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Perspective

A Perspective: Regulation of Ku70 Cytosolic Function

Roland P.S. Kwok*

*Departments of Obstetrics and Gynecology, and Biological Chemistry University of Michigan Medical School, Ann Arbor, MI 48109 USA

Corresponding Author: Roland P.S. Kwok, Departments of Obstetrics and Gynecology, and Biological Chemistry University of Michigan Medical School, Ann Arbor, MI 48109 USA; **E-mail:** rkwok@umich.edu

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Introduction

Ku70 was first discovered as an auto-antigen [1, 2]. The majority of Ku70 studies focused on its DNA binding activity in the non-homologous end joining (NHEJ) DNA repair mechanism in the nucleus [3, 4]. However, my laboratory has, for many years, been exploring the roles Ku70 in the cytosol, especially its role in regulating the proapoptotic activity of Bax [5, 6]. Here, I will discuss the roles of Ku70 in regulating Bax activity in the cytosol, integrating some of our key findings with others to illustrate a path forward in understanding the roles of cytosolic Ku70 in cells.

Model of Cytosolic Ku70 as a Survival Factor Against Bax-Mediated Cell Death

Because of its function as a DNA repair factor, the site of Ku70's activities was always considered to be in the nucleus. The first study, however, to show that Ku70 could be a cytosolic protein was from a yeast two hybrid screen searching for Bax binding proteins [7]. Bax is a pro- apoptotic protein belonging to the Bcl-2 family of proteins [8]. Activation of Bax plays an important role in both intrinsic and extrinsic apoptotic pathways. Bax's activity can be regulated by binding to other Bcl-2 proteins, such as Bcl-2 or Bcl-XL [9]. The yeast two hybrid study demonstrated that Ku70 binds to Bax and also suppresses Bax proapoptotic activity. This study was followed by a study showing that Ku70's binding to Bax is regulated by acetylation of two lysine resides at K539 and K542 of Ku70 [10]. When Ku70 is acetylated at these two lysine residues, Bax is released, resulted in its entrance into the mitochondria, triggering apoptosis. We have also established that depleting Ku70 triggers cell death, but cell death can be prevented by simultaneously depleting Bax, suggesting that Ku70 may be required to suppress Bax activity in cells such that Bax is activated without the suppressive effect of Ku70 [11].

To investigate how Ku70 acetylation is regulated, we used neuroblastoma (NB) neuroblastic (N-type) cells as a model, and established that by altering the acetylation status of cells, we could modulate Ku70-Bax complex formation or dissociation, protecting these cells from dying or inducing these cells to die, respectively [12]. We have confirmed the original observation by Cohen et al. [10] that the cAMP-response element binding protein (CREB) binding protein (CBP), a transcriptional activator and an acetyltransferase, acetylates Ku70 [11]. Mutation of K539 and K542 of Ku70 to arginine blocked histone deacetylase inhibitors (HDACIs) induced cell death and also blocked Bax release following HDACI treatment. Our studies showed that over expression of CBP induced cell death in a Ku70 dependent manner. In contrast, CBP depletion caused reduction of Ku70 acetylation, increasing the resistance of HDACI-induced cell death. Our results also indicated that p300, a homolog of CBP in human cells that shares many identical functions in cells with CBP [13], did not affect cell death when over expressed, suggesting that CBP plays a unique role in acetylating Ku70 in cells.

To further understand how Ku70 acetylation is regulated, we sought to identify the deacetylase that deacetylates Ku70 in the cytosol. Our results showed that HDAC6 bound to Ku70 and Bax [14]. HDAC6 is a class IIb HDAC containing two catalytic domains [15, 16]. HDAC6 is mainly localized in the cytosol and has been associated with many cell functions including tubulin stabilization, cell motility, and regulation of binding between Hsp90 and its cochaperone [17]. In NB cells, HDAC6 formed a complex with Ku70 and Bax, and depleting HDAC6 had similar effects to treatment with tubacin, a HDAC6 specific inhibitor [18]. Furthermore, depleting HDAC6 also increased Ku70 acetylation, releasing Bax from Ku70, causing cell death. Based on our findings, we proposed a model in which Ku70 may serve as a survival factor in suppressing Bax-induced cell death. We reasoned that, throughout life, cells continuously receive stimuli that affect cell viability. Some of these stimuli may be strong enough to trigger a high level of Bax activation leading to instant cell death while some minor stimuli may only activate a few molecules of Bax. As a protective measure and to conserve energy, cells may avoid dying when receiving weak signals that only activate a low level of Bax. Thus, to cope with these small sporadic Bax activation signals, cells may find ways to block these signals. We believe that Ku70 may act as one of these survival factors, blocking low level of Bax activation and thereby preventing premature cell death. While this model is compatible with the current data, it raises two important questions: 1. What is the stoichiometry of the binding between Ku70 and Bax? 2. Does this model apply to all cell types?

The model suggests that Ku70 needs to bind to activated Bax when activated Bax's level increases. But how much do cytosolic Ku70 and Bax bind to each other in cells at basal levels? One possibility is that Ku70 and Bax do not bind to each other at basal levels, and thus there is plenty of Ku70 available to bind Bax when Bax is activated. However, the study by Sawada et al. stated that "a large proportion of the Bax population is associated with Ku70 in normal cells." [7]

This statement is not consistent with published results reporting that Bax is inactive and monomeric in the cytosol [19]. Using gel filtration chromatograph and cross-linking techniques, we have shown that the majority of Bax is monomeric and the majority of Ku70 is in complex with other factors, including its DNA binding partner Ku80 [20]. There is only a small amount of Ku70 binding to a small amount of Bax at basal levels. Most important, however, is that there is no free Ku70 or monomeric Ku70 found in the cytosol. Where is the additional free Ku70 coming from when the rest of Ku70 in cells is in complex with other factors? Our model suggests that if Ku70 acts as a survival factor in rescuing cells from Bax-induced killing, Ku70 has to be released from complexes that contain Ku70 so that it is available to bind to activated Bax. If so, there has to be another level of regulation of Ku70 availability in the cytosol for Ku70 to act as a survival factor. Studies have shown that Ku70 binds to several factors in the cytosol [21–23]. For example, the FAAD-like interleukin-1-b-converting enzyme (FLICE)-inhibitory protein (FLIP) is an antiapoptotic protein that blocks caspase 8 activation by death receptors [24]. FLIP binds to Ku70 in an acetylation-dependent mechanism. However, unlike Bax binding to the carboxyl terminal of Ku70, FLIP binds to the Ku80 binding domain of Ku70. When Ku70 is acetylated at K539 and K542, the same two lysines when acetylated regulate the binding of Bax, causing FLIP to be released. It is then polyubiquitinated and degraded, allowing caspase 8 to be activated thereby inducing cell death via the extrinsic pathway [24]. It is not clear whether Ku70 binds to Bax and FLIP simultaneously, however. Thus, the weak incoming apoptotic signals have to achieve at least two things: 1. to activate Bax, and 2. to release Ku70 from its binding proteins so that Bax can be inactivated. This means that there is another level of regulation of Ku70 availability in the cytosol that releases Ku70 from Ku70-containing complexes. The relative affinities of Ku70 to various complexes are not yet known. Thus, it is difficult to predict which complex that Ku70 is released from and what the mechanism of regulation is.

While the notion that Ku70 is acting as a survival factor is intriguing, the question remains: does it apply to all cell types? Originally, we used the NB N-type SH-SY5Y cells to establish the model in which Ku70 is acting as a survival factor [11, 12]. Our results demonstrated that when Ku70 was depleted using Ku70 specific siRNA in SH-SY5Y cells, the cells would die in a Bax- dependent manner (rescued by simultaneously depleting Bax). However, previous studies by others have shown that in other cell types, such as HeLa and HEK293, depleting Ku70 using Ku70 specific siRNA did not induce cell death [7, 10]. Our results, together with the results in HeLa cells and HEK293 cells, suggest that there may be at least two cell types in terms of Ku70 regulating Bax function: one is Ku70-depletion sensitive, and one is Ku70-depletion less sensitive.

In a more extended study, we depleted Ku70 using Ku70-specific siRNA in a panel of N- type NB cells (including SH-SY5Y cells as a positive control), S-type NB cells (SHEP-1), HEK293T cells, and a couple of ovarian cancer cells [20]. We found that except for the N-type NB cells, depletion of Ku70 did not trigger cell death in these cells. More interesting, however, is the finding that the Ku70-depletion less sensitive cells were also less sensitive to the HDACI treatment. This was not due to the fact that Ku70 was not acetylated following treatment of these cells. In fact, Ku70 was also acetylated in these cells following HDACI treatment. However, even though Ku70 was acetylated, our results showed that Bax did not dissociate from Ku70, contrary to what we observed in SH-SY5Y cells: HDACI treatment induced Ku70 acetylation, separating Bax and inducing cell death. Our results can clearly distinguish between two types of cells in terms of their sensitivity to cell death after Ku70 depletion: one is sensitive and one is less sensitive.

In the Cohen et al. paper, they described that the two lysine residues (K539 and K542) on Ku70 that are important for regulating Bax binding are localized at the linker regions of Ku70, not within the Bax-binding domain at the carboxyl terminal of Ku70 (residues 578–609) [10]. Acetylation of these two lysine residues induces a conformational change of the Bax-binding domain of Ku70 resulting in Bax dissociation. Based on our current results in Ku70-depletion less sensitive cells, we suggest that some other factors in these cells must block the conformational change of Ku70 upon acetylation of these two lysine residues. Our gel filtration chromatograph data suggest that these factors must be small because the patterns of Ku70 and Bax gel filtration chromatograph are similar in HEK-293T cells and SH-SY5Y cells [20]. Another less likely possibility is that Ku70 depletion less sensitive cells may have higher levels of the anti-apoptotic Bcl-2 family of proteins that suppress activation of Bax and its association with Ku70 when Ku70 is acetylated. More work is needed to define these two cell types and how that knowledge can be used in targeted therapies in the treatment of cancer.

Conclusion

Our model that Ku70 acts as a survival factor for Bax-dependent cell death only in certain selected cell types is intriguing, and suggests that it could be beneficial to target Ku70-Bax complex as a therapeutic endpoint. It may be possible to manipulate the association or dissociation of Ku70 and Bax in these Ku70-depletion sensitive cells and preserve these specific cells from Bax-mediated cell death or to induce these cells to die, respectively, without affecting the Ku70-depletion less sensitive cells. A five-residue peptide corresponding to Ku70 (residues 596–600) has been demonstrated to bind Bax and block Bax-induced cell death [25]. Thus, this strategy provides a rationale for screening small molecules that mimic the Ku70-binding domain of Bax and block the interaction between Ku70 and Bax by competing with Bax for Ku70 binding, resulting in inducing cell death in these Ku70-depletion sensitive cells. Agents that block Bax binding to Kui70 resulting in cell death may be tested in clinical settings, either alone or in combination with radiotherapy or DNA damaging agents, to target cancer cells that are sensitive to Ku70 depletion, like that in N-type NB cells.

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