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Biochemical Pharmacology 66 (2003) 1371–1379
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Potentiation of cellular antioxidant capacity by Bcl-2: implications for its antiapoptotic function

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Received 1 February 2003; accepted 12 March 2003

Abstract

A substantial body of data from clinical and laboratory studies indicates that reactive oxygen intermediates are implicated in the pathogenesis of diverse human diseases, including cancer, diabetes, and neurodegenerative disorders. Oxidative stress induced by reactive oxygen intermediates often causes cell death *via* apoptosis that is regulated by a plenty of functional genes and their protein products. Bcl-2, which is an integral intermitochondrial membrane protein, blocks apoptosis induced by a wide array of death signals. In spite of extensive research, the molecular milieu that characterizes the antiapoptotic function of Bcl-2 is complex and not fully identified. Recently, there are several lines of evidence that Bcl-2 functions *via* antioxidant pathways to prevent apoptosis. Thus, *bcl-2*-over-expressing cells exhibit elevated expression of antioxidant enzymes and higher levels of cellular GSH compared with the control cells transfected with the vector alone. There has been increasing evidence supporting that the redox-sensitive transcription factor nuclear factor κB regulates the activity and/or expression of antioxidative and antiapoptotic target genes and promotes cell survival against oxidative cell death. This commentary focuses on the antioxidative functions of Bcl-2 and underlying molecular mechanisms in relation to its antiapoptotic property. The role of Bcl-2 in regulation of nuclear factor κB signaling pathways and possible cross-talk with mitogen-activated protein kinases are also discussed.

Keywords: Antioxidant enzymes; *bcl-2*; Glutathione; Mitogen-activated protein kinases; NF-κB; Oxidative cell death

1. Protective effects of Bcl-2 against oxidative cell death

Oxidative stress refers to the mismatched redox equilibrium between the production of reactive oxygen intermediates (ROIs) and ability of the cells to defend against them. Oxidative stress thus occurs when the production of ROIs increases, elimination of ROIs or repair of oxidatively damaged macromolecules decreases, or both. ROIs, such as superoxide anion, hydroxyl radicals, and hydrogen peroxide, are unwanted and toxic by-products formed during aerobic metabolism. These reactive species can also be produced by exogenous redox chemicals, physical agents (e.g. ultraviolet, X-ray, γ-ray, etc.), and viral/bacterial infection. Endogenously and/or exogenously produced ROIs can react with almost every critical cellular macromolecule, including DNA, lipid, protein, and carbohydrates, and cause functional as well as structural alterations in these biomolecules, which ultimately lead to cell death and tissue damage. There have been multiple lines of compelling evidence that ROIs are implicated as a major cause of cellular injuries in a vast variety of clinical abnormalities, including cancer, diabetes, rheumatoid arthritis, and neurodegenerative disorders [1].

ROIs can cause cell death *via* apoptosis and/or necrosis in many cell types, which can be blocked or delayed by various antioxidants and antioxidative proteins/enzymes [2–4]. The concentration of ROIs and the cellular microenvironment appear to be important in determining the mode of cell death [3]. Cells undergoing apoptosis exhibit mitochondrial depolarization, membrane blebbing, shrinkage of the nucleus, condensation of chromatin,

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Abbreviations: ROIs, reactive oxygen intermediates; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase; NF-κB, nuclear factor κB; GCL, glutamate-cysteine ligase; IKK, IκB kinase; BH4, Bcl-2 homology domain 4; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-*jun* N-terminal kinase.

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doi:10.1016/S0006-2952(03)00487-8

Table 1
Antioxidant effects of Bcl-2 against prooxidative damage induced by various stimuli

Markers of oxidative damage affected by Bcl-2	Treatment	Cell/tissue type	Reference
ROI accumulation			
Hydroxyl radicals	Quinoids (e.g. menadione, diaziquinone)	JB6	[10]
	3-Nitropropionic acid	Mouse brain	[11]
Peroxides	TGF-β	Hep3B	[12]
	β-Lapachone	HL-60	[13]
	Adriamycin	Hippocampal neuron	[14]
Lipid peroxidation	Dexamethasone	2B4	[8]
	H_2O_2 and β-amyloid	PC12	[15]
	Paraquat	32D	[16]
	Cyanide/aglycemia	GT1–7	[17]
	H_2O_2, serum withdrawal	NT-2, SK-N-MC	[21]
DNA modifications	H_2O_2	PC12	[18]
	H_2O_2	ROC	[19]
	UV irradiation	HL-60	[20]
	H_2O_2, serum withdrawal	NT-2, SK-N-MC	[21]
Protein carbonylation	H_2O_2, serum withdrawal	NT-2, SK-N-MC	[21]

and DNA degradation by endonucleases into fragments in multiples of 180–200 base pairs [5]. Apoptosis is a tightly regulated process, which involves changes in the expression of distinct sets of genes. One of the major genes responsible for regulating apoptotic cell death is the protooncogene *bcl-2* that encodes a 26-kDa integral membrane protein found in the nuclear envelope, parts of the endoplasmic reticulum, and the outer mitochondrial membrane. The *bcl-2* gene product has been shown to prolong the cell survival by blocking the cell death induced by a wide array of stimuli and treatment, including chemotherapeutic agents, radiation, hydrogen peroxide, growth factor withdrawal, neurotoxins, etc. [3,6,7]. In spite of extensive research, the exact molecular mechanisms by which Bcl-2 blocks apoptosis remain unresolved. The localization of Bcl-2 at the site of oxygen free radical generation, and evidence that ROIs are able to cause apoptosis in various cell lines have raised the possibility that Bcl-2 might prevent apoptosis by either acting as an antioxidant or by suppressing production of free radicals. Experimental data from *in vitro* and *in vivo* studies suggest that Bcl-2 may block apoptosis through regulation of cellular antioxidant defense mechanisms and, in this context, has been considered to act as a free radical scavenger [8,9]. For instance, the levels of hydroxyl radicals generated by quinone-producing agents and 3-nitropropionic acid were lowered in Bcl-2-overexpressing cells compared with the vector-transfected control cells [10,11]. Ectopic overexpression of Bcl-2 also attenuated TGF-β-, β-lapachone-, or adriamycin-induced peroxide accumulation [12–14]. Therefore, it is conceivable that induction or overexpression of *bcl-2* confers resistance to oxidant injuries. Bcl-2 inhibited lipid peroxidation and oxidative DNA and/or protein damage induced by a wide array of stimuli capable of triggering apoptosis [15–21]. Polyunsaturated fatty acids contain multiple carbon carbon double bonds and are susceptible to oxidative insult. Lipid peroxidation is initiated by ROIs and propagated through autocatalytic chain reactions. Bcl-2 overexpression attenuates lipid peroxidation induced by various kinds of agents [8,15–17,21]. ROIs induce DNA damage by direct chemical interactions and also by indirect interference with enzymes that can repair DNA damage. Bcl-2 prevents cells or facilitates their recovery from hydrogen peroxide (H_2O_2)-induced oxidative DNA damage, such as base modifications or single-strand DNA breaks [18,19]. In addition, Bcl-2 decreases the nucleotide excision repair capacity in human promyelocytic HL-60 cells after exposure to ultraviolet irradiation [20]. ROI-mediated oxidative attack on proteins produces carbonyls and other amino acid modifications which may ultimately lead to functional loss or alterations of critical enzymes/proteins responsible for maintaining cellular homeostasis. A superoxide dismutase (SOD) mutant cell line exhibited increased levels of lipid peroxidation, protein carbonyls, 8-hydroxyguanine, and 3-nitrotyrosine, which were attenuated by Bcl-2 overexpression [21]. The aforementioned protective effects of Bcl-2 against oxidative cell death are summarized in Table 1.

2. Regulatory roles of Bcl-2 in maintaining the cellular redox state

2.1. Cellular antioxidant defense against oxidative injuries

Besides several cellular genes that have been identified to control apoptosis, an array of cellular defense systems exist to counteract ROIs. These include enzymatic and non-enzymatic antioxidants that lower steady-state concentrations of ROIs and/or repair oxidative cellular damage. Endogenous antioxidant enzymes, such as SOD, catalase (CAT), thioredoxin reductase, and glutathione peroxidase (GPx) function, in concert, to detoxify ROIs and thus

rescues cells from oxidative damage [22]. SOD destroys superoxide anion by converting it to H_2O_2. There are two types of SOD (CuZn-SOD and Mn-SOD) which have similar functions but different subcellular localization. The majority of CuZn-SOD exists in the cytosol, while some appears in lysosomes, nucleus, the space between inner and outer mitochondrial membranes and peroxisome. Mn-SOD is largely localized in the mitochondria and extra Mn-SOD is detected in the cytosol. The relative catalytic activities of Mn-SOD and CuZn-SOD differ from tissue to tissue and depend on the cell type. The primary defense mechanisms against H_2O_2 are operated by CAT and GPx through the GSH redox cycle. CAT is present largely in the peroxisome fraction whereas GPx is found not only in the cytoplasm but also in the matrix of mitochondria. CAT reacts with H_2O_2 to form water and molecular oxygen. GPx detoxifies H_2O_2 by interacting with GSH producing water and GSSG which is recycled to GSH by glutathione reductase (GR). When a cellular GSH level is low, H_2O_2 can produce OH^- and more potent hydroxyl radical in the presence of transition metal ions by Fenton reaction. Overexpression of these antioxidant enzymes has been demonstrated to protect cells from deleterious effects induced by various kinds of prooxidative stimuli [23].

2.2. Upregulation of antioxidant enzymes by Bcl-2

Bcl-2-overexpressing cells have been shown to express relatively high levels of antioxidant enzymes and GSH [24–29] as schematically represented in Fig. 1. However, several lines of evidence suggest that the effects of Bcl-2 on the expression or activity of antioxidant enzymes are cell type-specific. For instance, Bcl-2 increased CAT activities in rat pheochromocytoma (PC12) cells but not in the hypothalamic GnRH cell line GT1–7 [24]. The activities of GPx and GR in Bcl-2-overexpressing PC12 and GT1–7 cells were similar to those of respective control transfectants. Astrocytes overexpressing *bcl-2* exhibited elevated SOD and GPx activities, but murine lymphoid hematopoietic FL5–12 cells showed no changes in any antioxidant enzyme activity when transfected with *bcl-2* [25]. *bcl-2*-transfected teratocarcinoma NT-2/D1 and neuroblastoma SK-N-MC cells displayed an increased CuZn-SOD activity, but not those of Mn-SOD, GPx, and GR [26]. Bcl-2 knockout mice have pathologies associated with defects in antioxidant enzymes [27]. Bcl-2 overexpression mimics the effect of SOD overexpression *in vivo* and *in vitro* [28], while the phenotype of SOD knockout mice is strikingly similar to that elicited in *bcl-2* knockout mice

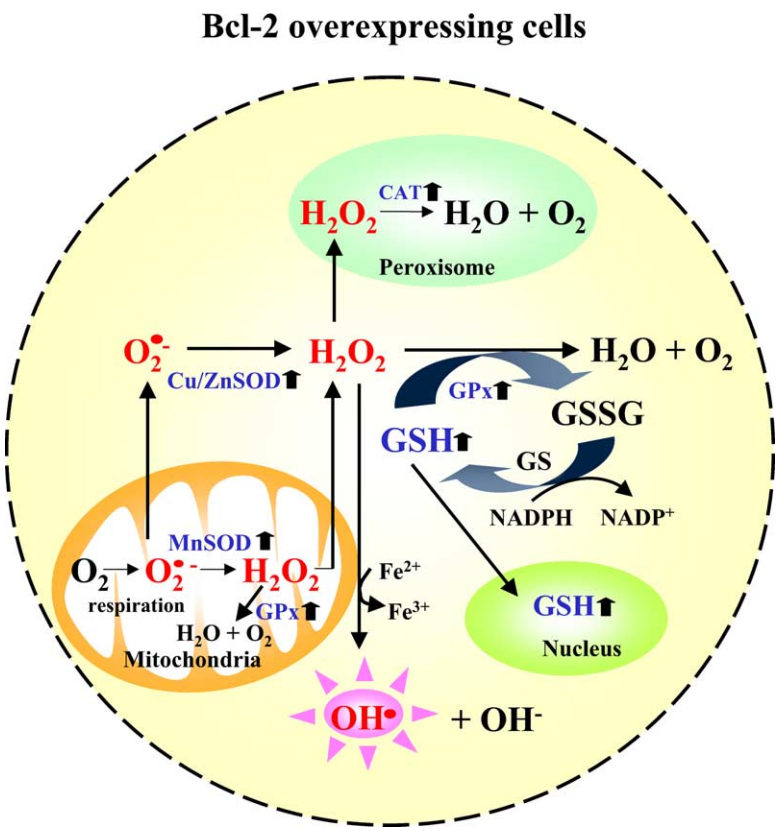

Fig. 1. Upregulation of antioxidant enzymes and elevation of cellular GSH levels in *bcl-2*-overexpressing cells. ROIs can be generated by various prooxidative insults and eliminated by antioxidative defense mechanisms. Superoxide anions are converted to H_2O_2 by SOD. Detoxification of H_2O_2 is normally mediated by GPx in the cytosol and CAT in the peroxisome. The latter produces water and GSSG which is reduced back to GSH by GR, thereby forming a redox cycle. Bcl-2-overexpressing cells exhibit elevated expression of antioxidant enzymes, such as SOD, CAT, and GPx, as well as increased levels of GSH. Bcl-2 also facilitates the nuclear translocalization of GSH, thereby enhances the activation of redox-sensitive transcription factors, such as NF-κB.

[29]. These findings suggest the distinct role of Bcl-2 in regulation of cellular redox state against oxidative stress.

2.3. Maintenance of cellular GSH redox status by Bcl-2

GSH, a ubiquitous tripeptide thiol, is a vital intra- and extracellular protective antioxidant against oxidative or nitrosative stress. Recent studies have suggested that GSH is important in cell proliferation, apoptosis, immune modulation, detoxification, and scavenging of free radicals [30]. GSH has been shown to inhibit or retard apoptosis triggered by many different stimuli, including oxidants, cytokines, and anti-Fas/APO-1 antibody [30]. A depletion of intracellular GSH has been reported to occur with the onset of apoptosis and frequently accompanied by a concomitant increase in the accumulation of ROIs. Bcl-2-overexpressing cells have elevated pools of GSH, and conversely, downregulation of Bcl-2 expression is associated with GSH depletion [24,31]. Therefore, Bcl-2 may block apoptosis through modulation of GSH metabolism. When PC12 and GT1–7 cell lines were transfected with *bcl-2*, their total GSH levels were elevated without any significant alterations in the levels of GPx and GR, indicating that the overexpression of *bcl-2* shifted the cellular redox potential to a more reduced state [24]. Furthermore, Bcl-2 can alter GSH compartmentalization. Thus, overexpression of Bcl-2 led to a relocation of GSH from cytosol to nucleus, which could change the nuclear redox potential, creating a highly reducing environment [32]. Nuclear GSH may act as a transcriptional regulator of nuclear factor κB (NF-κB), activator protein 1 (AP-1), and p53 by altering their nuclear redox state [33]. The transcriptional changes were observed in cells with different levels of Bcl-2 as determined by using DNA microarray, which suggests that GSH acts *via* a Bcl-2-dependent mechanism as a transcriptional regulator by altering the cellular redox environment [34]. Moreover, the altered GSH redox status also causes activation of antioxidant-protective genes, such as Mn-SOD and GPx, in certain epithelial cells [24,35].

3. Roles of NF-κB in protecting oxidative cell death: antioxidant enzymes and GSH as primary targets

ROIs can trigger the activation of multiple intracellular signaling pathways, possibly through modulation of nuclear gene expression [36,37]. The ubiquitous eukaryotic transcription factor NF-κB/Rel is known to regulate expression of numerous cellular and viral genes and is involved in immune and stress responses, inflammation, and apoptosis [38,39]. It has been suggested that ROIs can serve as common and crucial mediators of NF-κB activation signals [36,37,39–43]. NF-κB activation is regulated by the intracellular redox status, but the exact molecular mechanism underlying this regulation remains unresolved. Recent studies have revealed that NF-κB plays an important role in regulating the cell survival. Thus, overexpression of NF-κB/Rel promotes cell survival by suppressing induction of apoptosis [44]. The DNA binding and transcriptional activities of NF-κB were constitutively enhanced in selected clones of PC12 cells resistant to oxidative stress induced by amyloid β protein and H_2O_2 [45]. Conversely, NF-κB inhibitors have been found to augment the cell death by stimulating apoptosis [7,45–47]. Suppression of transcriptional activity of NF-κB with the synthetic glucocorticoid dexamethasone or by ectopic expression of a super-repressor mutant form of IκBα reversed the oxidative stress-resistant phenotype of above cells [35]. Inhibition of p65 nuclear translocation by the antioxidant pyrrolidine dithiocarbamate (PDTC) capable of blocking IκB phosphorylation, the peptide proteasome inhibitor, or the addition of unlabeled double-stranded antisense oligonucleotide containing a specific κB binding sequence reduced the NF-κB activity and increased apoptosis in PC12 cells [46]. The cytotoxicity of 6-hydroxydopamine in PC12 cells was exacerbated in the presence of parthenolide, a NF-κB inhibitor devoid of antioxidant effects [7]. Likewise, treatment with SN50, a cell-permeable inhibitor of NF-κB nuclear translocation and activity, sensitized multiple myeloma cells to TNF-α-induced apoptosis [47]. However, the molecular events and genetic programs activated in response to oxidative stress, and those involved in providing cells with resistance against oxidative insults are poorly understood.

Recently, increasing evidence supports the role of NF-κB in regulation of antiapoptotic gene expression and promotion of cell survival. The transcriptional regulation of antioxidant enzymes, such as CAT, SOD, and GPx, is mediated partially by NF-κB. Sequence analysis of the mouse GPx and CAT genes revealed putative binding sites for NF-κB [48]. The 5′-flanking region of human CuZn-SOD was cloned from the human genomic library, and the possible binding sites of transcriptional factors, such as NF-1, Sp-1, AP-1, AP-2, and NF-κB, were found [49]. Sequence analysis of the 5′-flanking region of Mn-SOD also revealed the existence of multiple potential regulatory elements, including several Sp-1 sites, two NF-κB sites, and an antioxidant-response element (ARE) [50].

GSH is another target molecule that is linked to antioxidative functions of NF-κB. GSH is formed by the consecutive actions of glutamate-cysteine ligase (GCL) and glutathione synthetase. The rate-limiting enzyme in GSH biosynthesis is GCL whose expression/activity is modulated by oxidants, antioxidants, growth factors, and inflammation-related agents [35]. The GCL holoenzyme is composed of heavy catalytic (GCL-HS, 73 kDa) subunit and light regulatory subunit (GCL-LS, 30 kDa), each encoded by a unique gene. The 5′-flanking regions of both human GCL subunits have been cloned and sequenced, and putative binding sites for NF-κB, Sp-1, AP-1, AP-2, metal-response element (MRE), and ARE/electrophile-response element (EpRE) have been identified in the promoter of the heavy subunit [51,52]. The promoter of the light subunit

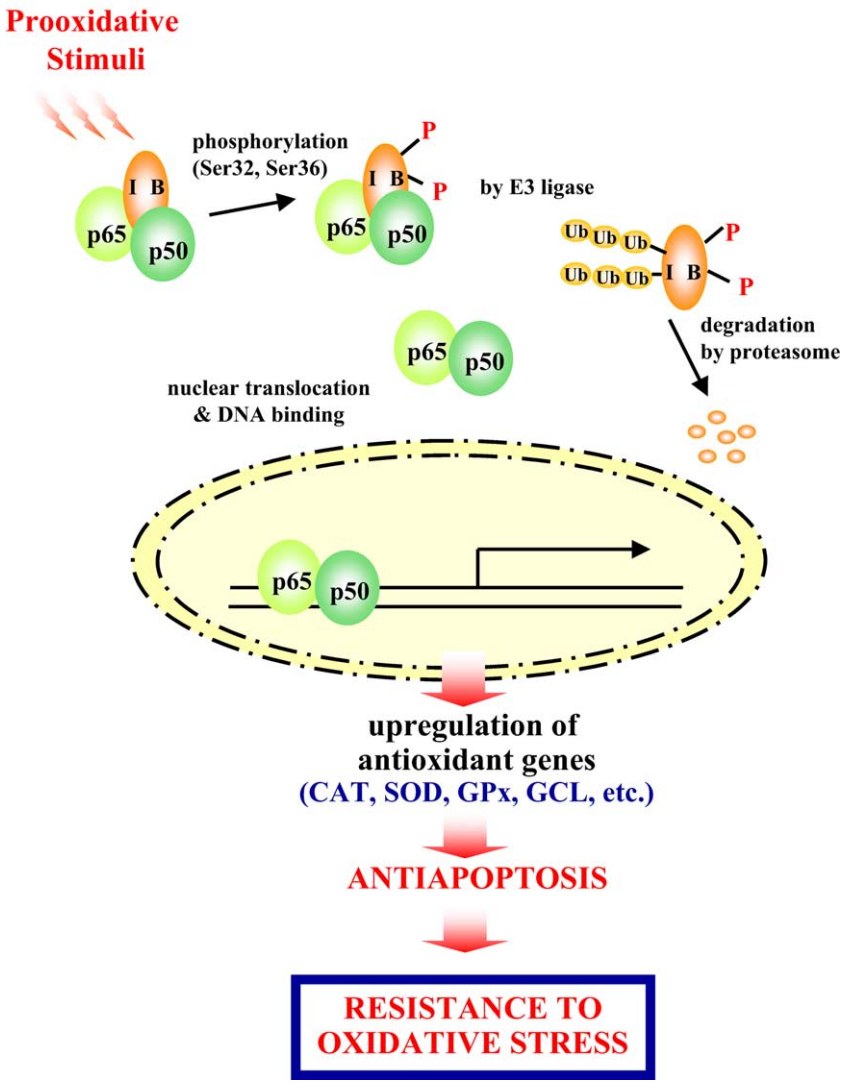

Fig. 2. Antiapoptotic functions of NF-κB in preventing oxidative cell death: possible involvement of antioxidant enzymes and cellular GSH. NF-κB exists in the cytoplasm in an inactive complex with inhibitory protein IκB. Phosphorylation of IκB at specific amino-terminal serine residues, ubiquitination by an E3 ubiquitin ligase and subsequent degradation by 26S proteosomes lead to translocation of NF-κB to the nucleus, where it binds to the promoter regions of target antioxidative genes (e.g. CAT, SOD, GPx, and GCL) to initiate their transcription.

also contains the consensus sequence for NF-κB, Sp-1, AP-1, AP-2, heat shock transcription factor (HSF), CCAAT enhancer binding protein (C/EBP), and ARE/EpRE binding [53]. Blocking activation of NF-κB by antisense strategies prevented the cytokine-induced increase in GCL-HS transcription in mouse endothelial cells [54]. Based on these findings, we can propose a plausible role of NF-κB in cellular protective or adaptive response against oxidative stress which may involve upregulation of antioxidative genes (Fig. 2).

4. Association between Bcl-2 overexpression and constitutive NF-κB activation

Interestingly, the DNA binding activity of NF-κB and its transcriptional activity are constitutively elevated in Bcl-2-overexpressing cells, compared with those in vector-transfected counterparts. Thus, overexpression of Bcl-2 in human embryonic kidney 293 cells [55,56] and myocytes [57] resulted in enhanced constitutive NF-κB DNA binding activities. The basal NF-κB-dependent transcriptional activity was markedly increased by ectopic expression of *bcl-2* in PC12 cells, as determined by the luciferase reporter gene assay [7]. Moreover, the elevated NF-κB DNA binding in *bcl-2*-overexpressing clones of human mammary cancer cells correlated with lower levels of the cytoplasmic inhibitor IκBα [58]. NF-κB, a homo- or hetero-dimer of Rel family (p50, p52, c-Rel, RelB, and p65/RelA), is normally sequestered in the cytoplasm as an inactive complex with an inhibitory IκB protein (IκBα, IκBβ, Bcl-3, p100, p105, IκBγ, or IκBε). Phosphorylation of specific serine residues in IκB results in multi-ubiquitination at lysine residues with subsequent degradation by

the 26S proteasomes. This allows the NF-κB dimers to translocate to the nucleus where it binds to the κB binding consensus sequences thereby regulating the expression of the target genes [59].

The critical regulatory step in the activation of NF-κB is the phosphorylation of IκBα and other IκB proteins, which is mediated by high-molecular weight multiprotein complex called IκB kinase (IKK). IKK consists of two catalytic subunits, IKK1 (or IKKα or CHUK) and IKK2 (or IKKβ), and a regulatory subunit IKKγ (also called NEMO or IKKAP1). The N-terminal region of IκB [Ser-32 and Ser-36 for IκBα, Ser-19 and Ser-23 for IκBβ, and Ser-157 and Ser-161 for IκBε] is rapidly phosphorylated by IKK [60]. The ability of Bcl-2 to interact with different signaling molecules suggests that altered levels of this antiapoptotic protein may regulate NF-κB activity *via* modulation of IκB function. In Bcl-2-overexpressing cells, the observed relatively high constitutive NF-κB activity might be associated with an enhanced degradation of IκB [55,56].[1] The N-terminal region of IκB was proposed to be an important regulatory site for Bcl-2. Thus, the N-terminal deletion mutant of IκB or the proteasome inhibitor lactacystin hampered the IκB degradation and attenuated NF-κB activation by Bcl-2 [55,56]. Bcl-2 homology domain 4 (BH4) of Bcl-2 plays a critical role in NF-κB activation as supported by the study using BH4 domain deletion and point substitution mutants [55,56]. However, the molecular mechanism by which Bcl-2 mediates NF-κB activation through interaction with IκB is not completely clarified. One possibility is that Bcl-2 directly or indirectly modulates IκB activity by interacting with one of cellular factors that are involved in the activation of IKK (Fig. 3). IKK is phosphorylated and activated by one or more of upstream activating kinases, which are likely to be the members of mitogen-activated protein kinase kinase kinase (MAPKKK) family of enzymes (also known as MAP3Ks and MEKKs). MEK kinase 1 (MEKK1) which phosphorylates the upstream kinase of mitogen-activated protein kinases (MAPKs) was shown to bind and phosphorylate IKK [61]. Alternatively, MEKK2 and 3 also have the potential to activate IKK and thereby stimulate the NF-κB activation [62]. Another evidence supports that regulation of the IκB activity by Bcl-2 may be mediated by a mechanism that involves the Raf-1/MEKK1 signaling pathway [57]. This study suggests that Bcl-2, through BH4 domain, interacts with Raf-1, leading to the downstream activation of MEKK1 and subsequent IKK-dependent NF-κB activation [57].

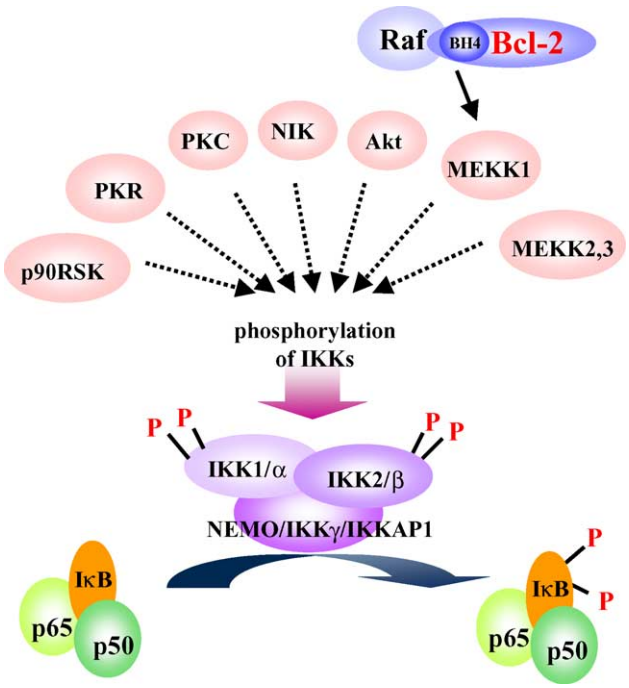

Fig. 3. Proposed molecular mechanisms underlying constitutively elevated NF-κB activation. The phosphorylation and degradation of IκB are tightly regulated events in which IKKs play a central role. IKKs are part of a larger multiprotein complex called 'IKK signalsome', which contains IKK1, IKK2, and IKKAP1. IKKs are phosphorylated and activated by upstream activating kinases, such as NIK, MEKK1, MEKK2/3, PKR, RSK, and Akt. Bcl-2 can stimulate the IKK activity by interacting with Raf-1 *via* the BH4 domain and activating MEKK1. Activated IKK phosphorylates IκB on serine residues in the N-terminal region and causes augmentation of NF-κB activation. Alternatively, Bcl-2 may facilitate IκB phosphorylation and degradation by modulating the signaling event downstream of aforementioned upstream kinases.

5. MAPKs: upstream targets for Bcl-2-induced NF-κB activation

Although Bcl-2-induced activation of NF-κB appears to be an important component of adaptive cellular response to oxidative stress, the underlying regulatory mechanisms of activation and its implication in controlling target gene expression are complex and ill-defined. Such complexity reflects the multiplicity of interacting proteins and the large number of cross-talk with other regulatory components. Possible upstream events responsible for constitutively elevated NF-κB activation in *bcl-2*-overexpressing cells and the role of IKK activation in this process are schematically proposed in Fig. 3. Recently, a number of upstream activators and regulators of IKK activity have been identified. These include NF-κB-inducing kinase (NIK), double-stranded RNA-activated serine/threonine protein kinase (PKR), 90-kDa ribosomal S6 kinase (p90 RSK), atypical PKC (zeta, iota/lambda; ζ, ι/λ) and Akt, which can be modulated by MAPKs and/or involved in the activation of MAPKs [63].

MAPKs encompass a large number of serine/threonine kinases involved in regulating a wide array of cellular responses, including proliferation, differentiation, stress adaptation, and apoptosis. Based on structural differences, they are divided into three multimember subfamilies: extracellular signal-regulated kinase (ERK), c-*jun* N-terminal kinase (JNK, also referred to as stress-activated protein

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kinase or SAPK), and p38 MAPK. However, the roles of MAPKs in cell death is controversial [64]. In general, activation of ERK occurs in response to growth factor stimulation whereas activation of JNK and p38 MAPK is triggered after exposure of cells to environmental stress, such as ROIs, ultraviolet irradiation, hyperosmolarity, and endotoxin. Although ERK has been regarded as an anti-apoptotic kinase, it can also control proliferation of certain types of cells, either positively or negatively, depending on the kinetics and duration of its activation [64,65]. Thus, transiently upregulated ERK participates in the induction of apoptosis, whereas basal, constitutive activity of ERK is required for the maintenance of cell survival. The ERK pathway is known to influence the expression of several genes which are mostly involved in cell proliferation. The ERK signaling cascade has been implicated in NF-κB activation through phosphorylation of inhibitory IκB [66]. The association between the ERK signaling cascade and NF-κB activation is also supported by the finding that the ERK-regulated kinase p90 RSK phosphorylates and thereby inactivates IκB in response to mitogenic stimuli [67]. The elevated NF-κB response was also observed in tumor promotion-resistant variants of mouse epidermal JB6 cells while a reduced level of NF-κB activation was detected in dominant-negative ERK2-expressing cells, suggesting that NF-κB is a target of ERK signaling [68]. Overexpression of ERK in T-cell enhanced NF-κB activation, lending further support to the above notion [69]. Furthermore, ERK activation can potentiate the antiapoptotic functions of Bcl-2 and cell survival [64]. Besides ERK, both JNK and p38 MAPK have been considered to be involved in NF-κB activation through phosphorylation of IκB [70,71]. Bcl-2 appears to exert its antiapoptotic function by repressing the JNK signaling pathway. For instance, overexpression of Bcl-2 was found to prevent JNK activation and to suppress apoptosis caused by a variety of agents [72]. However, exact roles of MAPKs in mediating Bcl-2-induced NF-κB activation are undiscovered yet. Further studies should be directed towards elucidation of the role of Bcl-2 in regulating the activation of MAPKs as well as their association with IKK-IκB-NF-κB signaling pathways.

6. Conclusion

This commentary addresses that the antiapoptotic functions of Bcl-2 are mediated by antioxidative mechanisms that involve constitutive induction of NF-κB and subsequent upregulation of antioxidative genes. However, the complete molecular events involved in potentiation of cellular antioxidant defense capacity through sustained constitutive activation of NF-κB remain to be elucidated. Bcl-2 has been reported to counteract apoptotic cell death through multiple mechanisms, which mainly target mitochondrial events, but attention has been recently focused on its role in maintaining or augmenting cellular antioxidant defense capacity that involve antioxidant enzymes (e.g. CAT, SOD, GPx, and GR) and an antioxidant molecule GSH. Understanding of cellular and molecular regulatory mechanisms of antiapoptotic functions of Bcl-2 may provide a new antioxidant therapeutic strategy for the management of a wide array of human diseases that are caused by oxidative stress. In addition, manipulation of Bcl-2 expression is useful for learning more about the apoptotic process as well as targeting the kinases that specifically modify key proteins in the apoptotic cascades. Continued attempts to identify the novel molecular targets of the Bcl-2 function and to clarify their cross-talk with upstream and downstream signaling molecules will pave the way to exploiting the cellular defence against oxidative stress.

Acknowledgments

This work was supported by the grant from Korea Research Foundation (ES0022) awarded to Y.-J. Surh.

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