Molecules and Cells

Minireview

New Insights into the Role of E2s in the Pathogenesis of Diseases: Lessons Learned from UBE2O

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Intracellular communication via ubiquitin (Ub) signaling impacts all aspects of cell biology and regulates pathways critical to human development and viability; therefore aberrations or defects in Ub signaling can contribute to the pathogenesis of human diseases. Ubiquitination consists of the addition of Ub to a substrate protein via coordinated action of E1-activating, E2-conjugating and E3-ligating enzymes. Approximately 40 E2s have been identified in humans, and most are thought to be involved in Ub transfer; although little information is available regarding the majority of them, emerging evidence has highlighted their importance to human health and disease. In this review, we focus on recent insights into the pathogenetic roles of E2s (particularly the ubiquitin-conjugating enzyme E2O [UBE2O]) in debilitating diseases and cancer, and discuss the tantalizing prospect that E2s may someday serve as potential therapeutic targets for human diseases.

Keywords: E2 ubiquitin-conjugating enzyme, E3 ubiquitin ligase, pathogenesis, ubiquitination, UBE2O

INTRODUCTION

Ubiquitin (Ub) is a highly conserved 76-amino acid (8.5 kDa) protein present in all eukaryotic cells. Ub is covalently conjugated to other proteins in a reversible manner, in order to alter its substrate's fate and biological function at multiple levels in a process called "ubiquitination" (Hershko et al., 1980). Ub can be attached to substrate proteins as a single molecule or in polymeric chains that are connected through specific isopeptide bonds to form a branched or forked structure (Komander and Rape, 2012). Ubiquitination is a dynamic, highly regulated process that involves successive steps of Ub activation, conjugation and ligation. First, Ub-activating enzymes (E1s) activate Ub in an ATP-dependent reaction (an energy-consuming step) to generate a thioester-linked E1~Ub conjugate. Second, the activated Ub is transferred via a transthiolation reaction to the cysteine residue of an Ub-conjugating enzyme (E2) (Ye and Rape, 2009). Finally, the C-terminal glycine of Ub is conjugated to a specific lysine on the target protein by Ub ligases (E3s) (Pickart, 2001). The Ub tags or chains can be removed from target proteins by a family of isopeptidases called deubiquitinases (DUBs), which reverse the function of ubiquitination (Komander et al., 2009).

As one of the most important cellular post-transcriptional mechanisms, ubiquitination is involved in a wide range of key physiological processes (Grabbe et al., 2011). Although it plays its best-known and best-characterized role in the mediation of targeted protein degradation via the 26S pro-

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teasome, many lines of evidence have shown that ubiquitination also acts as a crucial regulator of transcription, DNA repair, endocytosis, plasma membrane receptor recycling, intracellular trafficking, inflammatory signaling and angiogenesis (Popovic et al., 2014). This functional diversity is driven in part by the specific positions in the ubiquitin molecule of the lysine (K) residues (e.g., K6, K11, K27, K29, K33, K48, or K63) that are involved in monomer or polymeric chain formation (Komander and Rape, 2012). It has been repeatedly shown that deregulation of the ubiquitination process contributes to the pathogenesis of a wide range of diseases, including cancer, neurodegenerative disorders, immune disease, diabetes, muscle atrophy and other debilitating conditions.

While the action modes of E3s in various diseases are already the focus of much current research, limited attention has been paid to the pathogenetic role of E2s. In this review, we will explore recent advances in our understanding of the roles of E2 enzymes (in particular ubiquitin-conjugating enzyme E2O [UBE2O]) in the pathogenesis and progression of human diseases, and discuss the possibility that E2s could serve as potential targetsin therapeutic interventions.

E2 STRUCTURE AND SUPERFAMILY

To date, approximately 40 E2s have been identified, while 2 E1s and approximately 600~1000 E3s are known to be encoded in the human genome. A defining characteristic of all E2s (14~35 kDa at average mass) is a conserved catalytic "core" domain of 150-200 amino acids (the Ub-conjugating domain, or UBC); these domains of 14-16 kDa are ~35% conserved among different family members (Dikic et al., 2009). The E2 UBC domain typically adopts an α/β-fold with four α-helices and a four-stranded β-sheet (Fig. 1). Important loop regions constitute a portion of the E3-binding site (L1 loop and L2 loop) and the active site (L3 loop). Within the UBC, the catalytic cysteine residue required for Ub thioester formation is adjacent to an invariant asparagine residue, and together these compose the well-established Ub binding cleft; thus suppression of Ub conjugation and deconjugation for pharmacological intervention could prove a promising approach for disease therapy (as further discussed below).

E2s are classified into 4 different types: class I contains only the UBC domain, classes II and III have either N- or C-terminal extensions, respectively, and class IV E2s have both N- and C-terminal extensions (Fig. 2). These extra domains not only create E2s of diverse molecular size, including the largest E2s, UBE2O (1,292 amino acids) and BIRC6 (4,857 amino acids), but can also govern intracellular localization, confer regulatory properties, and enable specific interactions with particular E3s (Ye and Rape, 2009; Wenzel et al., 2011).

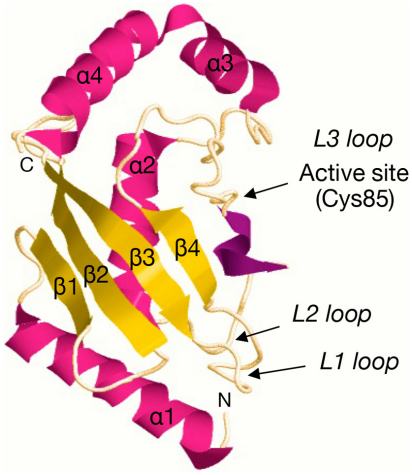

Fig. 1. Structural representation of E2s. Crystal structure of human UBE2D2(Protein Data Bank (PDB) code 2CLW) as a representative of the UBC fold conserved among E2s. The UBC core is comprised of four α-helices (pink colored) and an antiparallel β-sheet formed by four β-strands (yellow colored). The N- and C-termini of the domain are indicated. The location of the E3-binding site (L1 loop and L2 loop) and the active site cysteine (Cys85)(L3 loop) are also indicated.

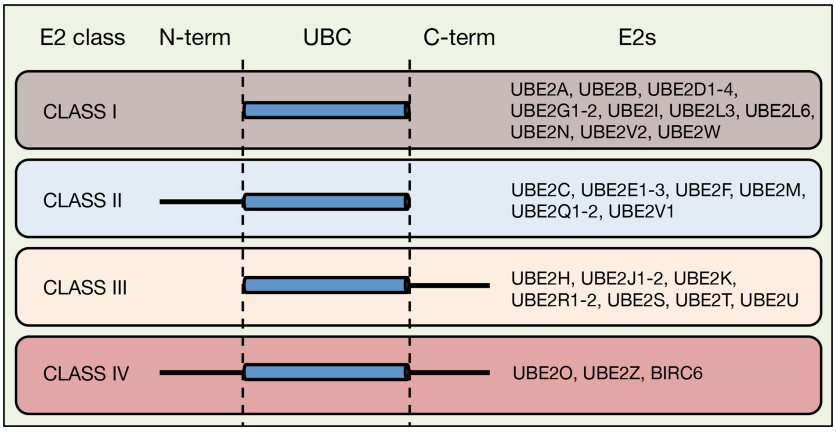

Fig. 2. Classification of E2s. Four classes of E2s are depicted in different colored boxes upon the absence (class I) or presence of additional extensions in the N- or C-terminal of the UBC domain (class II or class III, respectively) or in both termini (class IV).

E2 REACTIVITY

In general, E2s are engaged in Ub transfer reactions: (1) transthiolation (transfer from a thioester to a thiol group) and (2) aminolysis (transfer from a thioester to an amino group). The C-terminal carboxylate of Ub is conjugated to the E2 active site cysteine in an E1-catalyzed, ATP-driven reaction. E3s recruit E2s~Ub complexes (~ denotes a thioester linkage) and a substrate to promote Ub transfer, most commonly onto the ε-amino group of a lysine in the target protein, forming an isopeptide bond. Three classes of E3s have been identified to date - RING (really interesting new gene), HECT (homologous to E6AP C-terminus) and RBR (RING-between-RING) (Buetow and Huang, 2016). The RING E3s prime E2s~Ub for transfer by promoting a closed E2s~Ub conformation in which the thioester is activated toward nucleophilic attack on the substrate lysine (Plechanovova et al., 2012). Alternatively, Ub is transferred from E2s to the catalytic cysteine on the C-lobe of the HECT domain in a transthiolation reaction, and HECT~Ub is subsequently juxtaposed with a substrate lysine to which Ub is transferred (Kamadurai et al., 2009). Analyses of the crystal structures of RBR E3 have revealed that RING1 recruits E2~Ub and transfers Ub to the catalytic cysteine on RING2 to form a RING2~Ub intermediate; Ub is subsequently transferred to the lysine substrate on the target protein (Lechtenberg et al., 2016). E2-E3 interactions are also important because the interacting E2s dictate the specific type of inter-Ub linkage (i.e., K48 or K63) and thereby determine the ultimate fate of a substrate (i.e., targeted by the 26S proteasome for degradation or involved in signaling) (Stewart et al., 2016).

E2 ACTION INDEPENDENT OF E3

E2s can interact with and ubiquitinate substrates without the participation of E3s both *in vitro* and in intact cells. For example, UBCH5 and UBCH7 monoubiquitinate STS2 (or UBS3A) to promote its ability to regulate cargo (i.e., EGFR) sorting (Hoeller et al., 2006); in this case, E2s can bind to the ubiquitin-binding domain (UBD) of the target substrate via the Ub attached to its catalytic cysteine, prior to the Ub being passed to the lysine residue of the substrate. Another E3-independent mechanism, SETDB1, differs from coupled monoubiquitination in that it does not require a functional UBD. Instead, SETDB1 ubiquitination is directly catalyzed by UBE2E, which surprisingly acts as a functional E3 or UBD (Sun and Fang, 2016). UBE2A/B-EEA1 interactions likewise enable E3-independent mechanisms that decorate EEA1 with a mono-Ub moiety for endosome fusion and trafficking (Ramanathan et al., 2013).

Intriguingly, a few E2s (e.g., BIRC6 and UBE2O) could be described as parts of large multi-domain proteins, and act as E2/E3 hybrids. These atypical E2 members perform the second and third steps in the ubiquitination reaction, combining the activities of regular E2s and E3s. Once loaded with Ub from E1~Ub complex, these E2s can bind a substrate and transfer Ub to a lysine residue of that substrate protein. For example, BIRC6 (also known as BRUCE or Apollon) does not require any additional enzymes beyond E1 for ubiquitination of its substrate, Smac/DIABLO, in cell death pathways (Bartke et al., 2004). In this enzyme, the N-terminal BIR domain may mediate substrate binding in a manner analogous to E3s, whereas the UBC domain at the C-terminal end of the BIRC6 protein enables catalytic Ub-conjugation activity.

PATHOLOGICAL ROLES OF E2 IN HUMAN DISEASES

E2s have emerged as important pathogenetic factors for human diseases including neurodegenerative disorders, chromosome instability syndromes, immunological disorders and cancer (Table 1).

Table 1. Pathological roles of E2s in human diseases

Name	Synonyms	Biological roles	Relevant diseases
UBE2A	UBC2, HR6A, HHR6A, RAD6A	DNA repair (Koken et al., 1992); Transcription regulation	Cancer (Somasagara et al., 2017); Cognitive disability (Budny et al., 2010); Skeletal muscle atrophy (Polge et al., 2015a)
UBE2B	UBC2, HR6B, HHR6B, RAD6B, E2-17K	DNA repair (Xin et al., 2000); Spermatogenesis (Roest et al., 1996)	Idiopathic azoospermia (Mou et al., 2015); Skeletal muscle atrophy (Polge et al., 2015a)
UBE2C	UBCH10, DJ447F3.2, EC 6.3.2.19	Cell cycle progression (Townsley et al., 1997)	Cancer (van Ree et al., 2010; Psyrri et al., 2012)
UBE2D1	SFT, UBCH5, UBC4/5, UBCH5A	DNA repair (Schmidt et al., 2015); Iron transport (Gehrke et al., 2003)	Cancer (Shukla et al., 2014); Hemochromatosis (Gehrke et al., 2003)
UBE2D2	UBCH5B, UBC4	DNA repair (Schmidt et al., 2015); Parkin-mediated mitophagy (Geisler et al., 2014)	Parkinson disease (Fiesel et al., 2014; Geisler et al., 2014)
UBE2D3	UBC4/5, UBCH5C	DNA repair (Schmidt et al., 2015); NF-κB signaling (Shembade et al., 2010)	Parkinson disease (Fiesel et al., 2014; Geisler et al., 2014); Infectious disease (Pruneda et al., 2014)
UBE2D4	HBUCE1, UBCH5D	DNA repair (Schmidt et al., 2015)	Cancer (Ramatenki et al., 2017b)

(continued)

Table 1.

Name	Synonyms	Biological roles	Relevant diseases
UBE2E1	UBCH6	PTEN ubiquitination and transport (Chen et al., 2017b)	Cancer (Luo et al., 2016); Sjogren's syndrome (Espinosa et al., 2011)
UBE2E2	UBCH8, FLJ25157	Glucose homeostasis (Xu et al., 2016)	Diabetes (Yamauchi et al., 2010; Xu et al., 2016)
UBE2E3	UBCH9, UBCM2	NRF2 transport (Plafker and Plafker, 2015); Epithelial Na^+ transport (Debonneville and Staub, 2004)	Liddle's syndrome (Debonneville and Staub, 2004)
UBE2F	NCE2	Protein neddylation (Zhou et al., 2017)	Cancer (Zhou et al., 2017)
UBE2G1	UBE2G	Skeletal muscle protein regulation (Watanabe et al., 1996)	Skeletal muscle atrophy (Polge et al., 2015a)
UBE2G2	UBC7	ER-associated degradation (ERAD) (Liu et al., 2014)	Cancer (Menezes et al., 2014); Sjogren's syndrome (Barrera et al., 2016)
UBE2H	UBC8, UBCH, UBCH2, E2-20K	Histone and cytoskeleton ubiquitination (Kaiser et al., 1994)	Autism (Vourc'h et al., 2003)
UBE2I	UBC9, UBCH9	SUMO E2 (Yu et al., 2015)	Cancer (Yu et al., 2015)
UBE2J1	UBC6p, CGI-76, NCUBE1, HSPC153	ERAD (Burr et al., 2013); Spermiogenesis (Koenig et al., 2014)	Sjogren's syndrome (Barrera et al., 2016); Skeletal muscle atrophy (Polge et al., 2015a)
UBE2J2	NCUBE2, PRO2121	ERAD (Lam et al., 2014)	Cancer (Lam et al., 2014)
UBE2K	HIP2, LIG, UBC1, E2-25K	Aggregate formation of expanded polyglutamine proteins (de Pril et al., 2007)	Huntington Disease (de Pril et al., 2007)
UBE2L3	E2-F1, UBCH7, UBCM4	NF-κB signaling (Ikeda et al., 2011)	Lupus erythematosus and rheumatoid arthritis (Han et al., 2009; Stahl et al., 2010)
UBE2L6	RIG-B, UBCH8, MGC8489	Autophagy (Falvey et al., 2017)	Cancer (Falvey et al., 2017)
UBE2M	UBC12, UBC-RS2	Protein neddylation (Scott et al., 2017)	Hypertension (Schumacher et al., 2015)
UBE2N	UBCH-BEN, UBC13, MGC8489	DNA repair (Andersen et al., 2005)	Parkinson disease (Fiesel et al., 2014)
UBE2NL	Li174	Cell cycle progression (Ramatenki et al., 2017a)	Cancer (Ramatenki et al., 2017a)
UBE2O	E2-230K, FLJ12878, KIAA1734	AMPKα2 ubquitination and degradation (Vila et al., 2017); MLL ubiquitination and degradation (Liang et al., 2017); Erythroid differentiation and proteostasis (Nguyen et al., 2017; Yanagitani et al., 2017); Adipocyte differentiation (Zhang et al., 2013a); Endocytic trafficking (Hao et al., 2013)	Cancer(Mashtalir et al., 2014; Liang et al., 2017; Vila et al., 2017); Microcytic anemia (Nguyen et al., 2017)
UBE2Q1	GTAP, UBE2Q, NICE-5, PRO3094	β-catenin-EGFR-PI3K-AKT-mTOR signaling (Zhang et al., 2017)	Cancer (Zhang et al., 2017)
UBE2Q2		Apoptosis (Banerjee et al., 2007)	Cancer (Banerjee et al., 2007); Chronic kidney disease (Kottgen et al., 2010)
UBE2QL	FLJ25076, LOC134111	Unknown	Unknown
UBE2R1	CDC34, UBCH3, UBC3, E2-CDC34	Cell cycle progression (Ceccarelli et al., 2011)	Cancer (Ceccarelli et al., 2011); Parkinson disease (Fiesel et al., 2014)
UBE2R2	UBC3B, CDC34B	β-catenin degradation (Semplici et al., 2002)	Unknown
UBE2S	E2-EPF	Cell cycle progression (Garnett et al., 2009)	Cancer (Garnett et al., 2009); Parkinson disease (Geisler et al., 2014)
UBE2T	PIG50, HSPC150, FANCT	DNA repair (Machida et al., 2006)	Cancer (Yu et al., 2016); Fanconi anemia (Hira et al., 2015)
UBE2U	MGC35130, RP4-636O23.1	DNA repair (Guo et al., 2017)	Riddle's syndrome (Guo et al., 2017)
UBE2W	FLJ11011, UBC-16, UBC16	E2 for α-amino group ubiquitination (Vittal et al., 2015)	Fanconi anemia (Zhang et al., 2011)
UBE2Z	HOYS7, FLJ13855, USE1	FAT10 conjugation (Schelpeet al., 2016)	Coronary artery disease (Lu et al., 2017)
BIRC6	BRUCE, APOLLON, FLJ13726	Anti-apoptosis (Bartke et al., 2004); DNA repair (Ge et al., 2015)	Cancer (Bartke et al., 2004)

Neurodegenerative disorders

E2s are implicated in the pathogenesis of several neurological diseases. Mutations in genes encoding the Parkin E3 ligase are the most frequent causes of early-onset familial Parkinson disease (Kitada et al., 1998). Parkin performs an essential neuroprotective function by regulating mitophagy, which is key to the maintenance of mitochondrial homeostasis (Jin and Youle, 2012). Recent works have revealed that UBE2D2/3, UBE2L3, UBE2N and UBE2R1 regulate the activity and cellular compartmentalization of Parkin, and thereby impact the Parkin-mediated clearance of damaged mitochondria (Fiesel et al., 2014; Geisler et al., 2014). *UBE2A* has been identified as an intellectual disability gene and shown to act with Parkin to promote ubiquitination of mitochondrial proteins and mitophagy (Haddad et al., 2013). UBE2K interacts with and ubiquitinates huntingtin, the gene product of Huntington disease, and promotes aggregate formation of expanded polyglutamine proteins and apoptosis in polyglutamine diseases (de Pril et al., 2007). These results suggest that E2s play an important role in the pathology of neurological diseases.

Chromosome instability syndromes

Fanconi anemia (FA) is a rare genetic disorder affecting bone marrow function and hematopoiesis (Lobitz and Velleuer, 2006). The FA DNA repair pathway has become a paradigm for the physiological importance of Ub signaling in coordination of DNA repair pathways and the maintenance of genome stability (Ceccaldi et al., 2016). UBE2T was identified as the cognate E2 for monoubiquitination of the FA proteins FANCD2/FANCL, a key step in the signal transduction cascade of the FA DNA repair pathway (Machida et al., 2006). Furthermore, genomic analysis of FA patients has revealed that allelic alterations of *UBE2T* are associated with loss of function and deficiency of UBE2T protein (Hira et al., 2015). Overall, *UBE2T* is now recognized as a bona fide FA gene (and has been alternatively named *FANCT*).

Immunological disorders

Several genome-wide association studies have identified polymorphisms in the genomic locus of *UBE2L3* as high risk factors for developing systemic lupus erythematosus and rheumatoid arthritis (Han et al., 2009; Stahl et al., 2010). Notably, UBE2L3 can form specific E2-E3 pairs with the disease-associated E3 ligase LUBAC (linear ubiquitin chain assembly complex), a critical factor in the efficient activation of NF-κB signaling (Ikeda et al., 2011). This evidence suggests UBE2L3 may play a role in regulating inflammation and immunity signaling pathways in autoimmune diseases.

Cancer

Multiple studies have found deregulated expression of E2s in various cancers, and growing evidence indicates that during malignant transformation, many E2s promote DNA repair, cell cycle progression, and activation of oncogenic signaling pathways, while inhibiting apoptosis (Table 1). Thus, E2s may be key to identifying potential cancer susceptibility genes for diagnosis and prognosis, and could play a role in the design of novel therapies.

UBE2O AS A KICK-STARTER FOR DISEASES AND CANCER

Mechanisms of action of UBE2O

The large UBE2O, though a single entity, essentially operates as a combination of E2 and E3 enzymes (Klemperer et al., 1989; Berleth and Pickart, 1996); it is also capable of interacting cooperatively with the E3 RING ligase MAGE-L2/TRIM27, suggesting it can play multi-functional roles (Hao et al., 2013). It has been proposed that ubiquitination by UBE2O involves an intramolecular thioester relay mechanism, as this enzyme is inhibited by arsenites which can crosslink adjacent cysteines (Klemperer et al., 1989). UBE2O possesses three conserved regions (CR1, CR2 and CR3) and a coiled-coil (CC) domain (Fig. 3). The CR1 and CR2 domains are both believed to recognize the same targeted substrates, although their binding specificity varies (Mashtalir et al., 2014; Nguyen et al., 2017). Interestingly, Mashtair et al. (2014) have established a putative targeting consensus sequence for UBE2O (i.e., K/R and VLI patches: [KR][KR][KR]-X(1,3)-[VLI]-X-[VLI]-X-X-[VLI]) and have identified potential targets, several of which have tested positive experimentally for UBE2O-mediated ubiquitination. Like other E2s, UBE2O has a conserved core UBC domain in the C-terminus that can interact with multiple E3s. However, ubiquitination of most of the reported UBE2O substrates are catalyzed by UBE2O without E3s. While UBE2O mediates (multi-)monoubiquitination of SMAD6, WASH, BAP1 and α-globin (Nguyen et al., 2017;

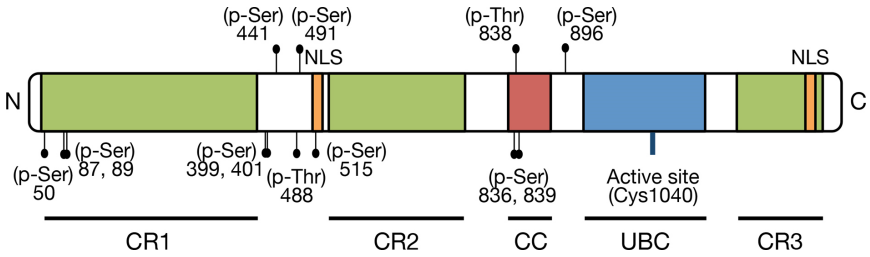

Fig. 3. Scheme of UBE2O functional domains. Shown are CR1 (conserved region 1), CR2 (conserved region 2), CR3 (conserved region 3), CC (coiled-coil domain) and UBC (ubiquitin-conjugating domain) containing an active site cysteine (Cys1040). Two putative nuclear localization signals and multiple predicted phosphorylation sites are also indicated.

Yanagitani et al., 2017), UBE2O is able to polyubiquitinate AMPKα2 and MLL, leading to their proteasomal degradation (Liang et al., 2017; Vila et al., 2017). UBE2O is ubiquitously expressed in mammalian tissues, but preferentially in brain, heart, skeletal muscle and liver tissue (Yokota et al., 2001). Although its cellular localization is predominantly cytoplasmic, UBE2O harbors two putative nuclear localization sequences (NLS) (Mashtalir et al., 2014). In addition, UBE2O contains potential sites for phosphorylation and its activity may be regulated by phosphorylation (Liang et al., 2017). Thus the specialized features and multifunctional domains within the UBE2O protein suggest that its role in disease pathogenesis includes a broad spectrum of molecular targets and functions.

A role for UBE2O in erythropoiesis and proteostasis

UBE2O is known to be strongly upregulated in terminally differentiating reticulocytes (Wefes et al., 1995), and an intriguing study recently demonstrated the crucial role it plays in erythroid differentiation (Nguyen et al., 2017)(Fig. 4). Mice expressing a truncated mutation in the *Ube2o* gene (known as *hem9*) exhibit hypochromic, microcytic anemia and their reticulocytes show a dramatic reduction in Ub-conjugated protein pools. Significantly, ribosomal proteins are the major targets of UBE2O once it has been reintroduced into *hem9* reticulocyte lysates. In a related finding, *hem9* reticulocytes exhibited elevated levels of ribosomal proteins such as RPL29 and RPL35, a phenotype apparently due to a defect in the elimination of ribosomes. UBE2O can similarly act as a self-contained quality control factor for the recognition and elimination of orphan subunits (Yanagitani et al., 2017), and can directly recognize juxtaposed basic and hydrophobic patches on unassembled proteins (e.g., α-globin that fails to assemble with β-globin in reticulocytes) as part of its role in mediating ubiquitination for the maintenance of protein homeostasis. In summary, UBE2O ubiquitinates free ribosomal proteins and unassembled proteins and targets them for degradation to the proteasome, thus playing an important role during terminal erythroid differentiation.

An oncogenic role for UBE2O in cancer: Spinning on the AMPK axis

A careful review of a large-scale genome analysis and associated expression profile data from a panel of human cancers has revealed that many cancers exhibit alterations in *UBE2O*, with amplifications occurring ata particularly high frequency (i.e., in ~20% of human breast, bladder, liver and lung cancers)(Vila et al., 2017). Notably, *UBE2O* is located in the 17q25 locus, the amplification of which is recurrent in human cancers. Several recent studies have demonstrated that UBE2O acts as an oncogene in various types of cancer. Significantly, Vila et al. (2017) generated the first *Ube2o* knockout mouse specifically to investigate the oncogenic role

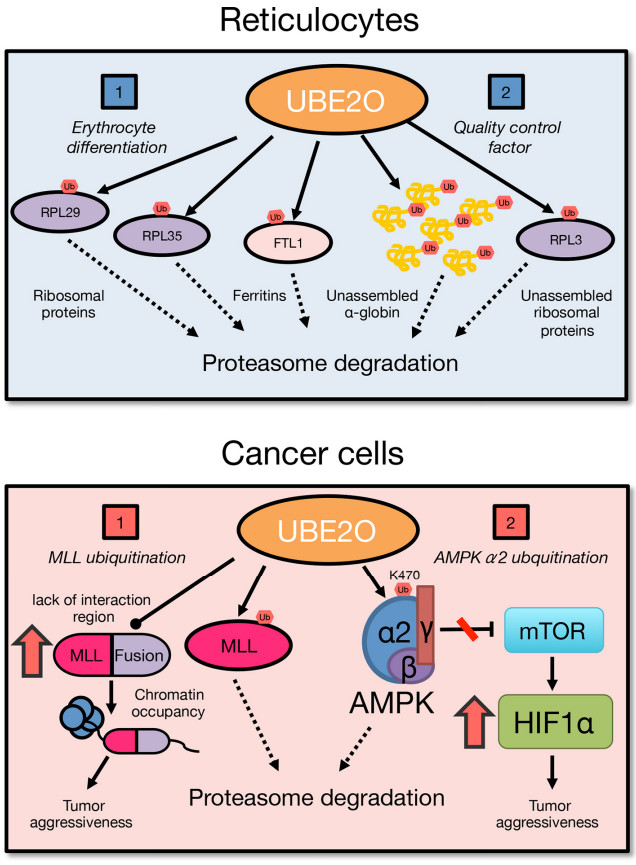

Fig. 4. Pathogenetic roles ofUBE2O. *(Top) A central role of UBE2O for erythropoiesis.* UBE2O confers the erythroid differentiation through promoting the ubiquitination of free ribosomal proteins (1) and unassembled α-globin (2) and thereby remodeling the differentiation-linked proteome. *(Bottom) An oncogenic role of UBE2O in cancer.* UBE2O ubiquitinates and promotes ubiquitination and degradation of wild-type MLL but not MLL fusion proteins in MLL leukemia (1). Furthermore, UBE2O promotes mTOR- and HIF1α-mediated tumorigenesis through selectively targeting the AMPKα2 protein (2).

of the protein, and in mouse models of prostate and breast cancer (TRAMP [transgenic adenocarcinoma mouse prostate] and MMTV-PyVT [MMTV-polyomavirus middle T antigen] transgenic mice, respectively), targeted deletion of *Ube2o* has dramatically delayed the onset of prostate and breast tumors and impaired invasion and distant metastasis.

Mechanistically, UBE2O directly interacts with and ubiquitinates AMPKα2 (AMP-activated protein kinase α2) to promote its proteasomal degradation, but leaves AMPKα1 untouched (Fig. 4). Accordingly, in two independent mouse models of lymphoma (Eμ-Myc mouse model of B cell lymphoma and *Pten* loss-driven T cell lymphoma) where AMPKα2 was not detectable, ablation of *Ube2o* did not show significant anti-tumor effects or confer a survival advantage. Moreover, UBE2O promoted activation of the mTOR (mammalian target of rapamycin complex 1)-HIF1α (hypoxia-inducible factor 1α) pathway and reprogrammed cancer cells toward aerobic glycolysis (or the Warburg effect) (Vander Heiden et al., 2009) and biosynthetic pathways in an AMPKα2-dependent manner. Finally, immunohistochemical and prognostic analyses of cancer patients have highlighted the clinical relevance of UBE2O regulation of the AMPKα2-mTOR-HIF1α axis.

Other pathological roles for UBE2O

UBE2O is also implicated in endosomal protein trafficking, an essential cellular process that is deregulated in several diseases and targeted by pathogens, through its ubiquitination of the WASH regulatory complex (Hao et al., 2013), and likewise plays a role in BMP7-induced adipogenesis via monoubiquitination of SMAD6 (Zhang et al., 2013a). UBE2O can also ubiquitinate the tumor-suppressive DUB BAP1 and regulate its subcellular localization and function in chromatin remodeling (Mashtalir et al., 2014). Other recent works have revealed a close association between UBE2O and interleukin-1 (IL-1) signaling: (1) UBE2O binds to TRAF6 and inhibit its K63-polyubiquitination, and thereby prevents the activation of NF-κB by IL-1 (Zhang et al., 2013b) and (2) UBE2O interacts with and induces degradation of wild-type mixed lineage leukemia (MLL) but not MLL fusion chimera proteins (Liang et al., 2017)(Fig. 4). Notably, IRAK4, which is activated by signaling from the IL-1 receptor, can phosphorylate UBE2O, leading to enhanced UBE2O-MLL interaction and subsequent degradation of MLL protein. This novel finding may pave a new way for treatment of MLL-rearranged leukemia via small-molecule inhibitors of IRAK4 or UBE2O.

E2S AS POTENTIAL DRUGGABLE TARGETS

The biological and clinical relevance of E2s to the pathogenesis and progression of diseases and cancer suggest that E2s potentially hold great therapeutic promise as druggable targets (Harper and King, 2011; Popovic et al., 2014). In this regard, new strategies to target E2s must be more effective and selective than those for E1s because E2s play critical roles in dictating the final Ub-product and the relevant substrate's ultimate fate. The recent advances in our knowledge of the structure and functions of E2s have revealed that they are essential for Ub~substrate specificity, and thus these conjugating enzymes have emerged as potential small-molecule therapeutic targets.

The discovery of the first small-molecule allosteric inhibitor of UBE2R1 (also known as CDC34) CC0651 underscores the feasibility of selectively inhibiting Ub transfer at the central step in the ubiquitination pathway (Ceccarelli et al., 2011). Treatment of human cancer cells with CC0651 leads to a lower proliferation rate without significant effect on the interactions between UBE2R1, E1s, and E3s. The recently developed small-molecule inhibitor NSC697923 targets UBE2N to inhibit proliferation and survival of neuroblastoma, and also diffuses large B-cell lymphoma (DLBCL) cells (Cheng et al., 2014; Pulvino et al., 2012). Despite recent progress in the development of additional small-molecule E2 inhibitors (Chen et al., 2017a; Morreale et al., 2017; Ramatenki et al., 2017a), no such E2-targeting therapy has yet made its way to clinical trials. Interestingly, arsenic, which can crosslink adjacent cysteines within the catalytic domains of UBE2O, could serve as the basis of an alternative approach to inhibiting E2 activity, and is currently being tested against various forms of cancer in clinical trials (https://clinicaltrials.gov) (Vila et al., 2017). It is also expected that E2-targeting therapeutics will be more efficacious against diseases and cancer when used in combination with current chemotherapy regimens.

CONCLUSION AND PERSPECTIVE

In the past, only the mechanisms common to all E2s, such as Ub transfer, were clearly understood; but our knowledge of E2s is now entering a second phase, in which researchers are uncovering the differences between E2s in specific physiological contexts, particularly those relevant to the pathogenesis of disease. Indeed, emerging evidence has persuasively demonstrated that deregulation of E2s can lead to debilitating disorders. Thus E2s could potentially serve as druggable targets in the treatment of disease. Although many groups across the globe, including several pharmaceutical companies, have initiated the development of new agents to target E2s, this mode of therapy has not yet made its way to clinical trials. A major obstacle to applying E2-targeting therapies in the clinic is the potential for off-target effects and side-effects due to the broad range of substrates and functions affected by E2s. Thus there is an urgent need for further research to both address the unique action modes of individual E2s in different biological contexts, and elucidate the pathogenesis of specific diseases. Ultimately, the development of novel strategies targeting individual E2s or specific interactions of E2-E3 pairs may lead to promising treatment options for cancer and other disorders in the years to come.

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REFERENCES

Andersen, P.L., Zhou, H., Pastushok, L., Moraes, T., McKenna, S., Ziola, B., Ellison, M.J., Dixit, V.M., and Xiao, W. (2005). Distinct regulation of Ubc13 functions by the two ubiquitin-conjugating enzyme variants Mms2 and Uev1A. J. Cell Biol. *170*, 745-755.

Banerjee, S., Brooks, W.S., and Crawford, D.F. (2007). Inactivation of the ubiquitin conjugating enzyme UBE2Q2 causes a prophase arrest and enhanced apoptosis in response to microtubule inhibiting agents. Oncogene *26*, 6509-6517.

Barrera, M.J., Aguilera, S., Castro, I., Cortes, J., Bahamondes, V., Quest, A.F.G., Molina, C., Gonzalez, S., Hermoso, M., Urzua, U., et al. (2016). Pro-inflammatory cytokines enhance ERAD and ATF6alpha pathway activity in salivary glands of Sjogren's syndrome patients. J. Autoimmun. *75*, 68-81.

Bartke, T., Pohl, C., Pyrowolakis, G., and Jentsch, S. (2004). Dual role of BRUCE as an antiapoptotic IAP and a chimeric E2/E3 ubiquitin ligase. Mol. Cell *14*, 801-811.

Berleth, E.S., and Pickart, C.M. (1996). Mechanism of ubiquitin conjugating enzyme E2-230K: catalysis involving a thiol relay? Biochemistry *35*, 1664-1671.

Budny, B., Badura-Stronka, M., Materna-Kiryluk, A., Tzschach, A., Raynaud, M., Latos-Bielenska, A., and Ropers, H.H. (2010). Novel missense mutations in the ubiquitination-related gene UBE2A cause a recognizable X-linked mental retardation syndrome. Clin. Genet. *77*, 541-551.

Buetow, L., and Huang, D.T. (2016). Structural insights into the catalysis and regulation of E3 ubiquitin ligases. Nat. Rev. Mol. Cell Biol. *17*, 626-642.

Burr, M.L., van den Boomen, D.J., Bye, H., Antrobus, R., Wiertz, E.J., and Lehner, P.J. (2013). MHC class I molecules are preferentially ubiquitinated on endoplasmic reticulum luminal residues during HRD1 ubiquitin E3 ligase-mediated dislocation. Proc. Natl. Acad. Sci. USA *110*, 14290-14295.

Ceccarelli, D.F., Tang, X., Pelletier, B., Orlicky, S., Xie, W., Plantevin, V., Neculai, D., Chou, Y.C., Ogunjimi, A., Al-Hakim, A., et al. (2011). An allosteric inhibitor of the human Cdc34 ubiquitin-conjugating enzyme. Cell *145*, 1075-1087.

Ceccaldi, R., Sarangi, P., and D'Andrea, A.D. (2016). The Fanconi anaemia pathway: new players and new functions. Nat. Rev. Mol. Cell Biol. *17*, 337-349.

Chen, H., Wu, G., Gao, S., Guo, R., Zhao, Z., Yuan, H., Liu, S., Wu, J., Lu, X., Yuan, X., et al. (2017a). Discovery of Potent Small-molecule inhibitors of ubiquitin-conjugating enzyme UbcH5c from alpha-Santonin derivatives. J. Med. Chem. *60*, 6828-6852.

Chen, M., Nowak, D.G., Narula, N., Robinson, B., Watrud, K., Ambrico, A., Herzka, T.M., Zeeman, M.E., Minderer, M., Zheng, W., et al. (2017b). The nuclear transport receptor Importin-11 is a tumor suppressor that maintains PTEN protein. J. Cell Biol. *216*, 641-656.

Cheng, J., Fan, Y.H., Xu, X., Zhang, H., Dou, J., Tang, Y., Zhong, X., Rojas, Y., Yu, Y., Zhao, Y., et al. (2014). A small-molecule inhibitor of UBE2N induces neuroblastoma cell death via activation of p53 and JNK pathways. Cell Death Dis. *5*, e1079.

de Pril, R., Fischer, D.F., Roos, R.A., and van Leeuwen, F.W. (2007). Ubiquitin-conjugating enzyme E2-25K increases aggregate formation and cell death in polyglutamine diseases. Mol. Cell Neurosci. *34*, 10-19.

Debonneville, C., and Staub, O. (2004). Participation of the ubiquitin-conjugating enzyme UBE2E3 in Nedd4-2-dependent regulation of the epithelial Na^+ channel. Mol. Cell. Biol. *24*, 2397-2409.

Dikic, I., Wakatsuki, S., and Walters, K.J. (2009). Ubiquitin-binding domains - from structures to functions. Nat. Rev. Mol. Cell Biol. *10*, 659-671.

Espinosa, A., Hennig, J., Ambrosi, A., Anandapadmanaban, M., Abelius, M.S., Sheng, Y., Nyberg, F., Arrowsmith, C.H., Sunnerhagen, M., and Wahren-Herlenius, M. (2011). Anti-Ro52 autoantibodies from patients with Sjogren's syndrome inhibit the Ro52 E3 ligase activity by blocking the E3/E2 interface. J. Biol. Chem. *286*, 36478-36491.

Falvey, C.M., O'Donovan, T.R., El-Mashed, S., Nyhan, M.J., O'Reilly, S., and McKenna, S.L. (2017). UBE2L6/UBCH8 and ISG15 attenuate autophagy in esophageal cancer cells. Oncotarget *8*, 23479-23491.

Fiesel, F.C., Moussaud-Lamodiere, E.L., Ando, M., and Springer, W. (2014). A specific subset of E2 ubiquitin-conjugating enzymes regulate Parkin activation and mitophagy differently. J. Cell Sci. *127*, 3488-3504.

Garnett, M.J., Mansfeld, J., Godwin, C., Matsusaka, T., Wu, J., Russell, P., Pines, J., and Venkitaraman, A.R. (2009). UBE2S elongates ubiquitin chains on APC/C substrates to promote mitotic exit. Nat. Cell Biol. *11*, 1363-1369.

Ge, C., Che, L., Ren, J., Pandita, R.K., Lu, J., Li, K., Pandita, T.K., and Du, C. (2015). BRUCE regulates DNA double-strand break response by promoting USP8 deubiquitination of BRIT1. Proc. Natl. Acad. Sci. USA *112*, E1210-E1219.

Gehrke, S.G., Riedel, H.D., Herrmann, T., Hadaschik, B., Bents, K., Veltkamp, C., and Stremmel, W. (2003). UbcH5A, a member of human E2 ubiquitin-conjugating enzymes, is closely related to SFT, a stimulator of iron transport, and is up-regulated in hereditary hemochromatosis. Blood *101*, 3288-3293.

Geisler, S., Vollmer, S., Golombek, S., and Kahle, P.J. (2014). The ubiquitin-conjugating enzymes UBE2N, UBE2L3 and UBE2D2/3 are essential for Parkin-dependent mitophagy. J. Cell Sci. *127*, 3280-3293.

Grabbe, C., Husnjak, K., and Dikic, I. (2011). The spatial and temporal organization of ubiquitin networks. Nat. Rev. Mol. Cell Biol. *12*, 295-307.

Guo, Y., An, L., Ng, H.M., Sy, S.M., and Huen, M.S. (2017). An E2-guided E3 Screen Identifies the RNF17-UBE2U Pair as Regulator of the Radiosensitivity, Immunodeficiency, Dysmorphic Features, and Learning Difficulties (RIDDLE). Syndrome Protein RNF168. J. Biol. Chem. *292*, 967-978.

Haddad, D.M., Vilain, S., Vos, M., Esposito, G., Matta, S., Kalscheuer, V.M., Craessaerts, K., Leyssen, M., Nascimento, R.M., Vianna-Morgante, A.M., et al. (2013). Mutations in the intellectual disability gene Ube2a cause neuronal dysfunction and impair parkin-dependent mitophagy. Mol. Cell *50*, 831-843.

Han, J.W., Zheng, H.F., Cui, Y., Sun, L.D., Ye, D.Q., Hu, Z., Xu, J.H., Cai, Z.M., Huang, W., Zhao, G.P., et al. (2009). Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. Nat. Genet. *41*, 1234-1237.

Hao, Y.H., Doyle, J.M., Ramanathan, S., Gomez, T.S., Jia, D., Xu, M., Chen, Z.J., Billadeau, D.D., Rosen, M.K., and Potts, P.R. (2013). Regulation of WASH-dependent actin polymerization and protein trafficking by ubiquitination. Cell *152*, 1051-1064.

Harper, J.W., and King, R.W. (2011). Stuck in the middle: drugging the ubiquitin system at the e2 step. Cell *145*, 1007-1009.

Hershko, A., Ciechanover, A., Heller, H., Haas, A.L., and Rose, I.A. (1980). Proposed role of ATP in protein breakdown: conjugation of

protein with multiple chains of the polypeptide of ATP-dependent proteolysis. Proc. Natl. Acad. Sci. USA *77*, 1783-1786.

Hira, A., Yoshida, K., Sato, K., Okuno, Y., Shiraishi, Y., Chiba, K., Tanaka, H., Miyano, S., Shimamoto, A., Tahara, H., et al. (2015). Mutations in the gene encoding the E2 conjugating enzyme UBE2T cause Fanconi anemia. Am. J. Hum. Genet. *96*, 1001-1017.

Hoeller, D., Crosetto, N., Blagoev, B., Raiborg, C., Tikkanen, R., Wagner, S., Kowanetz, K., Breitling, R., Mann, M., Stenmark, H., et al. (2006). Regulation of ubiquitin-binding proteins by monoubiquitination. Nat. Cell Biol.*8*, 163-169.

Ikeda, F., Deribe, Y.L., Skanland, S.S., Stieglitz, B., Grabbe, C., Franz-Wachtel, M., van Wijk, S.J., Goswami, P., Nagy, V., Terzic, J., et al. (2011). SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. Nature *471*, 637-641.

Jin, S.M., and Youle, R.J. (2012). PINK1- and Parkin-mediated mitophagy at a glance. J. Cell Sci. *125*, 795-799.

Kaiser, P., Seufert, W., Hofferer, L., Kofler, B., Sachsenmaier, C., Herzog, H., Jentsch, S., Schweiger, M., and Schneider, R. (1994). A human ubiquitin-conjugating enzyme homologous to yeast UBC8. J. Biol. Chem. *269*, 8797-8802.

Kamadurai, H.B., Souphron, J., Scott, D.C., Duda, D.M., Miller, D.J., Stringer, D., Piper, R.C., and Schulman, B.A. (2009). Insights into ubiquitin transfer cascades from a structure of a UbcH5B approximately ubiquitin-HECT(NEDD4L). complex. Mol. Cell *36*, 1095-1102.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., and Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature *392*, 605-608.

Klemperer, N.S., Berleth, E.S., and Pickart, C.M. (1989). A novel, arsenite-sensitive E2 of the ubiquitin pathway: purification and properties. Biochemistry *28*, 6035-6041.

Koenig, P.A., Nicholls, P.K., Schmidt, F.I., Hagiwara, M., Maruyama, T., Frydman, G.H., Watson, N., Page, D.C., and Ploegh, H.L. (2014). The E2 ubiquitin-conjugating enzyme UBE2J1 is required for spermiogenesis in mice. J. Biol. Chem.*289*, 34490-34502.

Koken, M.H., Smit, E.M., Jaspers-Dekker, I., Oostra, B.A., Hagemeijer, A., Bootsma, D., and Hoeijmakers, J.H. (1992). Localization of two human homologs, HHR6A and HHR6B, of the yeast DNA repair gene RAD6 to chromosomes Xq24-q25 and 5q23-q31. Genomics *12*, 447-453.

Komander, D., and Rape, M. (2012). The ubiquitin code. Annu. Rev. Biochem. *81*, 203-229.

Komander, D., Clague, M.J., and Urbe, S. (2009). Breaking the chains: structure and function of the deubiquitinases. Nat. Rev. Mol. Cell Biol. *10*, 550-563.

Kottgen, A., Pattaro, C., Boger, C.A., Fuchsberger, C., Olden, M., Glazer, N.L., Parsa, A., Gao, X., Yang, Q., Smith, A.V., et al. (2010). New loci associated with kidney function and chronic kidney disease. Nat. Genet. *42*, 376-384.

Lam, S.Y., Murphy, C., Foley, L.A., Ross, S.A., Wang, T.C., and Fleming, J.V. (2014). The human ubiquitin conjugating enzyme UBE2J2 (Ubc6). is a substrate for proteasomal degradation. Biochem. Biophys. Res. Commun. *451*, 361-366.

Lechtenberg, B.C., Rajput, A., Sanishvili, R., Dobaczewska, M.K., Ware, C.F., Mace, P.D., and Riedl, S.J. (2016). Structure of a HOIP/E2~ubiquitin complex reveals RBR E3 ligase mechanism and regulation. Nature *529*, 546-550.

Liang, K., Volk, A.G., Haug, J.S., Marshall, S.A., Woodfin, A.R., Bartom, E.T., Gilmore, J.M., Florens, L., Washburn, M.P., Sullivan, K.D., et al. (2017). Therapeutic Targeting of MLL Degradation Pathways in MLL-Rearranged Leukemia. Cell *168*, 59-72.

Liu, W., Shang, Y., Zeng, Y., Liu, C., Li, Y., Zhai, L., Wang, P., Lou, J., Xu, P., Ye, Y., et al. (2014). Dimeric Ube2g2 simultaneously engages donor and acceptor ubiquitins to form Lys48-linked ubiquitin chains. EMBO J. *33*, 46-61.

Lobitz, S., and Velleuer, E. (2006). Guido Fanconi (1892-1979).: a jack of all trades. Nat. Rev. Cancer *6*, 893-898.

Lu, D., Huang, J., Ma, X., Gu, N., Zhang, J., Zhang, H., and Guo, X. (2017). Rs46522 in the Ubiquitin-Conjugating Enzyme E2Z Gene Is Associated with the Risk of Coronary Artery Disease in Individuals of Chinese Han Population with Type 2 Diabetes. J. Diabetes Res. *2017*, 4501794.

Luo, H., Qin, Y., Reu, F., Ye, S., Dai, Y., Huang, J., Wang, F., Zhang, D., Pan, L., Zhu, H., et al. (2016). Microarray-based analysis and clinical validation identify ubiquitin-conjugating enzyme E2E1 (UBE2E1). as a prognostic factor in acute myeloid leukemia. J. Hematol. Oncol. *9*, 125.

Machida, Y.J., Machida, Y., Chen, Y., Gurtan, A.M., Kupfer, G.M., D'Andrea, A.D., and Dutta, A. (2006). UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. Mol. Cell *23*, 589-596.

Mashtalir, N., Daou, S., Barbour, H., Sen, N.N., Gagnon, J., Hammond-Martel, I., Dar, H.H., Therrien, M., and Affar el, B. (2014). Autodeubiquitination protects the tumor suppressor BAP1 from cytoplasmic sequestration mediated by the atypical ubiquitin ligase UBE2O. Mol. Cell *54*, 392-406.

Menezes, J., Acquadro, F., Wiseman, M., Gomez-Lopez, G., Salgado, R.N., Talavera-Casanas, J.G., Buno, I., Cervera, J.V., Montes-Moreno, S., Hernandez-Rivas, J.M., et al. (2014). Exome sequencing reveals novel and recurrent mutations with clinical impact in blastic plasmacytoid dendritic cell neoplasm. Leukemia *28*, 823-829.

Morreale, F.E., Testa, A., Chaugule, V.K., Bortoluzzi, A., Ciulli, A., and Walden, H. (2017). Mind the Metal: A Fragment Library-Derived Zinc Impurity Binds the E2 Ubiquitin-Conjugating Enzyme Ube2T and Induces Structural Rearrangements. J. Med. Chem. *60*, 8183-8191.

Mou, L., Zhang, Q., Diao, R., Cai, Z., and Gui, Y. (2015). A functional variant in the UBE2B gene promoter is associated with idiopathic azoospermia. Reprod. Biol. Endocrinol. *13*, 79.

Nguyen, A.T., Prado, M.A., Schmidt, P.J., Sendamarai, A.K., Wilson-Grady, J.T., Min, M., Campagna, D.R., Tian, G., Shi, Y., Dederer, V., et al. (2017). UBE2O remodels the proteome during terminal erythroid differentiation. Science *357*, doi: 10.1126/science.aan0218

Pickart, C.M. (2001). Mechanisms underlying ubiquitination. Annu. Rev. Biochem. *70*, 503-533.

Plafker, K.S., and Plafker, S.M. (2015). The ubiquitin-conjugating enzyme UBE2E3 and its import receptor importin-11 regulate the localization and activity of the antioxidant transcription factor NRF2. Mol. Biol. Cell *26*, 327-338.

Plechanovova, A., Jaffray, E.G., Tatham, M.H., Naismith, J.H., and Hay, R.T. (2012). Structure of a RING E3 ligase and ubiquitin-loaded E2 primed for catalysis. Nature *489*, 115-120.

Polge, C., Attaix, D., and Taillandier, D. (2015a). Role of E2-Ub-conjugating enzymes during skeletal muscle atrophy. Front. Physiol. *6*, 59.

Popovic, D., Vucic, D., and Dikic, I. (2014). Ubiquitination in disease pathogenesis and treatment.Nat. Med.*20*, 1242-1253.

Pruneda, J.N., Smith, F.D., Daurie, A., Swaney, D.L., Villen, J., Scott, J.D., Stadnyk, A.W., Le Trong, I., Stenkamp, R.E., Klevit, R.E., et al. (2014). E2~Ub conjugates regulate the kinase activity of Shigella effector OspG during pathogenesis. EMBO J. *33*, 437-449.

Psyrri, A., Kalogeras, K.T., Kronenwett, R., Wirtz, R.M., Batistatou, A.,

Bournakis, E., Timotheadou, E., Gogas, H., Aravantinos, G., Christodoulou, C., et al. (2012). Prognostic significance of UBE2C mRNA expression in high-risk early breast cancer. A Hellenic Cooperative Oncology Group (HeCOG). Study. Ann. Oncol. *23*, 1422-1427.

Pulvino, M., Liang, Y., Oleksyn, D., DeRan, M., Van Pelt, E., Shapiro, J., Sanz, I., Chen, L., and Zhao, J. (2012). Inhibition of proliferation and survival of diffuse large B-cell lymphoma cells by a small-molecule inhibitor of the ubiquitin-conjugating enzyme Ubc13-Uev1A. Blood *120*, 1668-1677.

Ramanathan, H.N., Zhang, G., and Ye, Y. (2013). Monoubiquitination of EEA1 regulates endosome fusion and trafficking. Cell Biosci. *3*, 24.

Ramatenki, V., Dumpati, R., Vadija, R., Vellanki, S., Potlapally, S.R., Rondla, R., and Vuruputuri, U. (2017a). Identification of New Lead Molecules Against UBE2NL Enzyme for Cancer Therapy. Appl. Biochem. Biotechnol. *182*, 1497-1517.

Ramatenki, V., Dumpati, R., Vadija, R., Vellanki, S., Potlapally, S.R., Rondla, R., and Vuruputuri, U. (2017b). Targeting the ubiquitin-conjugating enzyme E2D4 for cancer drug discovery-a structure-based approach. J. Chem. Biol. *10*, 51-67.

Roest, H.P., van Klaveren, J., de Wit, J., van Gurp, C.G., Koken, M.H., Vermey, M., van Roijen, J.H., Hoogerbrugge, J.W., Vreeburg, J.T., Baarends, W.M., et al. (1996). Inactivation of the HR6B ubiquitin-conjugating DNA repair enzyme in mice causes male sterility associated with chromatin modification. Cell *86*, 799-810.

Schelpe, J., Monte, D., Dewitte, F., Sixma, T.K., and Rucktooa, P. (2016). Structure of UBE2Z enzyme provides functional insight into specificity in the FAT10 protein conjugation machinery. J. Biol. Chem. *291*, 630-639.

Schmidt, C.K., Galanty, Y., Sczaniecka-Clift, M., Coates, J., Jhujh, S., Demir, M., Cornwell, M., Beli, P., and Jackson, S.P. (2015). Systematic E2 screening reveals a UBE2D-RNF138-CtIP axis promoting DNA repair. Nat. Cell Biol. *17*, 1458-1470.

Schumacher, F.R., Siew, K., Zhang, J., Johnson, C., Wood, N., Cleary, S.E., Al Maskari, R.S., Ferryman, J.T., Hardege, I., Yasmin, et al. (2015). Characterisation of the Cullin-3 mutation that causes a severe form of familial hypertension and hyperkalaemia. EMBO Mol. Med. *7*, 1285-1306.

Scott, D.C., Hammill, J.T., Min, J., Rhee, D.Y., Connelly, M., Sviderskiy, V.O., Bhasin, D., Chen, Y., Ong, S.S., Chai, S.C., et al. (2017). Blocking an N-terminal acetylation-dependent protein interaction inhibits an E3 ligase.Nat. Chem. Biol. *13*, 850-857.

Semplici, F., Meggio, F., Pinna, L.A., and Oliviero, S. (2002). CK2-dependent phosphorylation of the E2 ubiquitin conjugating enzyme UBC3B induces its interaction with beta-TrCP and enhances beta-catenin degradation. Oncogene *21*, 3978-3987.

Shembade, N., Ma, A., and Harhaj, E.W. (2010). Inhibition of NF-kappaB signaling by A20 through disruption of ubiquitin enzyme complexes. Science*327*, 1135-1139.

Shukla, S., Allam, U.S., Ahsan, A., Chen, G., Krishnamurthy, P.M., Marsh, K., Rumschlag, M., Shankar, S., Whitehead, C., Schipper, M., et al. (2014). KRAS protein stability is regulated through SMURF2: UBCH5 complex-mediated beta-TrCP1 degradation. Neoplasia *16*, 115-128.

Somasagara, R.R., Spencer, S.M., Tripathi, K., Clark, D.W., Mani, C., Madeira da Silva, L., Scalici, J., Kothayer, H., Westwell, A.D., Rocconi, R.P., et al. (2017). RAD6 promotes DNA repair and stem cell signaling in ovarian cancer and is a promising therapeutic target to prevent and treat acquired chemoresistance. Oncogene *36*, 6680-6690.

Stahl, E.A., Raychaudhuri, S., Remmers, E.F., Xie, G., Eyre, S., Thomson, B.P., Li, Y., Kurreeman, F.A., Zhernakova, A., Hinks, A., et al. (2010). Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat. Genet. *42*, 508-514.

Stewart, M.D., Ritterhoff, T., Klevit, R.E., and Brzovic, P.S. (2016). E2 enzymes: more than just middle men. Cell Res. *26*, 423-440.

Sun, L., and Fang, J. (2016). E3-Independent constitutive monoubiquitination complements histone methyltransferase activity of SETDB1.Mol. Cell *62*, 958-966.

Townsley, F.M., Aristarkhov, A., Beck, S., Hershko, A., and Ruderman, J.V. (1997). Dominant-negative cyclin-selective ubiquitin carrier protein E2-C/UbcH10 blocks cells in metaphase. Proc. Natl. Acad. Sci. USA *94*, 2362-2367.

van Ree, J.H., Jeganathan, K.B., Malureanu, L., and van Deursen, J.M. (2010). Overexpression of the E2 ubiquitin-conjugating enzyme UbcH10 causes chromosome missegregation and tumor formation. J. Cell Biol. *188*, 83-100.

Vander Heiden, M.G., Cantley, L.C., and Thompson, C.B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science *324*, 1029-1033.

Vila, I.K., Yao, Y., Kim, G., Xia, W., Kim, H., Kim, S.J., Park, M.K., Hwang, J.P., Gonzalez-Billalabeitia, E., Hung, M.C., et al. (2017). A UBE2O-AMPKalpha2 axis that promotes tumor initiation and progression offers opportunities for therapy. Cancer Cell *31*, 208-224.

Vittal, V., Shi, L., Wenzel, D.M., Scaglione, K.M., Duncan, E.D., Basrur, V., Elenitoba-Johnson, K.S., Baker, D., Paulson, H.L., Brzovic, P.S., et al. (2015). Intrinsic disorder drives N-terminal ubiquitination by Ube2w. Nat. Chem. Biol. *11*, 83-89.

Vourc'h, P., Martin, I., Bonnet-Brilhault, F., Marouillat, S., Barthelemy, C., Pierre Muh, J., and Andres, C. (2003). Mutation screening and association study of the UBE2H gene on chromosome 7q32 in autistic disorder. Psychiatr. Genet. *13*, 221-225.

Watanabe, T.K., Kawai, A., Fujiwara, T., Maekawa, H., Hirai, Y., Nakamura, Y., and Takahashi, E. (1996). Molecular cloning of UBE2G, encoding a human skeletal muscle-specific ubiquitin-conjugating enzyme homologous to UBC7 of C. elegans. Cytogenet. Cell Genet. *74*, 146-148.

Wefes, I., Mastrandrea, L.D., Haldeman, M., Koury, S.T., Tamburlin, J., Pickart, C.M., and Finley, D. (1995). Induction of ubiquitin-conjugating enzymes during terminal erythroid differentiation. Proc. Natl. Acad. Sci. USA *92*, 4982-4986.

Wenzel, D.M., Stoll, K.E., and Klevit, R.E. (2011). E2s: structurally economical and functionally replete. Biochem. J. *433*, 31-42.

Xin, H., Lin, W., Sumanasekera, W., Zhang, Y., Wu, X., and Wang, Z. (2000). The human RAD18 gene product interacts with HHR6A and HHR6B. Nucleic Acids Res.*28*, 2847-2854.

Xu, K., Jiang, L., Zhang, M., Zheng, X., Gu, Y., Wang, Z., Cai, Y., Dai, H., Shi, Y., Zheng, S., et al. (2016). Type 2 diabetes risk allele UBE2E2 is associated with decreased glucose-stimulated insulin release in elderly Chinese han individuals. Medicine (Baltimore) *95*, e3604.

Yamauchi, T., Hara, K., Maeda, S., Yasuda, K., Takahashi, A., Horikoshi, M., Nakamura, M., Fujita, H., Grarup, N., Cauchi, S., et al. (2010). A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at UBE2E2 and C2CD4A-C2CD4B. Nat. Genet. *42*, 864-868.

Yanagitani, K., Juszkiewicz, S., and Hegde, R.S. (2017). UBE2O is a quality control factor for orphans of multiprotein complexes. Science *357*, 472-475.

Ye, Y., and Rape, M. (2009). Building ubiquitin chains: E2 enzymes at work. Nat. Rev. Mol. Cell Biol. *10*, 755-764.

Yokota, T., Nagai, H., Harada, H., Mine, N., Terada, Y., Fujiwara, H., Yabe, A., Miyazaki, K., and Emi, M. (2001). Identification, tissue

expression, and chromosomal position of a novel gene encoding human ubiquitin-conjugating enzyme E2-230k. Gene *267*, 95-100.

Yu, B., Swatkoski, S., Holly, A., Lee, L.C., Giroux, V., Lee, C.S., Hsu, D., Smith, J.L., Yuen, G., Yue, J., et al. (2015). Oncogenesis driven by the Ras/Raf pathway requires the SUMO E2 ligase Ubc9. Proc. Natl. Acad. Sci. USA *112*, E1724-E1733.

Yu, H., Xiang, P., Pan, Q., Huang, Y., Xie, N., and Zhu, W. (2016). Ubiquitin-conjugating enzyme E2T is an independent prognostic factor and promotes gastric cancer progression. Tumour Biol. *37*, 11723-11732.

Zhang, Y., Zhou, X., Zhao, L., Li, C., Zhu, H., Xu, L., Shan, L., Liao, X., Guo, Z., and Huang, P. (2011). UBE2W interacts with FANCL and regulates the monoubiquitination of Fanconi anemia protein FANCD2. Mol. Cells *31*, 113-122.

Zhang, X., Zhang, J., Bauer, A., Zhang, L., Selinger, D.W., Lu, C.X., and Ten Dijke, P. (2013a). Fine-tuning BMP7 signalling in adipogenesis by UBE2O/E2-230K-mediated monoubiquitination of SMAD6. EMBO J. *32*, 996-1007.

Zhang, X., Zhang, J., Zhang, L., van Dam, H., and ten Dijke, P. (2013b). UBE2O negatively regulates TRAF6-mediated NF-kappaB activation by inhibiting TRAF6 polyubiquitination. Cell Res. *23*, 366-377.

Zhang, B., Deng, C., Wang, L., Zhou, F., Zhang, S., Kang, W., Zhan, P., Chen, J., Shen, S., Guo, H., et al. (2017). Upregulation of UBE2Q1 via gene copy number gain in hepatocellular carcinoma promotes cancer progression through beta-catenin-EGFR-PI3K-Akt-mTOR signaling pathway. Mol. Carcinog. *57*, 201-215.

Zhou, W., Xu, J., Li, H., Xu, M., Chen, Z.J., Wei, W., Pan, Z., and Sun, Y. (2017). Neddylation E2 UBE2F promotes the survival of lung cancer cells by activating CRL5 to degrade NOXA via the K11 linkage. Clin. Cancer Res. *23*, 1104-1116.