

Nek5 Association with Mitochondria Proteins and Functions: Is It a Nek Family characteristic?

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Short-Communication

The human kinome and all its associated signaling proteins comprise an important network that is crucial for the regulation on the majority of cellular functions. The NIMA-related kinases (NEKs) are a family of serine/threonine kinases involved in cell cycle control in fungi, mammals and other eukaryotes. In humans, eleven genetically distinct members were identified, named Nek1 to Nek11. Besides the well-established mitotic entry function, many other functions have been recently attributed to this class of proteins such as DNA damage response, cytokinesis, nuclear envelope breakdown and primary ciliary and mitochondrial functions [1-3]. Despite having highly conserved kinase domains, the selectivity and diversity of partners in the Nek family is mainly due to heterogeneity of the regulatory domain, the region in where many of these proteins interact.

Most of the new Nek functions have been discovered through interactome studies. After the Human Genome Project, researchers are focusing on building networks of protein-protein interactions, which can provide insight into the mechanisms of cell functions. The kinase interactome studies have been scarce due to the transient and weak nature of the protein-protein interactions and they may occur only in a specific cellular context or compartment [2,4].

Nek5 is one poorly studied member of the human Nek family and in our recent paper a yeast two-hybrid screening was performed with a human universal cDNA library using the C-terminal regulatory region of Nek5 as bait. Three mitochondrial proteins: metaxin-2 (MTX2), BCL2-associated transcription factor 1 (BCLAF1) and cytochrome C oxidase assembly protein 11 (COX11) were identified raising the possibility that Nek5 is involved in the regulation of mitochondrial functions [5].

Neks are mainly cytoplasmic proteins, but for the first time Nek5 was identified in mitochondria where we demonstrated its protective effects in cell death. Stable cells expressing Nek5 were treated with thapsigargin and regulated cell death by decreasing the level of Reactive Oxygen Species (ROS).

Previously, Nek1 had been also located at mitochondria where it has been shown to regulate mitochondrial cell death through phosphorylation of voltage dependent anion channel 1 (VDAC1) on serine 193 [6,7]. Furthermore, Nek1 depleted cells from homozygous *kat2j* mice (lacking Nek1 expression) presented exacerbated mitochondrial membrane permeability (MMP) and consequently high levels of cell death [6,7]. In cells depleted for Nek5 we showed a tendency also for the cells to be more prone to ROS formation and cell death [5]. In addition, some articles suggest that the depletion of NEK2 sensitizes HeLa cells to apoptosis [8] and in combination with

Paclitaxel and doxorubicin, Nek2 siRNA promote breast cancer cell apoptosis [9]. Nek7 was also recently associated to a special type of cell death: the pyroptosis, a form of programmed inflammatory cell death [10-13]. Shi et al. [13] showed that in macrophages Nek7 activates, in a kinase-independent manner, NLRP3 promoting inflammasome assembly in response to microbes and danger signals. During interphase, the infection induces caspase-1 activation as well as production of IL-1 β in a Nek7-dependent manner, but during mitosis the response was much lower, supporting the idea that the effects of Nek7 are non-redundant [13]. Nlrp3 inflammasome has been associated to many human diseases such as atherosclerosis, type 2 diabetes or Alzheimer's disease in a Nek-7 dependent process [10-12], suggesting that Nek7 and other members of the family may also be associated to these diseases.

Other members of the Nek family have recently been associated to cell death. Silencing or defects in Nek3 [14], Nek4 [15], Nek6 [16], Nek8 [17], Nek9 [18] and Nek11 [19] have also been related to an increase in cell death rates. Additionally, our group also observed the mitochondrial localization of the Nek4 [3]. The roles of the Neks during the cell cycle are clear, but now, those findings raise a new role of the family associated to mitochondrial cell death and the function of each member and their partners need to be re-evaluated.

Myogenic differentiation is driven by apoptotic signaling events such as caspase-3 activation [20]. Nek5 was identified in a screen for novel kinase substrates of caspase-3 [21]. C2C12 myoblasts over-expressing Nek5 promoted myogenic differentiation by its C-terminal cleavage driven by Caspase-3 [22]. As caspase-3 is one of the protagonists of the apoptosis pathway, the role of Nek5 in this process may be wider than expect.

The interaction of Nek5 with proteins associated to apoptosis is not restricted to caspase-3, MTX2, COX11 or BCLAF1. We also observed by an immunoprecipitation assay of Nek5 followed by Mass Spectrometry analysis the interaction with NADH dehydrogenase iron-sulfur protein 3; thioredoxin-dependent peroxide reductase; serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform and Endophilin-B1 (unpublished data), both latter proteins are related to cell death, and therefore give additional support to the idea of an involvement of Nek5 in this cell process.

As previously stated, many studies suggested an involvement of Neks in the cell death process, but the mechanisms in which they participate need to be investigated more deeply. Based on these works it is possible to infer that Neks have their function not exclusively related to cell cycle, DNA damage response or cilia. The mitochondria-related processes need to be analysed further. We think that ours and the works of other groups open a new perspective for the study not

only of the cell death mediated by the members of the Nek family of kinases but also their role in cancers. In conclusion, the knowledge about the function of each Nek as well as their mechanism of action may contribute to open new avenues for therapeutic strategies, where the combined treatment using Nek siRNA or kinase inhibitors and chemotherapeutic agents can be effective and serve as a novel therapeutic option for the treatment of cancer.

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