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Review

Molecular Cancer Research

Functional Roles of the E3 Ubiquitin Ligase UBR5 in Cancer

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Abstract

The Ubiquitin-Proteasome System (UPS) is an important regulator of cell signaling and proteostasis, which are essential to a variety of cellular processes. The UPS is disrupted in many diseases including cancer, and targeting the UPS for cancer therapy is gaining wide interest. E3 ubiquitin ligases occupy a key position in the hierarchical UPS enzymatic cascade, largely responsible for determining substrate specificity and ubiquitin (Ub) chain topology. The E3 ligase UBR5 (aka EDD1) is emerging as a key regulator of the UPS in cancer and development. UBR5 expression is deregulated in many cancer types and UBR5 is frequently mutated in mantle cell lymphoma. UBR5 is highly conserved in metazoans, has unique structural features, and has been implicated in regulation of DNA damage response, metabolism, transcription, and apoptosis. Hence, UBR5 is a key regulator of cell signaling relevant to broad areas of cancer biology. However, the mechanism by which UBR5 may contribute to tumor initiation and progression remains poorly defined. This review synthesizes emerging insights from genetics, biochemistry, and cell biology to inform our understanding of UBR5 in cancer. These molecular insights indicate a role for UBR5 in integrating/coordinating various cellular signaling pathways. Finally, we discuss outstanding questions in UBR5 biology and highlight the need to systematically characterize substrates, and address limitations in current animal models, to better define the role of UBR5 in cancer. *Mol Cancer Res; 13(12); 1523–32. ©2015 AACR.*

Ubiquitination and the Role of E3 Ubiquitin Ligases

Ubiquitination is one of the most widespread and frequent cellular posttranslational modifications (PTM) and is essential for normal cellular functions. Ubiquitination can regulate protein levels via degradation by the proteasome (1, 2), regulate protein–protein interactions, and modulating protein function and localization (3, 4). The small protein modifier Ubiquitin (Ub) forms an isopeptide bond with substrate proteins via acceptor lysine residues in a hierarchical enzymatic reaction catalyzed by an E3 Ub ligase (5). Much variety in Ub modification of substrate proteins arises from the ability of Ub to form either single conjugates (mono-ubiquitination) or chains (poly-ubiquitination) after repeated rounds of Ub attachment (6). Further complexity and diversity in Ub PTMs is generated by variation in Ub chain topology, depending on which Ub lysine residue is further ubiquitinated on the growing poly-Ub chain. Various poly-Ub chain topologies are known to effect different signaling outcomes via binding to specific adaptor proteins [reviewed elsewhere (4)]. Specificity of the Ubiquitin-Proteasome System (UPS) is largely determined by the approximately 617 (human) E3 Ub ligases (7), but significant pleiotropy and redundancy among E3 Ub ligases provides tissue specificity and fine-scale regulation of signaling outputs (6).

Correct functioning of the UPS is essential for many cellular processes such as cell-cycle progression and apoptosis. Consequently, the UPS is the subject of intense interest for its therapeutic potential in many diseases including cancer [reviewed elsewhere (8)]. Currently, only broad-acting proteasome inhibitors are in clinical use. Proteasome inhibitors bortezomib (Velcade) and carfilzomib (Kyprolis) have reached phase II clinical trials for use in mantle cell lymphoma (MCL) and multiple myeloma (9–12), although use is associated with peripheral neuropathy (12, 13). The E3 ligase modulator Lenalidomide (Revlimid, an analogue of Thalidomide), is also used in treatment of multiple myeloma but has associated resistance and toxicity (14).

The UPS targets a large range of proteins with important roles in cancer biology. Therapeutic benefit with reduced toxicity might be obtained by targeting more specific aspects of the UPS. For example, Ub-specific proteases, an extensive class of de-ubiquitinating enzymes, have promise as therapeutic targets (15). Of comparable interest and specificity are the E3 Ub ligases, especially as key proteins commonly associated with proliferation and cell-cycle arrest (such as p53, p27, Cyclins, and NF-κB) are specifically regulated by these enzymes (16).

One of the more intriguing members of the E3 ligase family is UBR5 [Ubiquitin protein ligase E3 component n-recognin 5, also known as EDD (E3 identified by Differential Display), EDD1, HHYD, KIAA0896, or DD5]. UBR5 is highly conserved (17) and is essential for mammalian development (18). UBR5 is rarely mutated in healthy somatic tissues, but is mutated and/or overexpressed in cancer (19, 20). UBR5 is known to modulate the DNA damage response (DDR) and transcription (see below for detailed discussion), and emerging roles for UBR5 in a number of biologic contexts have been identified using high-throughput, functional genomics screens. This review will summarize the

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characterized and emerging biologic roles of UBR5, its role in tumorigenesis, and its potential as a therapeutic target.

Unique Genomic and Protein Structure of UBR5

UBR5 is a homologue of hyperplastic discs (HYD), a *Drosophila melanogaster* tumor suppressor (21). Mammalian UBR5 is a HECT (homologous to E6-associated protein at the carboxy-terminus) E3 Ub ligase recognizing n-degrons (see Fig. 1; refs. 17, 22). The human *UBR5* gene has 59 exons, encoding an approximately 10 kb mRNA and >300 kDa protein with widespread expression in various cell types. One splice variant (loss of nucleotides 884–901, removing amino-acid sequence VLLLPL) has been identified in 26/26 cancer cell lines investigated (19), although numerous other splice variants are predicted in genome databases. The structural/functional consequences of these variants are not known. UBR5 is highly conserved, but appears to be restricted to the metazoan lineage, and based on phylogenetic analysis is grouped in a distinct class of HECT ligases (Class IV; ref. 23). Class IV likely evolved from a common ancestral gene duplication and includes UBR5/EDD and three other families that are highly divergent at the sequence level.

HECT E3 Ub ligases have a well characterized mechanism, forming a thio-ester linkage between Ub and the substrate protein, a reaction highly dependent on a conserved cysteine within the HECT domain (24, 25). The catalytically active HECT domain of UBR5 is made up of N- and C-lobes separated by a linker sequence as determined by high-resolution crystallography (26). However, the HECT domain of UBR5 has some unique structural features compared with other HECT ligases. The C-lobe of the UBR5 HECT domain does not have a surface for noncovalent binding of Ub, and it is unclear if this exists in the N-lobe (a feature seen in other HECT E3 Ub ligases such as NEDD4, and thought to keep Ub in close proximity for building poly-Ub chains; ref. 26). Instead, UBR5 interacts with Ub via the UBA domain (27). UBR5 also has a zinc finger Ubiquitin Recognin Box (UBR) domain, thought to be involved in N-rule substrate recognition (28), two nuclear localization sequences (29), and an MLLE/PABC domain [homologous to the C-terminal region of Poly-Adenylation Binding Protein (30); Fig. 1]. The MLLE/PABC domain is thought to be a protein–protein interaction motif (e.g., known to bind substrates such as PAIP2; refs. 31, 32) and may regulate ubiquitin transfer catalyzed by the HECT domain (33).

Expression of *UBR5* is responsive to progestin in human breast cancer cells, and shows varied expression in normal tissues (19). *UBR5* is highly overexpressed in breast cancer at the mRNA level with increases in UBR5 protein also evident in many samples (19). This indicates that regulation of UBR5 is likely at the transcriptional level, though regulation of activity may also occur through PTM. Many phosphorylation sites have been identified in UBR5 (see Fig. 1; refs. 34–41), and UBR5 is known to be a phosphorylation target of ATM (42), CHK (43), and ERK kinases (44). However, this phosphorylation has not as yet been directly linked to UBR5 activity.

UBR5 in Cancer

A number of studies have implicated UBR5 in various aspects of cancer biology and many of the molecular functions of UBR5 (e.g., DDR) are consistent with a role in cancer. Indeed, the *Drosophila* homologue *Hyd* was originally identified as a tumor suppressor gene (21). Human *UBR5* was originally identified in a screen for progestin-regulated genes in breast cancer cells (17). Amplification of *UBR5* has been reported in breast and ovarian cancer, mostly in the form of allelic imbalance resulting in increased *UBR5* mRNA levels (19). *UBR5* is located at genomic locus 8q22.3 (17), just downstream from *MYC* (8q22.4) but *UBR5* amplification is independent of microsatellites examined in the more distal regions of the 8q arm (19). Provisional data from The Cancer Genome Atlas (TCGA) clearly show *UBR5* amplification to be a common alteration in many cancer types (see Fig. 2A). UBR5 has been shown to mediate therapeutic resistance in ovarian cancer (20), likely through modulation of the DDR (see below). *UBR5* was also identified in a transposon mutagenesis screen for cooperating mutations and pathways in pancreatic adenocarcinoma (45).

Perhaps the most convincing evidence yet for an integral role for UBR5 in cancer comes from a recent study showing nonsynonymous mutations of *UBR5* in 18% of MCL cases. Most of these mutations affected the conserved cysteine of the HECT domain, which would be expected to disrupt E3 ligase activity (46). Unfortunately, the embryonic lethality observed in *Ubr5*-null mice has precluded their use in cancer models but the development of conditional knockout models should make these studies more feasible.

Functional mutations in *UBR5* may be more informative for inferring the biologic role of UBR5 in cancer. The COSMIC database contains details of published mutations throughout many cancer types (47). Point mutations occur frequently throughout the *UBR5* open reading frame in cancer, and frameshift mutations tend to occur toward the PABC/HECT region,

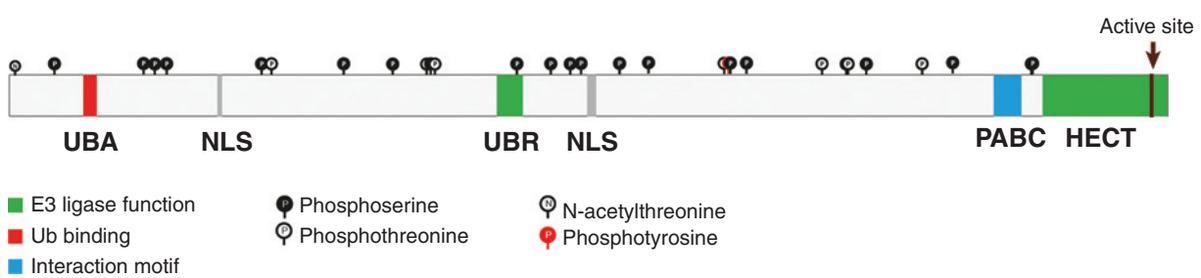

Figure 1.
Functional domains and known posttranslational modification sites of UBR5. Arrow in HECT domain indicates position of highly conserved catalytic cysteine. Other domains shown are Ubiquitin activation (UBA), nuclear localization sequence (NLS), the Ubiquitin Recognin Box (UBR), and the homologous to the C-terminal region of Poly-Adenylation Binding Protein (PABC/MLLE) domain.

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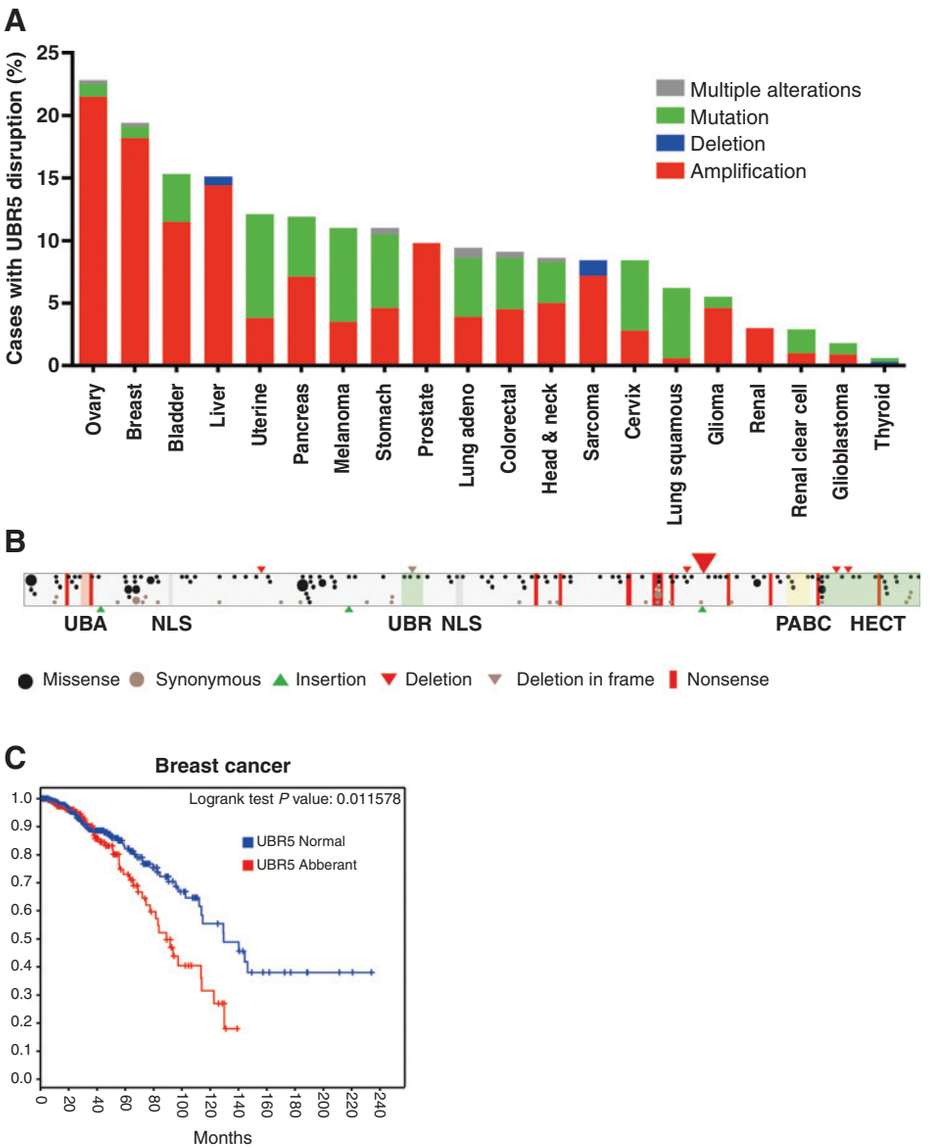

Figure 2.
UBR5 is commonly deregulated in many cancer types, with frequent amplification in breast cancer. A, UBR5 deregulation by cancer type [generated using cBioPortal analytical tool (97, 98) based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/, as of August 2014]. B, functional mutation spectrum of UBR5 by mutation type and location within UBR5 protein sequence, data from COSMIC (ref. 99; (cancer.sanger.ac.uk). Symbol size indicates relative mutation count. Note the tendency for frameshift and nonsense mutations toward carboxy-terminal region of UBR5 (associated with E3 Ub ligase function). C, survival analysis shows a strong survival disadvantage for patients with breast cancer with aberrations in UBR5 [generated using cBioPortal (97, 98) as above].

which would likely result in loss of E3 Ub ligase activity (see Fig. 2B). Loss of E3 Ub ligase activity in cancer suggests that UBR5-mediated ubiquitination has a tumor suppressive role. There is a clear overall survival advantage for breast cancer patients with normal *UBR5* expression (provisional TCGA data; Fig. 2C).

UBR5 Substrates and Other Interacting Proteins

UBR5 has been shown to directly interact with numerous proteins implicated in a wide variety of cellular processes, including the cell cycle, transcriptional and translational machinery, and the DDR. A list of currently known interacting proteins is shown in Table 1. Many of these are key to understanding the role of UBR5 in cancer development and progression.

Identification of E3 Ub ligase substrates is notoriously technically challenging and emerging systematic approaches hold great promise in this respect. Regardless, a subset of UBR5-interacting proteins have been validated as targets of UBR5 E3 Ub ligase activity, although many other interactions appear independent of E3 Ub ligase function, indicating that UBR5-mediated ubiquitination is tightly regulated and is likely context specific. Known targets of UBR5 ligase activity include the DDR proteins TopBP1 (48), RNF168 (49), and ATMIN (42), transcription factors β-catenin (50), pregnane X receptor (PXR; ref. 51), and E6-AP (52), translational machinery CDK9 (53) and PAIP2 (54), the KATNA1 subunit of the cell cycle–related protein KATANIN (55), and the rate limiting enzyme in gluconeogenesis PEPCK (56).

UBR5 Is Necessary for Development and Maintenance of Pluripotency

The first descriptions of a developmental role for UBR5 came from mutants of the UBR5 *Drosophila* homologue, hyperplastic

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Table 1. UBR5 interacting proteins and substrates

UniProt ID	Protein symbol	Protein names	Nature of interaction and confidence	Associated pathway	Reference
P25054	APC	Adenomatous polyposis coli protein (APC)	Screening of APC immuno-complexes by mass spectrometry identified UBR5 as a putative APC-interacting protein. Indirect immunofluorescent analysis demonstrated that APC and UBR5 co-localized in cytoplasm.	Wnt signaling.	(Ohshima *et al.*, 2007)
Q13315	ATM	Serine-protein kinase ATM, Ataxia telangiectasia mutated	UBR5 regulates p53 Ser15 phosphorylation. This was shown by knockdown and overexpression studies and IP western blot experiments.	Cell cycle, DNA damage and repair.	(Ling *et al.*, 2011)
O43313	ATMIN	ATM interactor	UBR5 ubiquitylates ATMIN following DNA damage, disrupting ATM/ATMIN binding. Interaction detected by IP-MS analysis. Validation using reciprocol CO-IP, ubiquitin pulldown, immunofluorescence, G_2–M checkpoint assay and radiosensitivity assay.	DNA damage and repair, transcription.	(Zhang *et al.*, 2014)
P50750	CDK9	CDK9	UBR5 associates with the CDK9 subunit of positive transcription elongation factor b (PTEFb) to mediate its polyubiquitylation in human cells. TFIIS also binds UBR5 to stimulate CDK9 polyubiquitylation. See TIIFS.	Transcription, Transcription regulation	(Cojocaru *et al.*, 2011)
O96017	CHK2	Serine/threonine-protein kinase Chk2	UBR5 and CHK2 interact through the CHK2 phosphopeptide-binding interface of the FHA domain. This interaction most likely requires phosphorylation of UBR5. UBR5-CHK2 interaction is required for CHK2 Thr^{68} phophorylation and kinase activity and for cell survival following DNA damage.	Apoptosis, cell cycle, DNA damage and repair.	(Henderson *et al.*, 2006)
Q99828	CIB1	Calcium and integrin-binding protein 1	Y2H screen identified CIB1 as a UBR5-interacting protein. Interaction confirmed by GST-CIB fusion protein pulldown. Pulldowns using UBR5 *in vitro* translated fragments identified CIB1 interacts with the carboxyl-terminal portion of UBR5. UBR5 and CIB1 interaction decreased following radiomimetic phleomycin treatment.	Calcium ion binding, Ras GTPase binding, cell adhesion.	(Henderson *et al.*, 2002)
P35222	CTNNB1	β-catenin	UBR5 ubiquitylates β-catenin, stabilizing and upregulating the protein. Methods used were Western blot, immunoprecipitation, pull down assays using GST-β-catenin or 6XHis-GSK-3β, immunofluorescence, *in vivo* ubiquitylation assay and siRNA knockdown experiments.	Cell adhesion, transcription, Wnt signaling.	(Hay-Koren *et al.*, 2011)
Q16531	DDB1	DNA damage-binding protein 1	see DYRK2.	DNA damage repair, UPS	(Maddika *et al.*, 2009)
Q92630	DYRK2	Dual specificity tyrosine-phosphorylation-regulated kinase 2	Used tandem affinity purification followed by mass spectrometry analysis to discover DYRK2 interacting proteins. UBR5, DDB1 and VPRBP were identified as major DYRK2 associated proteins. Confirmed interaction using co-immunoprecipitation, bacterially expressed GST-DYRK2 pulled down UBR5, DDB1 and VPRBP. Named this complex EDVP.	Apoptosis, UPS	(Maddika *et al.*, 2009)
P06463	E6	Protein E6 (from HPV type 18 virus)	Interaction detected by IP-MS analysis. Validation by *in vitro* and *in vivo* binding assays and siRNA depletion studies.	HPV infection, p53 regulation, HPV induced malignancy, transcription.	(Tomaic *et al.*, 2011)
Q05086	E6AP	Ubiquitin-protein ligase E3A	See E6	Host–virus interaction UPS.	(Tomaic *et al.*, 2011)
O75469	hPXR	Pregnane X receptor	PXR is phosphorylated by DYRK2, which induces UBR5 ubiquitylation of PXR for proteasomal degradation. UBR5 together with DYK2 regulate PXR protein stability. UBR5 regulation of PXR was identified by MS and a kinome-wide siRNA screen. Validation using knockdown experiments.	Transcription regulation	(Ong *et al.*, 2014)
P78318	IGBP1	alpha4 phosphoprotein (Immunoglobulin-binding protein 1)	Interaction detected by yeast two-hybrid screen with alpha4 phosphoprotein as bait. Validation using alpha4 phosphoprotein deletion mutants and IP-Western blot analysis.	B cell activation, negative regulation of apoptosis and transcription.	(McDonald *et al.*, 2010)
O75449	KATNA1	Katanin p60 (ATPase-containing) subunit A1	See DYRK2.	Cell cycle, mitosis.	(Maddika *et al.*, 2009)

(Continued on the following page)

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Table 1. UBR5 interacting proteins and substrates (Cont'd)

UniProt ID	Protein symbol	Protein names	Nature of interaction and confidence	Associated pathway	Reference
P52294	KPNA1	Importin subunit alpha-5	Using candidate gene and Y2H approaches to identify UBR5-interacting proteins and substrates identified a strong interaction between UBR5 and KPNA1. Interaction validated by GST pulldown and endogenous protein immune-precipitation experiments. Both UBR5 nuclear localization signals are necessary for the interaction to occur.	Protein transporter activity, nuclear localization sequence binding.	(Henderson *et al.*, 2002)
P28482	MAPK1	Mitogen-activated protein kinase 1	Using mutant ERK2 to search for Erk substrates and in vitro phosphorylation assays, it was found that in response to EGF, UBR5 is phosphorylated by ERK2 and in a MEK-dependent manner.	Apoptosis, cell cycle and transcription.	(Eblen *et al.*, 2003)
Q96EZ8	MCRS1	Microspherule protein 1	UBR5 and MCRS1 were shown to interact using *in vitro* and *in vivo* binding assays. In addition UBR5 negative regulates MCRS1 protein levels. However, UBR5 ubiquitylation of MCRS1 was not shown. Reducing expression levels of either UBR5 or MCSR1 affects levels of cyclins B, D, and E as well as cell cycle progression.	DNA damage and repair, transcription.	(Benavides *et al.*, 2013)
Q8IZQ8	MYOCD	Myocardin	UBR5 is an activator of smooth muscle differentiation through its ability to stabilize myocardin protein. Interaction detected using yeast two-hybrid screen with myocardin as bait to search for factors that may regulate the transcriptional activity of the myocardin.	Transcription, positive regulation of smooth muscle contraction.	(Hu *et al.*, 2010)
Q9BPZ3	**PAIP2**	**Polyadenylate-binding protein-interacting protein 2**	**Authors knocked out PABP and also UBR5 to demonstrate a mechanism of co-regulation that involves UBR5, which targets Paip2 for degradation. The turnover of Paip2 in the cell is mediated by UBR5 as shown by *in vitro* ubiquitylation assay.**	**Regulation of translation.**	**(Yoshida *et al.*, 2006)**
P35558	**PEPCK1**	**Phosphoenol-pyruvate carboxykinase, cytosolic [GTP]**	**UBR5 regulates gluconeogenesis acetylation dependent ubiquitylation of PEPCK1. IP-MS analysis identified UBR5 as a regulator of PEPCK1 activity. Validation involved siRNA, IP/Western blot and *in vitro* ubiquitylation experiments.**	**Gluconeo-genesis, metabolism.**	**(Jiang *et al.*, 2011)**
P54277	PMS1	PMSI protein homolog 1	PMS1 immuno-precipitation coupled with mass spectrometry identified UBR5-PMS1 interaction.	Postreplicative mismatch repair (MMR).	(Cannavo *et al.*, 2007)
P06401-1	PRB	Progesterone receptor isoform B	UBR5 contains five L*XX*LL domains and on account of this was tested for the ability to bind to PR-B and regulate its function. The authors used GST-PR fusion pulldowns and *in vitro* assays to confirm the interaction. A MMTV-luciferase reporter assay showed that UBR5 enhanced transcriptional activation by PR-B, in an E3 ligase independent manner.	Nuclear signaling, nuclear receptor transcription pathway.	(Henderson *et al.*, 2002)
Q8IYW5	**RNF168**	**E3 ubiquitin-protein ligase RNF168**	**UBR5 regulates transcriptional silencing and recruitment of DNA repair complexes at sites of DNA damage. UBR5 mediates this regulation by ubiquitylation of RNF168, together with the E3 ligase TRIP12. UBR5 and TRIP12 were identified by siRNA array, followed by depletion studies, WB experiments using inactive E3 ligases and IF experiments to validate the interactions.**	**Transcription regulation, DNA damage response, UPS.**	**(Gudjonsson *et al.*, 2012)**
P23193	TFIIS	Transcription elongation factor TFIIS.	Affinity purification of TAP-tagged TFIIS coupled with mass spectrometry identified TFIIS-UBR5 interaction. Validation using in vitro pulldown experiments using a TFIIS-GST fusion protein coupled with glutathione beads and a HEK 293 whole cell extract were used. Immunoblotting of the eluate with anti-UBR5 antibody revealed the presence of UBR5.	Transcription, Transcription regulation	(Cojocaru *et al.*, 2011)
Q92547	**TOPBP1**	**DNA topoisomerase II-binding protein 1**	**Identified UBR5/TopBP1 interaction by yeast two-hybrid screen. Confirmed TopBP1 ubiquitylation by UBR5 using *in vitro* reconstitution assay and immunofluorescence.**	**DNA damage response and repair.**	**(Honda *et al.*, 2002)**
Q14669	**TRIP12**	**E3 ubiquitin-protein ligase TRIP12**	**See RNF168**	**Transcription regulation, DNA damage response and repair, UPS.**	**(Gudjonsson *et al.*, 2012)**
Q9Y4B6	VPRBP	VPRBP (also known as DCAF1)	See DYRK2.	Transcription UPS.	(Maddika *et al.*, 2009)

NOTE: Proteins within **bold** boxes are validated UBR5 substrates.

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discs [Hyd, otherwise known as I(3)c43]. These display a range of developmental phenotypes, including imaginal disc abnormalities (57), larval lethality, sterility (21), premature photoreceptor differentiation (58), failed oogenesis (59), defective spermatogenesis (60), and plasma membrane gap junction deficiency (61). The severity of these phenotypes appears dependent on the relative level of HYD protein in mutant animals (21). Functional studies identified that Hyd negatively regulates expression of Hedgehog (*Hh*) and Decapentaplegic (*Dpp*) in eye and wing discs. However the mechanism is complex and context specific (58). Recent studies suggest that Hyd regulates differential outputs of Hh signaling by directing selective association of *Cubitis interruptus* (Ci) with different promoters (62). Further, Hyd can coordinate Hh signaling by regulating Hh and Ci expression in concert with *Shaggy* (Sgg), the *Drosophila* orthologue of GSK3β (63).

In mice, homozygous deletion of *Ubr5* causes embryonic lethality around E10.5 due to failed yolk sac and allantoic vascular development and failed chorioallantoic fusion (18). Although the molecular mechanism underlying this phenotype remains to be described, a cluster of UBR5-regulated genes was recently associated with vascular development. Specifically, vascular endothelial cell expression of *UBR5* is necessary for normal vessel formation through a repressive effect on the angiogenic factor ACVRL (64).

The involvement of UBR5 in regulation of hedgehog signaling has not been investigated in mammalian models; however, depletion of Indian hedgehog disrupts murine vascular yolk sac development (65), and paracrine hedgehog signaling is involved in tumor angiogenesis via modulation of VEGF (66). *Drosophila* studies showing a role for Hyd in controlling Hh and Dpp signaling may also be relevant in this context, as both Hh (65) and BMP (67) signaling are key regulators of yolk sac vascular development. It remains to be determined if the role of UBR5 in vascular development has functional relevance to tumor angiogenesis.

The importance of UBR5 in development is further reinforced by results from a functional genomics screen identifying UBR5 as crucial for embryonic stem cells (ESC) growth (68). UBR5 was subsequently shown to regulate ESC self-renewal via maintenance of the pluripotency factor OCT4 (69). A key outstanding issue in understanding the role of UBR5 in regulating stem cell biology and development is to determine the role of E3 ligase activity in mediating these functions. Aside from its high conservation across species, perhaps the strongest indication for the importance of UBR5 in development comes from a systematic survey of loss-of-function (LoF) variants in human protein-coding genes by the Exome Aggregation Consortium (ExAC). *UBR5* is one of the most highly conserved genes in this analysis, which identified just four LoF variants in *UBR5* at very low allele frequencies [less than 1/10,000; ExAC, Cambridge, MA (http://exac.broadinstitute.org), June 2015]. A *UBR5* missense mutation (c.5720G>A) was recently identified in affected individuals of a Japanese pedigree with familial adult myoclonic epilepsy, but not in any nonaffected family members or unrelated healthy residents of the pedigree's community (70). This mutation encodes a substitution at Arg^{1907} (Arg^{1907}His), which is highly conserved across species but does not occur in any known functional domains.

UBR5 and Regulation of Gene Expression

The UPS is intimately involved at every stage of transcriptional control and it is now evident that transcriptional control is a key aspect of UBR5 function (see Fig. 3). These stages include: initiation of RNA transcription by transcription factors and their associated co-activators, which recruit the transcriptional machinery (RNA polymerase II/general transcription factors) to gene promoters [reviewed elsewhere (71)]; RNA elongation, which requires chromatin remodeling through the removal and redeposition of histone proteins; and RNA processing and nuclear export [reviewed elsewhere (72)].

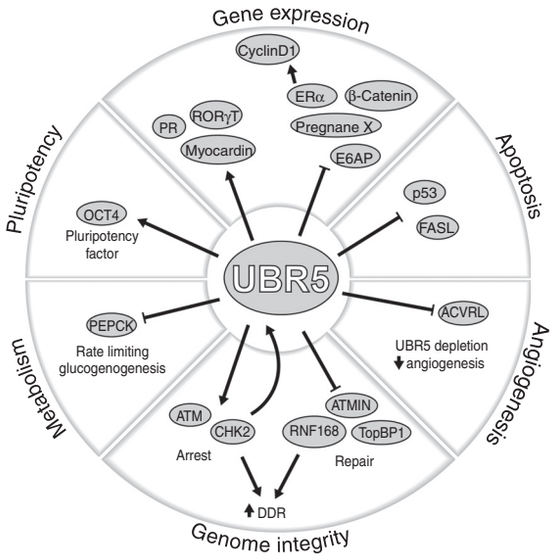

Figure 3.
Summary of well-characterized and emerging roles of UBR5. Arrows indicate upregulation or activation. Bars indicate downregulation or repression.

UBR5 was first identified as a target gene of the progesterone receptor (PR), a member of the nuclear receptor family of ligand (e.g., steroid hormones, retinoic acid, thyroid hormone, etc.) induced DNA binding transcription factors (29). Interestingly, UBR5 enhanced the transcriptional activity of the PR itself (29). It is now evident that UBR5 has similar effects on several other nuclear receptors through a variety of mechanisms, which in some (but not all) cases involve the E3 ligase activity of UBR5, and which either directly target the receptor or alternatively other proteins in the receptor transcriptional complex.

A screen of potential transcriptional modulators found UBR5 was a negative regulator of estrogen receptor α (ERα) protein levels and activity. This action was dependent on ubiquitination and an intact UBR5 HECT domain, but ubiquitination of ER was independent of ligand occupancy. Depletion of UBR5 in MCF-7 breast cancer cells resulted in increased endogenous ER levels, an estradiol-induced increase in transcription of *GREB1* and *CCND1* (ER target genes), as well as increased cell proliferation (73). UBR5 is also a negative regulator of PXR (an orphan nuclear receptor) transcriptional activity. UBR5 together with dual-specificity tyrosine phosphorylation-regulated kinase 2 (DYRKK2) are found in a multiprotein complex with PXR. Phosphorylation of PXR by DYRKK2 facilitates its ubiquitination by UBR5, resulting in reduced PXR protein levels and activity (51).

In T-helper (T_H17) cells, production of the pro-inflammatory cytokine IL17 is regulated by the transcription factor RORγt, an orphan nuclear receptor. Levels of RORγt are controlled by its proteasomal degradation, promoted by UBR5-mediated

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ubiquitination (74). Activity of UBR5 is regulated upstream by T-cell receptor signaling (deactivation) and TGF-B signaling (activating) through unknown mechanisms and by association between UBR5 and deubiquitin enzyme A (DUBA), which stabilizes the UBR5 protein possibly by inhibiting its auto-ubiquitlyation and consequent proteasomal destruction.

E6-associated protein (E6-AP) is a HECT domain ubiquitin ligase, which acts a co-activator for several nuclear receptors including ER, PR, AR, GR, and RARα [reviewed elsewhere (75)] as well as targeting the receptor co-activator SRC-1. As is the case for the UBR5 co-activation of PR (29), E6-AP's activity as a co-activator is not dependent on its HECT domain ubiquitin ligase activity. E6-AP itself is a ubiquitination substrate for UBR5 (52) and lowered UBR5 expression results in greatly increased levels and half-life of E6-AP. These results point to a possible role for UBR5 in regulating nuclear receptor transcriptional activity via its effects on E6-AP activity. Consequently, UBR5 affects the proteolytic activity of the E6-AP/E6 complex, which targets p53 and other substrates for ubiquitination. UBR5 knockdown in human papillomavirus (HPV) infected cells not only strongly reduced p53 levels but in line with UBR5's possible tumor suppressor function decreased apoptosis in response to etoposide-induced DNA damage and overcame S-phase arrest (52).

UBR5 also regulates other nonnuclear receptor transcription factors. β-catenin is ubiquitinated by UBR5, resulting in its stabilization, increased nuclear localization, and transcriptional co-activation of Wnt target genes (50). Ubiquitination is dependent upon phosphorylation of β-catenin by GSK-3B. Similarly, silencing of *UBR5* decreases β-catenin levels and inhibits β-catenin/TCF transcription. This is in contrast to the previously well-defined role of GSK-3B, which is to phosphorylate β-catenin when Wnt signaling is absent, resulting in ubiquitination by the SCF ubiquitin ligase complex and subsequent β-catenin degradation (76). UBR5 also binds and stabilizes the transcriptional co-activator Myocardin, upregulating smooth muscle gene transcription independent of E3 ligase function (77).

These findings represent both positive and negative regulation of genes associated with lineage-specific gene expression. Given the requirement for UBR5 in maintenance of pluripotency, this indicates that UBR5 may act as a molecular switch involved with coordinating differentiation of multiple cell lineages.

UBR5 as a Central Mediator of Genome Stability

A number of studies have implicated UBR5 in regulation of the cellular DDR (see Fig. 3), which is critical for maintenance of genomic integrity and suppression of tumorigenesis. These roles are likely partly responsible for the observed effects of UBR5 in mediating chemoresistance in ovarian cancer (see above).

ATM signaling is required for efficient cell-cycle arrest following DNA damage (78) and proteomic screens have identified UBR5 as a phosphorylation substrate of ATM, CHK1, and CHK2 (43, 79, 80). The functional consequences of phosphorylation on UBR5 function are not clear but there are reciprocal effects on ATM and CHK2 activity. For example, upon DNA damage UBR5 relocates to the nucleus and is required for ATM-mediated phosphorylation of CHK2 (81). The interaction with CHK2 is mediated via phospho-dependent binding to its FHA domain and is required for the activation of downstream DDR checkpoints. UBR5 depletion compromises the maintenance of cell-cycle checkpoints, leading to polyploidy and cell death via mitotic catastrophe (82). UBR5 also regulates ATM signaling via ubiquitination of ATMIN following DNA damage (42) and mediates TOPBP1 ubiquitination (83) via an interaction with the BRCT domain of TOPBP1 (48). The BRCT domain is a common feature of DDR enzymes (84, 85), indicating UBR5 may interact with other DNA damage-related proteins (48).

UBR5 also acts to restrict recruitment of DNA repair machinery at the sites of double-stranded breaks (DSB) by indirectly regulating ubiquitination of phosphorylated H2AX (γ–H2AX). In concert with TRIP12, UBR5 is required to control the nuclear pool of RNF168 (49). RNF168 is a chromatin-associated E3 Ub ligase, which promotes genome stability by acting with RNF8 to ubiquitinate γ–H2AX at the site of DSBs, promoting recruitment of DNA repair machinery (86–88). In the absence of UBR5, excess RNF168 activity promotes ubiquitination of γ–H2AX beyond sites of damage, causing hyperaccumulation of ubiquitin-regulated genome caretakers such as 53BP1 and BRCA1.

Hence, UBR5 plays a key role in homeostasis of ubiquitin-mediated signaling after DNA damage, emphasizing the potential importance of dysregulated *UBR5* expression and/or function in the evolution of cancer.

Emerging Functional Roles for UBR5

A number of large-scale functional genomics screens have identified putative novel roles for UBR5 in various biologic processes, including ciliogenesis, viral infection, apoptosis, and glucose homeostasis (see Fig. 3). Many of these remain to be validated but point to further roles for UBR5 and the UPS more generally in various aspects of cell biology and pathophysiology.

Primary cilia are microtubule-based organelles present on the apical surface of most vertebrate cell types (89), coordinating signaling pathways during development and in tissue homeostasis. Ciliopathy (defective cilia) is emerging as a major cause of various diseases and developmental disorders. UBR5 was identified in a whole-genome shRNA screen as a positive regulator of ciliogenesis (90), with UBR5 depletion causing decreased primary cilia formation. This screening hit was not further validated and the mechanism underlying this effect is yet to be determined, but the UPS is an established regulator of ciliogenesis (91).

A similar functional genomics approach was used to identify UBR5 (along with the transcription factor GRHL2) as a mediator of sensitivity to apoptosis. Depletion of UBR5 or GRHL2 increased sensitivity to Fas-ligand mediated apoptosis, and expression of both UBR5 and GRHL2 was slightly decreased in breast cancer cell lines less resistant to cell death (92). This indicates that UBR5 may play a role in suppressing apoptotic signaling.

The UPS is a well-established mediator of cellular response to viral infection and a number of studies have recently implicated UBR5 in regulation of viral infection. For example, UBR5 was identified in a siRNA screen for mediators of HIV replication (93). At the molecular level, UBR5 interacts with the HIV-1 VPR protein to regulate TERT activity (94) and also interacts with the HPV E6 protein and E6-AP (52).

Cross-talk between PTMs is emerging as an important regulatory mechanism in cell signaling (95, 96). An interesting example of this concept is demonstrated by the recent finding that acetylation of phosphoenolpyruvate caboxykinase (PEPCK) by P300 promotes its ubiquitination by UBR5, and subsequent

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proteasomal degradation (56). PEPCK catalyzes the conversion of oxaloacetate into phosphoenolpyruvate, a key step in gluconeogenesis. Phospho-dependent interactions involving UBR5 discussed above (e.g., CHK2 and DYRK2) are further examples of cross-talk between PTMs relevant to UBR5 function in cancer.

Conclusions and Future Perspectives

UBR5 is emerging as a key regulator of the UPS in cancer and development. However, it is unclear where UBR5 falls in the delineation between oncogene and tumor suppressor. In some cases, amplification and overexpression is clearly linked with chemoresistance and disease outcome (e.g., breast and ovarian cancer), whereas in other contexts, LoF mutations are clearly implicated in tumorigenesis (e.g., MCL). Development of better animal models allowing conditional modulation of UBR5 expression will be key to better understanding context-dependent roles of UBR5 in tumorigenesis and progression in various cancer types. These will be important not only in better understanding the biologic functions of UBR5 in cancer but in addressing the potential of UBR5 as therapeutic target. At this stage it is probably too early to define the potential utility of UBR5 as a therapeutic target in cancer.

UBR5 is a key regulator of cell signaling relevant to broad areas of cancer biology. However, despite insights from genetics, biochemistry, and cell biology, the precise mechanisms by which UBR5 contributes to tumor initiation and progression remain poorly defined. As an E3 ubiquitin ligase, many of the functions of UBR5 relevant to cancer are facilitated by its ubiquitylation of substrates. Many UBR5-interacting proteins have been identified and a number of these have been validated as substrates. However, there remains a clear need for systematic approaches to define the suite of UBR5 substrates in various contexts. This is a key knowledge gap in understanding the function of UBR5 and should be a priority for further research. It is also important to note that any development of UBR5 as a drug target will be heavily dependent on defining enzymatic substrates in a cancer context.

A clear outstanding question around UBR5 function in cancer is why the enzyme possesses many apparently divergent roles in multiple pathways in different tissue and disease contexts? Do these reflect discrete specific activities in different settings? An important consideration in this respect comes from the unique structure of UBR5, very high conservation of sequence, and restriction to the metazoan (i.e., multicellular) lineage. It is tempting to speculate that UBR5 evolved as a kind of signal integrator, coordinating extracellular signaling pathways (e.g., growth factor, hormonal, cilia) with regulation of cell-intrinsic pathways (e.g., proteostasis, DNA damage, etc.). The emergence of improved molecular tools, development of new model systems, and better definition of enzymatic substrates will provide a powerful platform for integrated approaches to define the function of UBR5 in normal cell biology and cancer pathology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Functional Roles of the E3 Ubiquitin Ligase UBR5 in Cancer

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