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AMBRA1: Orchestrating Cell Cycle Control and Autophagy for Cellular Homeostasis

Yang Wang[1], Goran Stjepanovic[1,*]

[1]Kobilka Institute of Innovative Drug Discovery, School of Medicine, The Chinese University of Hong Kong, Shenzhen, Guangdong 518172, China

[*]Correspondence should be addressed to Goran Stjepanovic, goranstjepanovic@cuhk.edu.cn

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Abstract

The Activating Molecule in Beclin-1-Regulated Autophagy (AMBRA1) is a scaffold protein involved in many cellular processes, including autophagy, apoptosis, cell growth and development. AMBRA1 functions as a substrate receptor of the DDB1-Cullin4-RBX1 ubiquitin E3 ligase complex that plays key roles in autophagy and the cell cycle regulatory network. Considering the crucial role of AMBRA1 in cellular homeostasis, structural and functional studies are important for understanding the mechanisms that coordinate these cell responses. Autophagy defects and impaired AMBRA1 function may contribute to the pathogenesis of several diseases, including cancer and neurodegenerative disorders. As a result, targeting AMBRA1 has gained interest as a potential therapeutic strategy. Due to the intrinsic disorder of AMBRA1, its structure has not been fully elucidated. A report by Liu *et al.*, provided new insights into the structure and function of AMBRA1 [1]. This mini-review aims to summarize the DDB1-AMBRA1 complex structure and regulatory mechanism and discuss future research directions.

Keywords: Autophagy, Cell-cycle control, Ubiquitination, Tumorigenesis, AMBRA1, E3 ligase

Main Text

Two major intracellular protein degradation pathways, the ubiquitin–proteasome system (UPS) and autophagy are crucial for protein quality control in eukaryotic cells [2]. In general, short-lived and damaged proteins, such as denatured, unfolded or misfolded proteins are targeted by UPS, whereas long-lived proteins, insoluble protein aggregates and organelles are degraded by autophagy. The UPS system depends on ubiquitination by the addition of a small protein ubiquitin to specific lysine residues on target proteins. Ubiquitination is a sequential three-step reaction involving E1 (a ubiquitin-activating enzyme), E2 (a ubiquitin-conjugating enzyme) and E3 (a ubiquitin ligase enzyme), resulting in covalent attachment of ubiquitin to the target protein. The human genome contains two E1 genes and approximately 40 E2 genes. Additionally, there are over 600 E3 ligase genes, and a single E3 ligase may target multiple substrates [3-5]. E3 ligases can be classified into four families: the HECT, RING-finger, U-box and RBR families [6]. The RING-finger family is the most abundant E3 ligase family and is characterized by the presence of a zinc-binding domain called RING (Really Interesting New Gene) [7]. The Cullin4-DDB1-RBX1 complex is a classic member of the RING-finger family [8]. Cullin4 (CUL4) serves as a scaffold, its N-terminus binds to a RING-box protein (RBX1) which brings the E2 and the substrate into close proximity, while the C-terminus interacts with an adaptor protein DDB1 (damage-specific DNA binding protein 1). DDB1 can recruit substrates to the E3 ligase by interacting with different substrate receptors known as DCAFs (CUL4-associated factors).

The activating molecule in Beclin-1-regulated autophagy (AMBRA1) is a DCAF and is reported to bind DDB1 as a substrate receptor for the CRL4 E3 ligase [9]. AMBRA1 was identified as an autophagy-associated protein that coordinates different cellular process including cell proliferation, autophagy, UPS, apoptosis and mitophagy [10-14]. AMBRA1 contains an N-terminal WD40 domain which mediates interaction with DDB1. In addition, AMBRA1 sequence contains a large

proportion of intrinsically unstructured regions (IDR). The conformational flexibility of AMBRA1 IDRs may facilitate multiple protein-protein interactions required for coordination of several biological processes [14,15]. AMBRA1 can positively regulate Beclin1-dependent autophagy through directly binding to Beclin1, which is needed for neurogenesis during the embryonic period [16,17]. Beclin1 and VPS34 are the components of the class III phosphatidylinositol-3-kinase complex, which initiates autophagosome formation by producing phosphatidylinositol-3-phosphate (PI3P) [18,19]. Mechanistically, AMBRA1 mediates K63-linked ubiquitylation of Beclin1, thereby promoting the interaction between Beclin1 and VPS34 and autophagy [20,21]. AMBRA1 is regulated by post-translational modifications, including phosphorylation and ubiquitination. Upon autophagy induction, Unc-51-like autophagy-activating kinase 1 (ULK1) phosphorylates AMBRA1 to promote it release from microtubules by inhibiting AMBRA1 interaction with dynein light chain (DLC)1 [22,23]. Subsequently, AMBRA1 re-localizes to the ER to enable autophagosome formation [23-25]. AMBRA1 also can serve as the substrate of CUL4-DDB1 and RNF2 (The ring finger protein 2) E3 ligase complexes which can mediate its ubiquitination and degradation [9,26].

Three recent reports have revealed that AMBRA1 mediates ubiquitination and degradation of cyclin D1 (**Figure 1a**) [13,27,28]. Cyclin D1 is a cell cycle regulator that controls G1 to S phase progression in many cell types, and a high expression level of this gene is related to cancer development [29]. Mechanistically, cyclin-dependent kinases CDK4/6 bind to cyclin D1 to form an active complex that phosphorylates and inactivates the tumor suppressor protein retinoblastoma protein (RB) [30-32]. Phosphorylated RB dissociates from the E2F transcription factor, and E2F regulates transcription of genes necessary for progression through the S phase and promotes cell cycle progression [33,34]. Loss of AMBRA1 leads to increased cellular levels of cyclin D1, resulting in increased cell proliferation, replication stress and tumorigenic activity [13,27,28] (**Figure 1a**). Moreover, loss of AMBRA1 promotes the formation of cyclin D1/CDK2 complexes, resulting in decreased sensitivity to CDK4/6 inhibitors [28]. These findings highlight the need for combined inhibition of CDK2/4/6 in cancer therapy. In addition to cyclin D1, AMBRA1 can regulate cell proliferation by activating protein phosphatase 2A (PP2A). PP2A dephosphorylates the proto-oncogene c-Myc at Ser62, thereby destabilizing c-Myc and decreasing cell proliferation and tumorigenesis (**Figure 1b**) [10,35]. Taken together, AMBRA1 functions as the platform for extensive crosstalk between cell cycle, proliferation, and autophagy through multiple protein-protein interactions.

Understanding the structural basis of the DDB1-AMBRA1 ^{WD40} complex is essential for elucidating its functional and regulatory mechanisms as well as exploring its therapeutic

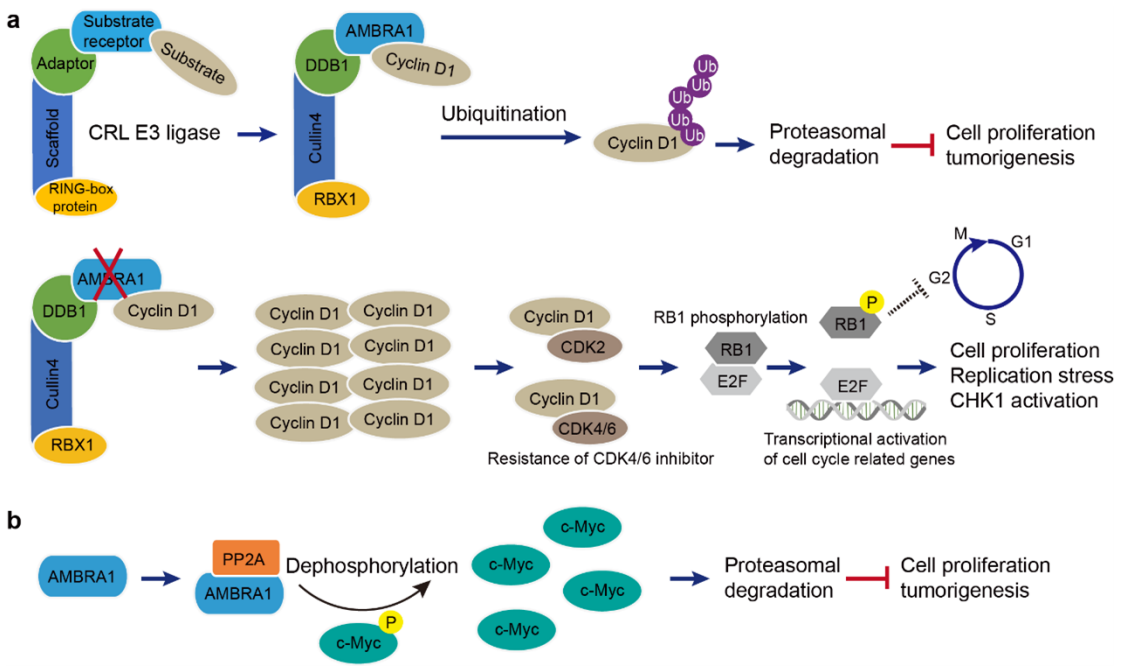

Figure 1. AMBRA1 links to cell cycle and tumorigenic activity. a. Cartoon schematic of the Cullin4-DDB1-RBX1-AMBRA1 complex and its regulation of the cell cycle and cell proliferation by ubiquitination of cyclin D1. The absence of AMBRA1 stabilizes cyclin D1, and results in the formation of the cyclin D1-CDK2 and cyclin D1-CDK4/6 complex, which phosphorylates RB1 and induces cell proliferation. **b.** The AMBRA1-PP2A complex activates the dephosphorylation of PP2A and destabilizes c-Myc.

potential [36,37]. A recent study by Liu et al. determined the cryo-EM structure of the human DDB1-AMBRA1 ^{WD40} complex and revealed a "split" WD40 domain formed by the N-terminal (residues 1-204) and C-terminal (residues 853–1044) regions [1]. DDB1 is composed of three seven-bladed WD40 β-propeller domains, BPA, BPB, BPC, and a C-terminal helical domain (CTD). The BPA and BPC domains are arranged in a double-β-propeller, forming a binding pocket for substrate receptors at the interface (**Figure 2a**). This DDB1 interface pocket is where the AMBRA1 ^{WD40} domain binds through the N-terminal helix-loop-helix motif. The key residues of AMBRA1 participating in the interaction with DDB1 include V10, L13 and W14 (**Figure 2a**). The CUL4-RBX1-DDB1-AMBRA1 ^{WD40} is able to bind the substrate Cyclin D1/CDK4 complex and mediate its ubiquitination. Mutating the key binding residues V10R and L13R in AMBRA1 disrupts the interaction with DDB1 and prevents ubiquitination of Cyclin D1. Disrupting the interaction between DDB1 and AMBRA1 or knocking down AMBRA1 dysregulates the cell cycle control and results in genomic instability [1,13,27,28]. Finally, interaction between AMBRA1 and CRL4 E3 ligase complex is essential for the induction of autophagy and mutations that disrupt the DDB1-AMBRA1 ^{WD40} interaction interface impair autophagy [38]. One notable feature of AMBRA1 is the presence of intrinsically disordered regions within its structure. These regions lack well-defined three-dimensional structures and exhibit high flexibility under the native physiologic conditions. This flexibility allows AMBRA1 to undergo conformational changes and function as a receptor protein in various cellular complexes. The stabilization of the AMBRA1 ^{WD40} domain by DDB1 likely serves to organize and bring together various substrates and CRL4 E3 ligase. This process assists in facilitating the transfer of ubiquitin, enabling AMBRA1 to coordinate multiple biological processes in response to different inputs. Recent studies of the AMBRA1 ^{WD40}-CRL4 E3 ligase complex provide a structural context in which to test how AMBRA1 loss can lead to uncontrolled cell growth and promote tumorigeneses and paving the way for development of novel therapeutic strategies targeting autophagy and cell cycle dysregulation in human diseases.

Conclusion and Future Perspectives

AMBRA1 functions as a scaffold protein to coordinate several cellular processes, including the cell cycle, cell proliferation, neurogenesis, autophagy and the UPS, and has wide implications for a variety of diseases in humans. AMBRA1 acts as a tumor suppressor and regulates two parallel pathways, the c-Myc pathway and the cyclin D–CDK4/6–RB–E2F pathway, which drive the G1–S transition [39]. Decreased AMBRA1 expression levels lead to accumulation of cyclin D, promote cell division and result in desensitization of CDK4/6 inhibitors [13,27,28]. RNF2 E3 ligase in complex with WASH can mediate the ubiquitination and degradation of AMBRA1 [9,26].

It is reported that AMBRA1 plays a key role in tumorigenesis and progression [40]. For example, deficiency of AMBRA1 promotes melanoma growth and invasion [11], induces lung adenocarcinoma in a mouse model, and is correlated with overall worse survival in lung adenocarcinoma patients [13]. Therefore, defects in AMBRA1 might be useful as cancer progression biomarker in oncology. In addition to the identified interactors, such as Beclin1 [41], LC3 [42], cyclin D1[13] and PP2A [10] (**Figure 2b**), a comprehensive proteomic study is needed to unravel the intricate network of AMBRA1 interactions and post-translational modifications. Molecular details of AMBRA1 interactions with different substrates and regulatory proteins are required to fully understand the substrate specificity and for the design of small molecule drugs targeting protein-protein interactions. Furthermore, it would be important to gain a deeper understanding of how cells regulate AMBRA1 expression levels and intracellular distribution and explore potential therapeutic strategies.

The recently reported structure of the DDB1-AMBRA1 ^{WD40} E3 ligase receptor complex provides the basis for further investigations into its therapeutic potential [1]. The molecular details of protein-protein interaction interfaces provide valuable information for identification of new substrates that bind to DDB1-AMBRA1 ^{WD40} and designing of small-molecule drugs. Targeted protein degradation (TPD) is an emerging therapeutic strategy to target dysregulated proteins that play key roles in diseases such as cancer and neurodegeneration. Molecular glues are small molecules that stabilize the interactions between proteins and can be used to reduce cellular levels of disease-causing proteins through TPD [43], enhance the interaction between protein of interest (POI) and the E3 ligase [44,45] and induce POI ubiquitination and degradation [46]. In addition, inhibitors could be designed to disrupt the interaction between E3 ligases which ubiquitinate AMBRA1, to decrease its degradation and potentially stimulate autophagy and/or maintain the cell cycle and proliferation at normal levels (**Figure 2c**). Whether some of these therapeutic strategies could be applied to AMBRA1 requires further research.

AMBRA1 plays a central role in the pathogenesis of multiple system atrophy (MSA) through interaction with α-synuclein and mediate its degradative dynamics. This raises the possibility that molecular modulation targeting AMBRA1 can be a promising candidate for the treatment of synucleinopathies [47]. Small molecules could be as well used to activate AMBRA1 by disrupting protein-protein interactions, such as DLC1 [22,23]. Disrupting the interaction between AMBRA1 and DLC1 can protect cells against apoptosis, induce autophagy and mitophagy in SHSY5Y cells, and enhance the switch from quiescence to proliferation in mouse neural stem cells. This strategy provides the potential to treat neurodegeneration [22]. Artificial Intelligence (AI) and Machine Learning (ML) algorithms have great potential for developing new drugs by improving efficiency, accuracy and speed of drug screening and discovery [48-50]. The employment of AI-driven techniques such as AlphaFold has

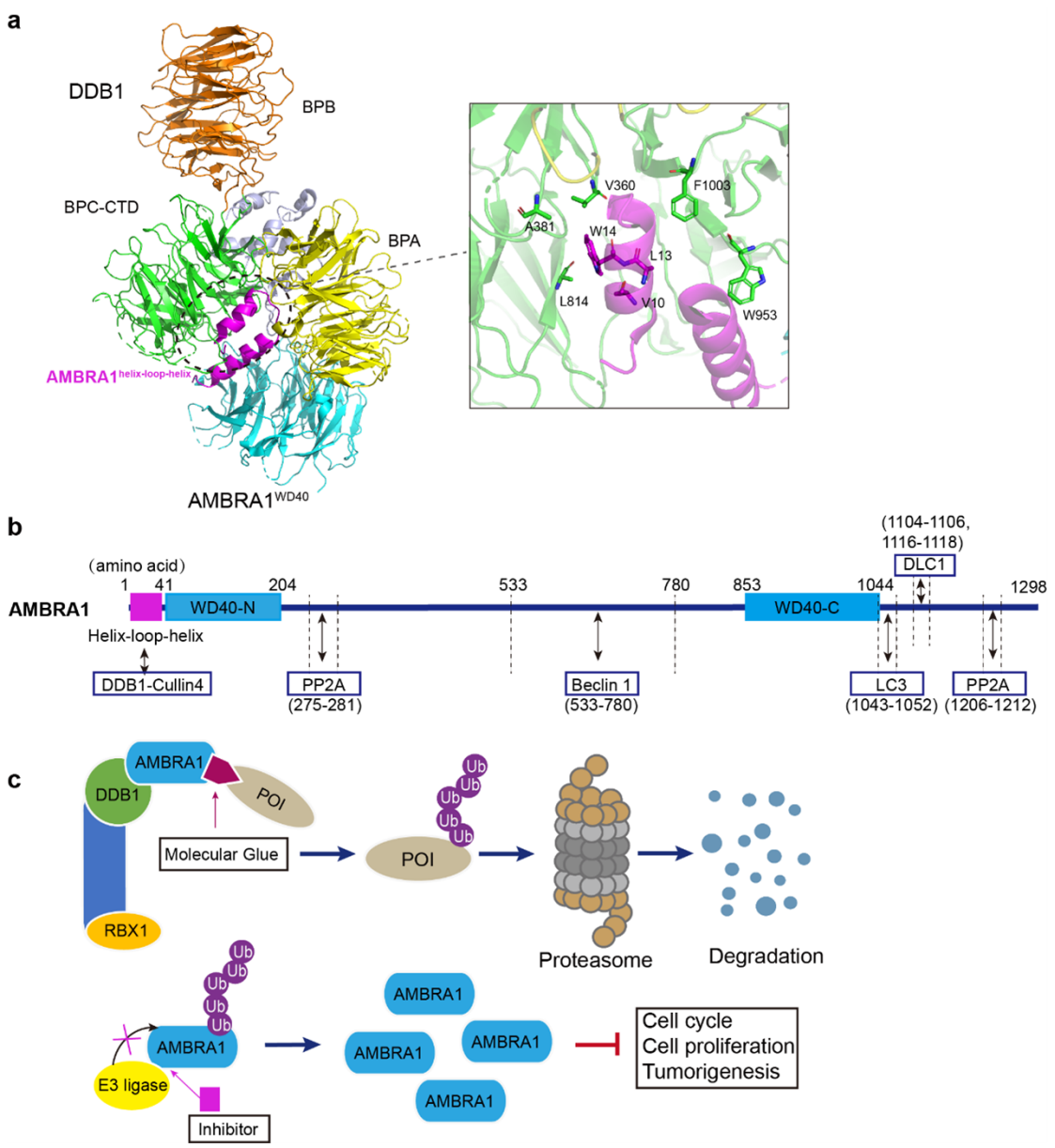

Figure 2. Model of the DDB1-AMBRA1^{WD40} complex and potential small molecule compound development. a. Model of the DDB1-AMBRA1^{WD40} complex and the binding interface (PDB 8WQR) [1]. The DDB1 domain and AMBRA1^{WD40} are colored as follows: BPA domain, yellow; BPB domain, orange; BPC-CTD domain, green and light blue; AMBRA1^{WD40}, cyan. **b.** The identified proteins that interact with AMBRA1 are shown in the corresponding binding region. **c.** The development of potential small-molecule compounds, including molecular glues and inhibitors. Molecular glue may enhance the interaction between the E3 ligase complex and the protein of interest (POI) and promote its degradation; the inhibitor interrupts the interaction between AMBRA1 and E3 ligase, reducing the ubiquitination and degradation of AMBRA1.

greatly aided in prediction and structure determination of AMBRA1, thereby offering deeper insights into its functional mechanisms and opening up new avenues for innovative therapeutic strategies [1,51,52]. The advancement of deep learning algorithms has revolutionized the study of proteins, empowering precise predictions of intricate structures of proteins, nucleic acids, and small molecules [53]. These computational approaches hold great promise in the field of drug discovery and development. AMBRA1 research is still evolving, and the development of therapeutic interventions targeting AMBRA1 is in the early stages. Further studies are necessary to fully understand the potential of AMBRA1 as a therapeutic target and to evaluate its safety and efficacy in various disease contexts.

Table 1. The identified interactors of AMBRA1.

Interactors	Binding region on AMBRA1 (based on canonical UniProt sequence)	Reference
Beclin1	Residue 533-780	[16,54]
DDB1-Cullin4 complex	Residue 1-41	[1]
PP2A	Residue 275-281 and 1206-1212	[10]
DLC1	Residue 1104-1106 and 1116-1118	[23]
TRAF6	Residue 618-623 and 681-686	[55]
Elongin C-Cullin5 complex	Residue 825-1298	[56]
LC3	Residue 1043-1052	[42]
ERLIN1	Residue 533-780 and 796-1298	[57]
ULK1	Residue 1-532 and 781-1298	[55,58]

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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