 give more detailed information about IKK target molecules/pathways and how they affect tumor development in various genetic cancer models. GSK3α/β, glycogen synthase kinase 3α/β; H3α, histone H3 alpha; RTK, receptor tyrosine kinase.

death [61]. Activation of the anti-apoptotic machinery in most cancers, especially in colorectal cancer (CRC), through induction of NF-κB-dependent gene expression requires IKKβ catalytic activity. In a colitis-associated colorectal cancer (CAC) model for tumorigenesis, $Ikk\beta^{\Delta IEC}$ (*Ikbkb*) mice were found to have reduced tumor burden due to increased apoptosis through downregulation of Bcl-x_L [62]. On the other hand, $Ikk\beta^{\Delta M\Phi}$ mice in a CAC model were found to have reduced tumor number and size due to decreased proliferation resulting from diminished macrophage-derived IL-6 synthesis [62]. Later, it was suggested that macrophage-derived IL-6 and inducible nitric oxide synthase promote tumor growth via inflammatory STAT3 [63,64], activation of which has been shown to regulate proliferation, survival and NF-κB signaling in a CAC tumor model [65,66].

IKKβ-driven inflammation not only provides proliferative or survival advantage but may also influence stemness and differentiation. As suggested in previous studies on different tissues, IKKβ-driven inflammation blocks the differentiation of adipocytes, osteoclasts and vascular endothelia to lock them in a more stem cell-like phenotype through various mechanisms involving regulation of Wnt signaling [67–69]. In our recent work, we have shown that non-stem cells from intestinal epithelia can convert to a stem cell-like phenotype in the presence of constitutive Wnt and NF-κB activation in spontaneous mouse models of colorectal tumorigenesis [70]. In the same work, activated NF-κB could induce Wnt signaling or vice versa through the cooperation of their transcription factors at related gene promoters [70]. However, the latter was not mechanistically illustrated and the proof for this mechanism came from a related study in which it was found that both NF-κB activation and ROS production are controlled by Rac1 in a Wnt-driven colorectal carcinogenesis model by loss of the *Apc* gene [59]. Accordingly, $Lgr5^+$ stem cell expansion in Wnt-driven tumors mechanistically required the activation of Rac1 to induce ROS production, and as a result, a marked activation of NF-κB to protect stem cells from apoptosis [59]. Other studies showed that IKKβ expression in cancer-associated fibroblasts (CAFs), also known as

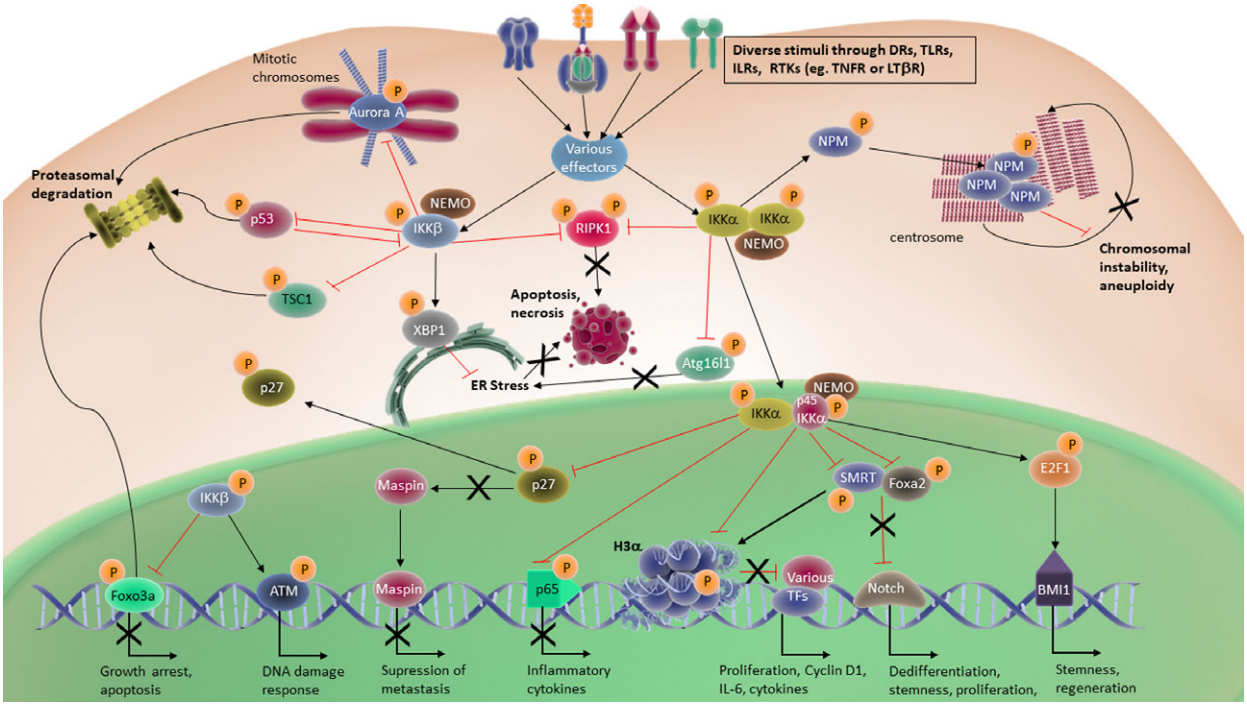

Fig. 3. NF-κB-independent functions of IKKs in tumor development. This figure summarizes some of the most common non-conventional IKK targets in different tumors. Again the regulation on these molecules is determined according to tissue type, tumor stage and other factors within the tumor microenvironment. Therefore, the outcome of any given regulation may show dramatic differences from one cancer to another. Table 2 gives more detailed information about non-conventional IKK targets in various malignancies. ATM1, ATM Serine/Threonine Kinase 1; BMI1, B cell-specific Moloney murine leukemia virus integration site 1; DR, death receptor; IL1R, interleukin 1 receptor; RTK, receptor tyrosine kinase; TF, transcription factor; TLR, toll-like receptor.

intestinal mesenchymal cells, may have potential roles in colorectal tumor cell plasticity [71,72]. Despite the fact that contrasting results were obtained in these studies, the oncogenic potential of IKKβ in CRC may be due to differences in tissue-specific ablation strategies that were constitutive [71] rather than inducible [72]. Although there are some critics requesting further proof on IKKβ-specific roles in CAFs and CRC [73], it is noteworthy that while germline mutations are often compensated during the course of development, studies with inducible models can illustrate better the sporadic nature of cancer.

It remains unknown how IKKβ-driven NF-κB activation triggers the metastatic shift in colorectal tumors. It was first shown that constitutively induced cytokines and a chronic inflammatory phenotype were not sufficient to drive inflammation in the intestine without mitogen-activated protein kinase (MAPK) signaling [74]. MAPKs are required for c-Myc stabilization upon activation of toll-like receptor signaling in Wnt-driven tumor models [75]. Hence, MAPKs and their downstream transcription factors regulate the expression of various cytokines and proliferative factors by co-operating with NF-κB at promoter regions of target genes [74,76]. However, in later studies, our colleagues have proven that this proinflammatory environment created by constitutive IKKβ activation is not only required for tumor initiation and progression but also for invasion and metastasis upon p53 loss [77]. Consequently, Twist1, an EMT marker, was transcriptionally activated leading to tumor invasion and lymph node metastasis [77].

IKKα regulates intestinal tumor cell plasticity through modulation of inflammation and cell to cell interactions within the tumor microenvironment. It is widely known that immune cells can suppress, tolerate or promote the growth of tumor cells in various cancers, yet the regulation of immune cells in the early stages of tumor development has been poorly described until recently. A recent study on the V12RAS-driven zebrafish melanoma model clearly demonstrated that myeloid cells were recruited to the tumor-initiating cells as soon as the oncogenic transformation occurred [78]. Interaction of both cell types is required for the tumor progression parallel to the wound healing response [78]. Similar to these observations, we have identified a unique intraepithelial localization of myeloid cells in the intestines of mice devoid

of IKKα activation (*Ikbka*$^{S181A/S185A}$ or simply *IKKα*$^{AA/AA}$) upon β-catenin-induced transformation [54]. Mechanistically, IKKα represses an NF-κB-dependent cytokine expression, required for enhanced recruitment of myeloid cells to the site of tumors [54]. In this model, IKKα-deficient myeloid cells showed M1-like polarization and actively produced IFNγ upon tumorigenesis. Additionally, the tumor suppressive function of myeloid cells in the absence of IKKα activation may not be limited to enhanced paracrine IFNγ secretion, which has been shown to be important in the anti-tumor response [79,80], and they may also phagocytize tumor cells [81] more efficiently, as their specific localization may suggest. Prior to our observations, it was reported that IKKα limits macrophage activation and contributes to the resolution of inflammation via preventing extended promoter binding of RelA and c-Rel [82]. IKKα was found to be crucial in limiting macrophage or dendritic cell activity [82–85]. Given that proinflammatory signaling governed by enhanced NF-κB activation may exacerbate inflammation in different diseases, IKKα-dependent feedback inhibition may prove to be indispensable for prevention of tissue damage in various pathologies [54]. Previously, it had been reported that nuclear localization of IKKα results in phosphorylation and release of the repressor protein SMRT from NOTCH1 target genes, leading to their aberrant expression upon malignant transformation in CRC [52]. In a recent report, Margalef and colleagues [86] showed that phospho-activated IKKα is first cleaved to a shorter form (p45-IKKα), and then this active and truncated IKKα is translocated to the nucleus where it phosphorylates and release the transcriptional repressor SMRT from the promoters of NOTCH signaling targets, preventing cancer cells from differentiating. The existence of cleaved-nuclear IKKα, a primary form in human patient samples, correlates with tumor stage and prognostic outcome in patients [86]. Thus, IKKα can govern stemness and proliferation of intestinal tumor cells through these non-conventional functions to further enhance their plasticity to tumor microenvironment changes.

Breast cancer

Breast cancer is another example where canonical and non-canonical functions of IKKs affect tumor cell plasticity in various settings related to tumor development and metastasis. One of the first pieces of mechanistic evidence showing that IKKβ can regulate non-canonical targets to promote tumorigenesis came from work with animal models of breast cancer [87]. This study demonstrated a physical FOXO3a–IKKβ interaction, followed by FOXO3a phosphorylation, ubiquitination and degradation [87], all of which occur independently of AKT. FOXO3a-depleted tumor cells grow better in mouse xenografts due to extensive NF-κB activation leading to survival of tumor cells. As a result, FOXO3a-positive breast cancer patients have improved survival due to activation of proapoptotic signaling while FOXO3a^{-}IKKβ^{+} patients had worse prognosis [87]. In another study, IKKβ was found to connect tumor-elicited inflammation to angiogenesis by directly phosphorylating the mechanistic target of rapamycin (mTOR) repressor TSC complex subunit 1 (TSC1)–TSC2 tumor suppressor complex to initiate angiogenesis within breast tumors [88]. Tumor xenografts with mutant TSC1 lacking a phosphoregulatory site grew poorly, whereas human patients positive for both phospho-TSC1 and phospho-S6K had worse prognosis [88]. Hence, IKKβ activation upon hypoxia-induced proinflammatory TNF expression is crucial in angiogenesis induction for further tumor growth and invasion in breast cancer. Additionally, IKKβ and NF-κB were shown to be essential for EMT and metastasis in breast cancer cells [89]. It subsequently became evident that NF-κB is required for stabilization of Snail or Twist1 in breast cancer cell lines to initiate EMT, invasion and metastasis of breast cancer [90–92]. IKKβ was also shown to be crucial for endothelial cell permeability, which is important for the extravasation of metastatic cells [68]. Park and colleagues [93] described that NF-κB-dependent granulocyte-colony stimulating factor secretion by metastatic breast cancer cells was directing osteoclast differentiation and osteolytic bone metastasis into skeletal tissue, which is frequently observed in breast cancer patients. Similarly, Chen and colleagues [94] observed that IKKβ implements a LIN28B/TCF7L2 feedback loop that promotes breast cancer cell stemness and metastasis with the help of *in vivo* xenograft and pharmacological inhibition studies. Briefly, IKKβ regulates tumor cell plasticity in breast cancer by regulating many facets of related mechanisms.

In the signaling believed to be involved in breast carcinogenesis, the non-canonical roles of IKK kinases, IKKα in particular, have not been fully investigated until recently. We now know that primary human breast cancer specimens and breast cancer cell lines exhibit aberrant IKKα and IKKβ kinase activities that may not involve NF-κB [95] or its downstream targets [96] to enhance transformation of breast cancer cells. IKKα/β kinases have been also shown to promote mammary tumorigenesis through inhibition of FOXO3a [97] and glycogen synthase kinase 3β by

the activation of mTORC2, which further regulates the phosphatidylinositol-3-kinase–AKT pathway [98]. Moreover, implication of a role for constitutively active IKK complex and NF-κB activation in breast cancer cell migration and metastasis has also been reported [99]. Given the important role of NOTCH1 [100,101] and IL-6 signaling in breast tumorigenesis [102], it was recently reported that IKKα and IKKβ, independent of NF-κB, control NOTCH-mediated IL-6/Janus kinase/STAT signaling in breast tumors cells [103]. IKKα was then discovered to be essential for oncogenic transformation in *in vivo* models of Her2/neu-induced breast tumorigenesis [104]. In this study, using two independent mouse models of mammary tumorigenesis, the authors provided evidence supporting the importance of IKKα kinase activity for the increased incidence and advanced onset of ErbB2/Her2-induced breast tumors [104]. This is reinforced with more recent findings as well [105]. In these studies, the enhanced, rather than totally inhibited, tumorigenesis was attributed to the kinase domain of IKKα, which maintains signals essential for the self-renewal potential of tumor-initiating cells. Currently, we know that IKKα regulates the expansion of these cells, in Her2-initiated tumors through a mechanism that involved NF-κB inducing kinase/IKKα axis induced p27/Kip1 phosphorylation and nuclear exclusion, contributing to augmented tumorigenesis [106]. On the other hand, IKKα was shown to be more efficient than IKKβ in activating NF-κB and the expression of downstream genes involved in cell invasion [107] in Her2-overexpressing breast cancer cells. The prometastatic activity of IKKα was stimulated by receptor activator of NF-κB (RANK) ligand (RANKL)/RANK to repress Maspin expression [108] and correlated inversely with nuclear p27/Kip1 in human invasive breast carcinoma specimens [106]. The studies summarized above indicate a protumorigenic and prometastatic role of IKKα in the mammary gland in which the kinase activity of IKKα stimulates proliferation of breast cancer cells and protects them from being killed by anti-cancer drugs. Consequently, IKKα can be considered an important regulator of breast cancer progression and a notable target for the development of therapeutic strategies targeting inhibition proliferation, metastasis and drug resistance in breast cancer cells.

Hepatocellular carcinoma

IKKβ plays opposing roles in different cells at different time points in hepatic cancer development. Since there were no hepatitis B virus or hepatitis C virus equivalents for generating a hepatitis-induced carcinogenesis in mice, *Mdr2* knockout mice, which develop cholestatic hepatitis followed by hepatic tumorigenesis, were used in early research [109]. By the use of this model, inflammatory cell-derived TNF is found to promote hepatocyte survival via activation of canonical anti-apoptotic NF-κB signaling [110]. Interestingly, using an IκB super-repressor model to silence NF-κB specifically in hepatocytes, no difference was observed in the tumor development in normal and mutant mice [110]. Only when the same model was used to inactivate NF-κB conditionally at a later point or when anti-TNF antibody was applied, was tumor growth seriously prevented due to apoptosis induction in the transformed hepatocytes [110]. In sharp contrast to these observations, in a mouse model of chemical carcinogen (diethylnitrosamine; DEN)-induced hepatocellular carcinoma (HCC), it was shown that IKKβ deletion in hepatocytes dramatically increased tumor burden while its deletion in macrophages inhibited tumor formation [111]. The observed differences in these two studies may be due to high regenerative abilities of hepatocytes in which IKKβ or NF-κB plays an important protective role against damage-induced cell death and resulting compensatory proliferation upon liver injury by DEN [111]. As shown in another study, NF-κB activates SOD to counteract ROS-induced stress in hepatocytes [112], whereas in the absence of NF-κB, ROS induces the activation of JNK and activator protein 1, which eventually leads to hepatocyte death via apoptosis [113]. Further work has clarified that ROS-damage-induced compensatory proliferation in HCC was driven by STAT3 activation in hepatocytes [114]. The contradictory NF-κB involvement in early and late stages of hepatocellular cancer was further supported by IKKγ/NEMO mutant mice, bearing IKKγ-deficient liver parenchymal cells. These mice spontaneously developed steatosis or multiple tumors in their livers [115]. The absence of IKKγ halts kinase complex formation and causes excessive cell death due to uncontrolled RIPK1 activation. Thus, RIPK1 ablation or RIPK1 kinase inactive knock-in models could prevent hepatocyte apoptosis and HCC in mice [115]. Later studies proposed that the control of RIPK1 to prevent hepatocellular necrosis or apoptosis relies on non-conventional functions of IKKs and differs from their roles in TNF-driven canonical NF-κB activation-related anti-apoptosis [58]. This observation partially explains the different observations between NF-κB super-repressor and IKKβ-deficient mouse models.

Aside from hepatocytes in HCC, the resident liver macrophages, Kupffer cells, when induced by apoptotic hepatocytes, secrete inflammatory cytokine IL-6, which in turn drives proliferation in hepatocytes through

activation of STAT3 [111,116]. Therefore, when tumor cell plasticity relies rather on myeloid cell-dependent activation of IKKβ in HCC, IKKβ in myeolid cells does not display a protective role in the late hepatic tumorigenesis as it does in hepatocytes at the earlier stage. In contrast, elevated IKKα levels have been associated with increased oncogenic potential in HCC models of tumorigenesis since IKKα has been found to be activated with elevated TNF expression during hepatitis [117]. Following this, IKKα translocates into the nucleus where it phosphorylates FOXA2 to suppress its downstream targets such as NUMB which in turn is a NOTCH1, an important transcription factor that regulates cell fate and differentiation during various developmental processes, inhibitor [117]. Therefore, this specific role for IKKα helps the proliferation of hepatocytes in an inflammation-driven and NOTCH1-dependent manner [117].

IKKα/β activation in HCC can be also induced through other pathways than tumor necrosis factor receptor (TNFR) signaling. Indeed, more recent studies with hepatitis-driven cancer models indicated that TNFR can be dispensable for NF-κB activation and its downstream effects in chronic hepatitis-driven HCC [118–120]. Upon observing that LTs and their receptor LTβR were highly upregulated in HCC, Haybaeck and colleagues [118] developed a chronic HCC mouse model (to simulate hepatitis B virus- and hepatitis C virus-driven chronic hepatitis and HCC) with LT-overexpressing hepatocytes. They showed that only an LT (α and β) response of hepatocytes leads to IKKα/β activation in tumor cells. Therefore, only LTβR activation is required for oncogenic transformation upon chronic hepatitis while TNFR1 was found to be dispensable. Later studies, with mouse models of high-fat-diet-induced steatohepatitis-driven HCC, showed that LTs or LIGHT (TNFSF14) produced by natural killer T cells or $CD8^+$ cells specifically signaled through LTβR on hepatocytes to induce steatohepatitis and hepatocyte damage through the activation of the canonical NF-κB pathway for hepatocellular tumorigenesis [119,120]. Hence, the differences in the observed results are probably due to differences in experimental models. More satisfactory conclusions about the upstream factors leading to IKK activation in hepatocellular tumorigenesis may be derived from advanced mouse models that better mimic the development of human disease.

As with other mechanisms, ER stress can alter the tumor microenvironment and tumor cell plasticity through modulation of immune responses, fueling tumor development [121]. Earlier studies proposed that IKKβ control on TSC1 and the mTOR signaling pathway may regulate glucose metabolism and insulin resistance through the regulation of insulin receptor substrate 1 in hepatocytes [122]. Similarly IKKβ has been shown to regulate glutamine deprivation, resulting from increased anabolic activity in highly dividing cancer cells, through its direct control on 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase isoform 3 in the glycolytic pathway [123]. Recent studies have provided solid connections between ER stress, inflammation and cancer in certain malignancies [124]. Especially in liver and colorectal cancers, elements of ER stress, nutrition and metabolism have been shown to be connected with tumor-elicited inflammation in tumor suppression and maintenance of the stem cell niche [125,126]. The important actors in ER stress, such as XBP1 and ATG16L1, have been shown to be associated with IKKα/β in the modulation of inflammation through protection from ER stress-induced apoptosis [57] and glucose homeostasis through their interaction [56]. All these observations indicate that IKKs can be the key regulators of ER stress- and tumor-elicited inflammation. Therefore, better understanding of IKK kinases in the mechanism of ER stress and cancer development is required to develop better treatment options for these cancers.

Skin cancers

IKKα has emerged as an innate surveillant in the skin controlling homeostasis and suppressing tumor formation. Mice with IKKα deletion exhibit numerous morphological abnormalities and die soon after birth, displaying defects in keratinocyte terminal differentiation and epidermal hyperplasia [127–129]. Reintroduction of an IKKα transgene in IKKα-deficient keratinocytes in mice induces terminal differentiation and rescues the phenotype of $Ikk\alpha^{-/-}$ (*Ikbka*) mice [130–132]. This function of IKKα in the formation of epidermis was independent of the kinase domain and NF-κB activities [130,131,133]. Decreased IKKα expression has been reported in several mouse [132–137] and human squamous cell carcinomas (SCCs) [133–136] suggesting that IKKα has a key role as a tumor suppressor in the skin. It has been proposed that IKKα expression level is important for skin tumor development. Liu *et al.* [132] highlighted the importance of IKKα in the development of squamous cell carcinoma in mice and humans. They showed that overexpression of an IKKα transgene in the epidermis (Lori-IKKα-Tg mice) enhanced terminal differentiation and inhibited carcinoma development and tumor metastasis in a 7,12-dimethylbenzanthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)

chemical model of skin carcinogenesis [132]. In the same study they identified novel IKKα mutations in human SCC samples, suggesting that the aggressiveness of SCC is inversely correlated with IKKα expression [132]. Moreover, elevated IKKα expression suppressed epidermal thickness and skin inflammation in a dose-dependent manner. In a follow-up study, the authors used two transgenic mouse lines with different expression levels of IKKα in the basal epidermis and demonstrated that higher IKKα levels are critical for mouse embryonic development and suppression of excessive keratinocyte mitosis [137]. Also, in DMBA/TPA-induced skin carcinogenesis IKKα hemizygotes developed more papilloma and carcinoma than the control group. Most of the carcinoma and some papilloma of $Ikk\alpha^{+/-}$ mice displayed reduced IKKα expression [138]. Furthermore, *Ikk*α deletion in basal epidermal keratinocytes (using keratin promoter 5) caused the same phenotype observed in $Ikk\alpha^{-/-}$ mice and was reversed by introduction of a keratinocyte-specific IKKα transgene [139]. Therefore, during SCC development the levels of IKKα are critically reduced, yet a sufficient dose is required for skin development and protection. In UVB-triggered carcinogenesis, IKKα reduction induced increased ERK activity and proliferation in the skin resulting in enhanced tumor numbers and p53 mutations [140]. Lastly, other studies suggested that inflammation-activated IKKα helps to protect skin cells against genomic instability by phospho-activation (at Ser125) of NPM oligomerization, activity of which blocks centrosome doubling [141]. As a result, in human SCC samples both IKKα and NPM are often observed to be decreased. Therefore, IKKα and NPM have profound effects in preventing aneuploidy and protecting genomic integrity in squamous skin epithelia [141].

In contrast, implication of IKKα in the malignant potential of skin tumors in the DMBA/TPA model has also been reported [142]. Although the essential role of IKKα in keratinocyte differentiation and stratification has been confirmed, an IKKα-overexpressing three-dimensional human skin equivalent (HaCaT and fibroblast cultures) has shown marked increase in keratinocyte proliferation and invasiveness [143]. In a chemically induced skin carcinogenesis model initiated by DMBA/TPA [144–146], IKKα reduced ERK activation affecting carcinoma development and vascular endothelial growth factor A (VEGF-A) expression impeding tumor invasion, independent of kinase activity and NF-κB activation [132,138]. Similarly, in non-melanoma skin cancer (NMSC) IKKα has shown protumorigenic roles depending on its subcellular localization [147]. NMSC mouse models were developed by the use of Tg.AC mice, expressing *v-Ha-Ras* oncogene, and these mice were mated to cytoplasmic or nuclear expressing human-IKKα transgenic mice. As a result, cytoplasmic IKKα-induced tumorigenesis was dependent on EGFR, VEGF-A and matrix metalloprotease 9, while nuclear IKKα transgene-induced oncogenicity was dependent on c-Myc, Maspin and integrin-α6 expression [147]. Consequently, IKKα exerts its oncogenicity in NMSCs while acting as a tumor suppressor in melanoma and papilloma, which further demonstrates its versatility in tumor cell plasticity, depending both on cell type and cellular localization. In contrast to numerous studies revealing the role IKKα, there are only a few associating IKKβ with skin tumorigenesis. A recent work showed IKKβ to act as a tumor suppressor in basal skin cancer models through regulation of p16-Ink4a (CDKN2a) [148]. The authors argued that IKKβ mediates resistance towards tumor development in epidermal cells but not in other cell types. Therefore, both IKKβ and IKKα may play a dual role in skin cancer depending on the cell type and interactions within the tumor microenvironment.

Lung, prostate and other cancers

Nicotine-derived nitrosamine ketone, a tobacco smoke byproduct, is a chemical carcinogen frequently used to generate lung cancer models in rodents. Although it was reported to induce inflammation and proliferation in bronchial epithelial cells through activation of NF-κB and cyclin D1 [149], previous studies using these models failed to report an association of tobacco smoke with increased tumor progression *in vivo*. However, a modified regime of tobacco exposure has been recently utilized to prevent desensitization of pulmonary cells to tobacco smoke [150] in a K-Ras (K-RasG12D)-driven pulmonary carcinogenesis model [151]. It indicated that chronic inflammation due to repeated tobacco smoke exposure promoted tumor growth in an IKKβ- and JNK-dependent manner. Consequently, tobacco smoke-driven chronic inflammation utilizes IKKβ for the activation of inflammatory signaling in macrophages leading to tissue damage and, following MAPK activation, to promote tumor growth via increased proliferation [150]. Later IL-6, a target of NF-κB activation in myeloid cells, was found to be a mediator of increased proliferation in inflammation-driven lung cancer [152].

The role of IKKα in macrophage regulation of lung tumorigenesis strikingly differs from that of colorectal cancer. These differences became more apparent in a study where IKKα kinase dead (KD) mutant animals

were generated [153] instead of the use of K-RasG12D-driven lung adenocarcinoma model. These mice produced sporadic lung tumors resembling human small cell carcinoma, marked by the upregulation of markers such as K5, p63 and Trim29 [153]. Reintroduction of transgenic K5 and wild-type bone marrow transplantation reversed the SCC-like phenotype in IKKα-KD mice [153]. Therefore, IKKα downregulation in bronchial epithelia triggers an inflammatory response that causes polarization of macrophages towards the tumor-promoting M2 phenotype. While both in colon and lung cancers IKKα plays an anti-inflammatory role to augment activation of NF-κB-driven inflammation, in lung cancer the absence of IKKα leads to excessive inflammation that arguably involves T-cell activation. As a result, the latter eventually leads to the recruitment of anti-inflammatory M2 macrophages into lung tumors.

Although most prostate cancers (CaP) are androgen-dependent and responds quite efficiently to androgen ablation, they become androgen-independent later on [154]. Therefore, the major risk for CaP patients is the development of drug resistance. While tumor initiation and progression clearly involve inflammatory processes, such as NF-κB and STAT3 activation, the role of inflammation in metastatic progression still awaits more evidence. In one study, IKKα was found to be an important mediator of CaP metastasis in a mouse model of prostate adenocarcinoma (transgenic adenocarcinoma of the mouse prostate, in which the SV40 large T antigen is specifically expressed in the prostate epithelium) that closely reflects the pathogenesis of human prostate cancer [155]. Using these mice, Luo *et al*. [156], demonstrated that IKKα kinase activity regulates the interplay between inflammation and prostate metastatogenesis. They suggested that IKKα activation and nuclear translocation are the critical events at the onset of metastasis since it is required for the repression of the metastasis suppressor Maspin. IKKα activation relies on the interaction of myeloid and lymphoid cells that are attracted into the tumors and secreted cytokines such as RANKL and LT [156]. The activation of IKKα in CaP cells was shown to be IKKβ-dependent but NF-κB-independent [156,157]. Leukocytes and B cells displaying IKKβ activation were recruited to the vanishing tumor and produced cytokines, such as LT, which in turn activated IKKα on the surviving CaP cells resulting in increased metastasis [157]. However, the effects of infiltrating B cells on IKKα activation were not limited to the metastasis but also involved in castration resistance and tumor recurrence in CaP through the regeneration of tumor cells [158]. In IKKα-deficient ($IKK\alpha^{AA/AA}$) CaP mice, tumor recurrence was considerably diminished. Mechanistically, upon activation by B-cell-derived LTs in CaP tumors, IKKα was localized to the nucleus where it associated with transcription factor E2F1 on the *Bmi1* promoter to activate a cancer stem cell renewal pathway [158].

In other cancers, such as ovarian and pancreatic, it has also been reported that myeloid-derived IL-6 is important in inflammation-induced tumorigenesis models through activation of STAT3 [159–161]. Therefore, IKKβ mostly, but not necessarily, exerts its protumorigenic function through canonical NF-κB signaling via activation of proinflammatory cytokine signaling in myeloid cells or anti-apoptotic pathways to protect tumor cells from stress-induced cell death. Indeed, proinflammatory cytokines such as TNF, IL-6, IL-11 and IL-22 activate proliferative STAT3 signaling that converges to canonical NF-κB signaling to orchestrate diverse mechanisms leading to tumor cell plasticity in solid tumors [37]. IKKα has tumor suppressive roles through suppression of destructive inflammation in skin, lung [139,153,162] and possibly in pancreatic cancer [163], but the recent literature has shown oncogenic roles of IKKα in driving tumor initiation, progression and metastasis in various other cancers [54,106,117,156]. The differential involvement of IKKα in various cancers can depend on upstream signals in a spatiotemporal manner and on variations in tumor microenvironment such as the microbiota or the cytokine milieu in different organs [54,164].

Perspectives

Since NF-κB signaling is constitutively activated in numerous cancers including hematological and solid malignancies, the pharmaceutical industry has aggressively sought to develop therapeutic strategies to target the NF-κB pathway [37]. There are as yet many constraints in developing effective IKK inhibitors that could make an ideal drug. They should (a) be very specific to NF-κB signaling (but not to other signaling pathways); (b) selectively target specific NF-κB components in a given disease, (c) be more effective on tumor cells than on any other cells; (d) have an effect that is transient and highly reversible to prevent harmful side effects on innate immunity and extended immunosuppression; and (e) have efficacy, dose, delivery method and delivery schedules that are carefully determined for a single agent or in combination with others [165]. With much effort put in by pharmaceutical companies, inhibitors targeting NF-κB, NEMO and IKKβ have been developed [165]. Early success with these was reached in a wide array of preclinical studies in

hematological malignancies, such as multiple myeloma, and some other solid malignancies where tumor development was largely dependent on NF-κB signaling [37]. However, none of this preclinical knowledge could be translated into clinical outcomes and none of these inhibitors could get clinical approval due to toxicity linked with systemic NF-κB suppression [166]. Due to the complexity of NF-κB regulation in various cells within the tumor microenvironment, systemic inhibition of any of the IκB kinases would lead to potential development of septic shock, as seen in many different animal cancer models [82,167,168]. Even indirect inhibitors, such as the proteasomal inhibitor bortezomib that targets multiple signaling pathways, could not avoid dose-limiting toxicities as their efficacy was significantly limited due to their interference with systemic NF-κB activities [169].

Although therapeutic approaches targeting the core elements of NF-κB signaling, such as IKKs, in principle have the potential to block cancer-promoting activity, they would also challenge the essential functions of this pathway in immunity, inflammation and anti-apoptosis [165]. It gets even more complicated when the effects of NF-κB signaling, being vastly tissue- and context-specific, have to be considered. That is the reason that many new studies are carried out with more specific strategies to only intervene with the NF-κB pathway in a cancer- and tissue-selective manner without affecting NF-κB's essential functions in other tissues [166]. Such approaches seem promising and indispensable to preserve non-conventional functions of specific IKK subunits. However, the biggest challenge is still how to develop IKKα- or IKKβ-specific inhibitors due to the great similarity in their catalytic domains. Over the past two decades, no such inhibitors have been developed [37] by the pharmaceutical industry.

Regardless of the pitfalls in developing effective targeting of the NF-kB pathway, the studies we presented in this review on IKKα and IKKβ involvement in tumor cell plasticity still support the idea that these proteins are potential therapeutic targets for many different malignancies. There are yet challenges in putting the theoretical knowledge into practice because of tumor cell plasticity. Problems include recurrence of tumors due to acquired resistance, and non-cancer stem cells gaining a stem cell-like phenotype [170]. Given that heterogeneity stemming from tumor cell plasticity makes the latter harder to control, the direct killing of cells by chemotherapeutic agents may lead to greater tumor stem cell propagation as a side effect [171]. Moreover, tumor cells cannot be targeted by one or more drugs efficiently due to coexistence of different subpopulations of them within tumors. On the other hand, intervention on EMT, as seen with many drugs, seems to increase colonization efficiency of circulating metastatic cells [20]. Due to the aforementioned obstacles, therapies aiming at immune cells within the tumor microenvironment may reverse drug resistance. We suggest that it could be essential for the future of clinical cancer research, such as reprograming blood cells for the initiation of anti-tumor activity [172] or cell-specific inactivation of inflammatory molecules like IKKs by means of gene therapy. In particular, to circumvent associated problems with the development of a specific inhibitor, one may propose the design of non-ATP competitive kinase inhibitors (for example small molecule inhibitors), which may offer better specificity and prevention of off-target effects [173,174]. For the design of such inhibitors, differences in structural information on catalytic domains of individual IKKs or structural differences in phosphorylation or docking sites of non-overlapping substrates targeted by these kinases can be used. Such information will be instrumental in identifying novel non-canonical targets. Since we have pointed out here that inflammatory pathways are the very source of plasticity within tumors, it is important to have a better understanding of regulatory pathways in inflammation-driven tumorigenesis to develop more advanced strategies to tackle cancer.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

SIG and MAD drafted the manuscript. TLC checked the manuscript and made critical changes and contributions. All authors read and approved the final manuscript.

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