doi:10.1016/j.jmb.2009.06.055 J. Mol. Biol. (2009) 391, 729–743

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Non-receptor Tyrosine Kinases c-Abl and Arg Regulate the Activity of C/EBPβ

Xiaorong Li[1,2]†, Xuan Liu[1]†, Guangfei Wang[1], Xiaohui Zhu[1], Xiuhua Qu[1], Xiaoming Li[1], Yao Yang[1], Li Peng[1], Chufang Li[1], Ping Li[1], Wei Huang[1], Qingjun Ma[1] and Cheng Cao[1]*

[1]*Beijing Institute of Biotechnology, Beijing 100850, China*

[2]*College of Pharmaceutical Sciences, Southwestern University, Chongqing 400715, China*

Received 11 November 2008; received in revised form 20 May 2009; accepted 14 June 2009
Available online 27 June 2009

The CCAAT/enhancer-binding protein beta (C/EBPβ) is a critical transcription factor that regulates gene expression during numerous biological processes, including differentiation, metabolism, homeostasis, proliferation, tumorigenesis, inflammation, and apoptosis. In this study, interactions between C/EBPβ and either the Abelson murine leukemia viral oncogene homolog 1 (c-Abl) or the Abl-related gene (Arg) were demonstrated *in vitro* and *in vivo* with a direct binding assay and by co-immunoprecipitation, respectively. The Y79 amino acid residue of C/EBPβ was phosphorylated by c-Abl or Arg. The phosphorylation of C/EBPβ resulted in an increased C/EBPβ stability and a potentiation of C/EBPβ transcription activation activity in cells. Expression of the C/EBPβ(Y79F) mutant in HEK293, and K562, and in other cell lines, resulted in less of a delay in the cell cycle compared to the wild type C/EBPβ; furthermore, the C/EBPβ (Y79F) mutant induced an increased apoptosis compared to the wild type C/EBPβ. These findings suggest that the c-Abl family non-receptor tyrosine kinases have a role in the regulation of the C/EBPβ transcription factor.

Edited by M. Yaniv

Keywords: c-Abl; Arg; C/EBPβ; tyrosine kinase; apoptosis

Introduction

CCAAT/enhancer-binding protein β (C/EBPβ), also known as nuclear factor-IL6 (NF-IL6) or liver-enriched activation protein (LAP), is a member of the CCAAT/enhancer-binding protein (C/EBP) family of basic leucine zipper (bZIP) transcription factors that bind DNA as dimers.[1] Initially identified as a nuclear factor that bound to the interleukin-1 (IL-1) response element in the human IL-6 gene,[2] C/EBPβ was found to be widely expressed in a number of cell types, including adipocytes, hepatocytes, enterocytes, keratinocytes, mammary gland, and hematopoietic cells. C/EBPβ was found to be involved in the regulation of multiple cellular biological processes, including differentiation, metabolism, homeostasis, proliferation, tumorigenesis, inflammation, and apoptosis.[3–5] Currently, more than 100 genes are reported to be regulated by C/EBPβ.[3]

As a member of the C/EBP family of transcription factors, C/EBPβ shares common structural features with other C/EBP proteins, including highly conserved leucine zipper domains and a basic DNA-binding domain at the carboxyl terminus. These domains confer protein dimerization and DNA-binding properties, respectively. In contrast to the bZIP domain, the divergent amino-termini of proteins in this family contain regulatory and transactivation domains. The amino-terminus of C/EBPβ contains three short sub-regions representing activation domains that interact with basal transcription elements to stimulate transcription.[6] The region between bZIP and the N-terminus masks the transactivation domain, resulting in the inhibition of the transcriptional activation functions.[7,8] C/EBPβ is expressed in several protein isoforms: a full-length

**Corresponding author.* E-mail address: caoc@nic.bmi.ac.cn.

† X. Li and X. Liu contributed equally to this work.

Abbreviations used: c-Abl, Abelson murine leukemia oncogene homolog 1; Arg, Abl-related gene; SH, Src homology; GST, glutathione-*S*-transferase; C/EBP, CCAAT/enhancer binding protein; IL6, interleukin-6; IR, ion irradiation; FBS, fetal bovine serum.

liver-enriched activation protein (LAP), an amino-terminally extended LAP*, and a truncated liver enriched inhibition protein (LIP). Both LAP* and LAP contain three transcriptional activation domains, while LIP lacks these transactivation domains and acts as a competitive inhibitor of LAP.[9–11]

Accumulating evidence has shown that the activity of C/EBPβ can be modulated by interaction with many other leucine-zipper family members and by site-specific phosphorylation. C/EBPβ is the substrate of several protein kinases, including protein kinase A (mouse Ser239, rat Ser240, human Ser288), calmodulin-dependent protein kinase (rat Ser276, human Ser325), the p42/p44 mitogen-activated protein kinase, Erk-1/2 (rat Thr189, human Thr235), the p90 ribosomal S6 kinase, RSK (rat Ser105, mouse Thr217), and cyclin-dependent kinase 2 (Cdk2) (rat Ser64 and Thr189).[12–17] The phosphorylation of C/EBPβ by these kinases augmented its DNA-binding activity and the transactivation of target genes. In contrast, phosphorylation by GSK-3β kinase (rat Thr189, Ser185, Ser181, and Ser177) in resting UMR106 osteoblasts decreased C/EBPβ DNA-binding activity and transactivation of target genes.[18] Although phosphorylation of C/EBPβ by the Ser/Thr kinases has been well documented, the regulation of C/EBPβ by tyrosine kinases is poorly understood.

The Abelson murine leukemia viral oncogene homolog 1 (c-Abl) is a non-receptor tyrosine kinase that is widely expressed in adult and fetal tissues, and its activities are tightly regulated in the cell. c-Abl and the Abl-related gene product (Arg) share ~90% identity in the N-terminal regions, including tandem SH3, SH2, and tyrosine kinase domains.[19,20] The SH3 domain of c-Abl preferentially binds proline-rich sequences that contain the consensus PXXP motif.[21–23] c-Abl and Arg were shown to be involved in the regulation of diverse cellular events, including cell proliferation, differentiation, survival, apoptosis, adhesion, migration, and inflammation. c-Abl and Arg interact with and phosphorylate substrate proteins, particularly under conditions of irradiation, exposure to genotoxic drugs, or during reactive oxygen species (ROS)-induced stresses.[21,24-25] In this study, we showed that c-Abl (and/or Arg) interacts with and phosphorylates C/EBPβ. Upon phosphorylation by c-Abl or Arg, C/EBPβ was stabilized. The phosphorylation status of C/EBPβ contributed to its regulation of the cell cycle and apoptosis.

Results

c-Abl and Arg associate with C/EBPβ

To demonstrate the association of C/EBPβ with c-Abl, MCF-7 cell lysates were immunoprecipitated with anti c-Abl antibody. Immunoprecipitation with IgG was used as a control. Analysis of these precipitates by immunoblotting and probing with anti-C/EBPβ showed that C/EBPβ formed complexes with c-Abl. In contrast, C/EBPβ was undetectable in the immunoprecipitates prepared with control IgG (Fig. 1a, left). The Arg tyrosine kinase is highly homologous to c-Abl. To determine whether Arg associated with C/EBPβ, lysates from MCF-7, cells were immunoprecipitated with anti-Arg. Again, immunoprecipitation with IgG served as a control. Analysis of these precipitates by immunoblotting and probing with anti-C/EBPβ showed that C/EBPβ also formed complexes with Arg (Fig. 1a, right). Further, lysates of 293T cells co-transfected with Flag-tagged c-Abl and Myc-tagged C/EBPβ were immunoprecipitated with anti-Flag. Analysis of these immunoprecipitates by immunoblotting and probing with anti-Myc demonstrated that c-Abl and C/EBPβ formed complexes. As a control, Myc-C/EBPβ had been co-transfected with the Flag-vector and immunoprecipitated with anti-Flag; in immunoblots probed with anti-Myc, no C/EBPβ was detected (Fig. 1b). In reciprocal experiments, lysates of 293T cells co-expressing Myc-c-Abl and Flag-C/EBPβ were immunoprecipitated with anti-Flag and examined by probing immunoblots with anti-Myc (Fig. 1c). These results further supported the association between c-Abl and C/EBPβ. Moreover, lysates of cells expressing Flag-Arg and Myc-C/EBPβ were immunoprecipitated with anti-Flag and immunoblots were probed with anti-Myc. The results showed that Arg also formed complexes with C/EBPβ (Fig. 1d). These findings indicated that both c-Abl and Arg associated with C/EBPβ.

To further demonstrate the association between c-Abl and C/EBPβ, lysates from 293T cells transfected with Flag-c-Abl were incubated with Sepharose beads that were conjugated with the glutathione-*S*-transferase (GST)-C/EBPβ fusion protein. The absorbates were analyzed by immunoblotting and probing with anti-Flag. The results showed that c-Abl bound to GST-C/EBPβ, but not to GST (Fig. 1e, upper panel). To eliminate possible indirect binding mediated by other components in the cell lysate, the Sepharose beads conjugated to GST-C/EBPβ were incubated with affinity purified ^{35}S-labeled Flag-c-Abl prepared by *in vitro* transcription and translation. The resulting absorbates were analyzed by SDS-PAGE followed by autoradiography. The results suggested that there was a direct interaction between c-Abl and C/EBPβ (Fig. 1e, middle panel). Similar results were obtained with Arg (data not shown).

To define the domains involved in the interaction between c-Abl and C/EBPβ, affinity-purified ^{35}S-labeled Flag-C/EBPβ was incubated with Sepharose beads conjugated to either GST-c-Abl-SH3 or GST alone and the absorbates were analyzed by autoradiography. The results demonstrated that C/EBPβ was bound to c-Abl SH3 domains. In the control, no binding of C/EBPβ to GST was detected (Fig. 1f). These findings demonstrate the direct binding of c-Abl and Arg to C/EBPβ through the SH3 domain.