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Review

Mitochondrial E3 ubiquitin ligase 1: A key enzyme in regulation of mitochondrial dynamics and functions

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ABSTRACT

Mitochondrial E3 ubiquitin ligase 1 (Mul1) is a multifunctional mitochondrial membrane protein with its RING domain exposed to the cytoplasm. On the one hand, Mul1 functions as a ubiquitin-ligase to ubiquitinate a bunch of signal molecules, such as mitofusin2 (Mfn2), Akt, p53 and ULK1, through its RING finger domain, leading to proteins degradation. On the other hand, Mul1 acts as a small ubiquitin-like modifiers (SUMO) E3 ligase to sumoylate certain proteins, such as dynamin-related protein 1 (Drp1), enhancing protein stabilization. Through the dual functions of ubiquitination and SUMOylation, Mul1 involves in regulation of many physiological and pathological processes, such as mitochondrial dynamics, cell growth, apoptosis and mitophagy. In addition, Mul1 can also directly activate or interact with some proteins, such as NF-κB and JNK, to take part in the regulation of cellular apoptosis. This review summarizes recent progress in relevant studies on the physiological and pathological functions of Mul1 and pays special attention to its role in regulation of mitochondrial dynamics.

Contents

1. Introduction

Mitochondrial E3 ubiquitin ligase 1 (Mul1), also named as mitochondrial-anchored protein ligase (MAPL) (Neuspiel et al., 2008), mitochondrial ubiquitin ligase activator of NF-κB (MULAN) (Li et al., 2008), growth inhibition and death E3 Ligase (GIDE) (Zhang et al., 2008) or Hades (Jung et al., 2011), is a multifunctional protein ligase embedded in the outer mitochondrial membrane with its RING domain facing the cytoplasm, where it can interact with other components of the ubiquitin and SUMO systems (Li et al., 2008; Neuspiel et al., 2008; Zungu et al., 2011). Phylogenetic analyses demonstrate that Mul1 has been present in the holozoan and viridiplantae lineages since their very beginnings (Li et al., 2008; Wideman and Moore, 2015). The mRNA expression of Mul1 is observed in many tissues, with the highest

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abundance in heart (Neuspiel et al., 2008). Endogenous Mul1 is mainly detected in mitochondria while it is not found in the other organelles such as for Golgi, endoplasmic reticulum and cytosol. It has been shown that the intact RING finger and proper subcellular localization are necessary for Mul1 to regulate the mitochondrial dynamics and functions (Li et al., 2008; Neuspiel et al., 2008). Transmembrane sequences with moderate hydrophobicity and net positive charge in flanking regions (called as signal-anchor or TMD2 domains) of Mul1 also influence its action to mitochondria. Mutation of two positively charged residues in C-terminal (R260A/K261A) of Mul1 leads to switching its target to the endoplasmic reticulum, while an N-terminal Flag tag in Mul1 results in its cytosolic localization. Mul1 mutant with TMD2 deletion (D242–259) or R260A/K261A mutation is defective in promoting perinuclear clustering of the organelle, although the TMD2-deleted mutant (D242–259) still remains partially localization to mitochondria. Isolated cytoplasmic domain (amino acids 260–352) of Mul1 does not affect the mitochondrial distribution or morphology. Collectively, an intact structure is required for Mul1 to play extensive roles in regulations of mitochondrial dynamics, cell growth, apoptosis and autophagy. This review aims to summarize recent findings in this regard.

2. Biological functions of Mul1

2.1. Mul1 and ubiquitination

The major biological function of Mul1 is ubiquitination, an enzymatic process that involves the binding of an ubiquitin protein to substrate proteins. The ubiquitin–proteasome system (UPS) is a primarily cytosolic multi-component system, which functions as post-translational modification of specific substrates with ubiquitin by the cooperative interaction of multiple enzymes and is important for many cellular processes (Livnat-Levanon and Glickman, 2011; Pickart and Eddins, 2004; Ross et al., 2015). Ubiquitin (Ub) is a small (76 amino acids) protein which is joined to amino groups on a substrate either at the free amino terminus or on an internal lysine residue. Ubiquitination of a protein with Ub can influence its biological half-life, cellular localization and/or the recognition by its binding partners. Generally, ubiquitination of a substrate is a cascade of ubiquitin-transfer reactions involving three sequential steps: (1) ubiquitin activation (E1), (2) carrier conjugation (E2), and (3) ligation of Ub to the target protein via a dedicated ligase (E3). Following the conjugation of ubiquitin to the target protein, the target protein is degraded by the proteasome and free ubiquitin is recycled (Glickman and Ciechanover, 2002; Komander and Rape, 2012). In this process, the E3 ligase plays a key role in controlling the specificity of target-protein selection and the amount of individual target proteins (de Bie and Ciechanover, 2011). To date, five large families of Ub ligases are classified by the following E3 signature motifs: HECT, RING, U-box, LAP and PHD (Fang and Weissman, 2004). Among them, enzymes with HECT and RING-finger domains are the two major classes of E3 Ub ligases. The RING-finger E3s, acting as scaffold protein, are capable of transferring the activated Ub directly from E2 to the substrates (Budhidarmo et al., 2012; Chasapis and Spyroulias, 2009; Deshaies and Joazeiro, 2009; Glickman and Ciechanover, 2002).

Mul1 belongs to the RING family of E3 Ub ligases and its enzymatic activity is dependent on the intact RING finger domain. It has been reported that Mul1 interacts with multiple signal molecules such as Mfn2, AKT, p53 and ULK1 through its RING finger domain (Bae et al., 2012; Jung et al., 2011; Li et al., 2015; Lokireddy et al., 2012; Yun et al., 2014). Mul1 ubiquitinates them to promote their degradation via the ubiquitin–proteasome system, and in turn takes part in various biologic processes including mitochondrial dynamic, cellular growth, apoptosis and autophagy, but the underlying mechanisms for these functions are unclear. It is likely that Mul1 interacts with E2 Ub conjugating enzymes, which forms a complex to bind and ubiquitinates specific substrates. Using the yeast two-hybrid system, Camilla T and his colleagues isolate and identify four different E2 conjugating enzymes (Ube2E2, Ube2E3, Ube2G2 and Ube2L3), which are able to interact with the cytosolic portion of Mul1 containing the RING domain (Ambivero et al., 2014). This study provides evidence that GABARAP, a member of the Atg8 family (Colecchia et al., 2012), is a specific interactor of Mul1–Ube2E3, which might be a potential mechanism for Mul1 to participate in mitophagy. It is not known, however, whether all of the four E2 conjugating enzymes involve in the Mul1-mediated ubiquitination of substrates. It is worth to point out, according to Braschi et al.'s report (Braschi et al., 2009), that the Mul1-catalyzed ubiquitination is supported only when the concentrations of the enzymes are in the micromolar range. As the cellular concentrations of the enzymes do not reach the micromolar range under physiological condition, it is unlikely that Mul1 provides significant ubiquitination activity under normal condition.

2.2. Mul1 and SUMOylation

Another biological function of Mul1 is SUMOylation, a post-translational modification process analogous to ubiquitination in terms of the reaction scheme and enzyme classes used (Elgass et al., 2013). Instead of conjugation by ubiquitin, SUMOylation involves addition of SUMO (small ubiquitin-like modifier), where SUMO is covalently conjugated to specific lysine residues of substrate protein (Eifler and Vertegaal, 2015; Flotho and Melchior, 2013). SUMOylation involves in diverse biological processes through regulations of protein stabilization, activity, localization and interaction. Similar to ubiquitination, SUMOylation is carried out through an enzymatic cascade including an E1 heterodimer, an E2 ligase (Ubc9), and an E3 ligase that controls the reaction specificity. To counteract the function of SUMO E3 ligase, SUMO isopeptidases (SENPs) can cleave off SUMO from specific substrates (Huang et al., 2015; Scorrano and Liu, 2009; Zungu et al., 2011).

The SUMOylation function of Mul1 has been identified by Braschi et al. recently (Braschi et al., 2009; Prudent et al., 2015). They have demonstrated that Mul1 functions as a mitochondrial SUMO E3 ligase. It is able to place SUMO1 on DRP1 in an ATP-dependent manner and to support SUMOylation on a synthetic peptide containing the SUMO consensus sequence or on isolated mitochondrial proteins. The identification of Mul1 as a mitochondrial SUMO E3 ligase dramatically expands the scope of enzymes for SUMOylation, supports the importance of non-nuclear SUMOylation and provides a platform to study the SUMOylation outside the nucleus. It has been reported that over-expression of Mul1 leads to mitochondrial fragmentation, which is dependent upon the RING-finger domain of Mul1, and that knockdown of Mul1 also perturbs mitochondrial dynamics, although it is not as evident as that in cells ectopically expressing the Mul1(Li et al., 2008), supporting a regulatory function of Mul1 in controlling the mitochondrial morphology. Interestingly, different from its ubiquitination activity, Mul1 is sufficient to sustain a robust SUMOylation reaction even its concentrations are in a nanomolar range, indicating that Mul1 preferentially acts as a SUMO E3 ligase (but not E3 Ub ligase) under physiological conditions (Braschi et al., 2009).

2.3. Mul1 and NF-κB

Mul1 has previously been identified as a mitochondrial ubiquitin ligase activator of NF-κB (MULAN) (Li et al., 2008; Matsuda et al., 2003; Tacchi et al., 2011, 2012; Zemirli et al., 2014), a transcription factor implicated in the regulation of a large number of genes, particularly for those encoding antiapoptotic proteins or proinflammatory cytokines (Espinosa et al., 2015; Napetschnig and Wu, 2013). NF-κB is normally sequestered by inhibitory proteins (IκBs) in the cytoplasm of non-stimulated cells. Phosphorylation of IκBs by inhibitor of NF-κB kinase (IKK), such as IKKα and IKKβ, results in IκBs Lys48 (K48)-linked ubiquitylation and proteasoma degradation, subsequently leading to NF-κB translocation to the nucleus where it initiates gene transcription,