Is BOK required for apoptosis induced by endoplasmic reticulum stress?

Yuniel Fernandez-Marrero[a], Francine Ke[b,c], Nohemy Echeverry[a,1], Philippe Bouillet[b,c], Daniel Bachmann[a], Andreas Strasser[b,c], and Thomas Kaufmann[a,2]

The B-cell lymphoma 2 (BCL-2)-related ovarian killer (BOK) shares sequence homology with the proapoptotic BCL-2 family members BAX and BAK. However, *Bok*$^{-/-}$ cells are not protected from classic apoptotic triggers and evidence for a proapoptotic role of BOK is derived mostly from overexpression studies (1). BOK localizes preferentially to the endoplasmic reticulum (ER) membrane, where it interacts with IP3-receptors (2, 3). Using cells from their newly generated *Bok*$^{-/-}$ mouse strain, Carpio et al. propose that BOK is a critical inducer of BAX/BAK-dependent apoptosis in response to ER stress (4). This proposal is in contrast to our earlier report, in which we showed that loss of BOK did not confer resistance toward ER stress in several cell types (2). Underlying reasons for this discrepancy may lie in the initial Sv129:C57BL/6 mixed genetic background of the strain used by Carpio et al. (4) [which may influence the phenotype despite backcrossing (5)] and their targeting strategy of the *Bok* locus. By targeting exons 2 (containing the START codon) and 3, alternative splicing of exon 1–4 is enabled and is indeed readily detectable based on the RT-PCR analysis in figure 1C of Carpio et al. (4). A resulting ∼1-kb transcript (exon1/4/5), which is not occurring naturally, contains several predicted ORFs and may influence the phenotype of these mice. In contrast, our *Bok*$^{-/-}$ strain was generated in a pure C57BL/6 genetic background, with no detectable transcript because of targeting of the exon 1 splice donor site along with exon 2 (1).

Based on figure 6B of Carpio et al. (4), it is also important to discuss that the SV40-immortalized *Bok*$^{-/-}$ mouse embryonic fibroblasts (MEFs), which they almost exclusively used for their studies, show a >50% reduction in the baseline levels of Bcl-2-interacting mediator of cell death (*Bim*) compared with WT controls (despite comparable *Chop* levels). Given the critical role of BIM in ER stress-induced apoptosis, this reduction of *Bim* may fully account for the reported resistance to ER stress. It is unclear whether this reduction of *Bim* is particular to these SV40 MEFs, which are prone to line-to-line variations within the one genotype, or whether this is also seen in primary cells (e.g., primary MEFs, which were used for some experiments) from these mice. Importantly, we did not observe significant changes in *Bim* levels in SV40 MEFs or tissues from our *Bok*$^{-/-}$ mice (Fig. 1*A*). Overall, our analysis of SV40 MEFs, primary MEFs, myeloid progenitors, mast cells, and primary neutrophils did not support a proapoptotic role of BOK downstream of ER stress (2) (Fig. 1 *B* and *C*). Furthermore, the rescue experiments in figure 5 of Carpio et al. (4) are in our view inconclusive, because BOK was overexpressed by transient transfection, a stress condition that induces BIM and PUMA (2).

Carpio et al. (4) conclude with in vivo data on *Bok*$^{-/-}$ mice being protected from thapsigargin-induced liver damage. Regretfully, however, no quantitative data on liver damage with statistical analysis were provided. Of note, we did not find any protection from ER stressors in *Bok*$^{-/-}$ primary hepatocytes or immortalized human hepatocytes rendered *BOK*-deficient (Fig. 1 *D* and *E*).

Taking these data together, we reason that BOK may not be essential in promoting ER stress-induced apoptosis.

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[a]Institute of Pharmacology, Medical Faculty, University of Bern, 3010 Bern, Switzerland; [b]Molecular Genetics of Cancer Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia; and [c]Department of Medical Biology, University of Melbourne, Melbourne, VIC 3010, Australia

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[1]Present address: Laboratory of Molecular Oncology, Clinic of Oncology, University Hospital Zurich, 8044 Zurich, Switzerland.

[2]To whom correspondence should be addressed. Email: thomas.kaufmann@pki.unibe.ch.

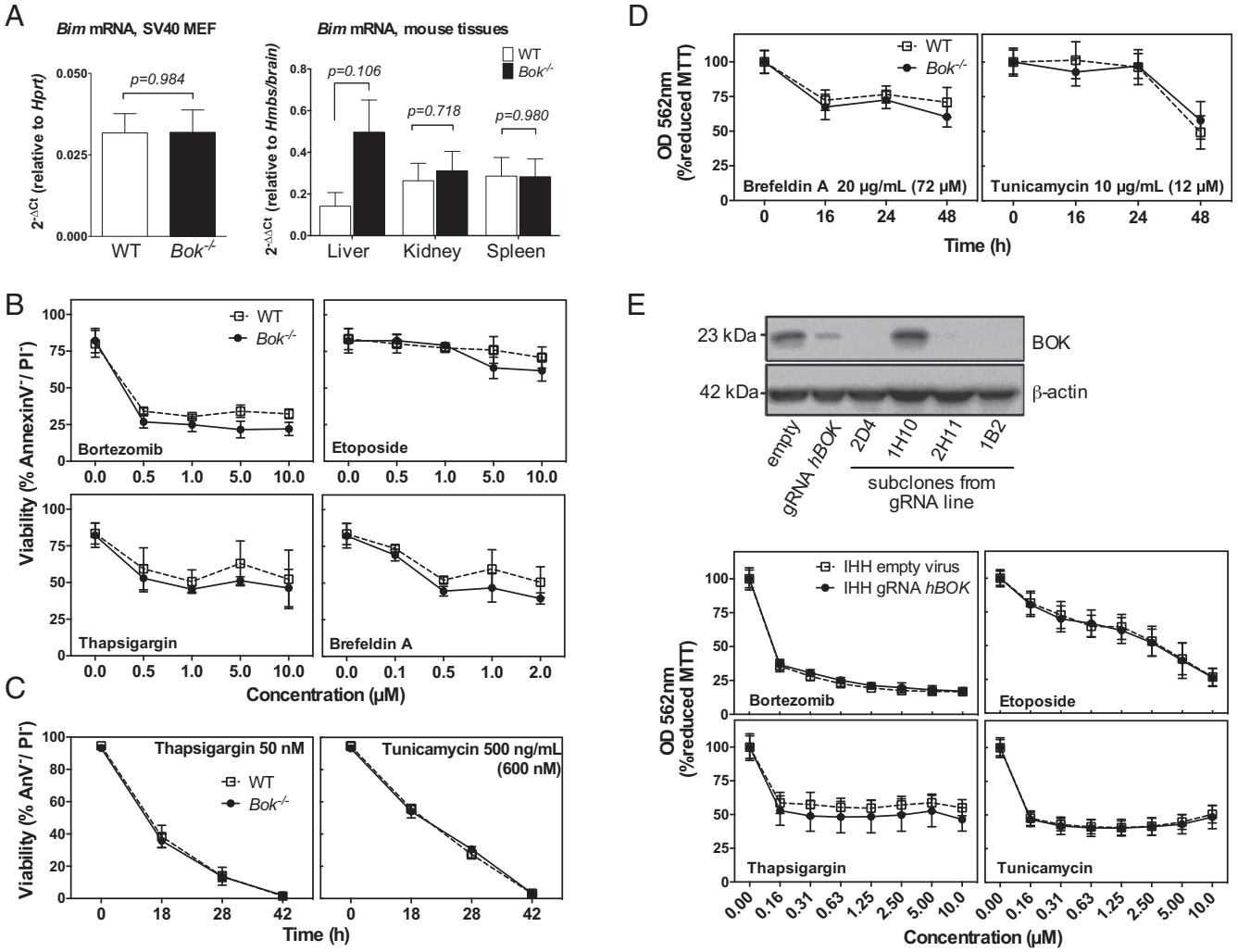

Fig. 1. (*A*) Loss of *Bok* does not affect *Bim* expression. Quantitative PCR analysis of *Bim* mRNA levels in SV40-transformed MEFs (mean ± SEM from three independent lines per genotype; *Hprt* was used as reference gene) or primary tissues isolated from WT and $Bok^{-/-}$ mice (mean ± SEM, four mice per genotype per tissue, *Hmbs* was used as reference gene). (*B*) Early passage primary MEFs ($t = 24$ h; mean ± SD from four independent lines per genotype) and (*C*) primary bone marrow-derived Gr-1^+ neutrophils from $Bok^{-/-}$ mice (mean ± SD, three mice per genotype) are not protected from ER stress-induced apoptosis. Note that primary MEFs were relatively resistant to etoposide-induced cell death. Cell viability was determined by flow cytometry using FITC-Annexin V/propidium iodide staining. (*D*) $Bok^{-/-}$ primary hepatocytes (mean ± SD, three mice per genotype) and (*E*) immortalized human hepatocytes (IHH) rendered *BOK*-deficient using CRISPR/Cas9 technology ($t = 24$ h; mean ± SD from three independent experiments; lentiCRISPR v2 was a gift from Feng Zhang, Broad Institute of MIT and Harvard, Cambridge, MA; Addgene plasmid #52961) are not protected from ER stress-induced apoptosis. Immunoblot (*E*) shows loss of BOK in IHH cells before subcloning (gRNA *hBOK*; BOK protein levels were reduced by 80%, as assessed by quantitative Western blot analysis with near-infrared fluorescence) and examples of derived subclones. Cell viability was determined by MTT assay performed in sextuplicates. Statistical analyses were performed using the unpaired *t* test.

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