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Molecules in focus

Death-associated protein kinase 2: Regulator of apoptosis, autophagy and inflammation

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ABSTRACT

Death-associated protein kinase 2 (DAPK2/DRP-1) belongs to a family of five related serine/threonine kinases that mediate a range of cellular processes, including membrane blebbing, apoptosis, and autophagy, and possess tumour suppressive functions. The three most conserved family members DAPK1/DAPK, DAPK2 and DAPK3/ZIPK share a high degree of homology in their catalytic domain, but differ significantly in their extra-catalytic structures and tissue-expression profiles. Hence, each orthologue binds to various unique interaction partners, localizes to different subcellular regions and controls some dissimilar cellular functions. In recent years, mechanistic studies have broadened our knowledge of the molecular mechanisms that activate DAPK2 and that execute DAPK2-mediated apoptosis, autophagy and inflammation. In this "molecules in focus" review on DAPK2, the structure, modes of regulation and various cellular functions of DAPK2 will be summarized and discussed.

1. Introducing DAPK2

DAPK2, also known as DAPK related protein 1 (DRP-1), was identified by database screening for EST sequences homologous to DAPK1 in two independent studies 15 years ago (Inbal et al., 2000; Kawai et al., 1999). The DAPK2 gene, which can only be found in vertebrates (Shoval et al., 2011), maps to mouse chromosome 9 and human chromosome 15q22 and translates into a 43 kDa protein of cytosolic localization (Kawai et al., 1999). The protein contains a N-terminal kinase domain with high homology to DAPK1 catalytic domain (Kawai et al., 1999), a conserved CaM-binding autoregulatory domain and a C-terminal tail with no homology to any known protein (Inbal et al., 2000). No overt abnormalities or infertility were reported in a mouse strain with genetic deletion of DAPK2 (Guay et al., 2014). Recently, an alternative splicing variant, denoted DRP-1β, was identified (Shoval et al., 2011). DRP-1β lacks the extra-catalytic domains of DAPK2, which are replaced by a single coding exon closely related to the extra-catalytic sequence of DAPK3. Hence, DRP-1β is a protein kinase of 55 kDa devoid of a CaM-binding domain, but possessing a leucine zipper-like motif.

Upon activation, DAPK2 and DRP-1β phosphorylate the regulatory light chain of myosin II (MLC) (Geering et al., 2014; Inbal et al., 2000; Kawai et al., 1999; Shoval et al., 2011) and induce membrane blebbing and autophagy (Inbal et al., 2000, 2002; Kawai et al., 1999; Shoval et al., 2011). Given the multitude of substrates identified for DAPK1 (for a recent review see Bialik and Kimchi (2014)) and the diverse cellular functions attributed to DAPK2, it is likely that DAPK2 and DRP-1β will possess many more, yet-to-be-identified in vivo substrates.

2. DAPK2 structure and cell biological workhorses

DAPK2 is defined by three structural features (Fig. 1). Pending on the start codon used during translation, DAPK2 is made of 360 (Inbal et al., 2000) or 370 (Kawai et al., 1999) amino acids, with the nomenclature based on 370 amino acids used in this article. At the N-terminus, ranging from residue 23 to 285, the enzyme contains its catalytic domain with 80% homology to DAPK1 kinase domain (Kawai et al., 1999). The crystal structure of the kinase domain was resolved a few years ago, suggesting that DAPK2 catalytic domains form dimers (Patel et al., 2011). This is in agreement with the identification of a key structural feature of the DAPK family of proteins, a short segment of mostly positively charged residues termed the basic loop (amino acids 55–66), which was shown to mediate DAPK homodimerization (Zimmermann et al., 2010). Lysine 52 within the catalytic domain is the ATP-binding site and mutation of K52 to an alanine residue renders DAPK2 inactive (K52A mutant (Kawai et al., 1999)) and shows dominant-negative behaviour towards wild-type DAPK2 (Inbal et al., 2000). Residues 288–330 form the Ca^{2+}/CaM-binding autoregulatoy domain, highly homologous to DAPK1. In the absence of CaM, the autoregulatory domain binds to the catalytic cleft, blocking access of exogenous substrates. Upon CaM binding,

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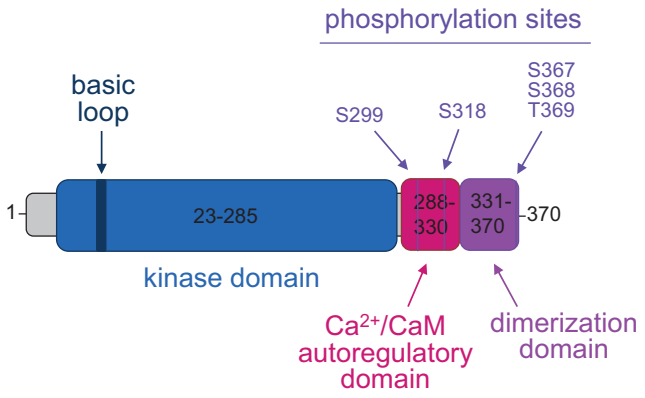

Fig. 1. Schematic diagram of DAPK2 protein structure, using amino acid numbering according to Kawai et al. (1999). The 43 kDa Ca^{2+}/CaM-regulated Ser/Thr kinase bears 3 domain structures. The kinase domain determines substrate specificity and allows for homodimerization via its N-terminal basic loop. The Ca^{2+}/CaM-binding autoregulatory domains dictates kinase catalytic activity, by de-blocking substrate access when bound to Ca^{2+}/CaM. Two phosphorylation events impact on catalytic activity, i.e. autophosphorlyation of S318 decreases DAPK2 activity whereas S299 phosphorlyation by cGMP-dependent protein kinase I enhances DAPK2 activity. The C-terminal dimerization domain allows for homodimerization. Phosphorylation of S367, S368, T369 was shown to decrease homodimerization.

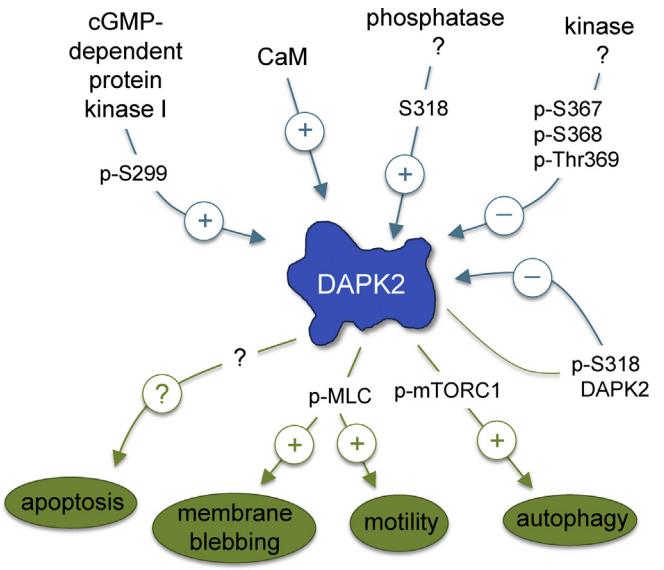

Fig. 2. Summary of DAPK2 signalling. Schematic representation of the molecular mechanisms regulating DAPK2 activity, coupled to DAPK2 effector functions. DAPK2 activity is regulated by post-translational modifications (autophosphorylation and phosphorylation by cGMP-dependent protein kinase I) and protein interactions (CaM). Increased DAPK2 activity was shown to mediate apoptosis, membrane blebbing, motility and autophagy, through direct phosphorylation of MLC and mTORC1 by DAPK2.

the autoregulatory domain is pulled away and substrates can be phosphorylated. Deletion of the CaM-binding domain results in a constitutively active DAPK2 variant, called ΔCaM mutant. At the C-terminus, DAPK2 possesses a 40-amino acid sequence with no homology to any known protein. This C-terminal tail was shown to mediate DAPK2 homodimerization. A DAPK2 mutant lacking the C-terminus (denoted DAPK2 Δ40 mutant) shows decreased DAPK2 activity (Inbal et al., 2000). For a recent in-depth review on the structure of all DAPK family members, see Shiloh et al. (2014).

3. Regulation of DAPK2 activity

The regulation of DAPK2 activity occurs at multiple layers, with spatial and temporal distinctive expression patterns due to DAPK2 promoter methylation and stimulation-dependent alterations in DAPK2 activity due post-translational modifications and protein:protein interactions.

At the transcriptional level, DAPK2 expression seems to be controlled – at least partially – by promoter methylation. Hence, low levels of DAPK2 in myeloid precursor cells could be increased by demethylating agents (Rizzi et al., 2007), and downregulation of DAPK2 expression in Hodgkin lymphoma was associated with DAPK2 promoter hypermethylation (Tur et al., 2009). The transcription factor SP1 was shown to be indispensable for basic DAPK2 promoter activity, with E2F1 and KLF6 transcription factors necessary for further DAPK2 regulation (Britschgi et al., 2008). Several other transcription factors have been implicated in the regulation of DAPK2 transcription. In malignant kidney and epithelial cells, β-catenin decreased DAPK2 expression through the regulation of the transcription factor Tcf-4 (Li et al., 2009), whereas PML-RARα was shown to be responsible for DAPK2 repression in acute promyelocytic leukaemia (Humbert et al., 2014). During granulopoiesis, DAPK2 mRNA and protein levels are elevated due to transcriptional activation by PU.1 and C/EBPα, which both directly bind to the DAPK2 promoter (Humbert et al., 2014). In addition to temporal differences in DAPK2 expression levels, also spatial differences were demonstrated. Northern Blot and quantitative PCR analysis of DAPK2 expression in various tissues revealed a highly abundant expression of DAPK2 mRNA in bone marrow, intermediate expression in lung and skeletal muscle, with lower expression levels in brain, ovary, placenta, and pancreas (Fang et al., 2008; Kawai et al., 1999).

At the post-translational level, DAPK2 activation is mediated by several interlinked steps and additional phosphorylation events (Fig. 2). Following cellular stimulation (e.g. by FAS or TNF ligation), dephosphorylation of DAPK2 at S318 (Shani et al., 2001) by a unknown phosphatase allows for Ca^{2+}-loaded CaM binding to the autoregulatory domain, releasing the auto-inhibitory binding of the domain to the catalytic cleft of DAPK2, thereby allowing for access of substrates. At the same time, dephosphorylation of S318 also enhances homodimerization of DAPK2 (Inbal et al., 2002). The nature and impact of homodimerization on DAPK2 activity is a matter of controversy. On the one hand, the resolution of the crystal structure of DAPK2 catalytic domain revealed that homodimerization of DAPK2 mediated by binding of two apposed catalytic domains to each other does not allow for substrate binding (Patel et al., 2011), implying decreased DAPK2 activity upon homodimerzation. On the other hand, C-tail deletion mutants demonstrated that homodimerization mediated by the C-terminus increases DAPK2 activity and is necessary for the DAPK2-mediated membrane blebbing (Inbal et al., 2000, 2002; Shani et al., 2001). The resolution of the full-length DAPK2 structure will hopefully solve this issue.

In addition to the described regulatory mechanisms, DAPK2 was recently shown to be phosphorylated by cGMP-dependent protein kinase I on S299 (Isshiki et al., 2012). Phosphorylation of S299, which lies within the CaM-binding autoregulatory domain, increased DAPK2 activity, independent of CaM binding, but by counter-acting the autoinhibitory mechanism resulting from S318 phosphorylation. Interestingly, a phoshomimetic mutant of the respective S289 in DAPK1 attenuated DAPK1 apoptotic activity, suggesting that the S289 phosphorylation, which was shown to be mediated by RSK, may decrease DAPK1 apoptotic activities (Anjum et al., 2005). These results indicate that DAPK1 and DAPK2 can be regulated by opposing mechanisms. Moreover, DAPK2 phosphorylation of the C-terminal S367, S368 and T370 residues by an unidentified kinase (of note, cGMP-dependent protein kinase I was shown to phosphorylate S367 and S368) allowed for binding