The IκB family member Bcl-3 stabilizes c-Myc in colorectal cancer

Dear Editor,

The proto-oncogene c-myc has been thought to play a critical role during the tumor-initiating process in multiple human cancers. Among others, colorectal cancer (CRC) is particularly associated with deregulated expression of c-Myc (Meyer and Penn, 2008; Wilkins and Sansom, 2008). Physiologically, Myc mRNA and protein levels are tightly regulated, and the Myc protein is highly unstable. The high levels of Myc protein in human CRC could be attributed to the altered Myc turnover and aberrant transcriptional activation of the myc genes (Ikegaki et al., 1986; Welcker and Clurman, 2008).

The atypical member of the IκB family Bcl-3 can bind to p50 and p52 homodimers on DNA, thereby positively or negatively regulating the expression of NF-κB target genes, depending on the context (Fujita et al., 1993; Wang et al., 2012). Recently, high levels of Bcl-3 have been noted in a variety of solid cancers including CRC (Puvvada et al., 2010; Maldonado and Melendez-Zajgla, 2011). However, the function of Bcl-3 in colorectal tumorigenesis remains to be elucidated.

We found that human CRC tissues exhibited increased levels of Bcl-3 compared with colorectal normal tissues (Figure 1A and B). In order to explore the role of Bcl-3 in human colorectal tumorigenesis, we transduced the human CRC cell line HCT116 with a tet-on lentiviral vector containing the shRNA against bcl-3 gene to establish the doxycycline (DOX)-inducible Bcl-3 knockdown cell line (HCT116/shBcl-3) (Supplementary Figure S1A). The in vitro cell growth was significantly slower upon Bcl-3 knockdown in HCT116 cells (Figure 1C and Supplementary Figure S2A). Bcl-3 knockdown also significantly suppressed the ability of HCT116 cells to form colonies in plate and soft agar (Supplementary Figure S2B and C).

The inhibition was associated with a block in the G1/S transition of cell cycle (Supplementary Figure S2D). The inhibitory effects of Bcl-3 knockdown on in vitro CRC cell growth were also observed in mouse CRC cell line CT26 WT (Supplementary Figures S1B, S3A–C), excluding that the growth inhibition was due to the off-target effects of Bcl-3 knockdown. These results indicate that Bcl-3 knockdown suppresses colorectal tumor cell growth in vitro.

To elucidate the mechanism by which Bcl-3 regulated the cell cycle, we compared the expressions of cell cycle-related genes between wild-type and Bcl-3 knockdown cells. We found that the level of c-Myc protein, but not the mRNA, was markedly decreased upon Bcl-3 knockdown. Accordingly, p21, which is negatively regulated by c-Myc, was increased. Bcl-3 knockdown, however, did not significantly affect the expression of other genes such as cyclin D1, cyclin E1, Skp2, p27, and IL-6 (Figure 1D and Supplementary Figure S4A). This suggests that reduced c-Myc protein level is associated with cell growth inhibition by Bcl-3 knockdown. We over-expressed c-Myc in Bcl-3 knockdown HCT116 cells, and found that the inhibited cell growth by Bcl-3 knockdown could be partially rescued by the over-expression of c-Myc (Figure 1E), indicating that reduced c-Myc protein contributes to reduced cell proliferation upon Bcl-3 knockdown.

To verify that Bcl-3 regulates c-Myc protein level, we determined the half-life of c-Myc protein after applying the protein synthesis inhibitor cycloheximide (CHX). c-Myc levels decreased faster in Bcl-3 knockdown cells than in control cells, while cyclin D1 levels decreased similarly in both cells (Figure 1F). Intriguingly, the inhibition of proteasomal function by MG-132 restored the decreased c-Myc protein in Bcl-3 knockdown cells to levels seen in control cells (Figure 1G). Moreover, Bcl-3 over-expression in a Bcl-3-deficient cell line significantly extended the half-life of c-Myc protein and reduced the levels of ubiquitinated c-Myc (Supplementary Figure S4B and C). The results above suggest that Bcl-3 regulates ubiquitination-mediated degradation of c-Myc.

c-Myc protein stability can be differentially regulated by phosphorylation at threonine 58 (Thr58) and at serine 62 (Ser62). The phosphorylation at Thr58 by GSK-3 leads to degradation of c-Myc, while the phosphorylation at Ser62 likely mediated by ERK1/2 is required for RAS-induced stabilization of the c-Myc protein (Sears et al., 1999, 2000). In this study, we did not observe any notable difference on AKT phosphorylation or the expression of its targets such as p27 and cyclin E1 upon the absence of Bcl-3 in HCT116 cells. By contrast, we consistently found reduced levels of c-Myc, p-c-Myc at Ser62 and p-ERK1/2 upon Bcl-3 knockdown (Figure 1H and Supplementary Figure S5). Collectively, our data indicate that Bcl-3 may stabilize c-Myc protein by enhancing the ERK1/2-mediated phosphorylation of c-Myc at Ser62.

Cell growth inhibition in vitro by Bcl-3 knockdown prompted us to investigate the effect of Bcl-3 on tumor cell growth in vivo. Bcl-3 knockdown induced by Dox significantly inhibited tumor cell growth in vivo when compared with tumor growth in control mice without Dox treatment (Figure 1I–K). There were significantly fewer Ki67 positive cells in xenograft tumors of Bcl-3 knockdown cells compared with controls (Supplementary Figure S6).

We also noted decreased levels of c-Myc protein and ERK phosphorylation, but not c-myc mRNA, in xenograft tumor tissue grown under Bcl-3 knockdown conditions (Figure 1L and Supplementary Figure S7). Similar inhibitory effects of Bcl-3...
Figure 1  Bcl-3 stabilizes c-Myc and promotes tumorigenesis in CRC. (A and B) Immunohistochemical staining for Bcl-3 was performed using multiple tissue microarrays containing 87 CRC tissues and 20 colorectal normal tissues. Representative images of Bcl-3 staining in human colorectal normal and cancer tissues were shown in A, and a summary of the scoring was shown in B. **400 × magnification.** (C) Cell growth of HCT116/shBcl-3 cells with or without Dox treatment (1 µg/ml) was quantified at the indicated time by counting the number of cells. (D) Protein and mRNA levels of c-Myc and other cell cycle-related molecules in HCT116/shBcl-3 cells with or without Dox treatment were determined by western blotting and real-time PCR, respectively. (E) HCT116/shBcl-3 cells were treated with Dox, and transfected with GFP control or c-Myc. Cell growth was quantified at the indicated time by counting the number of cells. (F) HCT116/shBcl-3 cells with or without Dox treatment for 6 days were treated with 20 µg/ml cycloheximide (CHX) for indicated periods. The protein abundance was measured by western blotting (left panel) and c-Myc protein levels...
knockdown on CRC cell growth in vivo and c-Myc protein were observed in CT26 WT cells (Supplementary Figure S8), confirming that knockdown of Bcl-3 results in decreased ERK phosphorylation and c-Myc protein level, and significantly suppresses tumor cell growth in vivo.

To address the relevance of Bcl-3 effects to human CRC development, we examined the expression of Bcl-3, phosphorylated ERK, and c-Myc in 283 CRC tissues. We found that there was a significant positive correlation between Bcl-3 and phosphorylated ERK levels (P < 0.0001), as well as between Bcl-3 and c-Myc levels (P < 0.0001) in human CRC tissues (Figure 1M and N). These data further support the notion that increased expression of Bcl-3 in CRC tissues. We found that there was a significant positive correlation between Bcl-3 and c-Myc protein levels (P < 0.0001), as well as between Bcl-3 and c-Myc levels (P < 0.0001) in human CRC tissues (Figure 1M and N). These data further support the notion that increased expression of Bcl-3 may lead to increased expression of c-Myc protein via ERK1/2-mediated phosphorylation of c-Myc, thereby promoting human CRC tumorigenesis.

Overall we identified a novel role for Bcl-3 in colorectal tumorigenesis. Our results demonstrate that Bcl-3 plays a critical role in stabilizing c-Myc protein via ERK activation. We show that Bcl-3 promotes CRC cell growth in vitro and tumorigenesis in vivo. These results provide a new insight into the human CRC development and identify Bcl-3 as a potential novel therapeutic target for the treatment of human CRC.

[Supplementary material is available at Journal of Molecular Cell Biology online. We thank Drs Y.E. Chin, B. Li, Q. Zhai, Y. Chen (SIBS), and Z. Lou (Mayo Clinic) for antibodies and plasmids and Y. Zhang and Q. Jing (SIBS) for support. This work was supported by grants from the National Natural Science Foundation of China (90919017, 30972695 and 31270937), the National Basic Research program (2011CB946102), Knowledge Innovation Project of Chinese Academy of Sciences (KSCX1-YW-22), and National Key Programs on Infectious Disease (2008ZX10002-014).]

Zhanjie Liu1,†, Yuhang Jiang1,†, Yinyong Hou2,†, Yiming Hu1, Xinwei Cao1, Yu Tao1, Chen Xu2, Sanhong Liu3, Shouli Wang4,†, Lunshan Wang1, Yufang Shi1, Ulrich Siebenlist2, and Xiaoren Zhang3,4

1Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Shanghai Jiao Tong University School of Medicine, Shanghai 200031, China
2Department of Pathology, Zhongshan Hospital, Fudan University, 180 Fenglin Road, Shanghai 200032, China
3Department of Pathology, Soochow University School of Medicine, Suzhou 215123, China
4Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
5These authors contributed equally to this work.

Correspondence to: Xiaoren Zhang, Tel: +86-21-54920601; E-mail: xrzhang@sibs.ac.cn

References