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Review

Ubiquitin at the crossroad of cell death and survival

Yu-Shan Chen and Xiao-Bo Qiu

Abstract

Ubiquitination is crucial for cellular processes, such as protein degradation, apoptosis, autophagy, and cell cycle progression. Dysregulation of the ubiquitination network accounts for the development of numerous diseases, including cancer. Thus, targeting ubiquitination is a promising strategy in cancer therapy. Both apoptosis and autophagy are involved in tumorigenesis and response to cancer therapy. Although both are categorized as types of cell death, autophagy is generally considered to have protective functions, including protecting cells from apoptosis under certain cellular stress conditions. This review highlights recent advances in understanding the regulation of apoptosis and autophagy by ubiquitination.

Key words Apoptosis, autophagy, BRUCE, caspase, NF-κB, p53, ubiquitin, ubiquitination

Failure in apoptotic cell death is one of the major causes of tumorigenesis. A primary strategy for cancer therapy is to specifically induce apoptosis in cancer cells, whereas the resistance of certain cancer cells to therapy can be at least partially due to the cytoprotective role of autophagy against apoptosis[1]. Autophagy not only inhibits the initiation of tumorigenesis by limiting cytoplasmic damage, genomic instability, and inflammation, but also promotes the survival of certain cancer cells by enabling adaptation to stressful metabolic environments. Ubiquitination is a post-translational modification that impacts almost all cellular activities, including protein degradation, cell cycle progression, apoptosis, and autophagy. This review highlights recent researches on the regulation of apoptosis and autophagy by ubiquitination, with particular emphasis on how this regulation affects tumorigenesis.

Targeting Ubiquitination and Related Pathways in Cancer Therapy

Ubiquitination is a process in which one or multiple ubiquitin moieties are covalently attached to a substrate through an enzymatic cascade involving ubiquitin-activating enzyme (E1), ubiquitin-carrier protein (E2), and ubiquitin-protein ligase (E3). Formation of a ubiquitin Lys48 chain on the ε-NH2 group of a substrate's internal Lys residue (polyubiquitination) can target the substrate for degradation by the 26S proteasome. Ubiquitin can also be attached to the free α-NH2 group in a substrate's N-terminus to promote proteasomal degradation[2]. The ubiquitin-proteasome pathway degrades most cellular proteins in eukaryotic cells. However, ubiquitination may not always target proteins for degradation. For example, polyubiquitination at Lys63 is involved in inhibitor of NF-κB (IκB) kinase (IKK) activation[3]. In addition, a linear polyubiquitin chain can be achieved by conjugating the C-terminal glycine of ubiquitin and the a-NH2 group of the N-terminal methionine of its neighbor ubiquitin[4]. Substrates can also undergo monoubiquitination or multi-monoubiquitination—adding one ubiquitin to one or multiple Lys residues, respectively. Recent evidence suggests that ubiquitin can be linked to Cys, Ser, or Thr residues in a substrate through thio- or oxy-ester bonds (i.e., esterification), though the physiological relevance of these modifications remains to be defined[5-7]. Ubiquitin moieties can be released from a substrate by deubiquitinating enzymes.

For an organism to function properly, proteins must be degraded after they undergo specific functions. Moreover, proteins that are misfolded or damaged during translation, folding, or translocation must be degraded and eliminated in time. Many regulatory proteins related to tumorigenesis are proteosomal substrates. Either blocked degradation of oncogenic proteins/growth-enhancing factors or accelerated degradation of growth-suppressing proteins may disrupt the pathways controlling cell cycle progression, cell death, or survival, leading to cancer development[8,9] (**Table 1**). For example, the tumor suppressor CYLD is mutated in several cancers, including cylindromatosis. The deubiquitinating activity of CYLD for IKKγ is critical for its cylindromatosis-suppressive function[10]. The ubiquitin ligase Itch promotes the polyubiquitination and degradation of large tumor suppressor 1 (LTSA1), which is closely related to enhanced cell growth and epithelial-to-mesenchymal transition.

Authors' Affiliation: Key Laboratory of Cell Proliferation and Regulation Biology, Ministry of Education, and College of Life Sciences, Beijing Normal University, Beijing 100875, P. R. China.

Corresponding Author: Xiao-Bo Qiu, College of Life Sciences, Beijing Normal University, 19 Xinjiekouwai Avenue, Beijing 100875, P. R. China. Tel: +86-10-58808117; Email: xqiu@bnu.edu.cn.

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Table 1. Deregulated ubiquitination of key substrates in different cancer types

Deregulated protein		Substrate	Modification	Tumors	Reference(s)
MDM2 (HDM2)	↑	p53	Polyubiquitination	Non–small cell lung cancer, breast cancer, soft tissue carcinoma, colorectal cancer	[71,72]
HAUSP	↓	p53, MDM2	De-ubiquitination	Non–small cell lung cancer, lymphoma	[73]
APC	↓	Cyclin B, securin	Polyubiquitination	Colorectal cancer	[8]
FANCL	↓	FANCD2	Monoubiquitination	Fanconi anaemia related cancers	[74]
CYLD	↓	IKKγ	De-ubiquitination	Cylindromatosis	[10]
IAP2	↓	BCL10	Polyubiquitination	MALT lymphomas	[75]
CBL	↓	RTKs	Multiple monoubiquitination	Lymphoma, AML, gastric carcinoma	[76]
pVHL	↓	HIF	Polyubiquitination	von Hippel-Lindau disease	[77,78]
E6-AP		p53	Polyubiquitination	Human papillomavirus-positive cancer	[79]
$SCF^{\beta\text{-}TRCP}$	↑	IκB	Polyubiquitination	Colon cancer, prostate cancer, melanoma	[80]
KLHL20	↑	PML	Polyubiquitination	Human prostate cancer	[81]
USP9X	↑	MCL1	De-ubiquitination	Diffuse large B-cell lymphomas, human follicular lymphomas	[82]
FBW7	↓	KLF5	Polyubiquitination	Breast cancer	[83]
ITCH	↑	LATS1	Polyubiquitination	Cancer cell lines (HeLa, MCF10A and MCF7)	[84,85]
SIAH2	↑	C/EBPδ	Polyubiquitination	Breast cancer	[86]
ASB2α	↑	Filamin	Polyubiquitination	Myeloid leukemia	[87]
FBXO11 (mutation)		BCL6	Polyubiquitination	Diffuse large B-cell lymphoma	[88]
Ubiquilin-1	↑	BCL2L10/BCLb	Monoubiquitination	Lung adencarcinomas	[32]

↑ stands for up-regulation, and ↓ for down-regulation. MALT, mucosa-associated lymphoid tissue; AML, acute myeloid leukemia.

Due to the critical roles of ubiquitination and the ubiquitin-mediated proteolysis in tumorigenesis and cell growth, targeting the components involved in these processes is a powerful approach for cancer therapy. Bortezomib is the first proteasome inhibitor for clinical use in human cancers[11]. It is a dipeptide boronate that specifically and reversibly blocks chymotrypsin-like activity of the proteasome in a variety of cancer cells[12]. Although bortezomib inhibits NF-κB activation and results in autophagy[13], this lethal effect of proteasome inhibition is probably due to loss of amino acid homeostasis[14]. Notably, bortezomib has been used successfully as an anticancer drug for multiple myeloma and mantle cell lymphoma in the clinic[12,15-17].

Because ubiquitination is generally substrate-specific, the components of the ubiquitination pathway might be more specific drug targets for cancer therapy than the proteasome. Cullin-RING ubiquitin ligases (CRLs) are involved in cellular processes such as cell cycle progression, cell death signaling, DNA damage, and stress responses[18]. NEDD8 is a ubiquitin-like protein that modifies Cullin and is required for the activity of CRLs[19]. Because NEDD8-activating enzyme (NAE) catalyzes the first step in the NEDD8 pathway, targeting CRLs via inhibition of NAE may be a promising anticancer strategy. Indeed, MLN4924, a selective inhibitor of NAE, has potent tumor-suppressing activity in a wide range of tumors, including acute myeloid leukemia and diffuse large B cell lymphomas[20,21].

Regulation of Apoptosis by Ubiquitination

Apoptosis (i.e., programmed cell death) is a cellular suicide process that is important for embryonic development and maintaining the size of cell populations. There are two primary apoptotic pathways: extrinsic and intrinsic. The extrinsic pathway involves members of the tumor necrosis factor (TNF) receptor gene superfamily, which bind extracellular ligands and transduce intracellular signals during cell destruction. This pathway involves several caspases, cysteine proteases with specific cellular targets[22]. The intrinsic pathway does not involve receptor-mediated intracellular signaling, but induces signaling in mitochondria. In mammals, the intrinsic pathway is regulated by the Bcl-2 family of proteins, the adaptor protein apoptotic protease-activating factor-1 (Apaf-1), and the caspases[23].

Bcl-2 family members include both anti-apoptotic (Bcl-2, Bcl-xL, Bcl-w, and Mcl-1) and pro-apoptotic proteins (Bax, Bak, Bad, Bid, and Bim). Caspases are crucial intracellular executioners of apoptosis. The release of cytochrome C from mitochondria causes the formation of the apoptosome (Apaf-1/caspase-9 complex), activates the downstream effector caspases, and finally results in cleavage of crucial substrates[24]. Degradation of anti-apoptotic members is necessary for apoptotic progression[25-27], whereas degradation of pro-apoptotic members is required for the suppression of apoptosis[28,29].

The levels of anti- and pro-apoptotic molecules can be regulated by ubiquitination and proteasomal degradation. Bcl-2 family proteins can be polyubiquitinated and degraded by the 26S proteasome. For example, Trim17-mediated ubiquitination and subsequent degradation of Mcl-1, an anti-apoptotic Bcl-2 family member, triggers neuronal apoptosis[30]. The pro-apoptotic Bcl-2 member Bax can be regulated by ubiquitination indirectly; the ubiquitin ligase Trim39 inhibits APC/C Cdh1-mediated ubiquitination and degradation of the Bax activator MOAP-1, thus enhancing Bax activation and apoptosis[31]. Moreover, the levels of Bcl2L10/Bclb, an anti-apoptotic Bcl2-like protein, are inversely correlated with survival in patients with several cancer types, including lung adenocarcinomas. Bcl2L10/Bclb can be specifically monoubiquitinated and stabilized by ubiquilin-1 (UBQLN1)[32] .

The inhibitors of apoptosis proteins (IAPs) have one to three baculovirus IAP repeat (BIR) domains and can block apoptosis by directly binding and inhibiting caspases[33,34]. Furthermore, almost all IAPs have ubiquitin ligase activity, which is required for the ubiquitination of certain substrates involved in apoptosis[35]. X-linked inhibitor of apoptosis protein (XIAP) catalyzes the ubiquitination and degradation of caspase-3[36,37]. cIAP1 promotes autoubiquitination and self-degradation[38]. Apoptosis inducing factor (AIF) is also a substrate of XIAP, and ubiquitination at K255 of AIF shows a non-degradable role of ubiquitination in caspase-independent cell death[39]. On the other hand, IAPs can be regulated by deubiquitinating enzymes. For example, ubiquitin-specific protease 19 (USP19) is responsible for the inhibition of TNF-α–induced caspase activation and apoptosis in a cIAP-dependent manner[40].

The activity of IAPs can be suppressed by pro-apoptotic factors, such as second mitochondria-derived activator of caspase (Smac)[41]. Conversely, some IAPs promote Smac ubiquitination and degradation[42]. BRUCE/Apollon is a large (528 kDa), membrane-associated, essential IAP in mammals. A decrease in BRUCE levels promotes apoptosis[43]. BRUCE inhibits the Smac-induced apoptosis by promoting Smac ubiquitination and degradation[44,45]. Furthermore, BRUCE/Apollon can be degraded in a ubiquitin-dependent manner by the ubiquitin ligase Nrdp1 during apoptosis.

The tumor suppressor p53 maintains the integrity of the genome and regulates cell cycle, DNA repair, and apoptosis. p53 promotes the activation of the pro-apoptotic Bcl-2 family proteins and the release of cytochrome C. Dysregulation of p53 is reported in numerous types of cancer. Several ubiquitin ligases, including MDM2, have been reported to promote ubiquitination and degradation of p53, while p53 is deubiquitinated and stabilized by ubiquitin-specific proteases (USPs). Evasion of apoptosis is a primary cause of tumorigenesis. Thus, inhibiting the activity of p53 ubiquitin ligases or activating p53 USPs can be a strategy for cancer therapy. Otub1 and nucleolin play direct roles in suppressing MDM2-mediated ubiquitination of p53[46,47]. HAUSP regulates the activities of MDM2 and p53 by deubiquitination, while vif1 and vif2 antagonize HAUSP and promote p53-dependent apoptosis[48]. Translationally controlled tumor protein (TCTP), which is down-regulated in tumor progression, inhibits MDM2 autoubiquitination and promotes MDM2-mediated ubiquitination and degradation of p53[49]. In addition, Fanconi anemia complementation group F (FANCF) monoubiquitinates FANCD2, which is involved in the FA/BRCA DNA damage response pathway. Silencing FANCF elevates p53 activation in mitoxantrone-treated breast cancer cells[50].

As a transcription factor involved in the extrinsic apoptosis pathway, NF-κB activates the expression of genes that contribute to cell proliferation, metastasis, and suppression of apoptosis. SHARPIN, a ubiquitin-binding and ubiquitin-like-domain-containing protein, promotes linear ubiquitination of NEMO/IKBKG, an adaptor of IKKs, and subsequent activation of NF-κB signaling[51]. IκB, which inactivates NF-κB under normal physiological conditions, can be phosphorylated by activated IKKβ, ubiquitinated by $SCF^{\beta\text{-}TRCP}$, and finally degraded by the proteasome in response to DNA damage[52,53]. Nrdp1 promotes ubiquitination and degradation of the epidermal growth factor receptor family member ErbB3, which is upstream of NF-κB activation[54,55]. In a word, ubiquitination plays an important role in the regulation of apoptosis, and the components involved in the ubiquitination of key substrates can be potential targets for cancer therapy (**Figure 1**).

Regulation of Autophagy by Ubiquitination

Autophagy, once categorized as programmed cell death type II, is a cellular process by which intracellular proteins, lipids, and organelles are degraded in the lysosomal compartment after delivery from other cellular compartments[56]. Autophagy can both suppress cancer initiation and promote the growth of established cancers[57]. There are three types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy. Although autophagy is generally thought to be non-selective, certain ubiquitinated proteins (e.g., catalase), organelles (e.g., peroxisomes and mitochondria), and invading bacteria have been shown to be selectively targeted for autophagic degradation[58].

Macroautophagy is mediated by a unique organelle—the autophagosome. To date, 18 autophagy-related proteins (Atgs) in yeast, namely Atg1–10, Atg12–14, Atg16–18, Atg29, and Atg31, have been found to play a role in autophagosome formation. Atg8, called LC3 in mammals, is a ubiquitin-like protein present on autophagic membranes as a phosphatidylethanolamine (PE)–conjugate. Ubiquitination plays important roles in selective autophagy. p62/SQSTM1 or NBR1 binds both ubiquitin and LC3, probably providing a selective link between ubiquitinated substrates and autophagy[59]. Nuclear dot protein 52 (NDP52), an autophagy receptor, targets intracellular ubiquitinated bacterial proteins for autophagic degradation[60].

Misfolded polypeptides are usually recognized by molecular chaperones and degraded by the proteasome following polyubiquitination by ubiquitin ligases, such as CHIP and Parkin. However, when misfolded proteins cannot be sufficiently removed by chaperone-mediated proteasomal degradation, protein aggregation occurs and may in turn inactivate the proteasome, resulting in cytotoxicity. Thus, p62/NBR1-mediated autophagic degradation may serve as an important compensatory mechanism for degradation of these ubiquitinated protein aggregates[59].

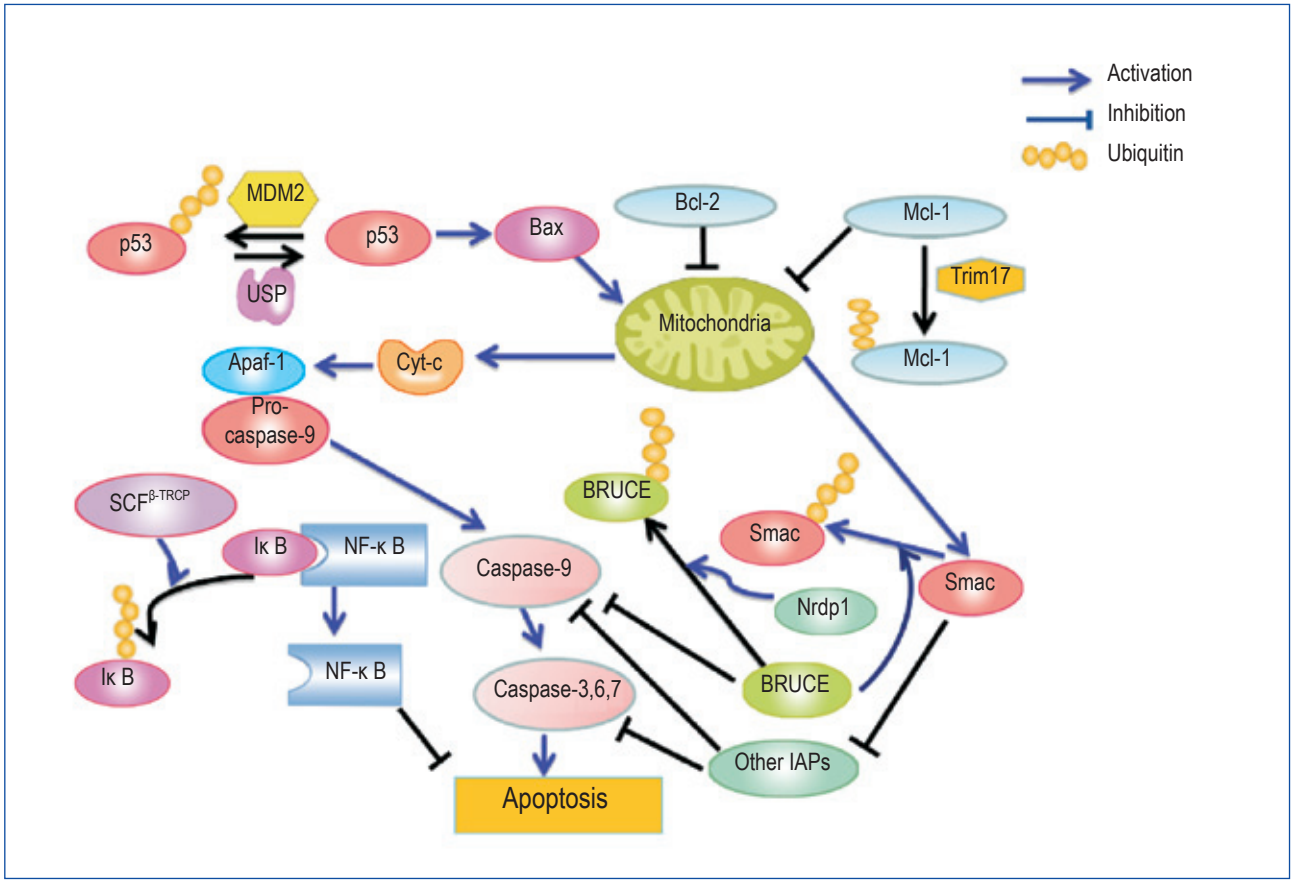

Figure 1. **Regulation of apoptosis by ubiquitination. Apoptosis is controlled by both pro-apoptotic and anti-apoptotic factors. Ubiquitination regulates almost all of these factors and promotes their proteasomal degradation. IAPs, inhibitors of apoptosis proteins.**

Role of Ubiquitination in Mutual Regulation of Apoptosis and Autophagy

The crosstalk between autophagy and apoptosis is necessary for controlling the balance between cell survival and death. These two processes share common stimuli and signaling pathways (**Figure 2**). Beclin 1, a mammalian Atg6 ortholog, is a subunit of the class III PI3-kinase complex. Beclin 1 interacts with Bcl-2 via the BH3 domain in Beclin 1 but can be released in starvation conditions to activate autophagy. This interaction can be terminated through c-Jun N-terminal kinase (JNK)–mediated phosphorylation of Bcl-2 and TNF receptor-associated factor 6 (TRAF6)–mediated ubiquitination of Beclin 1[61,62]. Phosphorylated Bcl-2 binds the pro-apoptotic protein Bax to inhibit apoptosis. Under extreme conditions that cannot be rescued by autophagy, JNK promotes hyperphosphorylation of Bcl-2, resulting in the release of Bax to execute apoptosis[63]. Caspase-mediated cleavage of Beclin 1 inhibits Beclin 1–induced autophagy, and the cleavage product, the C-terminal region (CT), enhances apoptosis by promoting the release of pro-apoptotic factors from mitochondria[64]. Beclin 1 can also indirectly affect the crosstalk between apoptosis and autophagy by controlling the levels of p53, a tumor suppressor that promotes apoptosis under genotoxic stress[12,65]. p53 induces the synthesis of mTOR and DRAM[66]. Inhibition of mTOR induces autophagy, whereas knockout of DRAM reduces autophagy[67,68]. Furthermore, p53 can down-regulate LC3 levels in starved cells, preventing the "autophagy burst" that may be dangerous for cells[69]. Under normal conditions, p53 is kept at low levels by the ubiquitin ligase MDM2[70]. However, p53 levels can also be controlled by Beclin 1 via regulating the deubiquitinating activity of USP10 and USP13[65].

Concluding Remarks

Dysregulation of ubiquitination can lead to the development of several types of cancer. Targeting ubiquitination is therefore a promising strategy for cancer therapy. Ubiquitination can occur on not only the ε-NH2 group of an internal Lys residue, but also the α-NH2 group of the N-terminal residue of a substrate. Moreover, recent evidence suggests that ubiquitin can be attached to Cys, Ser, or Thr residue on a substrate by esterification. These non-Lys ubiquitinations might provide another layer of the regulation of protein

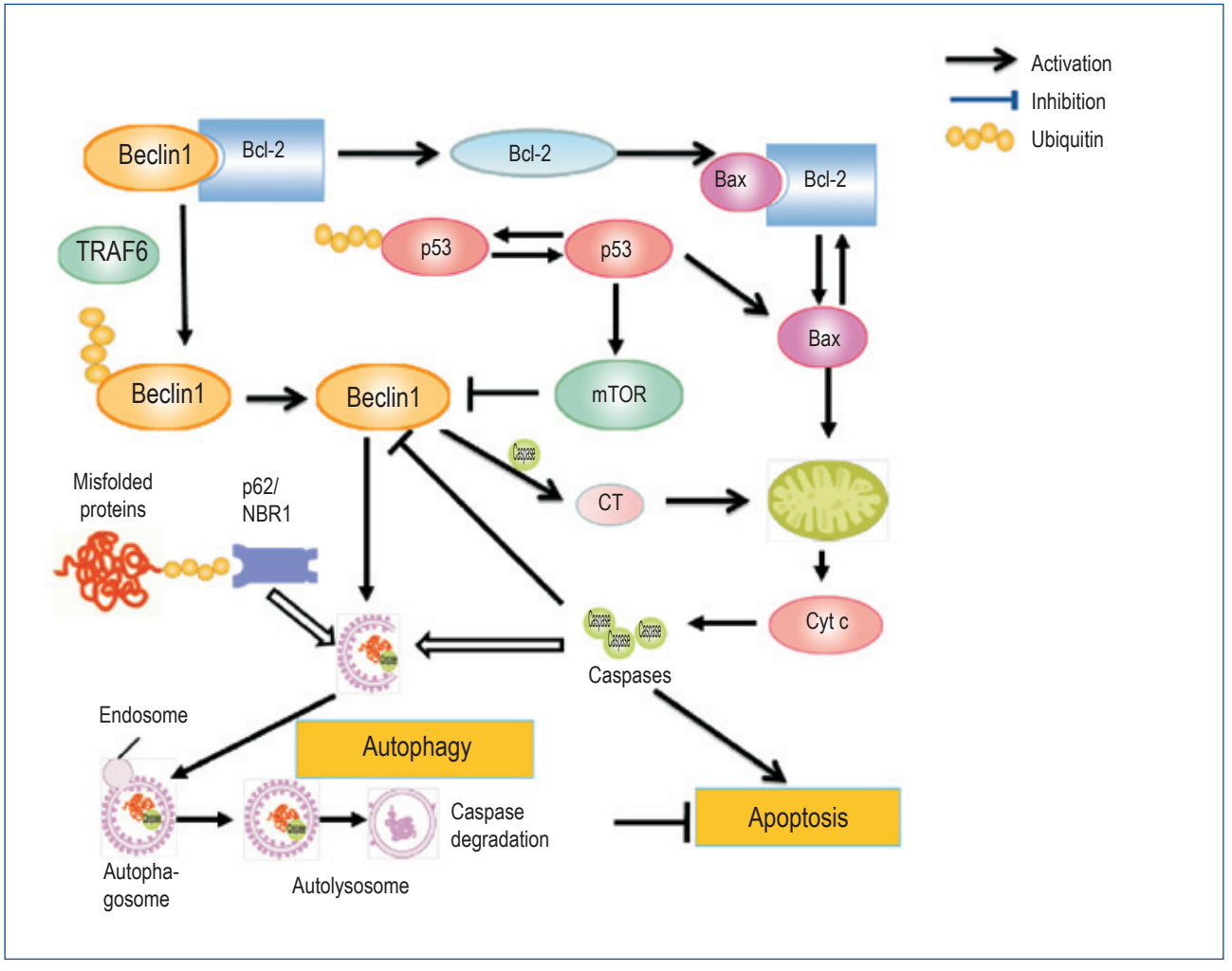

Figure 2. **Model for a role of ubiquitination in mutual regulation of apoptosis and autophagy. Autophagy can target ubiquitinated misfolded proteins, caspases, and other cargo (such as damaged mitochondria and invading bacteria) for degradation, probably through p62 and NBR1. Apoptosis and autophagy are counter-regulated in multiple steps, such as at p53, the Beclin 1/Bcl-2 interaction, the cleavage of Beclin 1 into the C-terminal region by caspases, and the autophagic degradation of caspases. Ubiquitination can promote degradation of both p53 and Beclin 1 and thus, controls the mutual regulation of apoptosis and autophagy.**

functions, and further studies should focus on the identification of relevant substrates and physiological roles of these modifications.

Future studies should also further explore how the ubiquitination of the critical proteins is involved in tumorigenesis and cancer therapy. Of course, these studies will require better understanding of tumorigenesis mechanisms. Recent research efforts on cancer stem cells and personalized cancer genome sequencing are expected to help in this regard. The mechanisms governing the selectivity in autophagy remain to be further explored. Because the cytoprotection of autophagy and the evasion of apoptosis contribute to resistance to cancer therapy, it is important to unravel how these two pathways are mutually regulated. The investigation on this issue has just begun and deserves more attention, especially with regard to how ubiquitination is involved in the counter-regulation of these critical processes.

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