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# The Druggable Target Potential of NF-κB-Inducing Kinase (NIK) in Cancer

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**Abstract:** NF-κB-inducing kinase (NIK) is primarily recognized for its role as the apical kinase that activates non-canonical NF-κB signaling and its involvement in immune system regulation. NIK is crucial for maintaining cellular health by regulating fundamental processes such as differentiation, growth, and survival. Emerging evidence suggests that dysregulated expression or function of NIK in non-lymphoid cells is a key factor in cancer progression. While NIK deficiency causes severe immune dysfunction, its overexpression or excessive activation is linked to inflammatory diseases, metabolic disorders, and cancer development. The development of small molecule inhibitors targeting NIK has sparked optimism for clinical intervention, positioning NIK as a promising druggable mediator for cancer. The ongoing progress in creating novel small molecule NIK inhibitors offers new opportunities for testing NIK-targeted cancer therapies, potentially advancing the clinical application of NIK-based cancer treatments.

**Keywords:** NIK; cancer; druggable target; NIK inhibitor



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## 1. Introduction

### *1.1. Nuclear Factor Kappa-B (NF-κB)*

Nuclear factor kappa B (NF-κB) is a family of transcription factors composed of polypeptide members from the Rel/NF-κB family. Initially discovered by Sen et al. in 1986 as a B cell-specific transcription factor, subsequent research has revealed that NF-κB is not limited to mature B cells but is widely expressed across eukaryotic cells [1]. The NF-κB transcription factor family comprises five members: p65 (RelA), RelB, p50, p52, and c-Rel. These members form a variety of homologous and heterodimers. The most biologically active components are the heterodimers p65/RelA and p50/RelA [2–6].

The NF-κB signaling pathway regulates the expression of over 150 target genes, including genes involved in cytokines, inflammatory chemokines, leukocyte adhesion factors, and effector enzymes [7,8]. Serving as a central hub for various signals, the NF-κB pathway links multiple signaling pathways and plays crucial roles in immune regulation. It inhibits cell apoptosis, promotes cell proliferation, supports the formation of new blood and lymphatic vessels, and is closely associated with conditions such as diabetes, chronic inflammation, cardiovascular diseases, and central nervous system disorders [9,10]. Furthermore, numerous studies have demonstrated that NF-κB is extensively involved in the initiation and progression of various cancers, where it promotes tumor cell infiltration and metastasis. The active components of NF-κB are not only present in hematologic cancers, including multiple myeloma, chronic lymphocytic leukemia, non-Hodgkin's lymphoma,

and Hodgkin's lymphoma, but also play a significant role in several solid tumors, such as gastric, breast, liver, ovarian, and pancreatic cancers, as well as glioblastoma and non-small cell lung cancer [7,10,11].

Activation of NF-κB is a complex, multi-cytokine-dependent process that involves several steps, including ubiquitin-mediated proteolysis triggered by the ortho-acidification of IκB proteins. Cells respond to specific signals, known as NF-κB agonists, which include growth factors (e.g., GM-CSF, NGF), inflammatory mediators (e.g., tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1)), immune receptors (e.g., CD40), bacteria, neurotoxins, metabolites such as lipopolysaccharide (LPS), and certain physical or chemical factors such as ultraviolet radiation [12,13]. These agonists bind to their corresponding receptors, initiating a cascade that first activates IκB kinase (IKK), which forms a trimeric complex consisting of IKKα, IKKβ, and IKKγ in the cytoplasm. The phosphorylation of Ser32/36 on IKKα or Ser19/23 on IKKβ leads to conformational changes in IκB. These changes expose hidden motifs, facilitating the ubiquitination of IκB at residues Lys21/22. This ubiquitination marks IκB for rapid degradation, which results in the dissociation of NF-κB from IκB, allowing NF-κB to translocate to the nucleus and initiate transcription [13,14]. Current research identifies two primary pathways for NF-κB activation: the canonical/classical signaling pathway and the non-canonical/alternative signaling pathway. These two pathways are differentiated based on their underlying mechanisms and the specific roles they play in cellular responses (Figure 1).

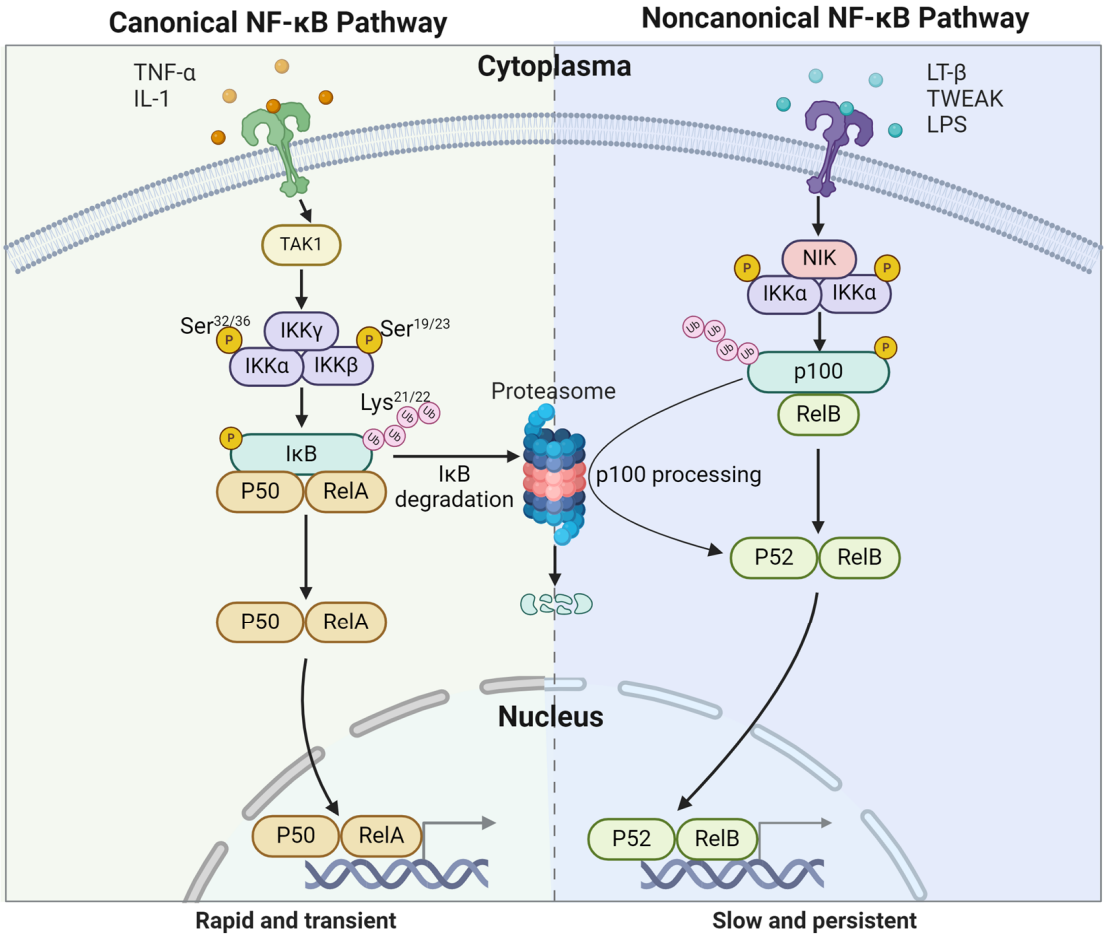


**Figure 1.** NF-κB signaling pathway.

#### 1.1.1. Canonical NF-κB Pathway

The canonical NF-κB pathway is a common mechanism of NF-κB activation, triggered by various extracellular agonists such as TNF-α and IL-1. These signals activate transforming growth factor β (TGFβ)-activated kinase 1 (TAK1), which in turn activates the IKKα/IKKβ/IKKγ trimer. This leads to the phosphorylation, ubiquitination, and subsequent degradation of IκB. As IκB is degraded, the NF-κB dimer that was bound by IκB is rapidly and robustly released, thereby activating the NF-κB signaling pathway [15–18]. During this activation process, IKKβ plays a central role (Figure 1, left panel).

#### 1.1.2. Non-Canonical NF-κB Pathway

The activation of non-canonical NF-κB signaling pathways primarily occurs in B cells, where it leads to the selective activation of p52/RelB dimers, resulting in NF-κB activation. Unlike the canonical pathway, which is activated by a variety of agonists, the non-canonical pathway is triggered by a smaller set of ligands, primarily tumor necrosis factor receptor (TNFR) family ligands lymphotoxin-beta (LT-β), tumor necrosis factor-like weak inducer of apoptosis (TWEAK), and LPS. Upon activation of NF-κB-inducing kinase (NIK), it selectively activates IKKα homodimers, which then phosphorylate the precursor protein p100, leading to its processing into p52 [19–23]. The p52 then binds to RelB to form heterodimers, undergoes nuclear translocation, and initiates NF-κB activation. Importantly, the non-canonical signaling pathway is dependent on IKKα activation and does not require IKKβ or IKKγ [19,24–26]. Recent studies have suggested that inhibiting the expression of this non-canonical pathway, particularly targeting NIK and IKKα, may aid in the development of novel anti-tumor therapies (Figure 1, right panel).

### *1.2. Role of NIK in the Non-Canonical NF-κB Pathway*

NIK, also referred to as MAPK kinase kinase 14 (MAP3K14), is the first MAP3K kinase to be discovered and is a serine/threonine kinase within the MAP3K family [27]. NIK is a key regulatory kinase in the non-canonical NF-κB signaling pathway. Due to the relative instability of NIK protein, its cellular levels are tightly regulated through ubiquitination by a complex consisting of tumor necrosis factor receptor-associated factor 2 (TRAF2) and 3 (TRAF3), as well as cellular inhibitor of apoptosis protein 1 and 2 (CIAP1/2) [25,26]. In unstimulated cells, NIK typically forms a complex with TRAF2, CIAP1/2, and TRAF3. Within this complex, NIK is highly unstable and undergoes degradation via the CIAP1/2-mediated K48 ubiquitination proteasomal pathway, leading to low cellular levels that prevent NF-κB activation. However, upon stimulation by ligands such as LTβ, TWEAK, and RANK, CIAP proteins mediate the ubiquitination and degradation of TRAF2 and TRAF3, resulting in the release of NIK into the cytoplasm. This release triggers an increase in NIK levels, which activates IKKα kinase. Activated IKKα subsequently phosphorylates the precursor protein p100, leading to its processing into p52. The p52 then associates with RelB to form a dimer complex, which translocates to the nucleus to activate NF-κB [27–29]. This activation regulates the expression of target genes, driving downstream biological functions (Figure 2).

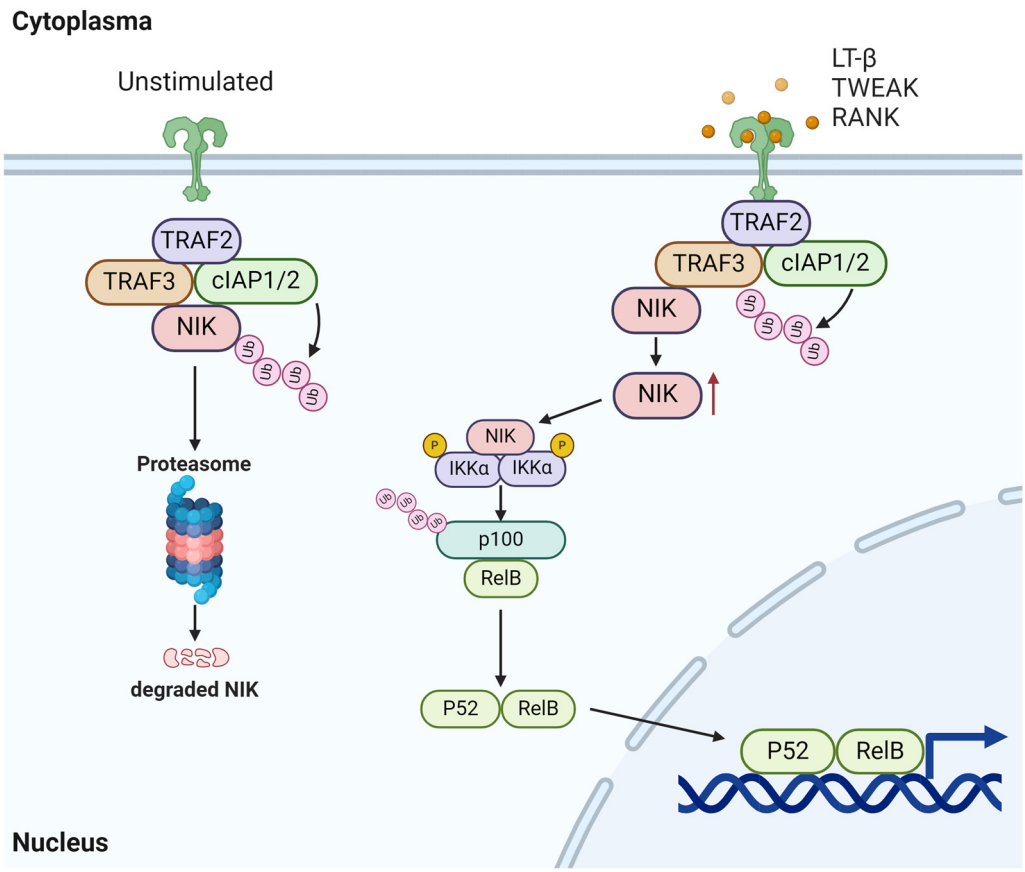


**Figure 2.** Role of NIK in the non-canonical NF-κB pathway.

### *1.3. NIK in Canonical NF-κB Pathway*

In addition to activating the non-canonical NF-κB pathway, NIK may also regulate the canonical NF-κB pathway in either a positive or negative manner. In the epithelial cell system, NIK mediates the expression of Map3k8 and the Map3k8-induced phosphorylation of RelA, as well as inflammation [30]. NIK/IKKα activation of NF-κB p50/RelA is essential for TNF-α-induced intestinal tight junction permeability [31]. In contrast, NIK can also suppress canonical NF-κB activation in primary cultured mouse cortical neurons by sequestering RelA in the cytoplasm, while in primary cultured mouse astrocytes, it increases NF-κB activity [32]. During T cell activation, NIK acts as a downstream mediator of Map3k8- and PKC-ζ-induced c-Rel transcriptional activation [33]. The reason for this discrepancy remains unclear. In acute myeloid leukemia cells, NIK stabilizes and activates the non-canonical NF-κB pathway while inhibiting the canonical NF-κB pathway [34]. Therefore, while NIK activates the non-canonical NF-κB pathway in many cell types, its effect on the canonical NF-κB pathway is context-dependent.

### *1.4. NIK Has Functions in NF-κB-Independent Pathway*

In recent years, researchers have discovered that in addition to its roles in the canonical and non-canonical NF-κB signaling pathways, NIK also possesses NF-κB-independent functions. One such function involves the regulation of transcription factor activity through NIK-mediated histone acetylation. For instance, TNF-α recruits NIK to the promoters of proinflammatory genes, inducing acetylation of histone H3 at lysine 9 (H3K9) in lung epithelial cells, although it remains unclear whether this modification actually regulates downstream gene expression [35]. Similarly, in LPS-stimulated macrophages, NIK promotes IKKα-induced phosphorylation of histone H3 at the COX2 promoter [36], but whether this phosphorylation directly influences COX2 transcription has not been explored. Beyond

NF-κB, NIK also activates other transcription factors. For example, NIK mediates tumor necrosis factor superfamily member 14 (TNFSF14)-induced phosphorylation and activation of Stat3 in cancer cells [37], regulates melanoma survival and growth through a β-catenin/T cell factor (TCF)-mediated pathway [38], and increases the phosphorylation and stability of the transcription factor cAMP response element-binding (CREB) in liver and primary mouse hepatocytes, promoting glucagon action and glucose production [39].

Outside the nucleus, NIK influences tumor invasion by regulating mitochondrial dynamics. NIK is essential for the phosphorylation and recruitment of the mitochondrial fission mediator Drp1 to mitochondria. Drp1 recruitment leads to mitochondrial fission and the redistribution of mitochondria to the cell periphery, providing local energy to promote cell migration [40]. Furthermore, in mouse embryonic fibroblasts and bone marrow-derived macrophages, NIK triggers stimulator of interferon genes (STING) activation in the viral DNA-driven interferon pathway in an oligomer-dependent manner, independent of the non-canonical NF-κB components NF-κB2 p100 and IKKα. This function is crucial for limiting DNA virus infection in vivo. NIK enhances STING stability by inhibiting the delivery of K48-linked ubiquitin chains to STING, preventing its proteasomal degradation. This stabilization promotes interferon-regulated transcription factor 3 (IRF3) phosphorylation by TBK1, followed by its nuclear translocation to stimulate interferon-β transcription [41].

## 2. The Structure and Regulatory Elements of NIK

NIK is a serine/threonine kinase in the MAP3K14 family. The human NIK sequence consists of 947 amino acids, which are organized into at least four distinct regions: the N-terminal TRAF3 binding domain and cIAP-binding motif (amino acid residues 30–120), the negative regulatory domain (NRD) (amino acid residues 121–318), the central serine/threonine kinase domain (amino acid residues 390–660), and the C-terminal non-catalytic region (NCR) that is involved in signal release. The NRD domain also contains a leucine zipper sequence (amino acid residues 127–146), a basic region (BR), and a proline-rich region (PRR) (amino acid residues 250–317). The C-terminal NRD domain plays a key role in the signal cascade amplification by interacting with important components such as the substrates IKKα and p100, as well as regulatory factors such as TRAF1, TRAF2, TRAF5, and TRAF6 [42–44]. IKKα phosphorylation sites are also contained in the C-terminal NRD domain (as shown in Figure 3).

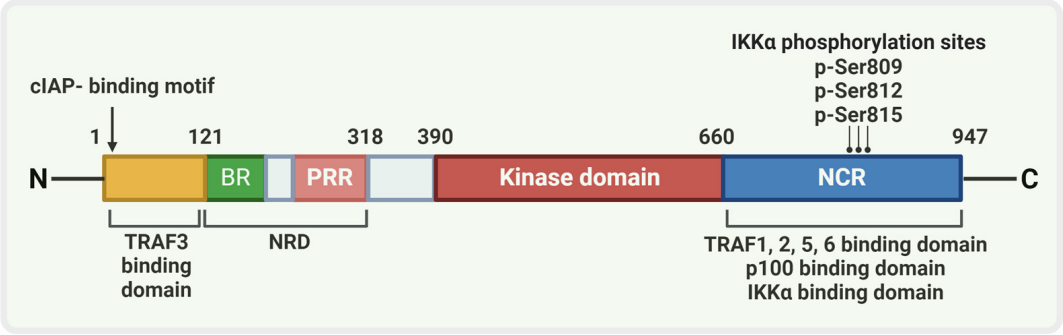


**Figure 3.** The structure and regulatory elements of NIK.

## 3. Role of NIK in Tumorigenesis

Studies have shown that NIK overexpression is closely associated with the onset of cancer and plays a significant role in immune regulation and inflammation [45].

### *3.1. Hematological and Immune System Tumors*

#### 3.1.1. Hodgkin Lymphoma

Many B-cell lymphomas, including HL, rely on the activation of the non-canonical NF-κB signaling pathway. Stella et al. reported that NIK was present in numerous Hodgkin lymphoma cell lines and that NIK shRNA could impact the viability of these tumor cells. This study also revealed that NIK was stably expressed in 49 out of 50 Hodgkin lymphoma patient biopsies. Furthermore, the NIK inhibitor 4H-isoquinoline-1,3-dione was shown to selectively kill Hodgkin lymphoma cells, without affecting other B-cell lymphoma cells [46,47]. These findings suggest that NIK-induced activation of the non-canonical NF-κB signaling pathway is a hallmark of Hodgkin lymphoma and that NIK inhibitors may offer a potential therapeutic strategy for treating this challenging and relapse-prone disease.

#### 3.1.2. Multiple Myeloma

Multiple myeloma is a kind of B-cell lymphoma malignancy originating from plasma cells in the bone marrow, which represent the final stage of B-cell development. Yulia et al. reported that two NIK inhibitors, described in patent WO2009158011 A1, demonstrated selective toxicity in multiple myeloma cell lines, with a 1 to 5 μM range, specifically targeting cells with mutations linked to NF-κB activation [48]. In the context of multiple myeloma, NIK is a crucial therapeutic target, as it contributes to the activation of non-canonical NF-κB signaling pathways in multiple myeloma cell lines [49,50].

#### 3.1.3. Peripheral T Cell Lymphoma (PTCL)

In 2013, Lina et al. discovered that NIK was overexpressed in a significant number of cell lines. In experiments using NIK knockout mice, they found that NIK-deficient PTCL cell lines exhibited reduced survival ability. Moreover, the survival of PTCL cell lines was shown to depend on the activation of both canonical and non-canonical NF-κB pathways, with NIK playing a crucial regulatory role in both [51]. These findings highlight NIK as a promising therapeutic target for peripheral T-cell lymphoma.

#### 3.1.4. Lymphoid Leukemia

In an in vitro mouse experimental model, Yasunori et al. found that while knocking out NIK did not significantly impact the growth of normal cells, it notably reduced the incidence of lymphoid leukemia [52]. Burley et al. reported that the NIK inhibitor CW15337 selectively targets the non-canonical NF-κB pathway and inhibits the transcription of downstream genes, effectively reversing B-cell leukemia/lymphoma 2 (BCL2) protein family-mediated tumor resistance in the context of CD40 Ligand (CD40L) stimulation [53]. Numerous studies have emphasized the important role of NIK in lymphoid leukemia, but the precise mechanisms through which NIK influences tumor cell development remain poorly understood.

Meanwhile, rising incidence of childhood leukemia is associated with activation of the NF-κB pathway [54]. Childhood acute lymphoblastic leukemia (cALL) is the most common pediatric cancer and remains the leading cause of disease-related mortality in children. Glucocorticoid (GC) resistance in cALL is particularly critical, as it is associated with the poorest prognosis. Specific modulation of the expression of key components in the NRAS/BRAF/NF-κB cascade and cell cycle pathways has been shown to effectively restore normal GC responses in GC-resistant pre-B cALL cell lines [55].

### *3.2. NIK in Solid Tumors*

In addition to hematological and immune system tumors, NIK has also been reported to be associated with the development of certain solid tumors.

#### 3.2.1. Lung Cancer

Yasunori et al. reported that NIK is aberrantly overexpressed at the pretranslational level in non-small cell lung cancer (NSCLC) cell lines. Silencing NIK through RNA interference significantly reduced nuclear NF-κB DNA binding activity and reporter gene expression [56]. Smac mimetic LCL161 induces lung cancer invasion and migration by activating the non-canonical NF-κB pathway and promoting the expression of interleukin-2 (IL-2) and matrix metallopeptidase 9 (MMP-9). In contrast, the OTU domain-containing 7B (OTUD7B) protein inhibits NIK by binding to and deubiquitinating TRAF3, thereby preventing non-canonical NF-κB activation and suppressing LCL161-induced lung cancer invasion and migration [57].

These findings highlight that NIK plays a crucial role in the constitutive activation of NF-κB in lung cancer cells, positioning NIK as a potential molecular target for lung cancer therapy.

#### 3.2.2. Colorectal Cancer

Kei et al. found that constitutive NF-κB activation was observed in 40% of colorectal cancer tissues and 67% of colorectal cancer cell lines. Inhibition of NF-κB through RNA interference reduced subcutaneous tumor growth by 23%, accompanied by a decrease in tumor vascularity. Tumor expansion was more significantly inhibited in IKKγ knockdown tumors when compared to wild-type (WT) tumors following treatment with 5-fluorouracil [58]. In colorectal cancer and cutaneous squamous cell carcinoma, studies on the IKK-independent non-canonical NF-κB pathway have focused on a truncated form of IKK, known as p45 IKK. This form of IKK is constitutively active and specifically localized in the nucleus, where it plays a role in tumorigenesis by influencing nuclear signaling pathways [59,60]. In colorectal cancer, the activation of the NF-κB1 and NF-κB2 pathways regulates the transcription of the downstream leucine-rich repeat-containing 8A (LRRC8A) gene. Upregulation of the NIK/NF-κB2/LRRC8A transcriptional axis has been shown to negatively impact the prognosis of colon cancer patients, contributing to metastasis and disease progression [61]. Ma et al. found that inhibition of NIK-mediated NF-κB2 activation and nuclear translocation prevents the active NF-κB2 complex from transcribing FAS, thereby enhancing the sensitivity of colorectal cancer cells to CD8+ T cell-induced apoptosis [62]. These findings suggest that targeting NF-κB could be an effective therapeutic strategy for colorectal cancer, particularly in cases with constitutive NF-κB activation, a process in which NIK plays a key role.

#### 3.2.3. Breast Cancer

Many studies have found that NIK expression is overactive in breast cancer. It has been found that NIK is linked to NF-κB activation in basal-like breast cancer. B-cell lymphoma 3 (Bcl3), which can form a DNA-binding complex with p52, has also been observed to be overexpressed in breast cancer samples [63]. The components IKK, RelB, and p52 have been associated with reduced cancer-specific survival in ER-positive breast cancer [64,65]. In human epidermal growth factor receptor 2 (HER2)-positive epithelial cells, nuclear IKK promotes tumorigenesis through p27 [66]. In transgenic mice, overexpression of p100/p52 led to delayed mammary gland development and the development of multiple tumors [67].

Additionally, constitutive RANK signaling results in elevated atypical NF-κB signaling in breast cancer cell lines, which stimulates cell proliferation by increasing transcription of cyclin D1 [68–70]. Immunohistochemical studies have shown that p52 subunits are expressed at higher levels in breast cancer tissues compared to adjacent normal tissues, and Western blotting of nuclear fractions from cancerous and adjacent normal breast tissues confirmed elevated p52 levels in tumor cells [71]. Recently, Hayashi et al. discovered that

NIK overexpression promotes tumor malignancy by driving the nuclear localization of NF-κB2 (p52) and RelB in highly malignant breast cancer cell lines. This activation triggers the activation of cancer-associated fibroblasts (CAFs) and partially enhances anti-apoptotic activity, contributing to tumor progression [72].

#### 3.2.4. Gastric Cancer

In gastric cancer, *Helicobacter pylori*-mediated NF-κB activation is believed to occur through an IKK-linked pathway that is independent of the non-canonical NF-κB pathway. This pathway involves both IKK and NIK, which work together to upregulate inflammatory infiltrates and promote tumorigenesis [73]. In patients with gastric cancer, central ferroptosis-related genes (FRGs) exhibit a significant positive correlation with the infiltration of activated CD4+ T cells, particularly Th cells. The NIK/NF-κB signaling pathway is highly activated in the gene signature of the high FRG score group, suggesting that central FRGs may mediate CD4+ T cell activation through the NIK pathway, thereby contributing to the progression of gastric cancer [74].

#### 3.2.5. Melanoma

Yee Mon et al. found that NIK promotes the expression of survival-related genes in melanoma cells by regulating β-catenin-mediated transcription. In NIK-depleted melanoma cells, expression levels of survival-related genes were significantly reduced, along with other β-catenin-regulated genes, such as c-MYC, mesenchymal-epithelial transition factor (c-MET), and Cyclin D2 (CCND2) [38]. This study suggests that NIK mediates both β-catenin- and NF-κB-regulated transcription to support melanoma cell survival and growth. Consequently, NIK may represent a promising therapeutic target for melanoma treatment. Thu et al. found that discoidin domain receptors (DDRs) in melanoma activate stromal-mediated drug resistance (MMDR), which subsequently promotes the targetable NIK/IKKα/NF-κB2 pro-survival pathway. This highlights the critical role of NIK in environmentally mediated drug resistance, offering valuable insights into its involvement in melanoma's resistance to therapy [38].

#### 3.2.6. Pancreatic Cancer

NIK overexpression has been observed in human pancreatic cancer samples, where the degradation of TRAF2 enhances NIK stability [75]. In pancreatic cancer, the non-canonical NF-κB pathway is activated and linked to increased cell proliferation [76]. NIK levels are elevated in pancreatic cancer and correlate with enhanced proliferation [77]. Additionally, the upregulation of RelB and p52 has been associated with mutant KRAS-driven pancreatic cancer [78], where IKK-dependent gene expression is also observed [79]. In gastrointestinal tumors, NF-κB2$^{DCT/DCT}$ mice spontaneously develop tumors, highlighting that p100/p52 plays a crucial role in driving tumorigenesis in this context [80]. The E3 ligase tripartite motif 16 (TRIM16) upregulates sinusoidal homeobox 1 (SIX1) by inhibiting its ubiquitination and degradation, a process mediated by NIK, an upstream regulator of SIX1. Inhibition of NIK can therefore suppress SIX1 expression, glycolysis, and metastasis in pancreatic cancer cells overexpressing TRIM16. Mechanistic studies reveal that TRIM16 competes with NIK's E3 ligase, TRAF3, thereby stabilizing NIK protein. This study identifies the TRIM16-NIK-SIX1 axis as a crucial regulatory pathway in aerobic glycolysis and pancreatic cancer metastasis, suggesting it as a promising therapeutic target for pancreatic cancer treatment [79].

#### 3.2.7. Brain Cancer

NIK plays tumor-promoting roles in gliomas, including aggressive high-grade gliomas such as glioblastoma, which are known for their aggressiveness and resistance to therapy. Duran et al. reported that NIK is associated with poor prognosis in glioblastoma [81].

Mouse orthotopic models have demonstrated that the upregulation of this pathway is linked to the more aggressive subtypes of glioblastoma [82]. NIK induces the upregulation of matrix metalloproteinase 14 (MT1-MMP) in invadopodia, promoting glioma cell invasion through an atypical NF-κB-dependent mechanism that is independent of canonical NF-κB signaling [81]. Moreover, overexpression of NIK significantly enhances tumor cell invasion and increases tumor size [83]. NIK also regulates cancer cell invasion by modulating mitochondrial dynamics and mitochondrial trafficking at the leading edge of migrating glioma cells [40]. Combination therapy of crizotinib with heptamethoxazole dye or near-infrared dye (IR-crizotinib) demonstrated enhanced intracranial chemotherapy delivery and tumor localization. This approach inhibited NIK and non-canonical NF-κB signaling, leading to reduced glioma growth both in vitro and in vivo and improved survival in preclinical rodent models [84].

Given these effects, therapy-induced NIK inhibition is emerging as a potential strategy for improving therapeutic outcomes in glioblastoma.

## 4. NIK in Cancer-Associated Cachexia

Recent discoveries on the role of a novel signaling pathway involving the ectodysplasin A2 receptor (EDA2R) and NIK revealed that NIK is involved in cancer-associated cachexia. Overexpression of NIK in the tibialis anterior muscle of mice also leads to the upregulation of atrophy-related genes and the induction of muscle atrophy [85]. Indeed, elevated NIK expression is linked to muscle loss caused by tumor growth or glucocorticoid administration. Interestingly, depletion of NIK in skeletal muscle protects against tumor-induced muscle atrophy. Muscle-specific NIK knockout mice maintain muscle mass and function after tumor inoculation. These mice show lower expression of atrophy-related genes, larger myofiber cross-sectional areas, and enhanced forelimb grip strength compared to wild-type controls [85,86]. Importantly, NIK inhibitors have been shown to block EDA2R-dependent myotube atrophy. To date, several NIK inhibitors have been developed and tested in preclinical studies for a range of diseases, including multiple myeloma and liver injury [48,87–89]. Such inhibitors should also be tested in models of cancer cachexia. However, clinical trials involving NIK inhibitors have not yet been conducted, as these inhibitors need to demonstrate better pharmacokinetics and lower toxicity profiles. Given the role of the non-canonical NF-κB pathway in the development of adaptive immunity, NIK inhibition may carry the risk of undesirable toxicities. Therefore, the development of inhibitors and modulators targeting the NIK pathway may offer new therapeutic options for the treatment of muscle atrophy in cancer-associated cachexia.

## 5. NIK Inhibitors in Anti-Cancer Progress

NIK is a member of the protein kinase family, which catalyzes the transfer of the terminal phosphate from ATP to substrates containing serine or threonine residues. Like all kinases, NIKs are structured with two subdomains that fold into a bilobed catalytic core. The ATP binding site is located in the deep cleft between the two lobes of the core structure [90–92]. Most kinase inhibitors discovered to date are ATP-competitive. These inhibitors typically bind to the kinase active site by forming 1–3 hydrogen bonds with residues in the hinge region, and they also engage in hydrophobic interactions within and around the region where ATP normally binds. This mechanism of inhibition effectively prevents ATP from interacting with the kinase, thereby blocking its enzymatic activity [93,94].

There are limited reports on the use of NIK inhibitors for cancer treatment, and many of these studies lack detailed biological activity data and structure-activity relationship (SAR) analyses. In 2010, Jérémie et al. unexpectedly discovered that the pyrazolo[4,3-c]isoquinolines, initially reported as NIK inhibitors, were neither inhibitors of NIK nor the

alternative NF-κB pathway. Instead, these compounds were found to inhibit another kinase, TGF-β-activated kinase 1 (TAK1), which is involved in the canonical NF-κB pathway [95]. Curcumin, the yellow pigment derived from Curcuma longa, is also a kind of NIK inhibitor. However, unfortunately, this compound also exhibits inhibitory activity against other kinases [96,97], which may limit their specificity.

To date, most preclinical studies have relied on genetic targeting of NIK. However, in recent years, several small-molecule NIK inhibitors have been discovered, showing promising potential for cancer treatment. These inhibitors offer an alternative approach to modulating NIK activity and could provide new therapeutic avenues for cancers driven by aberrant NIK signaling. Amgen and Genentech were pioneers in developing small molecule inhibitors targeting NIK, utilizing propargyl alcohol as a common motif to access the hydrophobic site behind the gatekeeper (gk) residue domain. Their approach is thoroughly detailed in a recent publication [98]. While one of these inhibitors (compound 2) demonstrated significantly improved potency and kinase selectivity, it lacked the stability required for in vivo assessment of NIK pharmacology. In a subsequent publication, the same group introduced compound 3, a potent and selective NIK inhibitor with optimal properties for advanced ADME and pharmacology studies [99]. This compound is expected to be a valuable tool for investigating the roles of NIK in the noncanonical NF-κB pathway in cancer.

## 6. Discussion

Recent research suggests that NIK is involved in a wide range of diseases. When NIK is defective, it can lead to conditions such as immunodeficiency or myeloid leukemia. On the other hand, when NIK is overactive, it is associated with diseases such as autoimmunity, cancer, sterile inflammation, and fibrosis. Growing evidence underscores NIK's critical role in the development and progression of various cancers, highlighting its potential as a therapeutic target.

As a key kinase in the non-canonical NF-κB signaling pathway, NIK plays a crucial role in various cellular processes, from immune regulation to tumorigenesis. While significant progress has been made in the over ten years since NIK was first cloned, many questions remain unanswered. Most notably, the full extent of NIK's regulatory function is still unclear. However, it is known that NIK is not a necessary component of the NF-κB canonical pathway, making it an attractive therapeutic target. Although NIK can regulate this pathway in response to certain specific agonists, normal physiological conditions do not require high levels of NIK expression. Overexpression of NIK can contribute to various diseases, including increased NIK stability in tumor cells, highlighting its potential as a promising target for the treatment of malignant tumors.

Moreover, the concurrent development of NIK inhibitors offers a unique opportunity to target NIK-IKK signaling through different approaches. While IKK and NIK can produce distinct patterns of gene expression, this distinction presents an intriguing alternative, especially in light of the limited success of IKK inhibition strategies to date. Kinase inhibitors with isoform selectivity will enable further refinement of the specific roles of NIK, IKK, and IKK in mediating transcriptional responses across various contexts. Pharmacological inhibition of NIK and IKK kinase activity, particularly in vivo, will provide valuable insights into whether these targets offer advantages in different tumor settings and open up potential therapeutic avenues for clinical application.

To date, medicinal chemistry efforts in academia are poised to drive research focused on targeting NIK in the coming years, offering innovative pharmacological strategies for what might otherwise be considered high-risk projects. As potent NIK inhibitors continue to emerge, they will provide valuable insights into the cellular signaling events

and gene induction profiles mediated by NIK. For instance, in the various hematological and immune system tumors and solid tumors contexts discussed earlier, these molecules will help determine whether targeting NIK can yield positive therapeutic outcomes while minimizing potential, yet unidentified, toxicities.

Although much of the current data on NIK comes from transgenic mice and cultured cells, recently developed small-molecule NIK inhibitors and other drugs targeting NIK are bringing us closer to the potential clinical treatment of diseases associated with increased NIK activity, as well as malignancies that have shown improvement in preclinical studies with NIK targeting. However, further preclinical studies are needed to assess the efficacy of these small molecule inhibitors in appropriate tumor models. Additionally, there is a lack of clinical data regarding the off-target effects, safety, and efficacy of NIK-targeted drugs. Specifically, the need for NIK in optimal hematopoiesis could pose challenges in the context of malignancies. Ideally, strategies should be developed to target NIK specifically to the cell types that activate it, in order to minimize side effects. Furthermore, once preclinical characterization of these new drugs is complete, clinical trials should be designed to evaluate their efficacy and safety for specific clinical conditions. While NIK as a druggable target holds great clinical potential, it also presents significant challenges that must be addressed.

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