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Molecules in focus

# Ubiquitin-conjugating enzyme E2C: A potential cancer biomarker

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ABSTRACT

The ubiquitin-conjugating enzymes 2C (UBE2C) is an integral component of the ubiquitin proteasome system. UBE2C consists of a conserved core domain containing the catalytic Cys residue and an N-terminal extension. The core domain is required for ubiquitin adduct formation by interacting with the ubiquitin-fold domain in the E1 enzyme, and contributes to the E3 enzyme binding. UBE2C N-terminal extension regulates E3 enzyme activity as a part of an intrinsic inhibitory mechanism. UBE2C is required for the destruction of mitotic cyclins and securin, which are essential for spindle assembly checkpoint and mitotic exit. The UBE2C mRNA and/or protein levels are aberrantly increased in many cancer types with poor clinical outcomes. Accumulation of UBE2C stimulates cell proliferation and anchorage-independent growth. UBE2C transgenic mice are prone to develop spontaneous tumors and carcinogen-induced tumor with evidence of chromosome aneuploidy.



**Molecule facts**

- A key component in the ubiquitin proteasome system by partnering with APC/C.
- The core domain forms ubiquitin adduct and the N-terminus regulates E3 enzyme activity.
- Assists in the destruction of cyclins and securin leading to anaphase onset and mitotic exit.
- Aberrantly increased in many cancer types with poor clinical outcomes.
- Transgenic mice are prone to develop spontaneous and induced tumors.

## 1. Introduction

In eukaryotes, the ubiquitin proteasome system (UPS) precisely regulates cell cycle at key checkpoints by targeting cell cycle regulators for proteasome-mediated degradation. The UPS requires the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2) and ubiquitin ligases (E3) to work in concert to facilitate ubiquitination of target proteins. While monoubiquitination regulates ubiquitin-dependent endocytosis, reorganization of protein complexes, DNA repair and transcriptional regulation; the labeling of target proteins for degradation requires polyubiquitination, *i.e.*, a chain of at least four ubiquitins is added to a single Lys residue (Lin et al., 2002; Ye and Rape, 2009).

*Abbreviations:* APC/C, anaphase-promoting complex/cyclosome; ATRA, all-trans retinoic acid; B1-4, β-sheets; BUB3, budding uninhibited by benzimidazoles 3; BubR1, BUB receptor 1; CCI-779, cell cycle inhibitor-779; CDC20, cell division cycle 20; Cdh1, cadherin-1; CDK, cyclin-dependent kinase; c-Myc, avian myelocytomatosis virus oncogene cellular homolog; CP1A/C, calpain-1 A/C; c-Rel, reticuloendotheliosis oncogene cellular homolog; D-box, destruction box; DUBs, deubiquitination enzymes; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E2F, elongation 2 transcription factor; E3, ubiquitin ligase; $G_1$, gap 1 phase; $G_2$, gap 2 phase; H1-4, α-helices; $3_{10}$, $3_{10}$-helix; JNK, Jun N-terminal kinases; KEN-box, the amino acid sequence KEN$(X)_n$P; M, mitosis phase; MAD 2, mitotic arrest deficient-like 2; MED1, mediator-1; Meis-1, myeloid ecotropic viral integration site 1; mRNA, messenger ribonucleic acid; NF-1, neurofibromin 1; NIH3T3, primary mouse embryonic fibroblast; N-terminal, amino acid-terminal; Pax-2, paxillin 2; QNP, motif, $Gln^4$, $Asn^5$ and $Pro^8$ motif; RING, finger domain, really interesting new gene finger domain; RWD, domain, domain find in RING finger-WD repeat domain containing protein; S, synthesis phase; SCF, Skp1-Cullin-Fox complex; Skp2, S-phase kinase-associated protein 2; SRF, serum response factor; UBE2C, ubiquitin-conjugating enzyme E2; UbcH10, human ubiquitin-conjugating enzyme 10; UPS, ubiquitin proteasome system; USP44, ubiquitin specific peptidase 44.

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Of the ubiquitin enzymes, the E1 is responsible for activating ubiquitin by attaching the molecule to an active site Cys and subsequently transfers the ubiquitin to the E2 active site Cys *via* a thioester linkage. The E2 then donates the ubiquitin from its Cys to Lys of the target protein through E3-mediated specificity. The E3 enzyme is also responsible for binding to the target protein destined for degradation. The human genome contains two genes coding for E1, ~38 genes for E2 and >600 genes for E3 (Ye and Rape, 2009). E3 enzymes are grouped according to their domains. The majority of E3s belong to the really interesting new gene (RING) domain group including the Skp1-Cullin-Fox (SCF) complex and the anaphase-promoting complex (also called cyclosome, APC/C) (Meyer and Rape, 2011). E3s have been the focus of most studies due to their deregulation being implicated in cancer. However, the crucial role of E2s in the regulation of cell cycle progression and in particular cancer development and progression has only been suggested recently.

Emerging evidence indicates that the ubiquitin-conjugating enzymes 2C (UBE2C) possesses oncogenic property. The UBE2C is highly conserved and its human homolog (also known as UbcH10) was cloned in 1997 (Townsley et al., 1997). UBE2C is essential for cell cycle progression, as mutation of the active site ($Cys^{114}$Ser) inhibits destruction of mitotic cyclins (Townsley et al., 1997). Accumulation of UBE2C stimulates cell proliferation and anchorage-independent growth *in vitro* and *in vivo* (Hao et al., 2012). UBE2C mRNA and/or protein levels are aberrantly increased in a wide range of human cancers, and high expression of UBE2C is associated with poor clinical outcomes in at least 6 cancer types (Hao et al., 2012; van Ree et al., 2010). Additionally, a tumor phenotype emerges when UBE2C is overexpressed in transgenic mice (Hao et al., 2012).

## 2. Structure

E2s are classified into four classes and all share a conserved core domain containing the catalytic Cys residue. Class I consists of the core domain only and requires the presence of an E3 for conjugation of ubiquitin. Class II and III has C-terminal and N-terminal extension from the core domain, respectively. These extensions may contribute to target protein recognition and regulate the conjugation of ubiquitin to target proteins. Class IV contains both N- and C-terminal extensions (Wing et al., 1995). UBE2C is a class III E2 enzyme.

The human *UBE2C* gene is located at 20q13.12. There are eight transcript variants through alternative splicing. UBE2C protein referred to in this article is the product of splice variant 1 with the longest reading frame. The full length UBE2C contains 179 amino acids with a molecular weight of 19.65 kDa (Lin et al., 2002; Townsley et al., 1997). The first 28 residues comprise an N-terminal extension with various motifs that will be discussed in detail later; the remaining residues form the core domain (Fig. 1A). The UBE2C protein structure (PDB: 1I7K) contains a four-stranded antiparallel β-sheet (B1-4), a conserved $3_{10}$-helix ($3_{10}$) and four α-helices (H1-4) (Fig. 1B). It is intriguing to envisage that a protein with a size of ~20 kDa is able to interact with 3–4 different proteins, *i.e.*, ubiquitin, E1, E3 and possibly the target protein (Jiang and Basavappa, 1999). Hence, a unique structural feature of UBE2C must be maintained to allow these interactions.

### 2.1. The E2 core domain

The catalytic $Cys^{114}$ responsible for ubiquitin adduct formation is located between B4 and the $3_{10}$. The three residues per turn geometry of the $3_{10}$ places the positively charged $Lys^{119}$ residue in close proximity to the active site $Cys^{114}$, contributing to ubiquitin adduct formation (Lin et al., 2002). The four β turns (1–4) provide the contact surface for the active site $Cys^{114}$. The residues $Gln^{36}$ (H1), $Leu^{39}$ (H1), $Leu^{59}$ (B2) and $Phe^{60}$ (B2) are predicted to regulate E2 thioester transfer by interacting with the E1 ubiquitin-fold domain (Olsen and Lima, 2013) (Fig. 1B). UBE2C interacts with the E3 through the Loop 1 (89–95), Loop 2 (122–127) and the N-terminal H1. The binding residues for APC/C include $Lys^{33}$, $Met^{43}$, $Met^{44}$ and $Asp^{47}$ in H1, $Phe^{53}$ in B1, $Tyr^{91}$ and $Ala^{124}$ in Loops 1 and 2 (Jin et al., 2008; Lin et al., 2002) (Fig. 1B). It remains to be determined if UBE2C is recruited by APC2 or APC11 (the core subunits of APC/C) or by the binding site created by APC2 and APC11 complex (Nagy et al., 2012; Summers et al., 2008). The degradation of UBE2C by ubiquitination also requires $Cys^{114}$ and a destruction box (D-box)-like motif involving residues 129–132 (Lin et al., 2002; Rape and Kirschner, 2004). UBE2C also contains a RWD domain, which includes a key $Glu^{38}$ residue in the H1 and a $Tyr^{89}Pro^{90}XXXPro^{94}$ motif. The UBE2C RWD domain is thought to be involved in the E2-E3 interaction and in E2 dimer formation (Lin et al., 2002; Nameki et al., 2004).

### 2.2. The N-terminal extension

The UBE2C N-terminal extension is not essential for ubiquitin-adduct formation but contributes to the regulation of APC/C activity as a part of an inhibitory mechanism (Lin et al., 2002). This is achieved mainly by enhancing the sensitivity of APC/C to its pseudosubstrate inhibitor Emi1 and BubR1, and by conferring D-box-APC/C engagement, which in turn limits the orientation or recruitment of target protein Lys near the E2 active site (Summers et al., 2008). In addition, the interaction between the QNP motif ($Gln^4$, $Asn^5$ and $Pro^8$) and the APC/C is required for negative regulation of APC/C activity. In the absence of the QNP motif, the UBE2C core domain strongly promotes APC/C activity. The initiation motif within the N-terminal extension promotes the autoubiquitination of UBE2C (Rape and Kirschner, 2004; Summers et al., 2008; Williamson et al., 2011). Specifically, the candidate residues for initiation motif, $Arg^6$, $Arg^{17}$ and $Lys^{18}$, are required for ubiquitin chain initiation (Williamson et al., 2011). Deletion of the N-terminal residues 1–27 impaired the formation of ubiquitin chains (Summers et al., 2008). The N-terminal residues 10–28 are also needed for its own degradation by binding to the 26S proteasome (Zhao et al., 2010) (Fig. 1A).

## 3. Biological function

UBE2C, as an exclusive partner of APC/C, participates in the degradation of a family of APC/C target proteins by initiating $Lys^{11}$-linked ubiquitin chains (Jin et al., 2008; Meyer and Rape, 2011). The human APC/C is composed of 14 subunits, and the catalytic core consists of a Cullin subunit (APC2) and RING domain subunit (APC11). The human APC/C has more than 55 reported target proteins in *Homo sapiens*. Of these, 37 are involved in cell cycle S and M phase (*e.g.*, cyclin A, cyclin B, p21 and securin), 11 are cell-cycle related in general (*e.g.*, E2-C, E2F1, JNK and Skp2), and 2 are APC/C co-activators (CDC20 and Cdh1) (Meyer and Rape, 2011).

### 3.1. Ubiquitin conjugation

The most common degradation motifs in APC/C target proteins are the D-box and KEN-box. The D-box is recognized by APC/C co-activators CDC20 and Cdh1, whereas the KEN-box is recognized by Cdh1 only (Meyer and Rape, 2011). Upon recruitment by APC/C, CDC20 and Cdh1 serve as D- and KEN-box receptors for various APC/C target proteins. Following binding to target proteins, $APC/C^{CDC20}$ and $APC/C^{Cdh1}$ provide a scaffold for UBE2C to be recruited and oriented, such that the UBE2C facilitates $Lys^{11}$-ubiquitin transfer from UBE2C to target proteins. A sequence

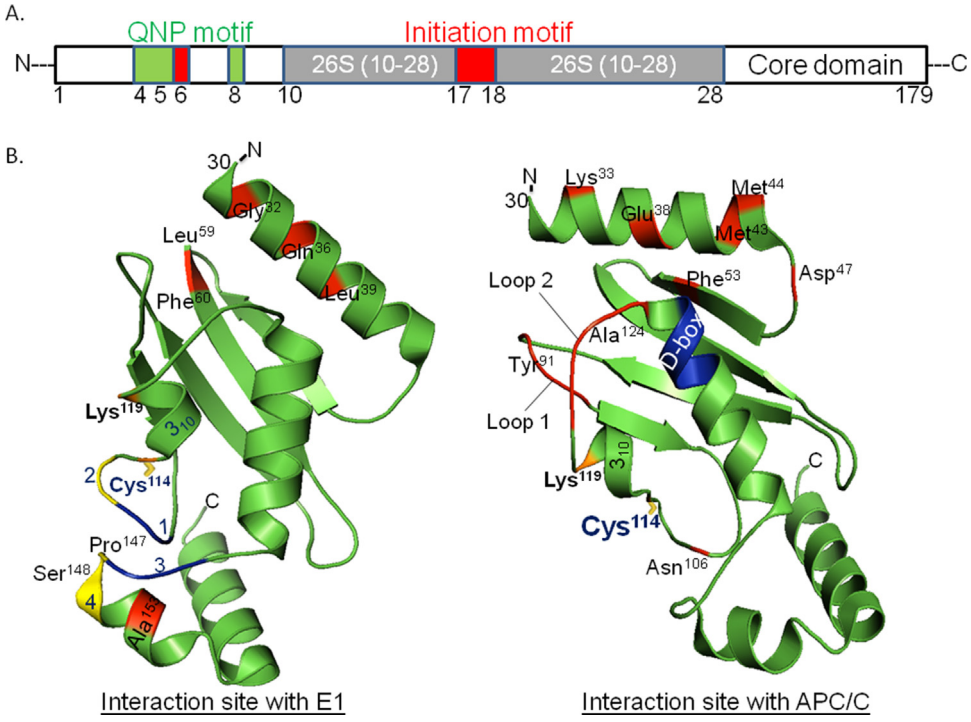


**Fig. 1.** The structure of UBE2C. (A) Predicted functional motifs in UBE2C (1–179). Green: QNP motif (Gln[4], Asn[5] and Pro[8]); red: initiation motif (Arg[6], Arg[17] and Lys[18]). (B) Secondary structure in core domain of UBE2C (30–175). Green: the α-helices, $3_{10}$-helix and β-sheets. Yellow stick: the active site Cys[114]. Orange: Lys[119]. The interaction site of UBE2C to E1 (left) is shown in red, the four β-turns near the active site are labeled 1–4 and marked as yellow or blue. The Loops 1–2 and the interaction sites of UBE2C to APC/C (right) are shown in red; the D-box like motif is shown in blue. This figure was created with PyMOL (PDB ID: 1I7K).

element named as the TEK box (~20 residues downstream of D- or KEN-box in target proteins) mediates the assembly of $Lys^{11}$-linked ubiquitin chains. The TEK-box forms a charged patch around $Lys^{11}$ and exposes Lys in target protein to the active site of UBE2C, directing $Lys^{11}$-ubiquitin chain transfer (Jin et al., 2008; Meyer and Rape, 2011). By mediating the binding of target proteins to APC/C, the positively charged initiation motifs in the N-terminus of UBE2C allow APC/C to fine-tune the timing of protein degradation (Jin et al., 2008; Williamson et al., 2011).

### 3.2. Degradation of key proteins regulating cell cycle progression

UBE2C is required for the destruction of mitotic cyclins and other mitosis-related substrates (Aristarkhov et al., 1996; Arvand et al., 1998; Rape and Kirschner, 2004; Rape et al., 2006). During metaphase, UBE2C promotes progression to anaphase by degradation of securin and cyclin B *via* $APC/C^{CDC20}$. Separase is a protease that degrades the cohesin rings that link the two sister chromatids together. Typically, securin inactivates separase by forming the securin-separase binding complex. By degradation of securin and the consequential activation of separase, sister chromatids are separated and thus UBE2C directly promotes anaphase onset (Hao et al., 2012) (Fig. 2). Cyclin B forms the complex with Cdk1. The cyclin B/Cdk1 complex remains active through the cell cycle until mitosis, where UBE2C-$APC/C^{CDC20}$ induces degradation of cyclin B rendering Cdk1 inactive. The Cdk1 inactivation is required to keep Cdh1 unphosphorylated and thus forming complex with APC/C by replacing CDC20. This is a prerequisite step for mitotic exit and $G_1$ and S progression (Arvand et al., 1998; Rape et al., 2006).

### 3.3. Regulation of mitotic spindle checkpoint

The metaphase to anaphase transition is tightly controlled by spindle assembly checkpoint (SAC) or mitotic checkpoint. The SAC proteins include MAD2, BUB3 and BubR1. The SAC is on to ensure proper kinetochores attachment and correct chromosome segregation. SAC proteins inhibit APC/C activity by sequestering CDC20. Upon proper kinetochores attachment, SAC function is switched off. UBE2C can also deactivate SAC by non-degradative ubiquitination of CDC20 causing its dissociation from other SAC proteins. Although this action is APC/C dependent, UBE2C plays an indispensable role in controlling the molecular switch of the SAC mechanism. Conversely, USP44, a deubiquitinating enzyme, mediates the deubiquitination of CDC20 thus preventing premature anaphase onset (Hao et al., 2012; Reddy et al., 2007; Williamson et al., 2011) (Fig. 2).

## 4. Expression, activation and turnover

### 4.1. UBE2C expression

The predicted transcription factors binding sites at the *UBE2C* promoter have been identified, including binding sites for C-Rel, Pax-2, Pax-2a, CP1A, CP1C (GeneCard: GC20P044442), NF-1, SRF, TGIF, Meis-1 and c-Myc (Hao et al., 2012). *UBE2C* can be an androgen receptor target gene in prostate cancer cells and is responsive to hyperphosphorylated MED1 (androgen receptor co-activator mediator-1) in androgen receptor negative prostate cancer cells (Chen et al., 2011). *UBE2C* expression can also be induced by the estrogen receptor in breast cancer cells (Wang et al., 2013). UBE2C mRNA and protein levels were also shown to be upregulated when EWS/FLI1 (a transcription factor highly expressed in Ewing's sarcoma and primitive neuroectodermal tumor) was transfected into NIH3T3 cells (Arvand et al., 1998).

UBE2C is localized in the nucleus and the cytoplasm and its levels are cell-cycle regulated. The levels of UBE2C are low in $G_1$ (van Ree et al., 2010; Walker et al., 2008), accumulate gradually during S and $G_2$ with a peak at mitosis, and then sharply decrease as cells exit from mitosis (Arvand et al., 1998; Nath et al., 2011;

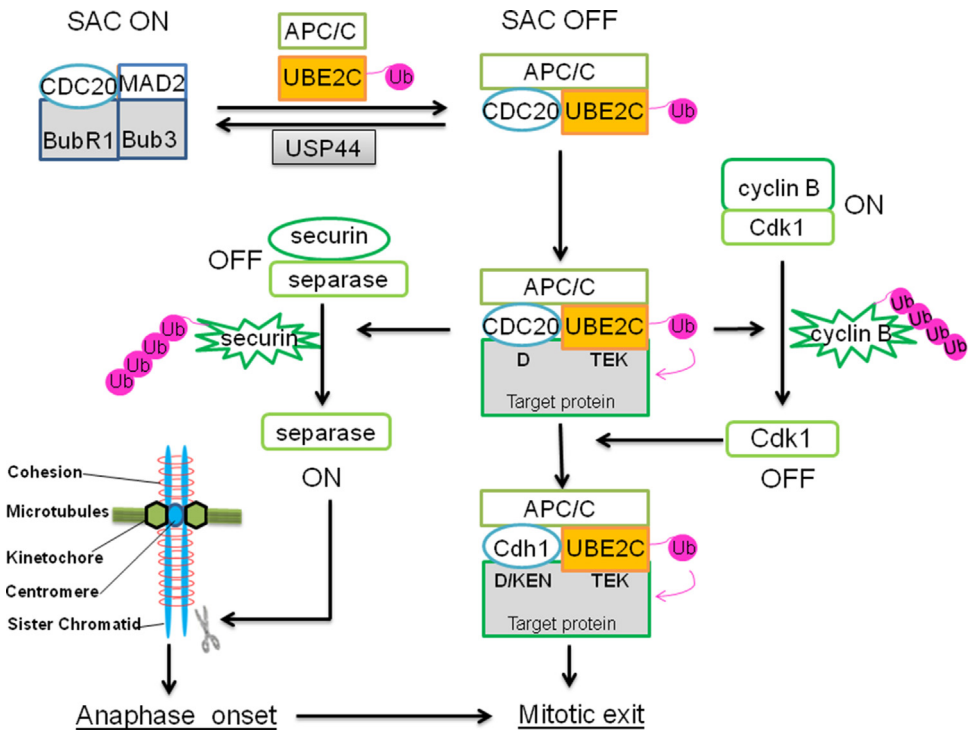


**Fig. 2.** The function of UBE2C. The CDC20 (a co-activator of APC/C) is inhibited by SAC proteins (MAD2, BUB3 and BubR1). APC/C and UBE2C facilitate the disassembly of SAC by ubiquitination. Whereas USP44 antagnises this ubiquitination process. The freed CDC20 complexes with APC/C and UBE2C, which in turn enhances the recruitment of protein for degradation *via* ubiquitination. Degradation of securin activates separase, which then degrades the cohesin rings, leading to separation of sister chromatids and anaphase onset. Degradation of cyclin B inactivates Cdk1, which is required to keep Cdh1 active and thus mitotic exit. D: D-box; KEN: Ken-box; TEK: TEK-box.

Walker et al., 2008). In quiescent cells induced by contact inhibition or serum deprivation, UBE2C mRNA and protein levels are low or undetectable (Arvand et al., 1998; Walker et al., 2008). After re-induction of serum for 14 h, UBE2C levels began to rise 2 h before cyclin A, the latter marks S phase transition (Walker et al., 2008).

### 4.2. *UBE2C activation*

Ubiquitin is activated by E1 in an ATP-dependent manner and then transferred to E2. Hence, when not bind to E3, free E2s are mostly loaded with ubiquitin (at least four $Lys^{11}$-linked ubiquitins are required for 26S proteasome degradation). Free E2s with pre-loaded ubiquitin are available for activation by E3s in order to transfer ubiquitin to a target protein (Ye and Rape, 2009). E3s induce conformational changes in E2 in the presence of target proteins, which positions UBE2C $Asn^{106}$ near the active site. This stabilizes an oxyanion intermediate in the transition state, causing the full activation of UBE2C (Rape et al., 2006; Ye and Rape, 2009).

### 4.3. *UBE2C turnover*

Interestingly, UBE2C is degraded in a process described as autoubiquitination, in association with $APC/C^{Cdh1}$ but not $APC/C^{CDC20}$ (Grutzmann et al., 2004; Rape and Kirschner, 2004). Throughout mitosis, the APC/C is saturated with targeting proteins, which spares UBE2C degradation as well as APC/C inactivation. After complete target protein destruction, the APC/C ubiquitinates UBE2C (Meyer and Rape, 2011; Rape and Kirschner, 2004). The degradation of UBE2C is a slow process because of deubiquitination enzymes (DUBs), which remove ubiquitin from UBE2C. Most DUBs are activated upon the dissociation of ubiquitin-labeled UBE2C from $APC/C^{Cdh1}$ (Rape et al., 2006). The exact DUB responsible for deubiquitination of $Lys^{11}$-ubiquitin chains is yet to be identified.

## 5. Medical application

UBE2C mRNA is barely detectable in the majority of normal tissues (Okamoto et al., 2003). In contrast, *UBE2C* mRNA and/or protein is expressed at high levels in leukemia, lymphoma and melanoma (TCGA data *via* cBioPortal:UBE2C), and cancers of the adrenal gland, bladder, brain, breast, cervices, colon and rectum, esophagus, liver, lung, nasopharynx, ovary, prostate (late-stage), pancreas, stomach, thyroid and uterus (Hao et al., 2012; Vasiljevic et al., 2013). An association between the levels of UBE2C and tumor grade/poor prognosis are reported in cancers of the adrenal gland, breast, colon, liver, lung and ovary (Hao et al., 2012; Okamoto et al., 2003; van Ree et al., 2010).

The mechanism of UBE2C upregulation in oncogenic condition is largely unresolved (Nath et al., 2011). The amplification of the *UBE2C* gene has been reported (Hao et al., 2012; Vasiljevic et al., 2013). Another possible mechanism is the overexpression or hyperactivation of proteins upstream of UBE2C, such as the androgen receptor and MED1 in advanced prostate cancer, the estrogen receptor in breast cancer and c-Myc in other cancer types. Out of 7949 unique cancer samples, 20 harbor somatic mutations in UBE2C gene (COSMIC: COSG4332). Based on the three-dimensional structure of UBE2C, the $Arg^{129}Ser$ mutation identified in lung squamous cell carcinoma tissue (COSMIC: COSM724441) could be biologically significant, because alterations in the UBE2C putative D-box motif have been shown to stabilize UBE2C against destruction. Conversely, anti-cancer drugs (*e.g.*, tamoxifen, ATRA and CCI-779) and expression of a tumor suppressor gene (C2ORF40) downregulate *UBE2C* mRNA levels leading to suppression of cancer cell proliferation *in vitro* and *in vivo* (Hao et al., 2012; Lu et al., 2013).

UBE2C transgenic mice are prone to develop a broad spectrum of spontaneous tumors as well as carcinogen-induced lung tumors with evidence of precocious degradation of cyclin B, extra centrioles, chromosome lagging and aneuploidy (van Ree et al., 2010).

In an *in vivo* study, *UBE2C* silencing decreased the invasiveness of advanced prostate cancer cells (Wang et al., 2011). Together, these results strongly suggest that UBE2C is causally involved in cancer development and progression.

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