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Chapter 6

Ubiquitin Signaling in Ovarian Cancer: From Potential to Challenges

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Abstract

Ubiquitin proteasome system (UPS) is an emerging arena in cancer intervention. Dysregulation of various UPS components has been implicated with many cancers, and this knowledge is starting to be exploited for its role in cancer initiation, progression, and therapeutics. UPS regulates both protein turnover and non-proteolytic regulatory function of the proteins involved in cell cycle, signal transduction, DNA repair, histone modification, and transcription. In addition, chromosomal aberrations and genomic alterations often present in the cancer cell genomes lead to excess of conformationally challenged aggregation-prone proteins and proteotoxic stress that make cancer cells more dependent on UPS-mediated protein degradation than normal cells. This proposition is the basis of the clinical use of proteasome inhibitor, Bortezomib, to treat multiple myeloma and mantle cell lymphoma targeting cancer cells and mostly sparing the normal cells. This chapter provides an overview of various components of UPS which are implicated in cancer and regulate ubiquitin-mediated oncogenic signaling in ovarian cancer.

Keywords: ovarian cancer, mutant p53, ubiquitin, proteasomes, deubiquitinating enzymes

1. Introduction

Ovarian cancer is the most lethal gynecologic malignancy with a high case-to-fatality ratio [1]. According to American Cancer Society, approximately 22,440 new cases of ovarian cancer will be diagnosed in the year 2017 and about 14,080 women in the United States will die from this deadly disease [2]. About 90% of ovarian carcinomas are heterogeneous epithelial neoplasms with distinctive biology and clinicopathologic features at cellular and molecular

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levels [1, 3]. The clinical management of ovarian cancer has addressed this heterogeneity and classified ovarian cancer into high-grade and low-grade serous, endometrioid, clear cell, and mucinous subtypes based on the histology, tissue of origin, prognosis, and genetic alterations that deregulate specific signaling pathways in these tumor cells [4, 5] (**Figure 1**). Of these, high-grade serous ovarian cancer (HGSOC) is the most prevalent and lethal subtype of ovarian cancer. It accounts for 70–80% of ovarian cancer deaths [1]. The low *five-year survival rate* of HGSOC patients is attributed to the late detection of extensively metastasized disease, especially to omentum, which is the primary site of ovarian cancer metastasis. Moreover, about 80–90% of HGSOC patients eventually develop chemo-resistant tumors, after an initial positive response to cytoreductive surgery and chemotherapy, which are important prognosticators of the survival of HGSOC patients [1, 3]. The initiation and development of HGSOC is known to proceed through the early acquisition of genetic alterations in the tumor suppressor gene *TP53* [3, 6]. About 96% of HGSOC patients carry gain-of-function (GOF) mutations in *TP53* gene [3]. It is believed that *TP53* mutations lead to the precursor lesions in fallopian tube fimbria, which develop into serous tubal intraepithelial carcinoma (STIC) and ultimately to HGSOC [7, 8]. The reduced risk of ovarian cancer in BRCA1 mutation carriers after salpingo-oophorectomy supports the theory of HGSOC origin from STIC [9]. Mutant p53 orchestrates a distinct pro-tumorigenic signaling network and confer chemo-resistance through transcription-dependent and independent mechanisms in cancer cells. A recent study in triple-negative breast cancer cells revealed the role of mutant p53-proteasome axis in regulating global effects on cancer cell's protein homeostasis, inhibiting tumor suppressive pathways or turning on the oncogenic signaling in cancer cells [10]. A growing number of evidences suggest the role of ubiquitin signaling in tumor progression and growth. This chapter discusses the role of ubiquitin-mediated signaling in ovarian cancer pathogenesis. The different components of ubiquitin proteasome system, which are involved in this regulation, will be highlighted.

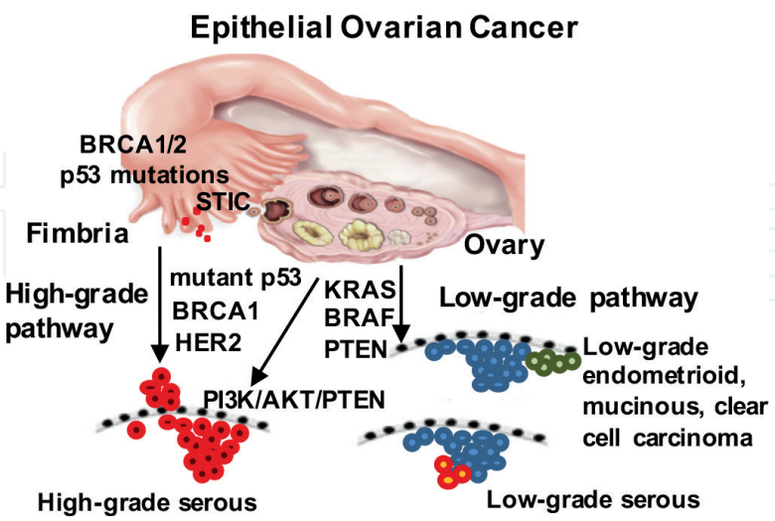


Figure 1. A schematic representation of molecular drivers of low- and high-grade ovarian cancer initiation and progression. Low-grade tumors are low malignant potential (LMP) tumors associated with KRAS or BRAF mutation and loss of PTEN. High-grade serous tumors frequently have mutated *TP53* gene as well as activated members of PI3K/Akt pathway. Highly invasive tumors originate from the fallopian tube precursor lesion, STIC, and spread to the ovary and other peritoneal surfaces. Genotoxic stresses in BRCA1/2 carriers predispose them to ovarian cancer.

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1.1. Conceptual overview of ubiquitin modifications

Protein ubiquitination is a dynamic multifaceted posttranslational modification (PTM), which is involved in nearly all biological functions in a eukaryotic cell. Similar to phosphorylation, it functions as a signaling device and can be activated by extracellular stimuli, DNA damage, phosphorylation, ligand-dependent receptor activation, and signal transduction. Ubiquitin is a highly conserved 76-amino acid protein, which is expressed in all cell types. It has seven lysine (Lys or K) residues, K6, K11, K27, K29, K33, K48, and K63. Each lysine residue can result in a linkage-specific ubiquitin chain of certain topology [11, 12], which when bound to the target protein (substrate) dictates the fate of the protein (**Figure 2**). For example, the most predominant K48-linked polyubiquitin chains, which have a compact conformation, lead to the proteasomal degradation of the bound substrate. By contrast, the second most abundant K63-linked chains, which have an open conformation, are involved in non-proteolytic regulatory functions [13]. The K11-linked ubiquitin chains act as an additional proteasomal degradation signal, particularly in cell-cycle regulation [13]. The functions of the other lysine-specific ubiquitin chains remain less well characterized. K6-linked chains are shown to be upregulated with UV genotoxic stress and are known to be associated with BRCA1/BARD1 complex [14]. Similarly, K27 chains act to serve as scaffolds for protein recruitment such as p53-binding protein 1 in the DNA damage response. In addition, ubiquitin chain of mixed topology with different linkage at succeeding positions is also seen as in NF-κB signaling or in protein trafficking (**Figure 2F**) [13]. Moreover, branched ubiquitin chains of unknown function are generated when a single ubiquitin is modified with multiple molecules [12, 13]. These ubiquitin chains creating a multitude of signals with distinct cellular outcomes are referred to as "ubiquitin code" [13]. New layers of the ubiquitin code are emerging, based on findings that revealed the modification of ubiquitin chains with small ubiquitin-like (Ubl) modifier such as SUMO, phosphorylation, and acetylation [13].

Box 1. The discovery of ubiquitin-mediated protein degradation in the late 1970s by Drs. Avram Hershko, Aaron Ciechanover, and Irwin Rose was awarded 2004 Nobel Prize in Chemistry. Their study highlighted the role of protein ubiquitination in selective protein breakdown, regulating the cellular functions by modulating the levels of key enzymes, regulatory proteins and removal of abnormal proteins that arise by biosynthetic errors or post synthetic damages. Ubiquitin was first isolated from bovine thymus in 1975 by Goldstein et al. (PNAS, 1975;72:11-15) [88] and found to be covalently attached to histone 2A (Goldknopf and Busch, PNAS, 1977;74:864-868) [89]. Subsequently, Drs. Hershko, Ciechanover, and Rose in a series of biochemical studies discovered and characterized the ATP-dependent, ubiquitin-mediated protein degradation using the reticulocyte lysate system (PNAS, 1979;76:3107-3110) [90].

Ubiquitination is an orchestrated enzymatic reaction of E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, and ubiquitin E3 ligase (E3). It is the most coordinated and conserved multistep process of covalently tagging a protein with mono- or polyubiquitin chain. The process begins with the ATP-dependent activation of ubiquitin by E1 ubiquitin-activating enzyme (E1s), which then transfers it to the active site cysteine of E2 ubiquitin-conjugating enzymes (E2s) forming a thioester linkage between ubiquitin and cysteine. Ubiquitin E3 ligases (E3s) have a central role in this process, as they recognize the specific protein substrates and facilitate the transfer of ubiquitin from the E2 onto the target protein [11, 12]. Deubiquitinating

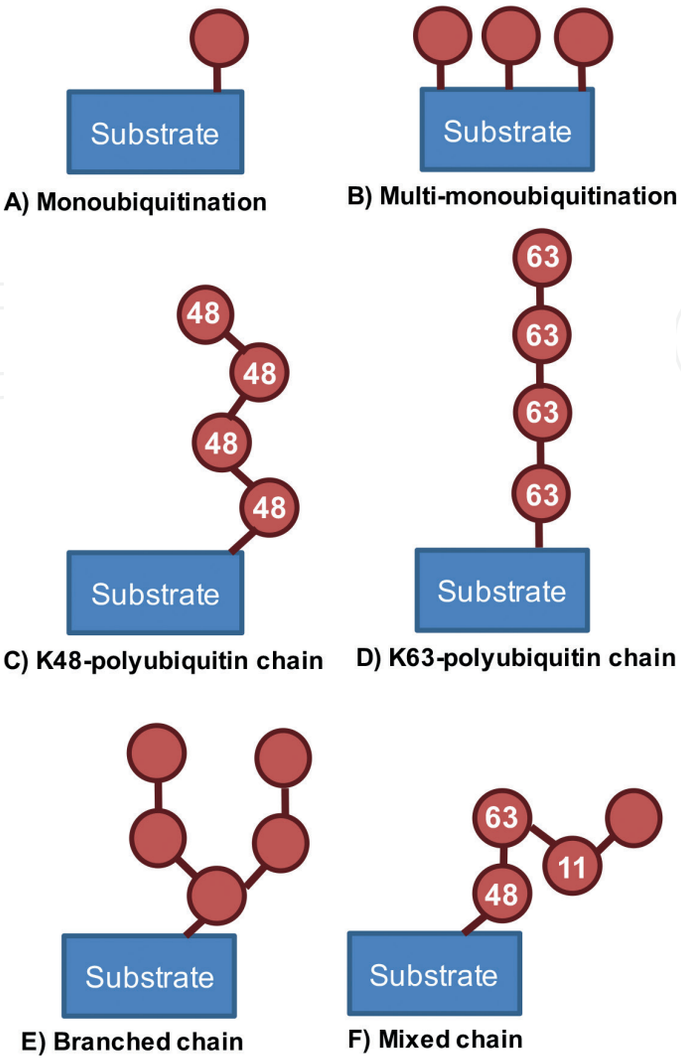


Figure 2. Linkage-specific ubiquitin chains of different topologies. Each circle represents one ubiquitin moiety. (A) Monoubiquitination, (B) multi-monoubiquitination, (C) K48-linked chain, (D) K63-linked chain, (E) branched chain, and (F) mixed chain.

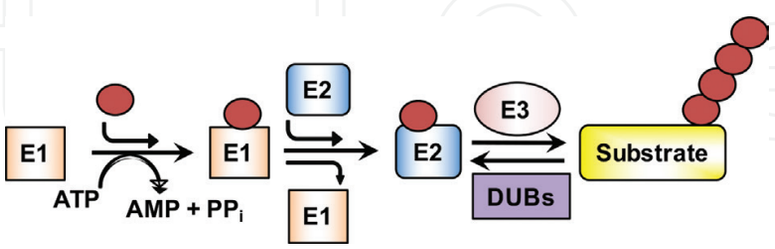


Figure 3. Enzymatic cascade of ubiquitin proteasome system. Ubiquitin is activated and conjugated to target protein by a conserved action of E1-ubiquitin-activating enzyme, E2-ubiquitin-conjugating enzyme, and E3 ubiquitin ligase.

enzymes (DUBs) are another class of enzymes, which removes or edits the ubiquitin chains attached to a protein, making this a highly reversible process and thus highlighting the dynamic regulation of ubiquitin signaling in the cell (**Figure 3**). These enzymes together with proteasomes, a cellular machinery involved in ubiquitin-mediated protein degradation, comprise the

ubiquitin proteasome system (UPS). UPS plays an indispensable role in regulating ubiquitin-mediated proteolytic and non-proteolytic regulatory signaling to control cellular homeostasis, protein stability, and a wide range of signaling pathways.

2. UPS components in ovarian cancer

Ovarian cancer is characterized by multiple genetic and epigenetic abnormalities and several major (about seven) activated signaling pathways, which are directly or indirectly implicated with UPS. Moreover, several UPS components, E1s, E2s, E3s, DUBs and proteasomes are known to be deregulated or mutated in cancer (**Table 1**), suggesting their role in cancer signaling and cancer progression. This section discusses each UPS component implicated in ovarian cancer and the role of key players of each component in regulating ovarian cancer signaling (**Figure 4**).

2.1. E3 ligases

E3 ligases (E3s) are the most heterogeneous class of enzymes in UPS as they facilitate ubiquitination with exquisite spatial, temporal, and substrate specificity. There are more than 600 E3s in a human genome, indicating the precise substrate specificity of E3s [15]. E3s can be classified into three main types, RING E3s, HECT E3s, and RBR E3s depending on the presence of type-specific domains and on the mechanism of ubiquitin transfer to the substrate protein. RING E3s are the most abundant type of ubiquitin ligases. They are characterized by the presence of zinc-binding domain called Really Interesting New Gene (RING) and U-box domain. RING E3s mediate a direct transfer of ubiquitin to substrate, functioning as a scaffold to orient the ubiquitin-charged E2, whereas E3s with homologous to the E6AP carboxyl

Gene.	Role	Effect	Cancer [references]
BRCA1	E3 ligase	Mutation, loss of tumor suppressor function	Ovarian and breast cancers [19, 20]
USP13	DUB	Amplification, oncogene	Ovarian cancer [41]
Mdm2	E3 ligase	Overexpression, loss of p53 tumor suppressor function	Ovarian cancer and various malignancies [63, 64]
USP7	DUB	Overexpression, oncogene	Ovarian cancer [42]
Skp2	E3 ligase	Overexpression, loss of tumor suppressor function of p27	Ovarian, breast, and prostate cancers [76–81]
UCHL1	DUB	Overexpression or methylation, role varies with cancer	Ovarian, breast, gastric, lymphoma, lung, Esophageal squamous cell carcinoma [44–48]
FBW7	E3 ligase	Mutation, loss of tumor suppressor function	Ovarian and endometrial cancer, leukemia [71]
VHL	E3 ligase	Mutation, loss of tumor suppressor function	Clear-cell carcinoma, lung cancer [49]

Table 1. Cancer-associated alterations in UPS.

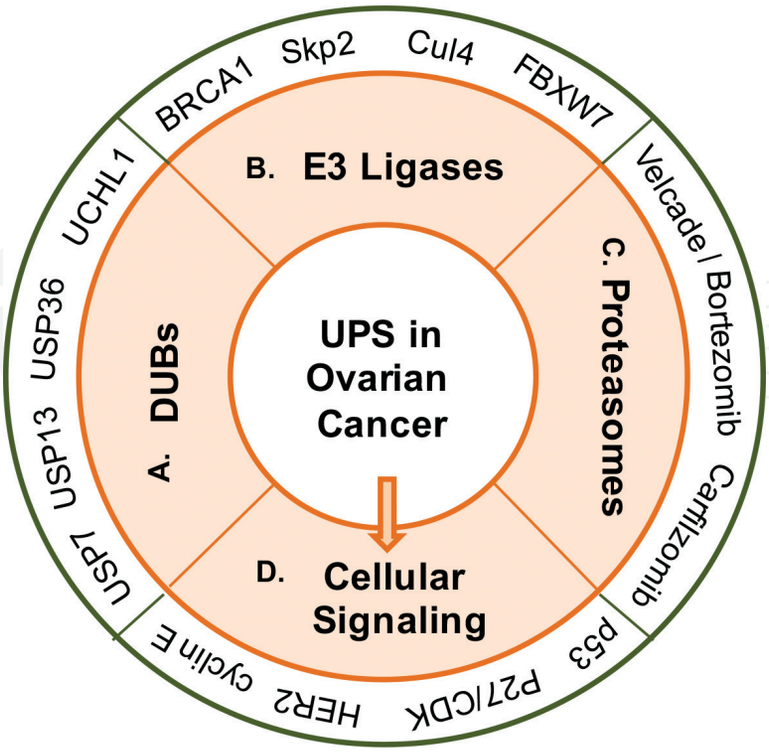


Figure 4. Key players of each UPS component involved in regulating ovarian cancer signaling. (A and B) DUBs and E3 ligases as candidate genes in ovarian cancer, (C) proteasomal activity and inhibitors in ovarian cancer, and (D) regulation of ovarian cancer oncogenic signaling by UPS.

terminus (HECT) domain transfer ubiquitin to the substrate in a two-step process—ubiquitin is first transferred to a catalytic cysteine on E3 and then to the substrate. Based on their N terminus extensions, HECTs are further classified into three subfamilies: Nedd4 family, HERC family, and other HECT that contain various domains. The RBR E3s are characterized by the presence of three RING domains, RING1 and RING2, separated by an in-between-RING (IBR) domain. RING1 recruits the ubiquitin-charged E2, RING2 possess catalytic cysteine. The IBR is called benign-catalytic domain as it lacks catalytic cysteine residue [15]. Given their cellular specificity and complexity, E3s are implicated in a number of pathophysiological conditions, which makes them an attractive therapeutic target in human diseases, including cancer [16].

2.1.1. BRCA1

The breast and ovarian cancer susceptibility gene, BRCA1, is a tumor suppressor gene [17]. Heterozygous mutations in BRCA1 gene predispose women to both familial and sporadic breast and ovarian cancers [18, 19]. Nonetheless, BRCA1 mutations are also associated with other cancers like stomach, pancreas, prostate, and colon [20]. BRCA1 acts as a hub protein, which participates in several different protein complexes to coordinate a diverse range of cellular functions including DNA repair, cell-cycle regulation, apoptosis, transcriptional regulation, and centrosome duplication to maintain genomic stability [17]. The structural analysis of BRCA1 protein suggested that it has a RING finger domain that harbors E3 ubiquitin ligase activity [14]. In addition, BRCA1 forms a heterodimer complex with BARD1, a protein with a RING finger domain [14]. BARD1 interaction stabilizes the proper conformation of BRCA1

RING domain for a potent E3 ligase activity and interaction with E2 UbcH5 [14, 21, 22]. BRCA1 E3 ligase substrate specificity is believed to depend on its phosphorylation-dependent binding to proteins containing phospho-SXXF motif such as CtIP, BACH1, and ABRA1 through a phospho-peptide recognition domain (BRCT) [23, 24]. The strong relation between BRCA1 tumor suppressor properties and E3 ligase activity is evident from the clustering of missense mutations that predispose to cancer in the Zn^{2+}-binding residues of BRCA1 RING finger domain crucial for its ubiquitin ligase activity [25]. The full range of function of BRCA1/BARD1 complex is not completely understood [18]. One of the most important functions of BRCA1 is to repair DNA double-strand breaks (DSBs). Following DNA damage, chromatin-associated histone H2AX phosphorylation by ATM and ATR at DNA damage site recruits an E3 ubiquitin ligase RFN8 and a phospho-module-binding mediator MDC1 at the damage site [17, 26–28]. RFN8 together with ubiquitin conjugase Ubc13, ubiquitinate histone H2A and H2B at chromatin lesions, which in turn translocate BRCA1 complex containing RAP80, a protein with ubiquitin-interacting motif (UIM), ABRA1, protein that interacts with BRCA1 BRCT domain and deubiquitinating enzyme, BRCC36 to Lys6- and Lys63-linked polyubiquitin chains at DSBs [17, 26, 29]. BRCA1 has also been implicated with the transcriptional activation of genes in response to DNA damage. The C-terminus of BRCA1 complexes with RNA polymerase II through RNA helicase, while N-terminus BRCA1/BRAD1 heterodimer binds to RNA polymerase II holoenzyme [30]. Identifying genes regulated by BRCA1 would shed a significant light on the transcriptional role of BRCA1. However, BRCA1 overexpression studies have shown induction in p53-responsive E3 ligase, mdm2, cell-cycle inhibitor, p21 and stress-response factor, and GADD45 in breast and small-cell lung cancer cell lines [31, 32]. Besides, BRCA1 also regulates G1/S, S-phase, and G2/M cell-cycle checkpoints through interactions with RAD3, ATM/ATR, and Chk1/Chk2 [26, 30, 33].

Over the last 10 years, significant information has been gained about the structure, function, and unique features of BRCA gene products, BRCA1 and BRCA2, which collectively contributes to the biological response to DNA damage through homologous recombination of DNA repair and regulation of cell-cycle checkpoints. BRCA1/2-deficient cancers, including ovarian cancer, are now recognized as the target for a class of drugs known as PARP (poly ADP-ribose polymerase) inhibitors [34]. PARP detects and initiates an immediate cellular response to metabolic or radiation-induced single-strand DNA breaks (SSB). It binds to DNA and synthesizes polymeric adenosine diphosphate ribose (poly ADP-ribose or PAR), which acts as signal to other DNA-repairing enzymes. PARP inhibition directly blocks the PARP enzymatic activity and subsequently leads to PARP accumulation on DNA, a process called PARP trapping, which converts an SSB into a double-strand DNA break through the collapse of replication fork [34]. BRCA-deficient tumor cells with impaired homologous recombination repair of double-strand DNA breaks are directed toward the error-prone repair process of non-homologous end joining which leads to genetic instability and cell death. Thus, BRCA1/2-deficient ovarian cancer cells with PARP inhibition undergo synthetically lethal cell death [34]. PARP inhibitor, Olaparib manufactured by AstraZeneca, is in phase I/II clinical trials for BRCA-deficient high-grade serous ovarian cancer [34]. Olaparib-treated ovarian cancer patients with BRCA1/2 mutation had a progression-free survival of 11.2 months compared to 4.3 months of patients receiving placebo [35]. In summary, BRCA is an ideal example of E3 ubiquitin ligase playing an essential role in ovarian cancer and its intervention.

2.1.2. Cullin-RING ligases: cullin 4

Cullin-RING ubiquitin ligases (CRLs), composed of CUL1, 2, 3, 4A, 4B, 5, and 7, are the largest family of E3s that ubiquitinate a wide array of substrates involved in cell-cycle, DNA-damage response, chromatin remodeling, and gene expression. Cullin (CUL) neddylation, a process of adding ubiquitin-like protein—NEDD8 to the cullin [36], is crucial for their activation. Neddylation is catalyzed by NEDD8-activating enzyme E1 (NAE), NEDD8-conjugating enzyme E2 (UBC12), and NEDD8-E3 ligase. The genome-wide analysis of human cancers revealed *CUL4A* amplification in 20% of the basal-like breast cancer subtype, characterized as "triple negative," and CUL4A levels were associated with aggressive growth and poor prognosis. Dysregulation of CUL4A in multiple tumor types leads to the hypothesis that CUL4A plays a role in promoting oncogenesis [36]. High CUL4A expression and activity in ovarian cancer is implicated with cancer cell proliferation and survival. NEDD8-activating enzyme inhibitor, MLN4924, which blocks cullin neddylation activation, is reported to induce cell-cycle arrest, apoptosis, and tumor cell growth in epithelial ovarian cancer cells. In addition, MLN4924 sensitized ovarian cancer cells to chemotherapeutic drug treatments [37].

The role of Skp2 and FBXW7 in ovarian cancer signaling is discussed in the next section.

2.2. Deubiquitinating enzymes

Reversibility is an important aspect of ubiquitin system, which is mediated by deubiquitinating enzymes or deubiquitinases (DUBs). DUBs are essential components of UPS that possess ubiquitin-isopeptidase activity and catalyze the removal of ubiquitin from the target proteins. Thus, DUBs play a crucial role in the regulation of ubiquitin-mediated regulatory and proteolytic signaling [11, 38]. DUBs activity affect the activation, recycling, localization, and turnover of multiple proteins, which in turn regulate cellular homeostasis, protein stability, and a wide range of signaling pathways [39]. DUBs also maintain ubiquitin homeostasis in the cell by generating free ubiquitin monomers, which is essential for ubiquitin-mediated regulation of cell function [38]. Consistent with this, an altered DUB expression or activity has been implicated with several diseases including cancer. Numerous DUBs have been characterized as oncogenes mediating cancer initiation and progression [11, 40]. Therefore, pharmacological interventions targeting DUB activity using small molecule inhibitors are being used as a rationale to search for novel anticancer drugs [11].

Box 2. About 98 DUBs are reported in human genome, which are mainly divided into five families based on their sequence and structural homology: Ubiquitin-specific protease (USP), ubiquitin carboxyl-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Machado Joseph disease proteases (MJD), and JAB1/MPN/Mov34 (JAMM) metallopeptidases. Most DUBs are cysteine proteases except JAMMs, which belong to catalytic class of metalloproteases. The recent discovery of DUBs with the selectivity of cleaving extended Lys-48-linked polyubiquitin chains belongs to new family of DUBs named Mindy. The DUB-substrate specificity somewhat depends on ubiquitin chain linkage and topology; however, by large, given the complexity of ubiquitin system, it remains unknown [38].

DUBs role is evident in several cancers including Fanconi anemia (USP1), prostate cancer (USP2), adenocarcinoma (USP4), non-small-cell lung carcinoma (USP7), glioblastoma (USP15), myeloma, and leukemia (USP9x) [11, 39]. Han et al. identified the role of USP13 as

the master regulator of ovarian cancer metabolism [41]. They reported the co-amplification of USP13 gene with PIK3CA (phosphatidylinositol-3-kinase catalytic subunit, α-isoform) in 29.3% of high-grade serous ovarian cancer patients and its association with poor clinical outcome. USP13 stabilized the protein levels of two key metabolic enzymes, ATP citrate lyase and oxoglutarate dehydrogenase, which in turn regulate the mitochondrial respiration, glutaminolysis, and fatty acid synthesis in ovarian cancer cells. USP13 inhibition suppressed ovarian tumor progression and sensitized the tumor cells to PI3K/AKT inhibitor [41]. Similarly, USP7 (also known as HAUSP, herpes virus-associated ubiquitin protease) plays a crucial role in ovarian cancer [42]. USP7 is a DUB for MDM2, which prevents MDM2 autoubiquitination, leading to its stabilization and consequent induction of p53 degradation. Treating an ovarian cancer xenograft model with a novel inhibitor of USP7, CDDO-Me suppressed tumor growth. CDDO-Me directly binds to USP7, which leads to a decrease in its substrate Mdm2, Mdmx protein levels [42]. USP4 overexpression is reported in invasive breast carcinoma, enhancing TGFβ signaling by stabilizing SMAD2/SMAD4 complex but not much is known about its role in ovarian cancer [11]. USP36 expression is increased in ovarian cancer cells compared to normal ovarian surface epithelium; however, further studies are needed to understand its role in ovarian cancer [43]. DUB UCHL1 (ubiquitin-carboxyl terminal hydrolase 1) plays a contradicting role in different cancers [11]; it is reported as a methylated tumor suppressor gene in ovarian cancer [44, 45], while it is overexpressed in lymphoma, esophageal squamous cell carcinoma, renal, lung cancers, and acts as an oncogene [46–48]. Under hypoxic conditions, UCHL1 is shown to deubiquitinate and stabilize HIF-1α and promote tumor metastasis [49, 50]. We for the first time identified the oncogenic overexpression of UCHL1 in high-grade serous ovarian cancer and association with poor clinical outcome (unpublished data). These studies suggest the emerging role of DUBs in ovarian cancer and the potential of DUB inhibitors in neo-adjuvant therapies for ovarian cancer.

2.3. Proteasomes

The efficient and selective degradation of cellular proteins is essential for protein quality control and maintenance of cellular homeostasis [51]. Impaired protein quality control and degradation is associated with many human diseases such as cancer, cardiovascular diseases, and aging-related pathophysiological conditions such as Alzheimer's and Parkinson's. UPS mediates targeted protein degradation under both normal and malignant conditions [52]. However, cancer cells are more dependent on UPS-mediated degradation to promote the degradation of tumor suppressors and various cell-cycle checkpoint proteins as well as to reduce proteotoxic stress accumulated due to genomic aberrations [53]. The 26S proteasome is a multi-subunit complex that contains one barrel-shaped 20S catalytic core particle (CP) and 19S regulatory particle (RP) that binds to one or both the ends of barrel-shaped CP. The active degradation of proteins is regulated by 20S CP harboring proteolytic active sites while 19S RP regulates substrate binding and target protein entry into 20S [52].

The amazing efficacy and clinical use of proteasome inhibitor Bortezomib (PS-341, Velcade) for the treatment of multiple myeloma and mantle cell lymphoma has encouraged researchers to explore the possibility of targeting other components of the UPS for cancer treatment [54]. However, Bortezomib has not demonstrated a significant activity against other solid

tumors [55]. This conundrum has spurred the development of next-generation proteasome inhibitors, including MLN9708 (Millennium Pharmaceuticals), Carfilzomib and ONX0912 (Onyx Pharmaceuticals, South San Francisco, CA), and CEP18770 (Cephalon, Frazer, PA) [56]. Although these compounds target the same 20S CP, they differ in targeted active site and enzyme kinetics, resulting in activity differences based on tumor type and tumor location. Bazzaro et al. reported elevated levels of ubiquitinated proteins and 19S and 20S proteasome subunits in both low-grade and high-grade ovarian carcinoma tissues and cell lines compared to benign ovarian tumors and immortalized normal ovarian surface epithelium controls. They reported an increased sensitivity to apoptosis in proteasome inhibitor, PS-341 treated cells, and a reduced growth of ES-2 ovarian carcinoma xenograft in immunodeficient mice [57]. In a similar study, proteasome inhibitor, MG132—a peptide aldehyde—showed an enhanced sensitivity of ovarian cancer cells, SKOV3 to cisplatin both *in vitro* and *in vivo* [58]. The effect of Bortezomib on ovarian cancer cells is also supported by the increased sensitivity of Bortezomib-treated chemoresistant ovarian cancer cells to TRAIL-induced apoptosis [59]. Together, these results indicate the essential role of proteasomes in mediating prosurvival signaling in cancer, which may also be due to altered proteasome composition resulting in an enhanced proteasomal activity [52].

3. UPS in ovarian cancer cellular signaling

Several important factors that are implicated in the molecular pathogenesis of ovarian cancer are known to be regulated by UPS, highlighting its significance in disease progression. Some of these factors are discussed subsequently.

3.1. Tumor suppressor p53 and Mdm2

Tumor suppressor protein p53 is a multifunctional sequence-specific transcription factor that plays a key role in cellular stress response. Abrogating p53 function is a key event in human cancers, leading to the deregulation of cell cycle, genetic instability, resistance to stress signals, and resulting in cancer development [60]. Due to its growth inhibitory properties, p53 is maintained at low levels in the normal cells. The E3 ubiquitin ligase Mdm2 promotes p53 ubiquitination and subsequent proteasomal degradation [61]. In addition, E4 ubiquitin ligase p300/CBP promotes polyubiquitination of p53 to accelerate its degradation by proteasomes [61]. Although Mdm2 is the predominant E3 ligase for p53, several other E3 ligases have been identified that can promote the degradation of p53, including C-terminus of HSP70-interacting protein (CHIP), murine double minute 4 (MdmX), and p53-induced protein with a RING H2 domain (Pirh2) [60]. In addition to proteolytic ubiquitination, p53 mono-ubiquitination mediates p53 nuclear export and activity [62]. Thus, UPS plays a crucial role in maintaining and regulating p53 functions.

Several cancers, including invasive breast cancer, pediatric rhabdomyosarcoma, and soft-tissue sarcoma, exploit Mdm2-p53 pathway to maintain low p53 levels under genotoxic or oxidative-stressed environment of cancer cell. Thus, Mdm2 gene amplification and overexpression

have been reported in many cancers [63]. In addition, the expression and activity of Usp7, a deubiquitinating enzyme for Mdm2, is increased in several cancers including breast and ovarian cancer, which prevents Mdm2 ubiquitination and promotes its stability. Reduced tumor growth was seen in an ovarian cancer xenograft model treated with Usp7 inhibitor [42]. On the other hand, when p53 acquires gain-of-function (GOF) mutations as in the case of nearly half of the cancers, it gains oncogenic functions and loses its wild-type tumor suppressor properties. Thus, in these cancer cells, several mechanisms stabilize mutant p53 through its activation or by inhibition of its degradation by disrupting Mdm2 and mutant p53 binding. Several splice variants of Mdm2 are reported in cancer, which lack a p53-binding domain and thus stabilizes mutant p53 expression [63]. In addition, GOF mutation-induced conformational changes in mutant p53 allow the binding of Hsp90 (heat shock protein 90) to mutant p53, which prevents Mdm2 binding and Mdm2-mediated degradation of mutant p53 [60]. It is now well established that elevated mutant p53 levels correlate with more aggressive tumors and poor prognosis. About 96% of high-grade serous ovarian cancer patients have GOF p53 mutations, which orchestrate a distinct pro-tumorigenic transcription and oncogenic programs. Knowledge of a UPS component responsible for mutant p53 stabilization, which could be chemically manipulated, will be useful in HGSOC. Nonetheless, Mdm2 is a great therapeutic target and prognostic factor for ovarian cancer with wild-type p53, such as clear-cell carcinomas [64].

3.2. Cyclin E

Genomic alterations in cell-cycle regulatory genes have been reported in almost every human carcinoma. Cyclins are the crucial regulators of cell-cycle progression [65]. A periodic increase in cyclin levels and their timed interplay with cyclin-dependent kinases (CDKs) is essential for the proper progression of cell cycle [65]. Their levels are regulated by a combination of transcription and ubiquitin-mediated degradation [18, 66]. About 30% of high-grade serous ovarian cancer patients have amplification of the CCNE1 gene, which encodes for G1/S-specific cyclin E. Cyclin E-CDK2 interactions commit the cell to S-phase genome duplication [3]. Aberrant accumulation and overabundance of cyclin E leads to premature entry of the cell into S-phase, resulting in chromosome instability and tumor formation [67]. Cyclin E amplification is likely to be an early event in the development of high-grade serous ovarian cancer [3]. This subclass of patients has no apparent defect in homologous recombination as seen in patients with BRCA1 and BRCA2 mutations with defect in DNA repair pathways [3]. The overexpression of cyclin E is an indicator of poor overall survival of ovarian cancer patients. Cyclin E protein levels are maintained by a multi-subunit SCF ubiquitin ligase, which mediates its ubiquitination and degradation [68]. Cyclin E auto-phosphorylation after its association with CDK2 is recognized by the SCF-associated F-box protein 7 (FBXW7), which binds to cyclin E and facilitates its ubiquitination and degradation [68, 69]. More than 30% of human cancers have a deleted FBXW7 gene located on chromosome 4q32. FBXW7 also regulates mTOR, Myc, and Notch1 degradation, depending upon the type of tumor [70, 71]. FBXW7 is known to be mutated in breast and ovarian cancer cell lines with high cyclin E levels [3]. The loss of cyclin E or CDK2 results in cell-cycle arrest or apoptosis in HGSOC cell lines [3], suggesting cyclin E inhibition as a novel therapeutic approach in ovarian cancer patients.

3.3. P27, a cyclin-dependent kinase inhibitor

Similar to cell-cycle regulatory proteins, cell-cycle inhibitors are frequently altered in cancer [72, 73]. $p27^{Kip1}$ inhibits cell-cycle G1 phase by interacting with CDK2/cyclin A or CDK2/cyclin E complexes [73, 74]. Low levels of $p27^{Kip1}$ protein are associated with tumor progression and growth resulting in poor prognosis of ovarian and breast cancer patients [74–76]. The evaluation of subcellular localization of $p27^{Kip1}$ in tissue microarray of late-stage ovarian cancer patients revealed that patients with nuclear-only expression of $p27^{Kip1}$ had a better overall survival than those with negative expression or cytoplasmic localization of the marker (p-value = 0.0002; n = 355) [77]. $p27^{Kip1}$ level is an important prognostic marker of malignant transformation. Genetically altered mice with $p27^{Kip1}$ haploinsufficiency are predisposed to cancer [78]. $p27^{Kip1}$ protein levels are regulated by SCF E3 ligase-associated protein Skp2. Skp2 binds to $p27^{Kip1}$ and mediates its ubiquitination and subsequent proteasomal degradation [79, 80]. Skp2 levels in different cancers correlate with tumor grade and inversely correlate with $p27^{Kip1}$ levels and cancer prognosis. Skp2 levels were upregulated in ovarian cancer patients and were associated with advanced FIGO stage III and IV and high grade of the tumor [81]. Skp2 levels were also associated with downregulation of both p27 and p21 in these patients, suggesting an important role of Skp2- $p27^{Kip1}$ pathway in ovarian cancer pathogenesis. A strong negative correlation between Skp2 levels and FOXO3a ($r = -0.743$; $p < 0.05$) in immunohistochemical analysis of ovarian cancer patients indicates that it is another potential target of Skp2 in ovarian cancer [82]. These findings and Skp2 overexpression or amplification in serous ovarian cancer characterize it as an oncogene and its inhibition a plausible approach in ovarian cancer management.

3.4. The epidermal growth factor receptor (also known as HER or ERBB) family

The EGFR family of receptor tyrosine kinases plays an important role in the pathogenesis of several cancers [83]. The four members: EGFR, HER2, HER3, and HER4 (or ERBB1–4), of EGFR family structurally consist of an extracellular ligand-binding domain, a single transmembrane-spanning region, and an intracellular tyrosine kinase domain. More than 30 ligands have been identified that bind to the EGFR family receptors, including EGF- and EGF-like ligands, transforming growth factor (TGF)-α, and heregulins (HRGs) [83]. The activated EGFR receptors undergo C-terminal phosphorylation of cytoplasmic tyrosine residues after receptor dimerization to mediate cell regulatory signaling. E3 ubiquitin ligase CBL binds to EGFR receptor at specific phosphotyrosine residues and mediates its ubiquitination subsequent internalization in clatherin-coated endosomes, which then lead to lysosome-mediated degradation of EGFR [84].

Amplifications and overexpression of various EGFR family members, including EGFR, Her2, and ErbB3, have been reported in epithelial ovarian cancer. Attenuated ubiquitination and HER2 gene amplification favor the formation of EGFR/HER2 heterodimers that recruit CBL to a lesser degree, thus stabilizing and recycling the receptor to cell surface [85]. BRCA1 mutations are known to be associated with an increased EGFR expression in serous ovarian cancer patients. EGFR expression was not only increased in BRCA1 mutated cancer tissues but was also high in BRCA1-mutated normal tissues compared to respective control tissues. These

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results were confirmed by knocking down BRCA1 in ovarian cancer cells [86]. However, inhibitors targeting this pathway have little effect on cancer cells as a single agent due to the presence of alternative pathways affecting the cancer phenotype, particularly the activation of the PI3K/Akt/mTOR and mitogen-activated protein kinases (MAPKs) pathway [83], suggesting a combined use of EGFR and PI3K inhibitors in ovarian cancer [87].

4. Concluding remarks

It is now well known that UPS not only mediates protein degradation but is also involved in the extensive regulation of cellular functions and signaling. A large number of studies in various cancers have uncovered the diverse and intricate role of ubiquitin in oncogenic signaling. The alterations in the genes involved in UPS support its role in cancer development and progression. However, the lack of information on DUBs specificity and multiple targets of E3s raise a question on the use of DUBs or E3s inhibitors in cancer treatment. One possible way forward is to characterize the cancer-specific and tissue-specific expression of DUBs as certain DUBs are predominantly expressed in certain tissues and cancer, suggesting the cancer-specific use of a DUB inhibitor. Moreover, most DUBs studied thus far appear to regulate a small number of targets. It is also possible that only a fraction of ubiquitinated proteins are regulated by a specific DUB family. Similarly, the E3s can be manipulated in cancer if their role is characterized in cancer-specific aberrant molecular signaling. Moreover, further characterization of mutations in DUBs or E3s in cancer patients can be used for cancer screening. In addition, proteasomes carry a great potential in cancer treatment. Although Bortezomib did not show promising results against solid tumors, the advent of next-generation proteasome inhibitors opens new possibilities. Currently, five different types of next-generation proteasome inhibitors are in phase I or phase IIb clinical trials. Moreover, understanding the regulation of proteasomal activity by altered proteasome composition may open novel ways to target proteasomes in cancer.

Compared to breast cancer, ovarian cancer is a rare but far more lethal cancer. It is estimated that 69% of all patients with ovarian carcinoma will succumb to their disease as compared with 19% of those with breast cancer [1]. Ovarian cancer heterogeneity is represented by several genetic (BRCA1/2), epigenetic, and signaling (p53, CDK/p27, CCNE1) alterations, and various UPS components are implicated in these ovarian cancer-specific alterations. Several studies have established a link between UPS and ovarian cancer. However, further studies are needed to identify potential inhibitors for proteasome-based or E3s/DUBs-based therapies in ovarian cancer, which can be taken to clinical trials.

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Abbreviations

CUL4A	Cullin 4A gene
DUBs	deubiquitinating enzymes
E1	E1 ubiquitin-activating enzyme
E2	E2 ubiquitin-conjugating enzymes
E3	ubiquitin E3 ligases
EGFR	epidermal growth factor receptor
GOF	gain-of-function
HER2	human epidermal growth factor receptor 2
HGSOC	high-grade serous ovarian cance.
K	lysine
Lys	lysine
Mdm2	murine double minute 2
OSE	ovarian surface epithelium
PTM	posttranslational modification
STIC	serous tubal intraepithelial carcinoma

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