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REVIEW ARTICLE

Ubiquitin pathway and ovarian cancer

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ABSTRACT

The ubiquitin–proteasome pathway is a common cellular process in eukaryotic tissue. Ubiquitin binds to proteins and tags them for destruction; this tagging directs proteins to the proteosome in the cell that degrades and recycles unneeded proteins. The ubiquitin–proteasome pathway plays an important role in the regulation of cellular proteins with respect to cell cycle control, transcription, apoptosis, cell adhesion, angiogenesis, and tumour growth. This review article discusses the various ways that the ubiquitin pathway is involved in ovarian cancer, such as modulating the ovarian-cancer-related gene *BRCA1* and tumour suppressor p53, and interfering with the ERK pathway, the cyclin-dependent cell cycle regulation process, and *ERBB2* gene expression.

KEY WORDS

Ubiquitin, protein degradation, ovarian cancer, gene expression, therapy

1. INTRODUCTION

Ubiquitin is present in almost all tissues of eukaryotic organisms. The ubiquitin–proteasome pathway plays an important role in the regulation of cellular proteins involving cell cycle control, transcription, apoptosis, cell adhesion, angiogenesis, and tumour growth.

Ubiquitin is a small regulatory protein of 76 amino acid residues that binds to proteins and tags them for destruction; this tagging directs proteins to the proteosome (a large protein complex) in the cell that degrades and recycles unneeded proteins[1]. Ubiquitin is an enzyme system with four critical enzymes[2,3]:

- E1, an ubiquitin-activating enzyme;
- E2, an ubiquitin-conjugating enzyme;
- E3, an ubiquitin ligase; and
- the 26S proteasome.

E1 binds to ubiquitin, activates it in an ATP-dependent process by thioester bond, and then passes it to E2. Ubiquitin is then transferred by E2 to a lysine residue in the substrate by terminal isopeptide bond through E3, a scaffold protein. E3 makes a ligation between the substrate and the ubiquitin-linked E2. The resulting covalent ubiquitin ligations form polyubiquitinated conjugates that are detected and rapidly degraded by the 26S proteasome. Polyubiquitin chains also serve as a signal for proteasome-dependent degradation; monoubiquitination is a signal for endocytosis or subnuclear trafficking. K63-linked polyubiquitin chains signal endocytosis, IKK activation, ribosome modification, or DNA repair. The E3 enzyme provides the substrate specificity in all of those processes. In this way, there is a specific E3 for each substrate[3]. Thus, the ubiquitin–proteasome pathway plays a critical role in the degradation of several proteins involved in the cell cycle. Dysregulation of this pathway leads to inhibition of cellular proliferation and the induction of apoptosis[4]. Ubiquitination and its downstream consequences have therefore been investigated intensively as targets for the development of drugs for tumour therapy.

Ovarian cancer is a neoplastic growth arising from various parts of the ovary, mainly the outer lining and the fallopian tube. Ovarian cancer is the fifth leading cause of death from cancer in women and the leading cause of gynecologic cancer death[5]. In this review article, we discuss the involvement of the ubiquitin pathway in the progress of ovarian cancer and possible targeting for ovarian cancer treatment.

2. ROLE OF UBIQUITIN PATHWAY IN OVARIAN CANCER

2.1 Ubiquitination in *BRCA* Gene Function and Ovarian Cancer

BRCA1 is a human tumour suppressor gene that produces a protein called breast cancer type 1 susceptibility

protein. *BRCA1* is expressed in the cells of breast, ovary, and other tissues. The BRCA1 protein plays a role in transcription, DNA repair of double-stranded breaks, ubiquitination, and transcriptional regulation, or destroys cells if the DNA cannot be repaired. If BRCA1 itself is damaged, and if the damaged DNA is not repaired properly, then the risk of cancer increases[6,7]. Mutations in the *BRCA1* gene are associated with an increased risk of breast and ovarian cancers[8]. Choi[9] demonstrated that BRCA1 protein may be degraded through the ubiquitin–proteasome mediated pathway. Kim *et al.*[8] reported the identification of receptor-associated protein 80 (RAP80) as a BRCA1-interacting protein in humans. A tandem ubiquitin-interacting motif domain in RAP80 is required for its binding with ubiquitin *in vitro* and its damage-induced foci formation *in vivo*. Moreover, RAP80 specifically recruits BRCA1 to the sites of DNA damage and functions with BRCA1 in G2/M checkpoint control. Considering those results together, Kim *et al.*[8] suggested the existence of an ubiquitination-dependent signalling pathway that is involved in DNA damage response. Cellular expression of BRCA1 is regulated in a cell cycle–dependent manner. BRCA1 interacts with BRCA1-associated RING domain protein 1 (BARD1) to generate significant ubiquitin ligase activity, which catalyzes nontraditional Lys-6–linked polyubiquitination. Hayami *et al.*[10] showed that the ubiquitin ligase activity of BRCA1–BARD1 is downregulated by cyclin-dependent kinase 2 (CDK2) and is thus involved in ovarian tumour progression. However, Shakya *et al.*[11] reported that E3 ubiquitin ligase activity is not required for BRCA1 tumour suppression; rather, phosphoprotein of C terminus of BRCA1 is involved in tumour suppression. Jensen *et al.*[12] suggested that deubiquitinating enzymes may play a role in BRCA1 function. They identified a novel protein Bap1 (nuclear-localized, ubiquitin carboxy-terminal hydrolase), which binds to the RING finger domain of BRCA1. They also suggested that Bap1 may act as a possible tumour suppressor in the BRCA1-related cell growth control pathway.

Individuals carrying a germline mutation of the breast cancer susceptibility gene *BRCA2* are predisposed to breast, ovarian, and other types of cancer. The BRCA2 protein has been proposed to function in the repair of DNA double-strand breaks. Schoenfeld *et al.*[13] found that a deubiquitinating enzyme, USP11, forms specific complexes with BRCA2. Moreover, BRCA2 was constitutively ubiquitinated *in vivo,* in the absence of detectable proteasomal degradation. They suggested that BRCA2 expression levels are regulated by ubiquitination in the cellular response to DNA damage and that USP11 participates in DNA repair functions within the BRCA2 pathway, independently of BRCA2 deubiquitination. Thus, the deubiquitinating enzyme USP11 may interfere with ovarian cancer by controlling *BRCA2* expression.

2.2 Ubiquitination in ERK Pathway and Ovarian Cancer

The extracellular signal-regulated kinase (ERK) pathway plays important roles in various cellular processes: for example, cell proliferation, growth, differentiation, and survival. The ERK signalling pathway relays on extracellular signals from ligand-bound cell surface tyrosine kinase receptors to gene transcription in the nucleus via the phosphorylation cascade[14]. Increased exposure to growth factors and overexpression or mutation of receptor tyrosine kinases Ras and Raf are responsible for aberrant activation of the ERK pathway, which has been implicated in various aspects of tumorigenesis such as cell proliferation, differentiation, invasion, angiogenesis, and apoptosis[15,16].

In ovarian cancer, constitutive activation of ERK has been associated with high tumorigenicity and chemoresistance[17]. Therefore, the ERK pathway is a crucial target for therapeutic intervention in ovarian cancer. Mitogen-activated protein kinase phosphatases (MKPs) can modulate the duration, magnitude, and subcellular compartmentalization of ERK1/2 activity, suggesting that these inhibitor proteins also play important roles in tumorigenesis[18,19]. Ubiquitination can effectively influence the expression of ERK1/2 and tumour progression by interfering with MKPs. Chan *et al.*[20] reported that loss of the MKP-3 expression (a negative regulator of ERK1/2) is significantly correlated with high ERK1/2 activity in primary human ovarian cancer cells. Interestingly, the loss of MKP3 protein is associated with ubiquitination or proteosome degradation. Conversely, enforced expression of MKP3 in MKP3-deficient ovarian cancer cells significantly reduced ERK1/2 activity and inhibited cell proliferation, anchorage-independent growth ability, and tumour development in nude mice. Chan *et al.*[20] suggested a molecular mechanism by which increased ubiquitination may cause the degradation of MKP3, which in turn leads to aberrant ERK1/2 activation and contributes to tumorigenicity and chemoresistance of human ovarian cancer.

2.3 Ubiquitination in Cyclin-Dependent Pathway in Ovarian Cancer

Cyclins are critical regulators of the cell cycle. Classical cyclins activate specific cyclin-dependent kinases (CDKs) to promote cell-cycle progression[21]. Cyclin G2 is degraded by the ubiquitin–proteasome pathway and contributes to ovarian cell proliferation[22], while the overexpression of cyclin G2 inhibits ovarian cancer cell proliferation. Degradation of cyclin E by phosphorylation in cells with increased ubiquitination has been reported in ovarian cancer by Brandt *et al.*[23]. Thus, ubiquitin-dependent cyclin E degradation may be a good approach to inhibit ovarian cancer.

2.4 Ubiquitination in *ERBB2* Gene Expression in Ovarian Cancer

Overexpression of the transmembrane receptor tyrosine kinase ErbB2 is common in multiple malignancies, including ovarian cancer. ErbB2 was found to be resistant to degradation mediated by c-Cbl, the E3 ubiquitin ligase. However the chaperone-binding ubiquitin ligase CHIP efficiently ubiquitinates and downregulates ErbB2[24].

2.5 Role of the Ubiquitin System in Ovarian Cancer Through p53-Dependent Pathway

Ubiquitination plays a key role in regulating the tumour suppressor p53. It targets p53 for degradation by the 26S proteasome. The ubiquitin pathway also regulates the activity and localization of p53. Ubiquitination requires ubiquitin-activating and ubiquitin-conjugating enzymes and ubiquitin ligases. In addition, ubiquitination can be reversed by the action of deubiquitinating enzymes. Allende–Vega and Saville[25] reviewed the role of components of the ubiquitin–proteasome system (UPS) in the regulation of p53 and suggested targeting those proteins to activate wild-type p53 for the treatment of cancer. The p53 pathway controls autophagy and the UPS and provides major cellular pathways for protein degradation. Hwang *et al.*[26] investigated whether p53 regulates the UPS in ovarian tumour cells. They established a reporter cell line (SKOV3-EGFPu) to measure UPS function against a constant genetic background. Compared with vector control, transient expression of either wild-type or mutant p53 in SKOV3-EGFPu cells reduced UPS activity. The authors suggested that expression of wild-type and mutant p53 protein equally impaired UPS function. Thus, it was postulated that p53 may regulate protein homeostasis by downregulating UPS function in response to cellular stress in ovarian cancer cells.

2.6 Ubiquitin-Like Activity of SHPRH Protein and Ovarian Cancer

Human SHPRH [small ubiquitin-related modifier-1 (SUMO-1) heptapeptide protein transduction domain for binding Rev (SHPR) of human gene] is located at the 6q24 chromosomal region, and loss of heterozygosity in this region is seen in a wide variety of cancers[27]. A member of the SWI/SNF family of ATPases and helicases, SHPRH possesses a C_3HC_4 RING motif characteristic of ubiquitin ligase proteins. In both of those features, SHPRH resembles the Rad5 protein in yeast, which, together with Mms2–Ubc13, promotes replication through DNA lesions by an error-free post-replicational repair system.

Genetic evidence in yeast has indicated a role for Rad5 as an ubiquitin ligase in mediating the Mms2–Ubc13-dependent polyubiquitination of proliferating cell nuclear antigen (PCNA). Unk *et al.*[27] showed that SHPRH is a functional homolog of Rad5 that physically interacts with the Rad6–Rad18 and Mms2–Ubc13 complexes and that SHPRH protein is an ubiquitin ligase indispensable for Mms2–Ubc13-dependent polyubiquitination of PCNA. Based on those observations, Unk *et al.*[27] predicted a role for SHPRH in promoting error-free replication through DNA lesions. Such a role for SHPRH is consistent with the observation that this gene is mutated in a number of cancer cell lines, including those from ovarian cancers, which raised the strong possibility that SHPRH is an important deterrent to mutagenesis and ovarian carcinogenesis in humans.

2.7 Role of Ubiquitin Pathway in Cisplatin-Mediated Ovarian Cancer Treatment

Cisplatin is among the most effective chemotherapeutic agents in the treatment of human ovarian cancer. The cytotoxicity of cisplatin results primarily from its ability to bind covalently to DNA and prevent DNA replication and transcription. The ubiquitin–proteasome pathway plays important roles in a broad array of basic cellular processes and makes ovarian cancer cell lines cisplatin-resistant. Lactacystin is a selective inhibitor of the proteasome that can inhibit the ubiquitin pathway. Li *et al.*[28] reported that lactacystin, at concentrations that do not appear to be harmful, increased cisplatin toxicity in three resistant human ovarian carcinoma cell lines by inhibiting the ubiquitin pathway. Checkpoint kinase 2 (Chk2) is one of the critical kinases governing the cell cycle checkpoint, DNA damage repair, and cell apoptosis in response to DNA damage signalling. Zhang *et al.*[29] demonstrated that Chk2 is degraded in response to cisplatin through the ubiquitin–proteasome pathway.

3. TARGETING THE UBIQUITIN PATHWAY FOR OVARIAN CANCER TREATMENT

Inhibition of the ubiquitin–proteasome pathway is regarded as a novel approach to the treatment of solid tumours[1]. Bazzaro *et al.*[30] suggested that the proteasomal and alternate histone deacetylase 6 (HDAC6)–dependent proteolytic pathways are both elevated in ovarian cancer. They also suggested the potential of combined inhibition of proteasome and HDAC6 as a therapeutic strategy for ovarian cancer. S-Phase kinase protein 2 (SKP2) targets cell-cycle regulators including CDK inhibitor p27Kip1 through ubiquitin-mediated degradation. SKP2 is frequently overexpressed in a variety of cancers. Uddin *et al.*[31] investigated the function of SKP2 and its ubiquitin–proteasome pathway in a large series (n = 156) of epithelial ovarian cancer patient samples, using a panel of cell lines and the nude mouse model. Their results suggested that SKP2 and ubiquitin–proteasome pathway would be a potential target for the treatment

of epithelial ovarian cancer. The tumour-associated Rpn13 protein, which interacts with ubiquitin and activates the deubiquitinating enzyme Uch37 at the 26S proteasome, has recently been identified as a novel target in ovarian cancer treatment[32].

4. SUMMARY

In recent years, the effects of the ubiquitin pathway on a variety of cellular machinery related to ovarian cancer have been studied, and a link between them has been established. However, various studies are required to evaluate the link properly. This alternative may be interesting to investigate for cancer therapeutics because targeting of the ubiquitin pathway has led to some successes in ovarian cancer therapy.

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6. CONFLICT OF INTEREST DISCLOSURES

The authors have no financial conflicts of interest to report.

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