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Review

NEDD4: The founding member of a family of ubiquitin-protein ligases

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ABSTRACT

Ubiquitination plays a crucial role in regulating proteins post-translationally. The focus of this review is on NEDD4, the founding member of the NEDD4 family of ubiquitin ligases that is evolutionarily conserved in eukaryotes. Many potential substrates of NEDD4 have been identified and NEDD4 has been shown to play a critical role in the regulation of a number of membrane receptors, endocytic machinery components and the tumour suppressor PTEN. In this review we will discuss the diverse pathways in which NEDD4 is involved, and the pathophysiological significance of this important ubiquitin ligase.

1. Introduction

Ubiquitination is a post-translational protein modification that is critical for a number of cellular processes. Ubiquitination involves the covalent attachment of the 8 kDa protein ubiquitin to one or more lysine residues in the substrate protein to signal proteins for degradation, altered localisation, trafficking or function. Substrate proteins can be mono-ubiquitinated, multi-monoubiquitinated or poly-ubiquitinated, with the type of ubiquitination determining the fate of the protein. Ubiquitin itself has seven lysine residues, allowing for different ubiquitin linkage types; for example the well-studied K48-linkage typically targets proteins for proteasomal degradation (Hershko and Ciechanover, 1998) whereas K63 linkages are associated with protein trafficking and lysosomal degradation (Hicke and Dunn, 2003).

Ubiquitin is covalently attached to a protein substrate via an energy dependent three step process, involving an E1 ubiquitin activating enzyme, an E2 ubiquitin conjugating enzyme and an E3 ubiquitin protein ligase. The E3 ubiquitin ligase largely determines the substrate specificity of the system and in mammals there are several hundred ubiquitin protein ligases (Hershko and Ciechanover, 1998). These can be grouped into two main classes; the RING (Really Interesting New Gene) E3s which mediate the direct transfer of ubiquitin to the substrate (Deshaies and Joazeiro, 2009), and the HECT (Homologous to E6-AP C-Terminus) E3s which are involved in the transfer of activated ubiquitin from the E2 to the substrate by forming an intermediate complex with the C-terminus of the E3 (Rotin and Kumar, 2009). This review will focus on the HECT type ubiquitin ligase NEDD4, one of the first HECT E3 ligases discovered, and the founding member of the NEDD4 family of HECT ubiquitin ligases.

2. History of NEDD4 discovery

The *NEDD4* gene was cloned in 1992 as one of a number of murine Nedd (Neural precursor cell expressed developmentally down-regulated) genes differentially expressed in the central nervous system (Kumar et al., 1992). At the time of its cloning, the predicted protein had only one known domain — an N-terminal calcium/lipid-binding domain (C2 domain). The presence of three partial repeats of approximately 40 amino acids containing two conserved tryptophan residues in the middle part of the protein was also noted. These repeats, now known to occur in numerous proteins, are widely known as WW domains (Bork and Sudol, 1994). Subsequently, in the following year the C-terminal region of NEDD4 was found to be similar to human E6-AP, the papilloma virus oncoprotein E6-associated protein. E6-AP was the first discovered ubiquitin-protein ligase and it was shown to be involved in the E6-mediated ubiquitination of p53 (Scheffner et al., 1993). The C-terminus of E6-AP comprising the catalytic domain was named HECT (homologous to the E6-AP C-terminus) (Huibregtse et al., 1995). E6-AP became the founding member of the HECT type of E3 ubiquitin ligases, of which now there are 29 human members. In yeast the first HECT ligase was Rsp5p/Npi1p from *Saccharomyces cerevisiae*, which was originally discovered as a suppressor of mutations in the *SPT3* gene (Huibregtse et al., 1995). NEDD4 and similar proteins discovered subsequently became a family of HECT ligases, comprising 9 human proteins including

Abbreviations: RING, Really Interesting New Gene; HECT, Homologous to E6-AP C-Terminus; Ndfip1, Nedd4 family interacting protein 1; AMPARs, alpha-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid receptors; EGFR, epidermal growth factor receptor; FGFR1, Fibroblast growth factor receptor 1; PTEN, phosphatase and tensin homologue depleted on chromosome ten; IGF-1, insulin like growth factor-1; ENaC, epithelial sodium channel; IGF-1R, insulin-like growth factor 1 receptor; PI3K, phosphoinositide 3-kinase

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NEDD4, NEDD4-2 (NEDD4L), ITCH, SMURF1, SMURF2, WWP1, WWP2, NEDL1 AND NEDL2 (Scheffner and Kumar, 2014). Rsp5 is also a member of the NEDD4 family, suggesting that these ligases are highly conserved.

3. NEDD4 orthologues and structure

As mentioned above, NEDD4 is a highly evolutionarily conserved protein from yeast to man, and was initially cloned as a highly expressed gene in the early embryonic brain (Kumar et al., 1992, 1997). There are 94 orthologues of NEDD4 in the NCBI database, all sharing the same modular structure consisting of an N-terminal C2 domain, 3–4 WW domains and a C-terminal catalytic HECT domain for ubiquitin protein ligation (Harvey and Kumar, 1999) (Fig. 1A). The C2 domain is a calcium-dependent lipid-binding domain around 116 amino acids in length that targets proteins to phospholipid membranes (Dunn et al., 2004), and can also be involved in protein–protein interactions (Morrione et al., 1999; Plant et al., 2000). The C2 domain-mediated membrane translocation is required for some cellular functions of NEDD4 (Dunn et al., 2004). The WW domains are protein–protein interactions domains, usually around 40 amino acids in length, containing two conserved tryptophans (W) residues that are 21 amino acids apart (Bork and Sudol, 1994). The WW domains interact with proline rich PPxY (PY) motifs and can also interact with phospho-serine/threonine residues in substrates (Sudol et al., 1995). The number of WW domains can vary between NEDD4 family members, and also between species i.e. the NEDD4 protein in human, chicken and *Xenopus* contains four WW domains, whereas mouse, zebrafish, *Drosophila* and yeast contains three WW domains (Yang and Kumar, 2010) (Fig. 1B). The HECT domain is a highly conserved domain that comprises around 350 amino acids, and contains a conserved cysteine residue that forms an intermediate thioester bond with the activated ubiquitin accepted from an E2, before catalysing the ubiquitination of a lysine in the substrate protein (Rotin and Kumar, 2009). There are a number of E2s that are able to transfer ubiquitin to NEDD4, including Ubc4, UbcH5B, UbcH5C, UbcH6 and UbcH7 (Anan et al., 1998; Fotia et al., 2006).

The human *NEDD4* gene is located on chromosome 15q21.3 and comprises 30 exons (HGNC:7727) shown to encode a ~120 KDa protein. There are five NEDD4 protein variants in the NCBI database, all of which share 100% identity from the first WW domain through to the end of the protein, only varying in the N-terminal region which includes the C2 domain. Recently it was reported that there is a 75 kDa NEDD4 isoform found exclusively in myotonic dystrophy type 2 muscle in addition to full length NEDD4 (Screen et al., 2014). NEDD4 protein is localised to the cytoplasm, mainly in the perinuclear region and cytoplasmic periphery of human cultured cells (Anan et al., 1998). NEDD4 is also found in exosomes when recruited by the NEDD4 family interacting protein Ndfip1 (Putz et al., 2008).

4. NEDD4 binding partners and targets

A number of *in vitro* binding studies and proteomic approaches have been used to identify potential NEDD4 substrates (see below for a summary and Table 1 for a list of interacting proteins).

4.1. Ion channels and membrane transporters

The epithelial sodium channel (ENaC) is a transmembrane ion channel that contains a PY motif in its cytoplasmic tail. Using yeast two hybrid studies, rat NEDD4 was shown to bind to the PY motif in ENaC via its WW domains (Staub et al., 1996). Furthermore, in response to increased intracellular sodium, NEDD4 binds and ubiquitinates ENaC to mediate the down-regulation of sodium channel activity (Dinudom et al., 1998).

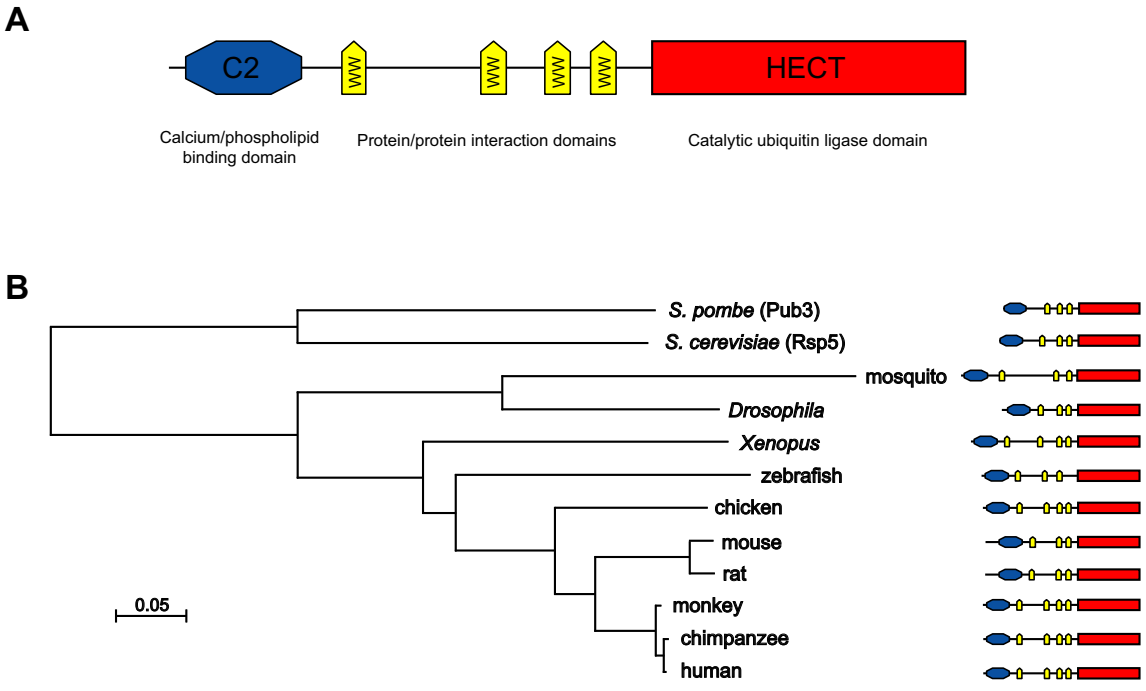

Fig. 1. (A) Schematic structure of the NEDD4 protein. Schematic of the modular structure of the human NEDD4 protein. The C2 calcium/phospholipid binding domain mediates NEDD4 binding to membranes, and is also involved in substrate recognition. The WW domains are protein–protein interaction domains that bind to conserved PY motifs in substrates and regulatory proteins. The catalytic ubiquitin ligase domain binds the E2 conjugation enzyme and forms a thioester bond with ubiquitin before transferring ubiquitin to the substrate. (B) Phylogenetic relationship of NEDD4 proteins from various species. NEDD4 sequences were obtained from the NCBI protein database as follows; *S. pombe* (*Schizosaccharomyces pombe Pub3*; NP_595793.1), *S. cerevisiae* (*Saccharomyces cerevisiae* Rsp5p; AAC03223.1), mosquito (*Anopheles gambiae*; XP_003436401.1), *Drosophila* (*Drosophila melanogaster*; NP_996116.1), *Xenopus* (*Xenopus laevis*; NP_001084258.1), zebrafish (*Danio rerio*; NP_001029358.1), chicken (*Gallus gallus*; XP_413791.3), mouse (*Mus musculus*; NP_035020.2), rat (*Rattus norvegicus*; NP_037118.1), monkey (*Macaca fascicularis*; XP_005559683.1), chimpanzee (*Pan troglodytes*; XP_523083.3),and human (*Homo sapiens*; NP_006145.2). Sequences were aligned using NCBI COBALT (Constraint Based Multiple Protein Alignment Tool) http://www.st-va.ncbi.nlm.nih.gov/tools/cobalt/re_cobalt.cgi (Papadopoulos and Agarwala, 2007) and the minimum-evolution Phylogenetic Tree output displayed. The individual domains on the NEDD4 schematics were identified using NCBI Conserved Domain Database search http://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi (Marchler-Bauer et al., 2011) and are drawn roughly to scale. The scale bar indicates evolutionary distance (Grishin, 1995).