[Cell Cycle 3:12, 1498-1501; December 2004]; ©2004 Landes Bioscience

Extra Views

Protein Phosphorylation as a Key Mechanism for the Regulation of BCL-3 Activity

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Received 10/20/04; Accepted 10/22/04

Previously published online as a *Cell Cycle* E-publication: http://www.landesbioscience.com/journals/cc/abstract.php?id=1328

KEY WORDS

BCL-3, NF-κB, phosphorylation

ACKNOWLEDGEMENTS

A.C. and M.P. M. are Research Associates at the Belgian National Funds for Research. A.C. is supported by a grant from the Belgian Federation against Cancer and TELEVIE.

The members of the laboratory are also supported by grants from the Belgian Funds for Research ("FNRS") and the "Centre anti-cancéreux".

ABSTRACT

Constitutive NF-κB activation, a hallmark of many human cancers, upregulates anti-apoptotic gene expression and therefore disrupts the balance between apoptosis and proliferation. In some lymphomas, this constitutive NF-κB activity is the result of point mutations or translocations of the genes coding for NF-κB inhibitors, namely *IκBα* or *p100*. The BCL-3 protein is another member of the IκB family and is overexpressed in a subset of human B-cell chronic lymphocytic leukemias because of a chromosomal translocation. This oncoprotein is phosphorylated by multiple kinases including GSK3 and this phosphorylation regulates BCL-3 function by modulating its oncogenic potential and by regulating the expression of a subset of its target genes. Therefore, deciphering the NF-κB/IκB protein phosphorylations is critical in order to better understand the molecular mechanisms of NF-κB-mediated oncogenesis.

INTRODUCTION

NF-κB is a critical family of proteins that play crucial roles in inflammation, immunity, cell proliferation and apoptosis. NF-κB exists in a latent stage in the cytoplasm bound to inhibitory proteins collectively called IκBs.[1] A variety of extracellular stimuli, including cytokines, pathogens or pathogens-related factors trigger signalling pathways that ultimately lead to the phosphorylation and subsequent proteasome-mediated degradation of the IκB.[1] Activated NF-κB subsequently migrate into the nucleus to regulate the expression of multiple target genes. As an ankyrin repeats-containing protein, BCL-3 is a member of the IκB family but its function is not similar to the other IκBs.

BCL-3 was originally identified by molecular cloning of the breakpoint of the t(14 ;19) chromosomal translocation from a subset of human B-cell chronic lymphocytic leukemias.[2] This translocation causes BCL-3 overexpression and presumably dysregulation of still mostly unknown downstream target genes involved in cell proliferation, apoptosis and differentiation.[3] Enhanced BCL-3 expression due to gene amplification is also a marker to differentiate t(2,5)-positive anaplastic large cell lymphoma from Hodgkin disease.[4] Moreover, high BCL-3 expression has also been described in solid tumors such as nasopharyngeal carcinomas[5] and breast cancers.[6]

BCL-3 IS A TRANSCRIPTION FACTOR

Unlike the other members of the IκB family, BCL-3 is a nuclear protein and has been described as a transcriptional activator or repressor through formation of heterocomplexes with the NF-κB proteins p50 and p52.[7-10] Although the molecular mechanisms underlying BCL-3-mediated transcription is unclear, BCL-3 harbors two transactivating domains located upstream and downstream the ankyrin repeats. BCL-3 activates transcription by removing inhibitory p50 homodimers from κB sites[9] while in other circumstances, it represses gene expression.[11] BCL-3 physically interacts with coactivators such as JAB1, Bard1 and Tip60[12] but also with transcriptional repressors such as HDAC1, -3 and -6[13,14] which could explain its dual role as a transcription activator or repressor. Although a clear demonstration of the physiological role of these interactions is still missing, a recent report demonstrated that the ability of BCL-3 to recruit HDAC1 is required to inhibit LPS-induced inflammatory responses in macrophages.[13] Another study even describes BCL-3 as a coactivator itself that is required for the expression of a subset of NF-κB target genes such as IP-10 in response to TNFα.[15] How could all these interactions with NF-κB proteins, coactivators and repressors be interpreted to better understand BCL-3 regulation

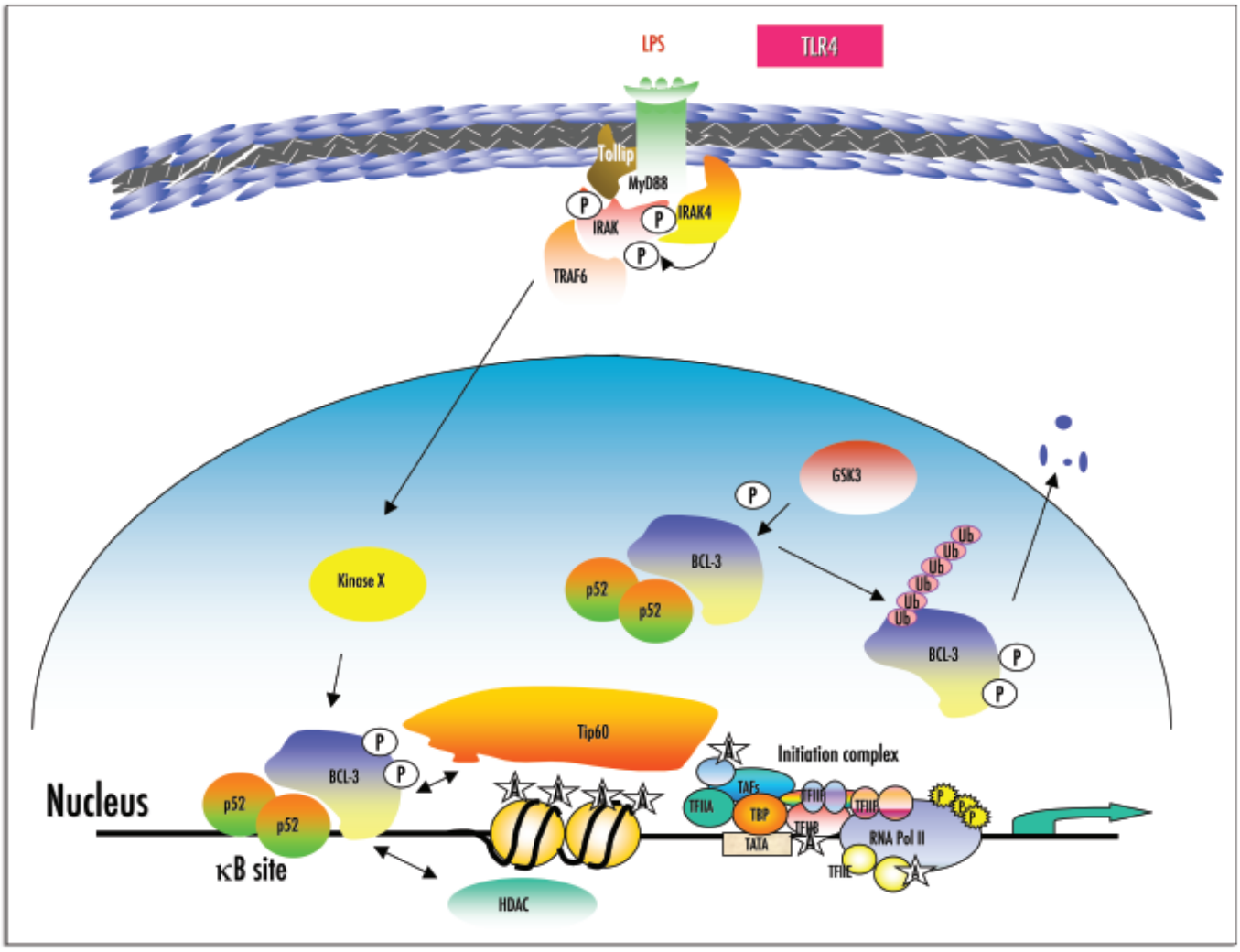

Figure 1. BCL-3 phosphorylation modulates its transcription potential. The kinase GSK3 phosphorylates BCL-3 on two residues within its C-terminal domain and this phosphorylation triggers ubiquitin linkage and proteasome-mediated BCL-3 degradation. In macrophages, LPS stimulation through the TLR4 triggers IRAK phosphorylation by the kinase IRAK4 and susbsequent recruitment of TRAF6 to the cell membrane. An LPS-inducible kinase subsequently phosphorylates nuclear BCL-3 but this activated kinase remains to be identified. In the nucleus, BCL-3 recruits either some coactivators such as Tip60 or HDAC proteins in order to activate or repress transcription, respectively.

of gene transcription? A study demonstrated that IL-1β stimulation triggers nuclear export of a specific corepressor complex and recruitment of a Tip60-containing activator complex by BCL-3 which is constitutively present within the promoter. As a result, a specific subset of NF-κB target genes are expressed upon cell stimulation.[16] However, this experimental model does not explain why BCL-3 activates but also represses gene transcription. This issue remains unclear to date but the answer may come from post-translational modification of BCL-3 itself. In any case, our preliminary results indeed indicate that, when overexpressed in NIH3T3 cells, BCL-3 induced the expression of about one hundred genes whereas twenty other candidates were repressed (unpublished results). Therefore, the dual role of BCL-3 regarding transcriptional activation or repression appears to be relevant.

BCL-3 ONCOGENICITY IS REGULATED BY PHOSPHORYLATION

Similarly to the NF-κB proteins, it is well accepted, although not yet proven, that the oncoprotein BCL-3 exerts its effect through direct regulation of gene expression. In this context, BCL-3 oncogenic potential has been recently demonstrated in vitro and in vivo.[14] Indeed, BCL-3 expressing NIH3T3 cells are transformed and form tumors when injected in nude mice. Moreover, the BCL-3 target genes that mediate this oncogenic potential have been identified[14] and include target genes such as *SLPI* which is known to promote the tumorigenic and metastatic potential of cancer cells.[17] Interestingly, BCL-3 oncogenicity is attenuated by GSK3-dependent phosphorylation on its C-terminal domain and this phosphorylation triggers BCL-3 degradation through the proteasome pathway. Therefore, this mechanism physiologically limits intracellular BCL-3 levels and prevents BCL-3 oncogenicity. Although the consequences of BCL-3 phosphorylation on gene transcription remain largely unexplored, we demonstrated that GSK3-mediated BCL-3 phosphorylation modulates its interactions with the HDAC proteins and regulates BCL-3 ability to induce a subset of its target genes.[14] However, the expression of many BCL-3 target genes is not regulated by GSK3-mediated BCL-3 phosphorylation, which does not rule out the possibility that BCL-3 phosphorylation by other

kinases may modulate BCL-3 transcriptional potential. Indeed, it has been known for many years that BCL-3 is heavily phosphorylated in several cell types including lymphoma cell lines.[8,14,18,19] Nuclear BCL-3 is phosphorylated upon LPS treatment[13] and we have recently identified a BCL-3 kinase distinct from GSK3 that is known to be activated by LPS (unpublished results). Therefore, at least two kinases phosphorylate BCL-3 in vivo. The biological consequences of this second phosphorylation on BCL-3 mediated gene transcription and on cellular transformation remains unknown but these issues are currently being addressed in our laboratory. It also remains to be determined whether BCL-3 is simultaneously phosphorylated by multiple kinases in any cell type or whether some cell specificity occurs.

Genetic disruption of the *bcl-3* gene in mice indicated that BCL-3 is required for antigen-specific priming of T and B cells since mutant mice are impaired in germinal center reactions and T-dependent antibody responses to influenza virus and have a partial loss of B cells which accounts for the immunological defects.[20] Interestingly, many but not all defects observed in these mice were also reported for the p52 KO mice,[21] therefore supporting the hypothesis that p52 and BCL-3 form a functional, physiologically relevant transcriptional complex and induce the expression of many common target genes. Although BCL-3 is required for the formation of B cell follicles, it is not clear whether BCL-3 phosphorylation critically regulates this function. Indeed, chimeric mice that had been generated by injecting bone marrow precursor cells from BCL-3 KO mice expressing either wild type or a BCL-3 mutant no longer phosphorylated by GSK3 did not exhibit any defects in B and T cells development (unpublished results). However, because of the decreased transgene expression in the transduced cells, we could not evaluate the effects of GSK3-mediated BCL-3 phosphorylation over a long period of time (3–4 months). This issue could be elegantly elucidated through the generation of knockin mice. As overexpression of BCL-3 in B cells causes lymphoproliferation and splenomegaly,[22] the knockin mice model could allow us to determine the exact role of BCL-3 in long term, sustained expression.

BCL-3 TARGET GENES

Very few BCL-3 target genes have been identified so far and include *cyclin D1* in breast cancer cells.[23] We recently identified about one hundred genes regulated by BCL-3 in NIH3T3 cells.[14] Although the recruitment of this oncoprotein to the corresponding promoter sequences remains to be experimentally demonstrated, the expression of some of these BCL-3 target genes such as *laminin-β 2* chain (unpublished results) was indeed previously shown to be altered in *Bcl-3* KO mice.[24] Moreover, other candidates such as *Cxcl1* were previously described as NF-κB target genes. We are currently investigating whether the expression levels of BCL-3 and its target genes can be correlated in lymphomas and breast cancer samples in order to identify genes that mediate BCL-3 oncogenic potential in vivo.

LINKING BCL-3 FUNCTIONAL DOMAINS WITH TARGET GENE EXPRESSION

As a member of the IκB family, BCL-3 harbors ankyrin repeats that mediate its interaction with p50 or p52. Moreover, these domains are required for interaction with the HDAC proteins. Whereas the C-terminal transactivating domain harbors all the phosphorylated residues identified so far, the N-terminal domain displays a couple of lysine residues that are critical for BCL-3-mediated transcriptional activation.[14] Indeed, a BCL-3 mutant lacking these residues failed to activate most of the BCL-3 target genes. Why are these N-terminal lysine residues critical to mediate BCL-3 effects? Several hypothesis can be made but all of them require experimental validation. First, these lysine residues are targeted for ubiquitin linkage and several studies indeed demonstrated that some DNA-bound transactivation factors are targeted for ubiquitination within their transactivation domain, this signal being required for transcriptional activation.[25,26] Therefore, there may be a link between BCL-3 ubiquitination and activation. In any case, the molecular mechanism of BCL-3 ubiquitination and the identity of the ubiquitin E3 ligase remain unknown. Beside ubiquitination as a mechanism for transcriptional activation, the same lysine residues could also be targeted for acetylation, a post-translational modification associated with gene activation.[26] We are currently investigating whether BCL-3 is a substrate of histone acetyltransferases. As a preliminary observation, some but not all histone acetyltransferases known to acetylate several transcription factors, enhances BCL-3-mediated transcriptional activation (our unpublished results). Therefore, BCL-3 may recruit these proteins in order to activate transcription. However, direct acetylation of BCL-3 by histone acetyltransferases still needs to be experimentally demonstrated.

CONCLUSION

Although BCL-3 was identified more than fifteen years ago, most of its biological roles remains unexplored. The increasing interest for the role of NF-κB/IκB proteins in cancer will certainly help to better understand the BCL-3-dependent molecular mechanisms. It is also likely that phosphorylation will emerge as a critical mechanism for the regulation of BCL-3 activity.

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