Cell Research (2007) 17:283-285

www.nature.com/cr

COMMENTARY

p53 and Bad: remote strangers become close friends

Peng Jiang[1], Wenjing Du[1], Mian Wu[1]

[1]*Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Anhui 230027, China*

Cell Research (2007) **17:**283-285. doi: 10.1038/cr.2007.19; published online 3 April 2007

The transcription factor p53 is a key regulator of the DNA damage response to genotoxic stress in higher eukaryotes. Mutation/inactivation of p53 has been implicated in the pathogenesis of many tumor types [1]. p53 responds to cellular insults, such as DNA damage or hypoxia, either by arresting the cell cycle so that DNA damage could be repaired, or by triggering apoptosis if DNA repair is futile. Transcriptional activation of apoptosis-related genes by p53 is critical for the induction of programmed cell death. In search of target genes of p53 involved in cell-cycle arrest and/or apoptosis, numerous p53-inducible genes have been identified over the past 15 years. Among them are genes such as *Bax* [2], *Puma* [3], *Noxa* [4], *Bid* [5], *p21waf1* [6], and many others. These apoptosis-related factors function in different cell organelles including the cytosolic membrane, the mitochondria and the cytosol. However, none of these candidate effectors alone can be accountable for the complicated mechanisms underlying the p53 transcription-dependent apoptotic signaling pathways. Earlier work implicated that Bad may contribute to p53-induced mitochondria-mediated apoptosis, yet no known molecular mechanism had been ascribed to this observation. Our recent study published in *Molecular and Cellular Biology* revealed that a functional p53-binding element exists at the human *bad* genomic locus, residing roughly 6.6 kb upstream of the Bad translation start codon [7]. The fact that this p53-binding element is relatively far away from the Bad promoter could be the reason why it has not been recognized as a p53-binding element until today. Interestingly enough, we found that a similar p53-binding region is well conserved approximately 7 kb upstream of the murine *bad* locus (our unpublished data).

Moreover, recent studies have shown that cytoplasmic accumulation of the transactivition-deficient knock-in p53 (QS) can sufficiently elicit substantial apoptosis upon cell exposure to hypoxia [8], implying that there may exist an apoptotic signaling pathway triggered by a p53 transcription-independent mechanism. In addition, p53 is able to translocate into mitochondria to interact with Bcl-2 or Bcl-XL, thereby activating Bax directly or indirectly, and leading to caspase-dependent apoptosis. All these experimental evidence has shown that the extranuclear role of p53 in inducing apoptosis is through mechanisms involving Bcl-2 family proteins. Bcl-2 family members are the main factors that mediate mitochondria dysfunction and cell apoptosis. Bad, one of the Bcl-2 family members, is a BH3-only protein that can induce mitochondria disruption by interacting with and inhibiting the anti-apoptotic function of Bcl-2 and Bcl-XL [9]. The apoptotic activity of Bad is largely determined by its phosphorylational status. In unstressed cells, Bad is hyperphosphorylated by several protein kinases including PKA and PKB (AKT) and is gripped by 14-3-3 [10]. However, upon apoptotic stimuli, Bad is rapidly dephosphorylated and targets to the mitochondria where it induces cell death. This observation might be better explained in light of our recent data [7]. We demonstrated that p53 can form a complex with dephosphorylated Bad thereby converting it to a pro-apoptotic player. However, unlike 14-3-3, the p53/Bad interaction appears to be independent of the phosphorylation of Bad at Ser-112, -136 and -155 (PJ and MW, unpublished result). Obviously, the tripartite nexus between 14-3-3, Bad and p53 is far more complex than expected. Therefore, how the phosphorylation status of Bad may control its binding partner selection between 14-3-3 and p53 deserves further investigations. Nonetheless, for the first time we provide convincing evidence that unphosphorylated Bad can direct

Correspondence: Mian Wu
Tel: +86-0551-3606264
E-mail: wumian@ustc.edu.cn

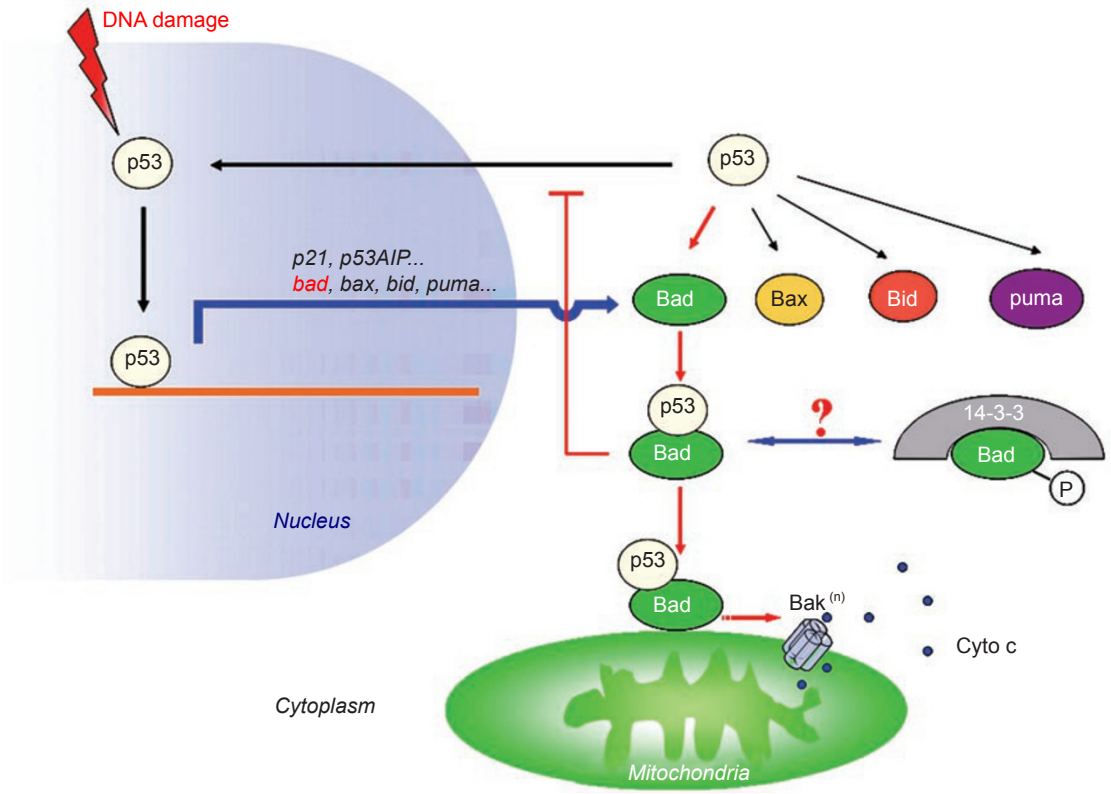

Figure 1 Bad plays dual roles in p53 transcriptional-dependent and -independent pathways. Upon treatment with a DNA damaging agent such as etoposide, upregulated p53 directly binds to the upstream promoter region of many target genes, such as *Bad*, *Bax*, *Puma*, *Noxa* and *Bid* to transactivate their gene expression. When enough Bad has been translated in the cytoplasm, it in turn associates with p53 to prevent the latter from entering nucleus. Bad expression is thus to be kept at a physiological level; moreover, dephosphrrylated Bad is able to direct cytosolic p53 to mitochondria and promotes apoptosis via activating Bak oligomerization and cytochrome *c* release.

p53 to mitochondria to induce apoptosis via the release of cytochrome *c*. To define an alternative contribution of the association between p53 and Bad, we examined the feedback effect of Bad on p53. As we described in this work, association of p53 with Bad within the cytosol prevents p53 from entering the nucleus to further transactivate Bad expression. This creates a negative feedback loop that maintains Bad at a physiological level to avoid unnecessary apoptosis in healthy cells.

Responding to DNA damage, p53 directly binds to the bad promoter region and upregulates Bad transcription. The resultant cytosolic Bad serves two distinct functions. On one hand, when accumulated Bad goes beyond the level needed for maintaining normal cell physiology, p53 is neutralized by the excess Bad to prevent the former from entering the nucleus, hence reducing further transcription of Bad. On the other hand, dephosphorylated Bad, which is no longer able to bind 14-3-3, can heterodimerize with p53 and translocate to mitochondria. They jointly activate Bak oligomerization which results in cytochrome *c* release and subsequent apoptosis (Figure 1).

Our current data provide the first evidence that Bad plays dual roles in both p53 transcription-dependent and -independent pathways. However, the results also raise a series of questions. For instance, will p53 and Bad act in the same way in all types of tumor cells? We have noticed that in some cell lines such as the human lung cancer cell line H460, Bad's expression does not comply with the activation of p53. This may be explained by the different genetic backgrounds (in this case, p16 is mutated) which could lead to a cell type-dependent variation in p53-induced Bad transcription. Another more challenging question is: how is the heterodimerization of p53 and Bad regulated? Particularly, what kind of post-translational modifications

of Bad favors the association with p53 or visa versa? These queries await further convincing answers.

Annotation

We apologize to those authors whose relevant work could not be cited owing to space restrictions.

References

1. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997; **88**:323–331.
2. Miyashita T, Reed JC. The tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 1995; **50**:293-299.
3. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell 2001; **7**:683-694.
4. Oda E, Ohki R, Murasawa H, *et al*. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science 2000; **288**:1053-1058.
5. Sax JK, Fei P, Murphy ME, Bernhard E, Korsmeyer SJ, El-Deiry WS. BID regulation by p53 contributes to chemosensitivity. Nat Cell Biol 2002; **4**:842-9.
6. El-Deiry WS, Tokino T, Velculescu VE, *et al*. WAF1, apotential mediator of p53 tumor suppression, Cell 1993; **75**:817-825.
7. Jiang P, Du WJ, Heese K, Wu M. The Bad Guy Cooperates with Good Cop p53: Bad is transcriptionally up-regulated by p53 and forms a Bad/p53 complex at the mitochondria to induce apoptosis. Mol Cell Biol 2006; **26**:9071-82.
8. Johnson TM, Hammond EM, Giaccia A, Attardi LD. The p53QS transactivation- deficient mutant shows stress-specific apoptotic activity and induces embryonic lethality. Nat Genet 2005; **37**:145-52.
9. Yang E, Zha J, Jockel J, Boise LH, Thompson CB, Korsmeyer SJ. Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. Cell 1995; **80**:285-291.
10. Datta SR, Katsove A, Hu L, *et al*. 14-3-3 proteins and survival kinases cooperate to inactivate BAD by BH3 domain phosphorylation. Mol Cell 2000; **6**:41-51.