

Review Article

The complex interplay between autophagy and NF- κ B signaling pathways in cancer cells

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Abstract: Tight regulation of both the NF- κ B pathway and the autophagy process is necessary for maintenance of cellular homeostasis. Deregulation of both pathways is frequently observed in cancer cells and is associated with tumorigenesis and tumor cell resistance to cancer therapies. Autophagy is involved in several cellular functions regulated by NF- κ B including cell survival, differentiation, senescence, inflammation, and immunity. On a molecular level, autophagy and NF- κ B share common upstream signals and regulators and can control each other through positive or negative feedback loops, thus ensuring homeostatic responses. Here, we summarize and discuss the most recent discoveries that shed new light on the complex interplay between autophagy and NF- κ B signaling pathways; this certainly has functional relevance in tumorigenesis and tumor responses to therapy.

Keywords: Autophagy, NF- κ B, cancer, signaling pathways

1. Macroautophagy

In this section, we do not intend to cover the detailed signaling pathways involved in macroautophagy (referred to as autophagy hereafter) but to briefly summarize the regulation of autophagy in cancer cells. Readers interested in details of autophagy signaling pathways and regulation are referred to the several excellent and extensive reviews [1-7].

In eukaryotes, autophagy is a lysosomal process that induces the degradation of cytoplasmic constituents such as proteins, lipids, and organelles [8]. Autophagy is a pivotal regulator of several important physiological processes including development, cell survival, differentiation, and senescence (**Figure 1**) [2, 9]. This process is also involved in regulating inflammation and innate and adaptive immune responses [10-12]. Dysfunction of autophagy is linked to the development and the progression of human diseases (**Figure 1**) such as cancer, neurodegenerative disorders, liver diseases, inflammatory pathologies (such as Crohn's disease), and infectious diseases [13-15].

Autophagy contributes to the maintenance of cellular homeostasis by inducing the recycling of damaged organelles and toxic components such as aggregation-prone proteins [16-19]. Recent studies using tissue-specific deletion of autophagy-related genes have revealed the accumulation of ubiquitinated proteins and aggregates in autophagy-impaired tissues emphasizing the importance of basal autophagy in tissue homeostasis [20, 21]. By inducing degradation of cellular material, autophagy plays also an essential role in tissue remodeling during cell differentiation [2]. In response to starvation, autophagy is induced to provide nutrients and energy necessary to cope with changes in metabolism thus ensuring cell viability [21, 22]. In this sense, autophagy is essential for survival during the early neonatal starvation period [23]. Autophagy is also induced in response to stressful conditions including oxidative stress, hypoxia, endoplasmic reticulum stress, hormonal imbalance, pathogen-associated molecular patterns, DNA damage, and anti-cancer therapies [6, 24-26]. Under stress conditions, autophagy mostly plays a cytoprotective role; however, autophagy has been also shown to induce a cell

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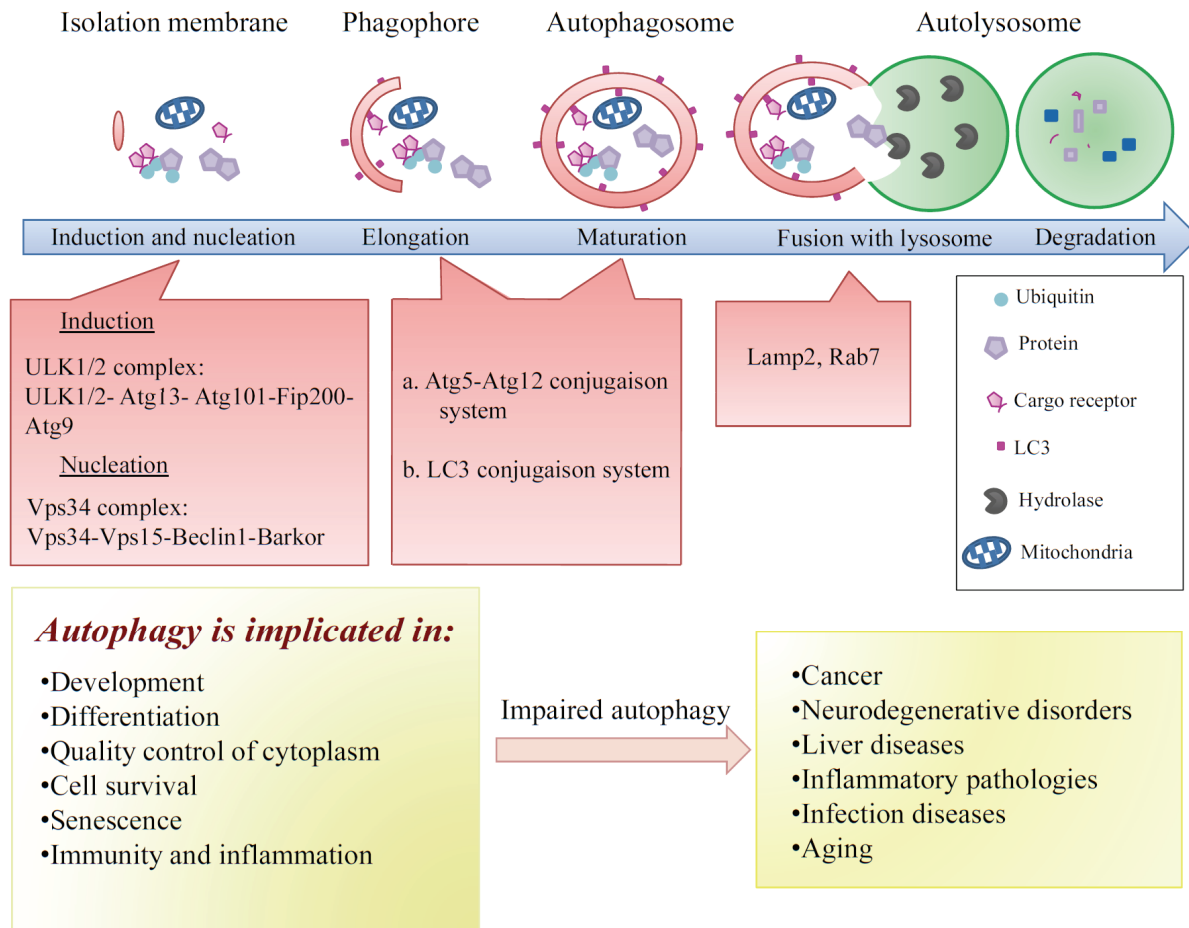


Figure 1. Schematic representation of autophagy in mammals. The first step in the induction of autophagy is the formation of a multi-membrane structure, known as a phagophore which further elongates to form an autophagosome before enclosing cytoplasmic material. The autophagosome then fuses with the lysosome to form the autophagolysosome, in which the sequestered material is degraded by lysosomal hydrolases. The ULK1/2 complex and the class III phosphatidylinositol 3-kinase (Vps34) complex drive the first step of autophagosome formation (induction and nucleation). The elongation and the closure of the autophagosomal membranes (maturation) require the recruitment of two conjugation systems, Atg5-Atg12 and Atg8/LC3-phosphatidyl ethanolamine, to the phagophore. The lysosomal membrane protein Lamp 2 and the GTPase Rab7 together mediate the fusion of autophagosome with the lysosome. The major cellular functions of autophagy are shown. Depicted are the consequences of impaired autophagy in the development of certain human pathologies. For details, see text.

death program [27-29].

Autophagy core machinery

Autophagy starts with the formation of a multi-membrane structure, the preautophagosomal membrane also called the phagophore (**Figure 1**). This membrane elongates to form the autophagic vesicle, known as autophagosome, before enclosing cytoplasmic components. When the autophagosome fuses with the lysosome, the autophagosomal contents are degraded and

recycled to be used for the synthesis of macromolecules and the production of energy [1, 3, 21, 30]. Genetic studies of autophagy in the yeast have led to the identification of autophagy-related genes (Atg genes) whose products orchestrate the steps of autophagosome formation, namely nucleation, expansion, uncoating, and completion. The core machinery of autophagy that drives autophagy in mammals involves three complexes formed by Atg proteins and lysosomal-associated membrane protein 2 (Lamp-2) and Rab7 proteins (**Figure 1**). The

complex involved in the initiation of autophagy process is the Atg1/unc-51-like kinase (ULK) complex (ULK1/2, Atg13, Atg 101, Fip200 and Atg9). Next, the class III phosphatidylinositol 3-kinase (Vps34) complex (Vps34, Vps15, Atg6/Beclin1 and Barkor) generates phosphatidylinositol 3-phosphate (PI3P) by inducing the activity of PI3-kinase, a key event that subsequently leads to the elongation of autophagosomal membrane. Two conjugation systems comprised by Atg5-Atg12 and Atg8/microtubule-associated protein 1 light chain 3 (LC3) phosphatidylethanolamine are required for the elongation and the closure of the autophagosomal membranes. Finally, the fusion of autophagosome with the lysosome requires the lysosomal membrane protein Lamp2 and the GTPase Rab7 [31-34]. The functions of the autophagy machinery core complexes are tightly regulated by different factors that favor or repress the induction of autophagy. For example, autophagy is positively regulated by the interaction of Atg6/Beclin 1 with Atg14L, ultraviolet radiation resistance associated gene (UVRAG), Bif-1, and Activating molecule in Beclin 1-regulated autophagy (Ambra1). Conversely, the binding of Beclin 1 with the proto-oncogene B-cell lymphoma 2 (Bcl-2) inhibits autophagy in several experimental settings [32].

Although autophagy has long been considered a non-selective process, a number of recent studies have revealed that autophagy can selectively degrade certain cytosolic cargos including protein aggregates, damaged organelles (such as mitochondria and peroxisomes), intracellular pathogens, and virus [17-19]. By regulating the turnover of damaged cellular constituents, autophagy prevents the accumulation of reactive oxygen species (ROS) and oxidative damage to DNA and may thus be a tumor-suppressive mechanism. In selective autophagy, cargos are recognized through interaction with specific receptor proteins. For example, p62 and NBR1 are cargo receptors for degradation of ubiquitinated proteins and Bnip3 (NIX) is required for the clearance of mitochondria (mitophagy) and cell death in response to hypoxia [19, 35].

Signaling pathways regulating autophagy

A variety of cellular stress signals converge to the mammalian target of rapamycin 1 (mTOR) complex 1 (mTORC1) to regulate autophagy upstream of the core machinery. During depriva-

tion of nutrients and growth factors, autophagy is induced through inactivation of mTORC1, which drives the initial step of autophagy by triggering the activation the ULK1/2 complex [31]. Upstream of mTOR, several tumor suppressor genes, including LKB1, AMPK, TSC1/TSC2 and PTEN have been shown to inhibit mTORC1 and lead to the stimulation of autophagy. In contrast, tumor promoter pathways initiated by Class I PI3K, PKB/Akt, insulin and IGF receptors, Rheb and NF- κ B activate mTORC1 and thereby repress autophagy [3, 25, 36-38]. Some other tumor regulatory molecules that control autophagy include oncoproteins Bcl-2 and Bcl-x_L (which inhibit Beclin 1-dependent autophagy), Ras (via class I PI3K), and hypoxia-inducible factor-1 (Hif-1) and tumor suppressors such as p53, DAPK, E2F-1, and FoxO proteins [6, 39].

Autophagy and cancer

The relationship between autophagy and cancer is complex and depends on the tumor's nature, stage, and context. Autophagy can function as a tumor-suppressive mechanism or conversely as a tumor-promoting process [3, 38, 40-42].

Several genetic studies have established the link between the autophagy machinery and tumorigenesis [13]. For example, a monoallelic deletion of the essential gene *Beclin 1* is frequently observed in ovarian, breast, and prostate cancers [43]. Defects in essential autophagy genes *Atg5* and *Beclin 1* promote tumorigenesis of certain cell lines. Allelic loss of *Beclin 1* and deficiencies in expression of two other autophagy-regulating genes, *Atg4C* and *Bif-1*, render mice prone to tumor development [43-46]. Moreover, as mentioned above, the regulation of autophagy is tightly controlled by signaling pathways that regulate tumorigenesis. Indeed, inactivating mutations or allelic deletions in tumor suppressor genes whose products positively regulate autophagy (e.g., PTEN, TSC1/TSC2, and p53) are frequently observed in human tumors, emphasizing the tumor-suppressive function of autophagy. On the other hand, some tumors have aberrant activation of genes like *Bcl-2*, *Akt*, *Class I PI3K*, and *NF- κ B* whose products have tumor promoter functions and repress autophagy [3, 38, 40-42]. However, in some instances oncogenes can promote autophagy stimulation as it has been shown for H-Ras (V12) and K-Ras (V12) [47].

Several mechanisms have been proposed to explain the tumor-suppressive role of autophagy. As stated above, one of them concerns the function of autophagy in the selective removal of toxic cellular components that produce ROS, a mechanism that would limit the incidence of DNA mutation and prevent chromosome instability and subsequent tumorigenesis [48]. Autophagy may limit cell proliferation as demonstrated by results showing that enforced expression of *Beclin 1* represses the growth of cancer cell xenografts [43, 49] and that Ras-induced downregulation of *Beclin 1* expression enhances proliferation of malignant cells that remain viable upon their detachment from extracellular matrix [50]. In addition, through its ability to promote rapid protein turnover, autophagy facilitates the acquisition of the senescence phenotype, a tumor suppression mechanism that prevents tumor development through cell-cycle arrest, cellular remodeling, and secretion of specific cytokines [51]. The prevention of chronic tumor cell death mediated by autophagy may represent another tumor-suppressive function of this process. Indeed, in apoptosis-defective tumors exposed to metabolic stress, autophagy prevents chronic necrosis, limiting undesirable inflammatory responses that create a pro-tumorigenic environment [48]. The role of autophagy in the suppression of inflammation is also supported by the fact that defective autophagy enhances susceptibility to some inflammatory diseases such as Crohn's disease and pancreatitis [11].

On the other hand, autophagy contributes to tumor progression by maintaining the survival of established tumor cells in starvation conditions and in hypoxic microenvironments. This phenomenon might occur when cancer cells are located in the core of a solid tumor and are poorly vascularized; under these contexts, the cells have to survive under conditions of low oxygen (hypoxia) and reduced nutrients. Metabolic stress-induced autophagy may also render cancer cells more resistant to anti-cancer therapies [21, 52]. Moreover, the release pro-inflammatory factors such as the damage-associated molecular pattern molecule HMGB1 by stressed or dying tumor cells has been shown to favor the survival of stressed cells in tumor microenvironment by a mechanism that depends on the induction of autophagy [53, 54].

Autophagy, thus, appears to have a dual role in tumorigenesis: on the one hand, autophagy restricts tumor development by inhibiting inflammation, cell proliferation, and accumulation of cellular damages. On the other hand, autophagy sustains viability of established tumors exposed to environmental changes or subjected to anti-cancer treatments [3, 38, 40-42]. Depending on the context, autophagy may also promote or inhibit metastasis by limiting anoikis during extracellular matrix detachment or by promoting anti-tumor inflammatory responses and maintaining cancer cells dormancy, respectively [55].

Autophagy, cancer therapies and cell death

A variety of anticancer agents can induce autophagy; these include tamoxifen, rapamycin, temozolomide, histone deacetylase inhibitors, and a subset of DNA-damaging agents such as camptothecin, etoposide, and ionizing radiation [25, 26]. In most cases, the stimulation of autophagy by these agents results from a cytoprotective mechanism and confers tumor resistance to therapies. However, in some instances autophagy induced by anti-cancer therapy may facilitate the activation of cancer cell death programs [27, 29, 56]. Interestingly, there are molecular interconnections between autophagy and different cell death pathways. For example, the interplay between autophagy and apoptosis is demonstrated by extensive molecular cross-talk between these two pathways [27, 56, 57]. Several mediators of death receptor-induced apoptosis, such as TRAIL, TNF, FADD, and DAPK, activate autophagy. Moreover, some pro-apoptotic regulators, such as RIP, JNK, ARF, E2F1, and Foxo, have recently been shown to positively regulate autophagy [27, 57]. p53, another apoptosis-regulatory protein, represses autophagy when localized in the cytoplasm; its nuclear localization leads to the activation of autophagy through transactivation of *damage-regulated autophagy modulator (Dram)* and *Sestrin 2* genes [58]. Conversely, several anti-apoptotic proteins can repress autophagy by either forming a complex with Beclin 1 or Atg3 (Bcl-2/Bcl-X_L and Flip, respectively) or by activating the mTOR pathway (Akt/PKB and NF- κ B) [32, 36, 56, 59, 60]. Moreover, some of the autophagy core components, including Beclin 1, Atg5, and Atg4D, are cleaved during apoptosis, and the cleaved fragments induce cell death; this suggests that apoptosis events may change

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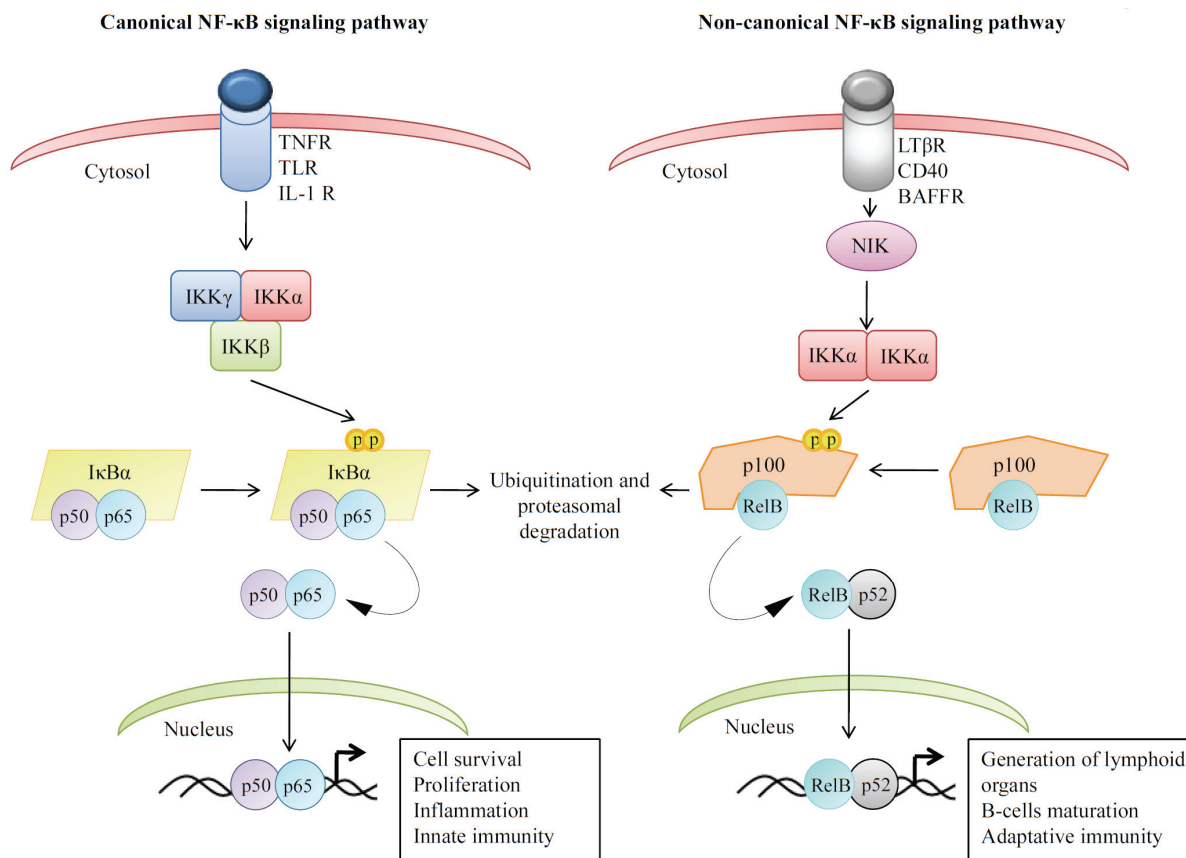


Figure 2. Schematic model for the activation of NF- κ B signaling pathways. In the canonical NF- κ B pathway, stimulation of pro-inflammatory receptors activates the IKK complex (comprised of IKK α , IKK β and IKK γ) leading to the phosphorylation of I κ B α and its proteasomal degradation. This induces the translocation of p65/p50 dimers into the nucleus where the dimer binds to the promoter regions of a subset of target genes. In the noncanonical NF- κ B pathway, stimulation of BAFF-R, CD40, and lymphotoxina- β receptor (LT β R) leads to the stabilization of NIK and results in the activation of the IKK α homo-dimers. Active IKK α , in turn, induces the phosphorylation of the NF- κ B family member NF- κ B2/p100 leading to its processing by the proteasome to generate p52. This allows the translocation of the p52/Rel B dimer into nucleus where it regulates the transcription of target genes. For details, see text.

the function of autophagy-related proteins [56, 59, 61, 62].

2. NF- κ B transcription factors

In this section, we briefly summarize the regulation of NF- κ B transcription factor in cancer cells. Readers interested in details of NF- κ B signaling pathways and regulation are referred to the following excellent reviews [63-69].

NF- κ B family members

The NF- κ B family of transcription factors regulates the expression of a broad range of genes involved in development, cell proliferation, sur-

vival, differentiation, and senescence. These transcription factors also play a pivotal role in regulating inflammation and the innate and adaptive immune responses [63, 70, 71]. The mammalian NF- κ B family has five members: RelA (called also p65), c-Rel, Rel B, p50, and p52 [66, 71, 72]. These proteins contain the Rel homology domain that is essential for their homo- and hetero-dimerization and their binding to a specific DNA response sequences in the promoters of target genes called κ B sites. Of note, each of the five members induces the activation of a selective set of genes that depend on the cell type and the stimulus. In most cells, the NF- κ B dimers are sequestered in the cytoplasm as latent complexes through their binding

to a member of the inhibitor of NF- κ B (I κ B) protein family including I κ B α , I κ B β , I κ B ϵ and the I κ B-like inhibitors p100 and p105 [67].

NF- κ B signaling pathways

Depending on the stimuli and the cell type, NF- κ B activation can be promoted by two distinct pathways: the canonical and noncanonical pathways (**Figure 2**) [27, 63]. In both pathways, the activation of NF- κ B is mediated by the activation of I κ B kinase (IKK) complexes formed by either the catalytic kinases IKK α and IKK β and the regulatory protein NEMO (IKK γ) or by IKK α homo-dimers [73, 74]. The canonical NF- κ B pathway is activated primarily by stimulation of pro-inflammatory receptors such as TNF receptor family members, the Toll receptor family member, and antigen receptors [68, 69]. Certain anti-cancer therapies also activate this pathway [75-78]. These stimuli activate the IKK complex promoting the phosphorylation and subsequent proteasomal degradation of I κ B α protein. This allows the translocation of the liberated NF- κ B dimers into nucleus where they bind to the promoter regions of target genes that control cell survival, proliferation, inflammatory and innate immunity (**Figure 2**) [63-65, 76].

The noncanonical NF- κ B pathway is activated by stimulation of specific TNF receptors, including LT β R, CD40, and BAFFR, and plays a key role in the generation of lymphoid organs, in B-cell maturation, and in adaptive immunity [73, 79]. In the noncanonical pathway, the stabilization and the accumulation of NF- κ B-inducing kinase (NIK) is an essential signal that triggers the activation of the IKK α homo-dimer [80, 81]. Conversely, stimulus that induces the proteasomal degradation of NIK promotes the negative regulation of the noncanonical NF- κ B signaling events [80]. Once activated, IKK α induces the phosphorylation of NF- κ B2/p100 (commonly associated with Rel B) leading to its processing by proteasome to generate p52. This allows the translocation of the p52/Rel B dimer into nucleus where it regulates the transcription of target gene (**Figure 2**) [79-81].

In context of normal cells, the stimulus-dependent activation of NF- κ B is mostly transient due to establishment of feedback loops that terminate the activation. In both canonical

and noncanonical pathways, one of the mechanisms that cells invoke to prevent the persistent activation of NF- κ B is the inactivation of IKK. This can occur through IKK-mediated phosphorylation of its upstream activators (NIK in the noncanonical NF- κ B pathway and RIP in canonical pathway), which subsequently induces their loss of function and promotes their proteasomal degradation. Of note, IKK can also inhibit itself by inducing its proper phosphorylation [73, 74]. Other repressors of NF- κ B activation are A20 and CYLD, two proteins that mediate the K-63 deubiquitination and subsequent inactivation of signaling molecules like tumor necrosis factor receptor-associated factor (TRAF), RIP, and IKK γ that are required for NF- κ B activation [82, 83]. Interestingly, these NF- κ B repressors, as well as I κ B family members (for example I κ B α), are target genes of NF- κ B activation and are therefore function in negative feedback mechanisms [82, 83]. Moreover, post-translational modifications of the p65 subunit of NF- κ B (e.g., phosphorylation, ubiquitination, and methylation) have been shown to affect IKK/ NF- κ B signaling events [76, 84]. In addition to the above mentioned regulators of NF- κ B pathways, the activity of NF- κ B in cancer cells is under the control of several tumor-regulatory pathways including Ras, EGF receptor, mTOR, Akt, STAT3, and p53 [67, 68, 85]. Therefore, different signaling pathways converge to NF- κ B, and the process is tightly regulated by a counterbalance between activators and repressors to maintain appropriate cellular responses. The disruption of feedback inhibition mechanisms of NF- κ B are linked to the development and the progression of several human diseases including inflammatory disorders and cancers [68, 81, 85, 86].

NF- κ B and cancer

It is now well-established that NF- κ B plays a critical role in the initiation, promotion, and progression of certain cancers due to the ability of this pathway to upregulate genes responsible for cell survival, invasion, angiogenesis, and metastasis [69, 71, 74, 81, 85, 87, 88]. NF- κ B-mediated prevention of apoptosis is certainly one key event that favors the development of tumors and that plays a pivotal role in resistance to cancer therapies [75, 77, 78, 88-90]. Studies focused on identifying the mechanism involved in the anti-apoptotic role of NF- κ B have shown that activation of this transcription factor

results in an increase in expression of genes encoding for anti-apoptotic proteins like Bcl-2 family members, c-Flip, and IAPs (inhibitor of apoptosis) [87]. NF- κ B activation also upregulates expression of antioxidant proteins that suppress ROS-dependent apoptosis and necrosis [88, 89].

Constitutive activation of NF- κ B is observed in various types of malignancies. Activation may be due to alterations in expression of genes encoding NF- κ B family members or their negative regulators or to persistence of NF- κ B inducers such as pro-inflammatory cytokines (mostly, TNF and IL6) in the microenvironment of tumor cells [68, 69, 78, 81, 87, 88, 91, 92]. The first evidence of the oncogenic role of NF- κ B emerged after NF- κ B subunits p50/p105 were cloned; this revealed homology of the cellular homologue c-Rel to the oncogene v-Rel encoded by the avian reticuloendotheliosis virus [85, 91]. Later, the over-activation of NF- κ B members in a wide variety of types of tumors was demonstrated. For example, mutations in the gene encoding NF- κ B2/p100 were detected in B and T cell lymphomas that resulted in loss of the inhibitory effects of the p100 protein and gain of transcriptional activity of the truncated p52 product [91, 92]. The transcriptional activity of p52 and p50 complexes is also enhanced by the Bcl-3 oncoprotein, a product of chromosomal translocation in B cell chronic lymphocytic leukemia. The overexpression of Bcl-3 has also been observed in various solid and lymphoid tumors, and patients with high levels of Bcl-3 have poor prognosis [93]. Amplification of c-Rel is observed in diffuse lymphoma with a large cell component, non-Hodgkin's B cell lymphoma, and in some solid tumors [92]. Mutations or deletions in *I κ B α* and *I κ B ϵ* have been also detected in a subset of Hodgkin lymphomas, although their roles in the development of the disease is still not clear. Several experiments indicate that inhibition of *I κ B α* degradation using a repressor form of this protein promotes apoptotic cell death and inhibits tumorigenesis in different animal models. In support to this tumor-suppressive role, constitutive degradation of *I κ B* has been observed in various types of tumor cells [94, 95].

Although aberrant activation of IKKs has been observed in hematological and solid malignancies, no oncogenic mutations of the IKK genes have been yet discovered. Several lines of evi-

dence support the idea that constitutive activation of IKK is due to the upregulation of IKK upstream activators or inactivation of its repressors. One example comes from recent studies in myeloma cell lines that led to the identification of mutations in genes encoding proteins that lead to the stabilization and accumulation of NIK, an upstream kinase of IKK α [96, 97]. The turnover of NIK is tightly regulated by IAP1 and c-IAP2, two ubiquitin ligases that target NIK for ubiquitination and thereby enhance its proteasomal degradation [98]. Mutational inactivation of *c-IAP1* and *c-IAP2* genes also occurs in myeloma cells and is correlated with the stabilization of NIK. Moreover, gain-of-function mutations in the *NIK* gene have been also observed in myeloma cells that may contribute to the accumulation of NIK protein. As stated above, A20 and CYLD, two proteins belonging to deubiquitinase family, can also attenuate the NF- κ B responses by targeting signalling proteins that are required for IKK activation [82, 99]. Inactivating genetic alterations of A20 have been observed in various hematological malignancies and are linked with chronic NF- κ B activation in these cancers [99]. Moreover, the overexpression of A20 in A20-deficient lymphoma cell lines inhibits both NF- κ B activity and cell growth and triggers apoptotic cell death. Together, these data suggest that A20 has a tumor suppressor function [99]. Similarly, loss of CYLD1 function has been shown to promote tumorigenesis by enhancing NF- κ B activation [82].

Although chronic activation of NF- κ B is observed in the majority of cancer cells, genetic alterations in NF- κ B members and regulatory signalling components are rare except in certain lymphoid malignancies [68, 81, 87]. This supports the idea that cancer cells have evolved other mechanisms to activate NF- κ B. In fact, mutations in growth factor signalling pathways (e.g. k-Ras, EGF receptor, mTOR) or changes due to oncogenic viruses (T-cell leukemia virus type I, Epstein-Barr, Kaposi sarcoma-associated herpes virus) constitute other manners of persistent activation of NF- κ B [68, 81, 100, 101]. Moreover, a link between NF- κ B and inflammation-associated cancer progression has been demonstrated in several carcinogen/inflammation *in vivo* models by using conditional IKK knockout mice [72, 86]. These studies revealed that IKK β -dependent NF- κ B activation in premalignant-infiltrating immune cells leads to the production of cytokines that stimu-

late proliferation and enhance survival of malignant cells. The cytokines produced by immune cells, in turn, activate NF- κ B in cancer cells to induce chemokines that attract additional inflammatory cells prolonging tumor-associated inflammation. IKK β is therefore a key factor in inflammation-associated cancer progression and its inhibition may reduce tumor growth and enhance susceptibility to anti-cancer therapies. However, in some specific circumstances IKK β and NF- κ B act as tumor suppressors indicating that their inhibition would not always be beneficial to cancer patients [71].

3. The interplay between NF- κ B and autophagy signalling pathways

3.1 Degradation of NF- κ B signaling components through autophagy

Inhibition of Hsp90 induces autophagic degradation of IKK and NIK

IKK α , IKK β , and IKK γ are targets for degradation by autophagy when heat shock protein 90 (Hsp90) function is inhibited (**Figure 3a**) by geldanamycin [102, 103]. Hsp90 is a chaperon protein, required for the correct folding and maturation of certain signaling proteins thus preventing their degradation under conditions of stress. Interestingly, geldanamycin-mediated degradation of IKK is prevented when autophagy is inhibited; but the clearance of Akt/PKB, another substrate of Hsp90 known to be degraded through the proteasomal pathway, is not affected by inhibition of autophagy. Thus, Hsp90 substrates are specifically degraded by either autophagy or proteasomal pathways. One example of such regulation has been recently reported in NB4 leukemia cells treated with selenite, a common dietary form of selenium with chemopreventive potential against various cancers [104]. Selenite treatment promotes downregulation of Hsp90, which leads to inactivation of NF- κ B pathway and inhibition of autophagy through downregulation of *Beclin 1* expression. In NB4 cells, Hsp90 is a key regulator of the cell signaling pathway that switches cells treated with selenite from the autophagy program to apoptosis. Interestingly, in addition to induction of autophagy, specific inhibition of mitochondrial Hsp90 by gamitrinib elicits the organelle unfolded protein response (UPR), which also contributes to the repression of NF- κ B activity [105].

NIK, an activator of IKK α in the non-canonical pathway, is another Hsp90 interacting protein. Disruption of the NIK/Hsp90 interaction by geldanamycin targets NIK for degradation by autophagy leading to the inhibition of both p100 processing and NF- κ B activity (**Figure 3a**) [106]. Thus, both the proteasomal system and the autophagy pathway are involved in the degradation of NIK and the regulation of the NF- κ B activity [98]. Taken together, these studies showed that Hsp90 is a novel regulatory bridge between autophagy and IKK/NF- κ B pathways [37, 107]. Further studies are needed to determine the mechanisms involved in selective degradation of IKKs and NIK by autophagy. Interestingly, the activation of NF- κ B by heat shock stress has been shown to activate, in turn, autophagy pathway [108] which underscores the notion that NF- κ B and autophagy are mutually regulated under heat shock treatment. This will be further discussed in the chapter 3.2.

Keap1-dependent autophagy degradation of IKK β

One clue in the mechanisms responsible for the control of autophagy-dependent degradation of IKK came from study that led to the identification of Kelch-like ECH-associated protein 1 (Keap1) [109], as an interactor of IKK beta. Keap1 interacts with the kinase domain of IKK β through its C-terminal domain. This domain is also required for the binding of Keap1 to the transcription factor NF-E2-related factor 2 (Nrf2), which controls expression of certain antioxidant target genes. In response to TNF, Keap1 negatively regulates activation of NF- κ B through inhibition of the IKK β phosphorylation and induction of IKK β degradation by autophagy pathway (**Figure 3b**). Moreover, Keap1 functions as an E3 ligase to mediate proteasomal degradation of IKK β . Thus, Keap1 mediates degradation of IKK β by both autophagy and proteasome pathways. However, Keap1 does not interact with IKK α , which is also degraded by autophagy implying another, not yet identified mechanism. Recently, the NF- κ B family member p65 was shown to interact with Keap1 [110]. Moreover, current data suggest that a functional interplay between NF- κ B components (IKK β and p65) and Keap1 are necessary for the tight regulation of the transcription activity of both NF- κ B and Nrf2 [111]. However, although various somatic mutations of Keap1 have been detected in some malignancies [112], a causal link be-

tween these mutations and the aberrant NF- κ B activity observed in these tumors was not yet established. Interestingly, recent report has identified the inhibitor of NF- κ B, I κ B α as a new autophagic substrate. At the first phase following stimulation with TNF treatment, I κ B α is degraded through proteasome-dependent mechanism while at later phase it is cleared through the autophagic pathway [113]. Autophagy can thus lead to either the termination of NF- κ B by inducing the clearance of IKK or the persistent activation of NF- κ B through degradation of I κ B α [113].

Ro52-dependent autophagy degradation of IKK β

The E3 ubiquitin ligase Ro52 is another signaling molecule that targets IKK β for degradation through the autophagy pathway [114]. In cells infected with HTLV-1, Tax oncoprotein is responsible for the persistent phosphorylation of IKK β and constitutive activation of NF- κ B activity (Figure 3b). Once activated, IKK β interacts with Ro52, which mediates its monoubiquitination, a signal necessary for targeting IKK β to autophagosomes. Inhibition of autophagy suppresses Tax/IKK β -induced NF- κ B activity suggesting that Ro52-mediated autophagy degradation of IKK β represents a feedback mechanism for the termination of NF- κ B activation by Tax. Interestingly, Ro52-mediated monoubiquitination of IKK β occurs only under conditions of the persistent phosphorylation and activation of IKK β (observed in response to Tax), which allows the formation of a stable interaction between Ro52 and IKK β . Indeed, the monoubiquitination of IKK β is not detected in response to TNF (another inducer of NF- κ B activity) probably because the phosphorylation of IKK β by this cytokine is transient and does not lead to the formation of a stable IKK/Ro52 complex. However, it is not yet clear whether the Ro52-dependent monoubiquitination of IKK β is a general response that occurs during persistent activation of IKK β or a specific response triggered in cells expressing Tax.

Together, these results indicate that Hsp90, Keap1, and Ro52 are novel partners of IKKs, (especially IKK β) which mediate the interplay between NF- κ B and the autophagy pathway. In response to distinct stimuli, their specific interactions with IKKs regulate NF- κ B activity through their ability to activate or repress degra-

dation of IKKs through autophagy. It will be important to determine whether these interactions are mutually exclusive or whether some of these proteins cooperate to regulate IKK β degradation. Determining this mechanism is essential to understanding how IKK/NF- κ B activity can be switched on or off in response to different stimuli.

Regulation of NF- κ B signaling components by autophagy-regulatory proteins

In addition to the direct effects of autophagy on NF- κ B signaling components like IKKs and NIK, some proteins known to regulate autophagy have been reported to modulate NF- κ B activity. For example, p62 (also known as sequestosome-1 or SQSTM1), a cargo receptor for degradation of ubiquitinated proteins through the autophagy pathway, activates NF- κ B in response to several stimuli including TNF, IL-1, receptor activator for NF- κ B (RANK) ligand, and nerve growth factor [115]. Intriguingly, the impairment of both autophagy and apoptosis in immortal baby mouse kidney (iBMK) cells, an epithelial tumor cell line, results in the accumulation of p62, which is sufficient to inhibit NF- κ B activity and to promote tumorigenesis [116]. The reason for seemingly opposite effects of p62 on the regulation of NF- κ B is yet not clear.

Another protein involved in the formation of autophagosome with a link to the NF- κ B pathway is Atg5. Atg5 interacts with Fas-associated protein with death domain (FADD), an upstream regulator of NF- κ B [117-119]. This interaction is required for IFN γ -induced autophagy-dependent death in HeLa cells and contributes to the regulation of autophagy and activation of caspase 8 during mitogenic stimulation of T lymphocytes. However, whether NF- κ B regulation was affected in HeLa cells treated by IFN γ was not explored. Beclin 1 is another essential autophagy protein that has been shown to regulate NF- κ B activity in context of cells exposed to cigarette smoke extract. The partial loss of *Beclin 1* in fibroblasts from *Beclin 1* heterozygous knockout mice increases p65 phosphorylation and prevents apoptosis mediated by cigarette smoke extract. However, the mechanism by which Beclin 1 regulates NF- κ B, has not been elucidated [120].

Several recent studies have revealed molecular crosstalk between tuberous sclerosis proteins

TSC1 and TSC2 (upstream inhibitors of mTOR activation and activators of autophagy) and NF- κ B pathway [121-124]. NF- κ B signaling and cell survival are attenuated in TSC1- and TSC2-deficient cells stimulated by DNA damage or TNF [121]. The addition of rapamycin, an inhibitor of mTOR activity, restores NF- κ B activation and cell survival in TSC2-deficient cells suggesting that TSC2-mediated mTOR inhibition contributes to NF- κ B activation [121]. Interestingly, IKK β in turn, can activate the mTOR pathway in TNF-treated cells through a mechanism that involves its interaction with phosphorylated TSC1 [122]. Moreover, the IKK α subunit is required for activation of mTOR in PTEN-deficient cancer cells and in cells exposed to insulin [123, 124].

3.2 Regulation of autophagy by NF- κ B

NF- κ B activation occurs in response to various signals such as ligation of death receptors or pathogen recognition receptors (PRRs), oncogenes, virus, and DNA damage [68, 101, 125]. Recently, several studies have highlighted the regulation of autophagy in response to inducers of NF- κ B-mediated signaling.

Regulation of autophagy by NF- κ B activity

In response to TNF, NF- κ B is activated in most cells through the canonical NF- κ B pathway and leads to the upregulation of several anti-apoptotic and anti-oxidants genes [90, 126]. TNF-dependent activation of NF- κ B represses autophagy in Ewing's sarcoma, breast, and leukemia cancer cell lines, whereas loss of NF- κ B activation in these cells triggers the re-activation of autophagy [36, 127] (**Figure 4a**). The repression of autophagy in TNF-treated cells is associated with the activation of mTOR pathway, a negative regulator of autophagy. This is consistent with studies showing that IKK activation is required for mTOR activation in certain cell types [122, 123, 124]. The negative regulation of autophagy by NF- κ B is also supported by a study showing that inhibition of NF- κ B activity results in an enhancement of starvation-induced autophagy in cells derived from myelodysplastic patients with poor prognosis [128]. Similarly, the suppression of prolonged NF- κ B activity in macrophages exposed to *Escherichia coli* promotes autophagy and increased cell survival whereas NF- κ B-proficient macrophages

undergo to cell death under the same condition [129] (**Figure 4a**). It should be mentioned that autophagy can be an effector of the innate immune response by targeting some pathogens for degradation by lysosomes [10-12]. These results support the idea that, in response to certain pathogens, cells initiate a regulatory circuit to override the repression of autophagy, and this protects them against the microbe.

The induction of autophagy by TNF in the absence of functional NF- κ B activity requires the accumulation of ROS (mainly superoxide), as activation of antioxidants responses do not occur when NF- κ B is inhibited [36, 127]. The link between autophagy and NF- κ B inactivation and ROS accumulation is supported by a study showing that sex-related differences in autophagy in the Syrian hamster are controlled by ROS production through a mechanism that relies on the activities of NF- κ B and p53 [130]. The key role of ROS in the regulation of autophagy has been also demonstrated under starvation conditions and in response to several anti-cancer therapies [24, 131]. Although the superoxide is assumed to be the major ROS species involved in modulation of autophagy [131], hydrogen peroxide also acts as a sensor for the regulation of autophagy. For example, in senescence cells, the overproduction of hydrogen peroxide due the NF- κ B-dependent upregulation of manganese superoxide dismutase (MnSOD) antioxidant results in the induction of both autophagy and cell death [132]. Thus, depending on the cellular context, the duration and the intensity of NF- κ B activity, the redox-regulating function of NF- κ B can act either as an inhibitor or an activator of autophagy. Interestingly, ROS, in turn, can activate NF- κ B activity and promotes autophagy. For example, upon hypoxia, MCF7 epithelial cancer cells activate the oxidative stress pathway in adjacent cancer-associated fibroblasts resulting in the activation of both NF- κ B and Hif-1 α [133]. This activation is sufficient for the induction of autophagy and leads to the degradation of Caveolin-1 (one component of caveolae membranes involved in receptor-independent endocytosis). The clearance of Caveolin 1 mediated by NF- κ B/Hif-1 α prevent the death of adjacent cancer cells suggesting that caveolin-1 may be a relevant biomarker for certain types of cancer cells (**Figure 4a**).

Additional evidence for the positive regulation of

autophagy by NF- κ B came from study that investigated the consequences of NF- κ B activation during the recovery period after heat shock treatment. Upon heat shock, NF- κ B is activated through a mechanism that is independent of I κ B kinase activity and I κ B α degradation but dependent on the thermolability of the I κ B/NF- κ B complex (**Figure 4a**). NF- κ B activation enhances autophagy and increases the likelihood of cell survival, probably by inducing the degradation of misfolded and aggregated proteins that accumulate during heat shock stress [108]. In addition to this pro-survival function of NF- κ B-dependent induction of autophagy, in the context of ganglioside-induced astrocyte activation, NF- κ B activation is involved in the induction of cell death through an autophagy-dependent mechanism [134].

Upon recognition of pathogen infections or tissue damage, PRRs (e.g., Toll-like receptors, TLRs) and NOD family proteins activate multiple signaling pathways, including NF- κ B-mediated signaling, which trigger the induction of the innate immune responses to defend against pathogen infections [10-12]. The link between Toll-like receptor-mediated autophagy and NF- κ B signaling has been recently investigated in a macrophage cell line subjected to the TLR4 agonist lipopolysaccharide (LPS) [135]. TLR4 stimulation relies on the recruitment of Beclin 1 into the TLR4-signaling complex and requires the K63-linked ubiquitination of Beclin 1 by TRAF6, an upstream activator of NF- κ B signaling pathway. The deubiquitination enzyme A20 (a target gene product of NF- κ B) inhibits the ubiquitination of Beclin 1 and limits autophagy in response to TLR4 signaling (**Figure 4a**). Thus, TRAF6 and A20 can stimulate or inhibit autophagy by regulating the ubiquitination and deubiquitination of Beclin 1, respectively. Since the duration and intensity of TRAF6 activation and the A20 levels vary depending on the cell type and the stimulus, this can explain, at least in part, why NF- κ B can act as either an inhibitor or an activator of autophagy. Of note, several primary cell lines fail to activate autophagy upon PRR engagement but, as of yet, TRAF6 and A20 activities have not been determined in these cells. The signaling pathways that lock the cascade linking NF- κ B stimulation and autophagy induction is a matter for investigation in further studies.

Transcription regulation of autophagy-regulatory

genes by NF- κ B

The analysis of promoters of murine and human *Beclin 1* genes led to the identification of several NF- κ B consensus sequences suggesting that NF- κ B regulates *Beclin 1* expression. p65, a member of NF- κ B family, specifically interacts with the κ B site of *Beclin 1* as revealed by *in vitro* and *in vivo* studies [136]. As a consequence, p65 induces the upregulation of *Beclin 1* mRNA that is associated to the activation of autophagy in several cellular settings including T cell receptor-dependent activation of Jurkat cells (**Figure 4b**). Of note, p65-dependent *Beclin 1* upregulation is dependent on the cellular context and the stimulus; p65 overexpression does not upregulate *Beclin 1* expression in some cell lines and *Beclin 1* mRNA levels are not increased in response to TNF α (unpublished data of our laboratory) or heat shock, which activate the p65/NF- κ B signaling pathway [108].

Interestingly, NF- κ B not only induces the upregulation of *Beclin 1* but promotes deubiquitination of Beclin 1 by inducing the expression of A20 [135]. As stated above, induction of A20 expression, at least in response to TLR engagement, is thought to be the mechanism for the termination of NF- κ B-dependent activation autophagy. However, it is unclear whether this kind of negative feedback loop occurs specifically in response to TLR activation or represents a general mechanism for interrupting the initial induction of autophagy.

In addition to the direct effect on the transcription of the pro-autophagic gene *Beclin 1*, NF- κ B family members function cooperatively with other transcription factors to control the expression of genes involved in the regulation of autophagy. For example, *S-phase kinase-associated protein 2* (*Skp2*) is an NF- κ B target gene whose product functions as the receptor component of the Skp1/Cul1/F-box ubiquitin ligase complex that induces the degradation of p27^{Kip1}, an activator of the autophagy pathway [137]. In fact, silencing of the p52 NF- κ B subunit inhibits *Skp2* expression and results in increased levels of p27^{Kip1} and an induction of autophagy. Following DNA damage, *Skp2* is also a direct target of the p53 tumor suppressor, which in concert with NF- κ B regulates *Skp2* expression. This regulation is dependent upon the activity of the Akt and the glycogen synthase kinase 3 β (GSK3 β) pathways. When Akt is active, the p52

NF- κ B subunit and p53 cooperatively induce Skp2 expression whereas the inactivation of Akt leads to the phosphorylation of p52 NF- κ B subunit by GSK3 β and thereby the repression of Skp2 expression (**Figure 4b**). Thus, the GSK3 β -dependent phosphorylation of p52 plays a critical role in the regulation of autophagy and apoptosis in response to DNA damage. Another example is the NF- κ B-dependent transcriptional regulation of *Bnip 3*, an important regulator of mitochondrial turnover via autophagy and cell death [35]. Under basal condition, p65 blocks binding of E2F-1 to the *Bnip3* promoter thus inhibiting *Bnip3* transcription (**Figure 4b**). Conversely, loss of NF- κ B activity (which occurs, for example, during hypoxia) de-represses the *Bnip3* promoter, *Bnip3* is transcribed and cell death occurs [138]. Moreover, E2F-1-dependent *Bnip3* transcription is epigenetically regulated by the histone deacetylase HDAC1 [139]. Thus, NF- κ B and HDAC1 serve as molecular switches that regulated E2F-1-dependent *Bnip3* expression, thereby regulating cell death mediated the E2F-1 tumor suppressor.

Regulation of autophagy by NF- κ B signaling components

Molecules known to signal both up- and downstream of NF- κ B are also sensors in the regulation of autophagy. Transforming growth factor- β -activating kinase 1 (TAK1), an upstream kinase of the IKK complex, has been shown to activate autophagy in breast epithelial cells subjected to Trail treatment [140]. TAK1-induced autophagy is dependent on AMP-mediated inhibition of mTORC1, a potent repressor of autophagy, and leads to cell protection against Trail-induced cytotoxicity (**Figure 4c**). Similar results were obtained with IL-1, which activates autophagy through a TAK1/AMPK-dependent mechanism. Interestingly, despite its ability to activate TAK1, TNF fails to activate AMPK, suggesting that TAK1 is essential but not sufficient for the effective activation of both AMPK and autophagy. Thus, TAK1 acts in conjunction with not-yet-identified regulatory factors to induce autophagy. It is not yet known whether NF- κ B activation is required for TAK1-mediated autophagy.

The IKK subunits, critical sensors of the NF- κ B signaling pathway, have been shown to regulate autophagy. Of note, IKKs can also control signaling pathways that are independent of the NF- κ B [64, 141]. IKK subunits are necessary for

the optimal induction of autophagy in response to starvation and rapamycin, the best known autophagy-inducing stimuli [142, 143] (**Figure 4c**). Moreover, the BH3-mimetic ABT737, a molecule that disrupts the interaction between Beclin 1 and Bcl-2/Bcl-X_L, has been also shown to stimulate autophagy through a mechanism that involves IKK [144]. In response to starvation, rapamycin and ABT737, constitutively active IKK subunits signal autophagy through a canonical pathway that involves AMPK activation, mTOR inhibition, c-Jun N-terminal kinase (JNK) phosphorylation, p53 depletion, and dissociation of Beclin 1 from Bcl-2 [142-144]. Disruption of NF- κ B activity, either due to a mutant form of I κ B α that represses IKK-induced nuclear translocation of p65 or in p65-null cells, fails to prevent IKK-induced autophagy, indicating that IKK can operate through a mechanism that does not involve NF- κ B. The investigation of autophagy responses in mice displaying a liver-specific conditional knockout of IKK β revealed a defect in autophagy in liver but not in the other organs in response to starvation or rapamycin treatment. This *in vivo* data support the idea that IKK β can function as a positive regulator of autophagy.

Under periods of prolonged nutrient deprivation, IKK activity promotes the upregulation of three essential autophagic genes *LC3*, *Atg5*, *Beclin1*, through an NF- κ B independent mechanism (**Figure 4c**). This constitutes a positive feedback loop mechanism for the regulation of autophagy under starvation [145]. In starved cells, IKK activity also controls another pathway leading to the activation of both canonical and non-canonical pathways of NF- κ B, thus resulting in the upregulation of NF- κ B-dependent anti-apoptotic genes (e.g., *Bnip 3*, *Bcl-xl*, and *cIAP-2*) [145]. The identification of additional IKK-dependent substrates will lead to a better understanding of how IKK activates autophagy in both a transcriptionally-dependent and -independent manner.

Conclusion

The studies mentioned in this review shed light on the mutual control that exists between the autophagy and the NF- κ B signaling pathways. Autophagy may constitute a pathway through which NF- κ B signaling components are specifically degraded which results either in the termination of the initial NF- κ B activation or con-

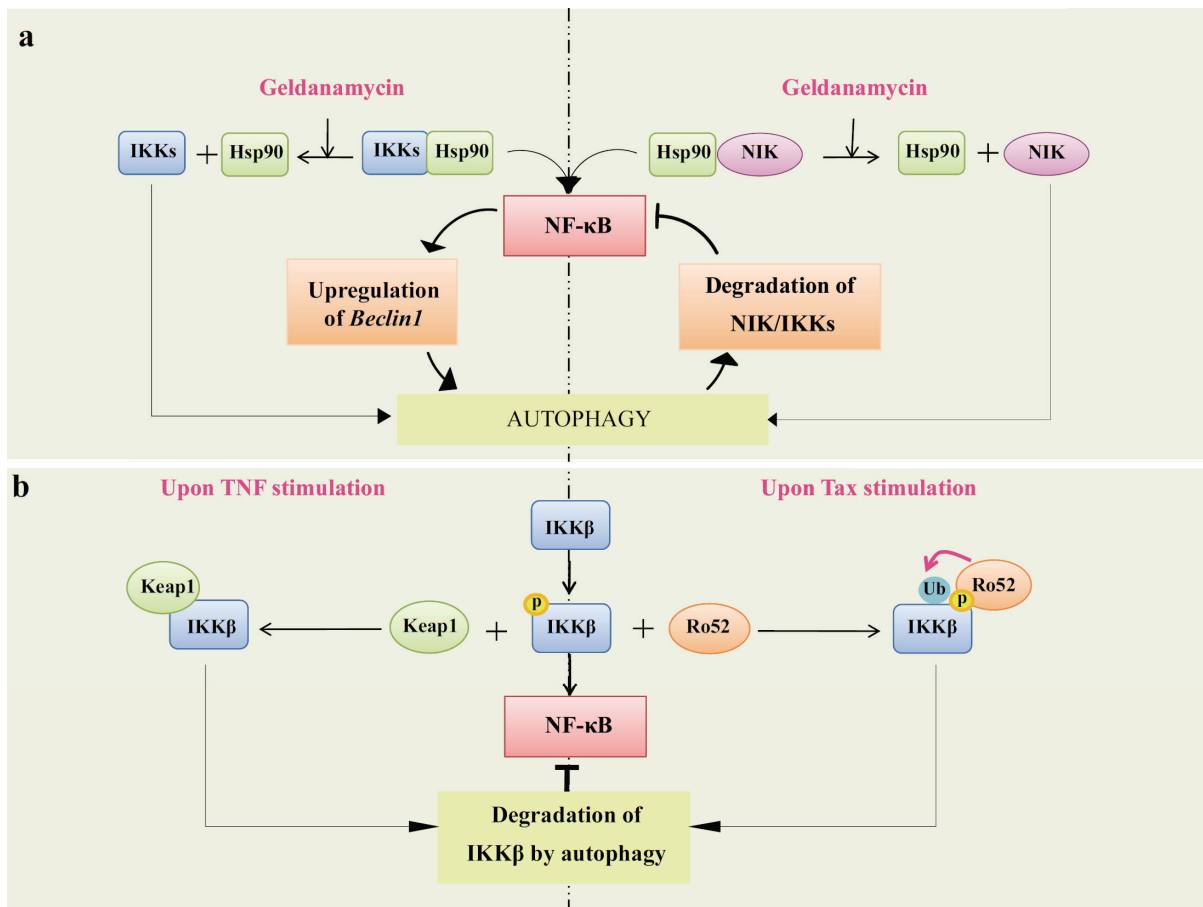


Figure 3. Autophagy mediates degradation of NF-κB signaling components. **a)** *NIK* and *IKKs* are targets for degradation by autophagy upon inhibition of *Hsp90*. This leads to inactivation of NF-κB pathway and, in turn, inhibition of autophagy through downregulation of an essential autophagy gene *Beclin 1*. The feedback regulatory loop between autophagy and NF-κB pathways is depicted. **b)** *IKKβ* degradation by autophagy through a mechanism that involves *Keap1*. In cells treated by TNF, *Keap1* is responsible for the negative regulation of NF-κB through inhibition of the *IKKβ* phosphorylation and induction of *IKKβ* degradation by the autophagy pathway. **c)** *IKKβ* degradation by autophagy through a mechanism that involves *Ro52*. In cells infected with HTLV-1, Tax activates *IKKβ*-dependent NF-κB activation. Active *IKKβ* subsequently interacts with *Ro52*; this induces monoubiquitination of *IKKβ* and leads to its degradation by autophagy resulting in the termination of NF-κB activation. Ub represents ubiquitin. For details, see text.

versely in the persistent activation of this process (Figure 3). In turn, NF-κB signaling can regulate autophagy through different routes that depend on the cellular context and the stimulus: NF-κB members can either activate or inhibit signaling pathways that lead to the induction of autophagy and regulates the transcription of a subset of pro-autophagic-regulating genes as well. Moreover, NF-κB signaling components can promote autophagy independently of NF-κB through stimulation of signaling pathways that

activate autophagy or by inducing the transactivation of a subset of autophagy-related genes (Figure 4).

Since the regulation of both autophagy and NF-κB pathways overlap with that of multiple pathways that control tumorigenesis, a more detailed molecular dissection of the cross-regulatory circuits that exist between autophagy and NF-κB is required to better understand how these pathways interact to regulate tumor-

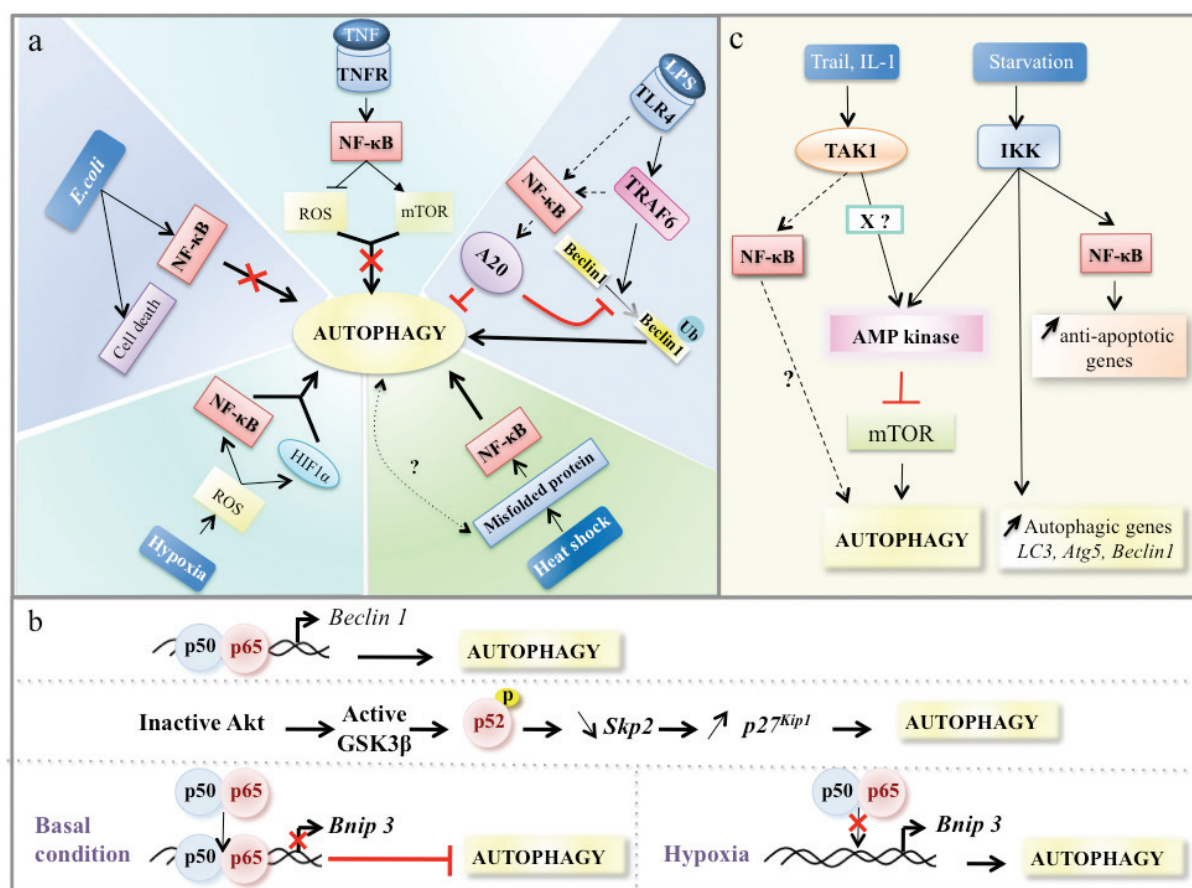


Figure 4. Regulation of autophagy by NF- κ B signaling pathways. **a)** NF- κ B activity regulates the extent of autophagy in cells through NF- κ B inducers that regulate autophagy either in a positive or a negative manner. TNF-mediated NF- κ B activation represses autophagy through both ROS inhibition and mTOR activation. In macrophages exposed to *E. coli*, NF- κ B activation inhibits autophagy and triggers cell death program. TLR4 stimulation activates autophagy by inducing the ubiquitination (Ub represents ubiquitin) of Beclin 1 by TRAF6, an upstream activator of NF- κ B. In turn, NF- κ B activation upregulates the expression of A20, a factor that inhibits the ubiquitination of Beclin 1 and thereby limits autophagy. Upon hypoxia, ROS-mediated the activation of both NF- κ B and Hif-1 α is responsible for the induction of autophagy. In response to heat shock stress, autophagy is induced through a mechanism that involves NF- κ B activation. **b)** NF- κ B regulates the transcription of autophagy regulatory genes. In some experimental settings, p65, a member of NF- κ B family, upregulates *Beclin 1* mRNA level and induces autophagy. In response to DNA damage, the p52 subunit of NF- κ B subunit controls autophagy by modulating the expression of both *Skp2* and *p27^{Kip1}*. The p65 subunit of NF- κ B blocks E2F1-dependent *Bnip 3* promoter binding and *Bnip 3* transcription, thus inhibiting mitochondrial turnover via autophagy. **c)** NF- κ B signaling components regulate autophagy. TAK1 enhances autophagy through AMP-mediated inhibition of mTORC1. X represents an as-yet-unidentified factor that is required for autophagy induction by TAK1. Under starvation condition, active IKKs induce autophagy through an AMPK inhibition-dependent of mTOR. In starved cells, IKK activity also promotes the upregulation of several essential autophagy genes and a subset of anti-apoptotic genes through an NF- κ B independent or dependent mechanism, respectively.

related responses. It is worth noting that several anti-cancer therapies that have been used in cancer clinical trials have the ability to influence both the autophagy and the NF- κ B signaling pathways (Table 1). Fundamental insights into the functional interplay between autophagy and

NF- κ B pathways may provide clues for developing new strategies for combining drugs that target both pathways to improve effectiveness of cancer therapies. One example of such a strategy is the use of hydroxychloroquine (an potent inhibitor of autophagy) in combination with bor-

Table 1. Examples of drugs that interfere with the autophagy and NF- κ B signaling pathways

Drugs	Mode of action	Effect on NF- κ B	Effect on autophagy	References
Drugs that target autophagy				
Rapamycin and analogs (CCI-779, RAD001, AP23573)	Inhibition of mTOR	inhibition / activation	Activation	[25, 121, 146-148]
Hydroxychloroquine	lysosomotropic	Inhibition/activation	inhibition	[26, 149-151]
Drugs that target NF-κB				
Bortezomib (PS-341, Velcade)	Inhibition of proteosomal degradation of I κ B	inhibition	inhibition/activation	[26, 152-154]
Natural products (curcumin, resveratrol)	Inhibition of IKK activity	inhibition	activation	[75, 155-157]

tezomib (a molecule that targets the NF- κ B pathway) in patients that manifest relapsed-refractory myeloma; this combination is being evaluated in an ongoing Phase I-II clinical trial [26].

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Declaration of conflicts of interest: None

Abbreviations: AMP kinase, adenosine monophosphate-activated protein kinase; Atg, autophagy-related genes; Bcl-2, B-cell lymphoma 2; CYLD1, cylindromatosis; DAPK, Death associated Protein Kinase; Dram, damage-regulated autophagy modulator; FADD, Fas-Associated protein with Death Domain; Fox, forkhead box; GSK3 β , glycogen synthase kinase 3 β ; Hif-1, hypoxia-inducible factor; HMGB1,

high-mobility group box 1; Hsp, heat shock protein; IKK, I κ B kinase; iBMK, immortal baby mouse kidney; IL-1R, interleukin receptor; JNK, c-Jun N-terminal kinase; Keap1, kelch-like ECH-associate protein 1; LC3, microtubule-associated protein 1 light chain 3; Lamp, lysosomal-associated membrane protein; LKB1, liver kinase B1; LPS, lipopolysaccharide; LT β R, lymphotoxin- β receptor; TNF-R, tumor necrosis factor receptor; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; NF- κ B, nuclear factor κ -light-chain-enhancer of activated B cells; Nfr-2, NFE2-related factor 2; NIK, NF- κ B inducing kinase; MnSOD, manganese superoxide dismutase; PRR, pathogen recognition receptor; PI3P, phosphatidylinositol 3-phosphate; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PTEN, phosphatase and tensin homolog; RANK, receptor activator for NF- κ B; RIP, receptor-interacting proteins; ROS, reactive oxygen species; TAK1, transforming growth factor- β -activating kinase 1; TLR, Toll-like receptor; TNF, tumor necrosis factor; TSC, tuberous sclerosis complex; TRAF, tumor necrosis factor receptor-associated factor; TRAIL, TNF-related apoptosis-inducing ligand; Vps34, vacuolar protein sorting 34, ULK, Unc-51 like kinase 1; UVRAG, ultraviolet radiation resistance associated gene.

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