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Review Article

Chemoresistance and targeting of growth factors/cytokines signalling pathways: towards the development of effective therapeutic strategy for endometrial cancer

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Abstract: Endometrial cancer tends to be an aggressive malignancy. Although the disease prognosis can be good at the early stages of disease, the advanced condition is not curable. Chemotherapy regimens and hormone-based therapy in combination with surgery are major approaches for the management of endometrial cancers. However, intrinsic chemoresistance reduces the success rate and increases the possibility of disease relapse. Investigation of underlying mechanisms revealed altered activation of PI3K/AKT, MAPK, fibroblast growth factor (FGF), mTOR and WNT pathways and reduced gene expression of tumor suppressor p53 in recurrent endometrial cancer. A PTEN mutation, deletion or degradation induces positive p-AKT expression, while PI3K knock-down increases the level of pro-apoptotic proteins and decreases the level of anti-apoptotic ones in cancerous cells. Additionally, RAS proteins trigger both the RAF-MEK-ERK and PI3K-PTEN-AKT signalling mechanisms, thus conferring resistance to anti-tumor agents. FGF up-regulates angiogenesis via receptor-mediated tyrosine kinase activation. Single nucleotide polymorphism, gene amplification or missense mutations of FGFR2 are associated with endometrial cancer. The mTOR complex integrates the nutrient and mitogen signals via AMPKs, S6 kinase 1 (S6K1) and eukaryotic initiation factors, causing unrestricted endometrial cellular proliferation. WNT signalling molecules, such as frizzled receptors, β-catenin, PORCN, RSPO3 and DKK1 undergo dysregulation, and drugs targeting these pathways are under clinical trials in patients with endometrial cancer. Common therapies for endometrial tumor include platinum-based anti-neoplastics, taxanes, nucleoside analogues, immune modulators, FGFR and tyrosine kinase inhibitors, small-molecule mTOR inhibitors and drugs that trigger cell cycle arrest in the G1 phase. Taken together, the current review elucidates the mechanism underlying endometrial cancer, existing therapies and chemoresistance, and points towards the need for novel therapeutics that may promote disease-free survival.

Keywords: MAP kinases, Wnt pathway, fibroblast growth factors, mTOR pathway, chemotherapy

Introduction

Endometrial cancer that accounts for more than 95% of cases of uterine cancer is one of the most prevalent forms of gynaecological cancers. The disease is predominantly diagnosed in the developed countries and Western World [1]. In the United States, around 50-60,000 cases of endometrial cancer were reported annually in the years 2015-2017, resulting in 20-30% of lethal cases [2]. Risk factors of endometrial cancer include menopause, sterility, obesity, diabetes and history of colon cancer, breast cancer and pelvic radiotherapy [3]. However, hormonal imbalance characterized by significantly increased estrogen level in comparison to the level of progesterone appears to be the leading cause. This imbalance is linked to an abnormally thickened epithelial glandular lining and the commencement of pre-malignant phase [4].

Like most other cancers, endometrial cancer at stages I and II responds well to surgical interventions, but the disease at stages III and IV has poor prognosis with low survival rates [5]. The diagnosis tends to be established only at advanced stages of disease that are character-

ised by invasive tumors and metastases. This results in a survival rate of less than 5 years even after chemotherapy [6]. Other existing treatments for endometrial carcinoma, such as radiotherapy and hormonal therapy, seem to be less efficient. They fail after few rounds of administration, ending in a marked disease revival and recurrence [7]. Additionally, malignant endometrial cells that originally responded to the chemotherapy often turn refractory with time, leading to a situation of chemoresistance, i.e. lack of response to chemotherapy [8]. Owing to the low effectiveness of the currently available chemotherapeutics, the prevalence of endometrial cancer is predicted to increase sharply in the next 10-20 years [1]. Hence, new research in this field are essential to discern the disease mechanism and to identify effective therapeutic targets for attenuating the growth and survival of endometrial cells. This review analyses the common approaches to the treatments of endometrial cancer, the causes for chemoresistance in endometrial cancer and the role of newer anti-survival signalling pathways in the development of therapeutics that may restrict the recurrence of disease.

Conventional therapies for endometrial cancer

The mainstream treatments for endometrial cancers primarily include surgical removal of tumor mass. If needed, secondary or tertiary surgeries are performed to ensure the complete elimination of remaining tumor from the endometrium [9]. In the cases of localized and non-metastatic endometrial carcinomas, radiation therapy often follows the surgery, especially for the stage I endometrial cancer [10]. However, the method fails to have a significant impact in the patients who have already undergone cytoreductive surgery and are at the advanced stages, particularly at stages III-IV with a survival chances of only around 7% [11]. Hormonal therapies that target the receptors of estrogen and progesterone and luteinizing hormone releasing hormone were attempted for reducing the metastases [12]. However, the hormonal therapies demonstrated significant dependence on the target receptor types and activation status, receptor mutations, hormonal signals, cancer stages, patient heterogeneity and chemoresistant status [13]. Chemotherapy seems to be the most potent strategy for the removal of rapidly proliferating malignant endometrial cells [11]. Approved platinum-based anti-neoplastics, such as cisplatin, carboplatin, oxaliplatin and nedaplatin, as well as diterpenes from the class of taxanes, such as paclitaxel (Taxol) and docetaxel (Taxotere), are the most frequently used chemotherapeutics that improved survival and reduce the risk of disease recurrence [14]. Other anti-malignant drugs used in the treatment of endometrial cancer include cyclophosphamide, gemcitabine, topotecan and vinorelbine [15]. Although chemotherapy shows a good response to endometrial and gynaecological cancers, chemoresistance often emerges and results in eventual treatment's failure. In fact, chemoresistance is currently a key impediment in the therapy of endometrial cancer associated with disease recurrence and failed recovery [16]. Thus, targeting different pathways is essential for reducing chemoresistance and promoting the cancer cell death. In this context, the key target pathways that received attention include 'mammalian target of rapamycin' (mTOR), fibroblast growth factor (FGF) and Wnt signalling. These pathway are envisaged not only to reduce the cell division and proliferation, but to trigger the cancer cell death and reduce their survival [17].

Chemotherapy and chemoresistance in endometrial cancer

Chemotherapeutic agents for the endometrial cancer acted through various mechanisms, culminating in cancer cell apoptosis and death. Platinum-containing drugs cross-link with purine bases of DNA, triggering DNA degradation and damage, suppressing DNA repair and promoting cell death via Bax/Bcl2 pathway of apoptosis [14]. Taxanes generate poorly developed spindles and deregulate microtubule dynamics. This alters the normal cell cycle progression, causing atypical mitosis, BCl2 phosphorylation and the ultimate cell death [18]. Doxorubicin intercalates into double-stranded DNA, forms DNA adducts alters the DNA and chromatin topology, induces torsional stress and nucleosome instability, and triggers topoisomerase II poisoning, oxidative stress and ceramide overproduction [19]. Cytoxan (cyclophosphamide) undergoes biotransformation in the liver with the formation of an active alkylating component, which restricts DNA duplication

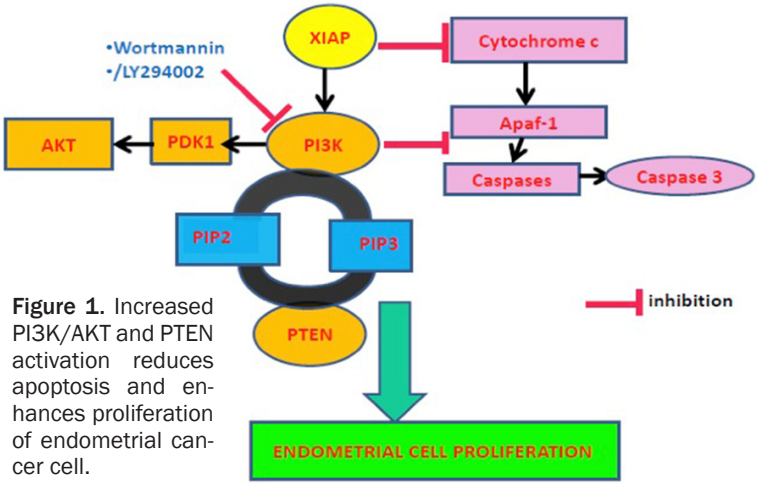

Figure 1. Increased PI3K/AKT and PTEN activation reduces apoptosis and enhances proliferation of endometrial cancer cell.

and RNA production [11]. Gemcitabine controls DNA replication and arrests tumor growth [20]. Topotecan functions by altering intercalation in the topoisomerase-I cleavage complex, ultimately disrupting DNA replication and DNA repair [21]. Vinorelbine interacts with tubulin, restricts mitosis at metaphase and deregulates the cyclic AMP and glutathione metabolic pathways, lipid synthesis axonal microtubule polymerization and Ca-dependent ATPase activity, thus inducing cellular antitumor mechanisms [22]. Combination therapy that includes platinum-based drugs, taxanes and doxorubicin have been tried as a first-line treatment for endometrial cancer, arbitrating an improved response with about 50% recovery rate [23].

Multifactorial chemoresistance is one of the major reasons for around 90-95% lethality in endometrial cancers, even after appropriate treatment [9]. Several mechanisms of chemoresistance were proposed, including enhanced efflux pump activity, β-tubulin mutations, undesired DNA repair and non-targeted signalling pathways [11]. An overexpressed multidrug-resistance gene (MDR-1) that encodes the efflux pump, P-glycoprotein (P-gp), restricts the build-up of chemotherapeutic agents in the cells and reduces their cytotoxicity [24]. The p-gp expression undergoes significant up-regulation in endometrial cancer and adenomatous hyperplasias and, to a certain extent, in the benign endometrium as well. On the other hand, the expression of motility-related protein 1 (MRP-1) is markedly increased in endometrial carcinomas, with no changes observed in the normal endometrium [25]. The acquisition of resistance to platinum-based compounds in endometrial cancer involves a 22-56% reduction in the copper influx, which is associated with an enhanced expression of the copper export pumps (ATP7A or ATP7B). This result in a 54-73% decrease in copper accumulation compared to platin-sensitive endometrial malignant cells [26].

Participation of DNA repair pathways in the development of chemoresistance in endometrial carcinomas is well acknowledged. The mechanisms of DNA repair include *Nucleotide excision repair (NER), Base excision repair, Gene specific repair, Strand-specific Mismatch repair and Homologous recombination deficiency.* Chemoresistance to cisplatin involves an augmented platinum-DNA adduct elimination and nucleotide excision activity in the endometrial cancer model systems, mainly due to *NER* [27]. The *excision repair* of bulky cisplatin DNA adducts appears to be mediated by the mutated Excision repair gene cross-complementing (ERCC) and xeroderma pigmentosum (XP) proteins, chiefly involving the helicases encoded by ERCC2, ERCC3 and ERCC6 genes. The simultaneous expression of ERCC and XP enzymes further facilitates the elimination of cisplatin-induced DNA damage [28]. Homologous recombination during double-stranded break repair in the carriers of BRCA-1 or BRCA-2 germ line mutations is accompanied by an amplified risk for endometrial cancer. It has also been shown that the blocking of BRCA pathway increases the responsiveness to cisplatin, and a defective repair in this pathway triggers cisplatin resistance. It was proposed that the *mismatch repair* deficiency in endometrial cancer results from the loss of MLH1 gene, which facilitates cell division and proliferation even in the presence of the platins and attenuates cellular apoptosis [29].

Endometrial cancinomas are frequently characterized by increased activation of the pro-survival PI3K signalling (**Figure 1**), usually downstream of tyrosine kinase (TK) signalling, promoting growth and proliferation of cancer

cells [30]. Activated PI3K stimulates the phosphoinositol lipids (PIP2 and PIP3), which trigger the serine-threonine kinase AKT mechanism and thereby glucose metabolism [31]. Additionally, an activated PI3K/AKT induces phosphorylation of pro-apoptotic proteins like caspases, BAD, Ikappa-B kinase and others, causing reduced cell death. This pathway plays a contributory role in chemoresistance of endometrial cancer [32]. The inhibition of 3-phosphoinositide-dependent protein kinase 1 (PDK1)-AKT pathway enhances the cisplatin-mediated endometrial cell apoptosis. It is associated with an increased generation of cytochrome C and expression of cleaved poly (ADP-ribose) polymerase (PARP). Its impact is enhanced through the use of AKT inhibitors, Wortmannin, and LY294002 [33]. Reduced expression and activation of the tumor suppressor PTEN (phosphatase and tensin homolog) that carries a mutation in endocrine cancer has a significant association with AKT phosphorylation, particularly during the development of endometrial chemoresistance. In fact, an additive effect of PIK3CA and PTEN mutations appears to be a leading cause for increased proliferation of endometrial cancer cells and resistance to anti-cancer drugs [34]. A cisplatin-mediated caspase-3 activation triggers PTEN cleavage as a downstream mechanism, which often culminates in the enhanced expression of p-AKT in endometrial cancer cells, thus suggesting a possibility of chemoresistance induced via PTEN-AKT activation [35].

A direct link between AKT and cell survival also involves the activation of the protein kinase mTOR that promotes endometrial cell stability, multiplication, malignancy and survival. The enhancement of mTOR pathway is currently being studied as a key cause for endometrial cancer drug resistance [32]. The AKT isoforms AKT1, AKT2 and AKT3 contribute differently towards chemoresistance. Decreased activation of AKT1 and AKT2 enhances the sensitivity of endometrial cells to cisplatin through activation of pro-apoptotic caspases, while up-regulation of AKT1 and AKT2 results in increased chemoresistance and reduced cell death [36].

The proteins XIAP (X-linked inhibitors of apoptosis) and P53 serve as important decisive factors for cisplatin resistance, where the activation of PI3/AKT pathway appears to be essential. The dominant negative form of AKT2 retains the functions of platins, even when XIAP and P53 are active [37]. It has also been observed that the link between AKT and p53 lies in the mitochondrial apoptotic pathway, ultimately influencing the responsiveness to cisplatin [38]. Platins inhibit XIAP and activate Bax, Bcl-xl and Fas-L in the endometrial cancer cells. As observed in the cisplatin-resistant subclones, the increased XIAP has an opposite effect on the pro-apoptotic proteins, thereby inducing chemoresistance [35].

An AKT-mediated chemoresistance has been reported to involve the modulated cisplatin-induced ubiquitination of p53-dependent protein FLIP (Fas-associated death domain-like interleukin-1 beta-converting enzyme (FLICE)-like inhibitory protein), while the inactivation of AKT signalling promotes FLIP degradation [39]. Cisplatin stimulates the p53 up-regulated modulator of apoptosis (PUMA), which involves p53 induction and seems to be indispensable for the platin-mediated apoptosis. It was also observed that AKT stimulates P53 phosphorylation and attenuates PUMA induction, thus modulating the sensitivity to platins and their effect on endometrial apoptosis [40].

The activation of Ras-Raf-MEK-ERK pathway also has a significant impact on the propagation of endometrial cancer and its chemoresistance. Although prolonged activation of JNK/P38 signalling stimulates the transcription of death inducer Fas ligand, a transiently activated status serves as a key reason for platin-induced chemoresistance and reduced endometrial cell apoptosis [35]. A decreased activation of ERK and P38 in association with cytoplasmic tyrosine kinase FAK (Focal adhesion kinase), promotes integrin-directed cell signalling and cell adhesion, causing the induction of chemoresistance [41]. The ERK inhibitor, mitogen-activated protein kinase phosphatase (MKP)-3, contributes towards the responsiveness to the platins in endometrial cancer. The proteosomal degradation of MKP3 mediated by reactive oxygen species causes an atypical ERK1/2 activation and increases endometrial cell proliferation, despite the platin treatment [42].

A significant cross-talk between JNK, P38, Ras-ERK, AKT2 and PI3K-mTOR signalling occurs in endometrial cancers, and simultaneous mutations in these pathways could also attenuate

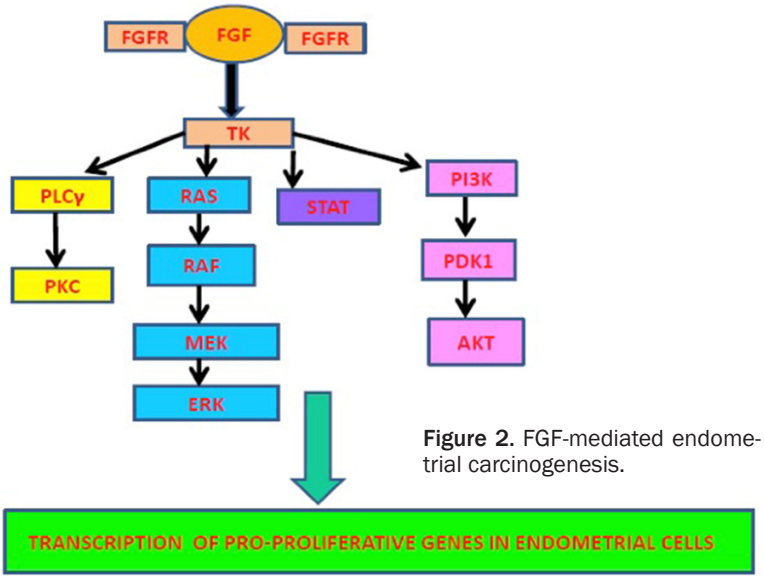

Figure 2. FGF-mediated endometrial carcinogenesis.

the cytotoxicity of chemotherapeutics in ovarian and endometrial cancers [43]. The activated TK-dependent epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor-2 (HER-2) cellular oncogene not only promote the growth of highly aggressive variants of endometrial cancer but also lead to the development of drug chemoresistance. In reality, MEK/ERK-dependent PI3K/AKT signalling emerges as the downstream component of EGFR, attenuating endometrial cell apoptosis. The estrogen and progesterone receptors have been found to possess the prognostic importance for endurance of endometrial cancer. On the other hand, it has been observed that estrogen enhances the chemoresistance to paclitaxel via a modulated expression of apolipoprotein clusterin that mediates the clearance of endometrial cell debris following apoptosis [44].

The molecular chaperone and glucose-regulated protein GRP78 that regulates apoptosis plays an important role in attenuating the ER-mediated endometrial cell death. The increased GRP78 attenuates the expression levels of cleaved caspase-3 and PARP in endometrial cancer cells even after treatment with paclitaxel and cisplatin [45]. Estrogen could also attenuate the paclitaxel-mediated endometrial cell degradation via reduced JNK activation and increased association of AKT and apoptosis signal-regulating kinase 1 (ASK1), which results in enhanced phosphorylation of serine-83 of ASK1. As a result, the estrogen receptor antagonist ICI182,780 and AKT inhibitor LY294002 could restrict the estrogen-mediated activation of AKT-ASK1 and promote sensitivity to pro-apoptotic drugs targeting endometrial cancer [46].

Inhibiting the functions of the tumor suppressor gene p53 that plays a key function in coordinating important cellular events, such as cell cycle arrest, metabolism, metastasis and interaction within the malignant microenvironment following the DNA damage, leads to reduced apoptosis in endometrial cancer cells [47]. Missense and somatic mutations and P53 gene aberrations in P53 are associated with the high-grade endothelial tumors, causing peritoneal infiltration and metastatic action. Attenuated apoptosis of endothelial cells, even following the treatment with chemotherapeutic adjuvants, cisplatin and carboplatin, was observed in the presence of P53 mutations [48].

The transcriptional repressors of the Snail family that are active during the epithelial mesenchymal transition and embryonic development undergo an up-regulation following chemotherapy, which ultimately results in aggressive cancer micrometastases and macrometastases and chemoresistance via reduced P53 expression. These transcriptional suppressors not only attenuate the transactivation of p53 inducers, but also activate the expression of self-renewal genes, causing increased tumor metastases and propagation. In reality, the Snail and slag expression confers a stem cell-like property to the endothelial cancer cells, attenuating their responsiveness to chemotherapeutics [49].

The nitric oxide synthases, eNOS, iNOS and nNOS, play a determining role in the modulation of P53 levels. An increased endogenous eNOS/nNOS activity fails to up-regulate P53 and restricts the loss of endothelial tumor. Conversely, iNOS promotes P53 expression, inducing sensitivity to chemotherapeutics in gynaecological cancers, and a significant accu-

mulation of iNOS enhances the apoptosis in ovarian and endothelial cancers [50].

FGF pathway

FGF activation and endometrial cancer: The binding of FGF receptor subunits FGFR1-R4, which possess intracellular TK activity, to FGF triggers angiogenesis in endometrial carcinoma [51]. The FGFR-FGF interaction leads to the dimerization of receptor and phosphorylation of the TK domain. This, in turn, sets a signalling cascade that involves mitogen-activated protein kinase (MAPK), PI3K/AKT, phospholipase C-gamma (PLCγ) and Signal Transducer and Activator of Transcription (STAT) pathways [52] (**Figure 2**). PLCγ accentuates the activation of MAPK pathway and enhances the transactivation property of cell cycle activator MYC. PI3K maintains the MAPK activity by inducing cell growth and multiplication and attenuating apoptotic cell death. The STAT molecules also induce transcription of cell proliferating genes and transrepression of pro-apoptotic ones. All these factors together lead to the propagation of endometrial carcinoma, tumor angiogenesis and an ultimate unrestricted cell survival, migration and motion, which was initiated by the primary FGFR activation.

However, the FGF-induced signalling pathways in endometrial and other gynaecological cancers can be checked by the degradation of FGFR and the promotion of FGF deregulatory pathways, such as SPRY, SPRED1 and 2 and SEF, that typically inhibit the Ras/Raf/MEK/ERK pathway as negative growth factors [53, 54]. Interestingly, FGFs also interact additively and synergistically with the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) pathways, and the three pathways together cause a marked stimulation of endometrial tumorigenesis, via the initiation and activation of the receptor TK pathway [55]. An interdependent autocrine/paracrine relation exists between FGF, PDGF and VEGF and their receptors' functioning in the endometrial cancer cells, and over-expression or silencing of these ligands in endometrial cells leads to an increased or suppressed tumor angiogenesis, tumor volume, malignancy and lymph node metastasis respectively [56].

In order to restrict the activation of these TK pathways, FGF, VEGF and PDGF mechanisms are targeted. However, it has been observed that, unlike the FGF inhibitors, resistance to VEGFR and PDGFR inhibitors in endometrial cancer is very common and develops within a short span of treatment time [57]. Thus, the FGF pathway is generally targeted in combination with the VEGFR and PDGFR inhibitors, and quite often the FGF inhibitors alone are used as endometrial cancer therapeutics [55].

Mutations in the FGF receptors, particularly the receptor FGFR2, are observed in around 20% of the endometrial cancer cases. They are associated with the microsatellite instability that essentially functions as an identifier of a defective DNA mismatch repair [58]. For this reason, not only the typical FGF inhibitors, such as PD173074 and TKI258, but also the silencing, knock out or knock down of FGFR2 attenuates the unrestricted endometrial cell viability [59-61]. FGFR2 mutation is an inherent condition and is mainly linked to the early-stage endometrial carcinoma. It causes a significantly higher activation of receptor that fails to respond to strong FGF inhibitors, ultimately leading to reduced chances of survival or disease-free status. In fact, FGFR2 mutations are irreparable following a surgery or aggressive chemotherapy as well [62]. The N550K mutations in FGFR2 appears as a key reason for the resistance of endometrial cancer cells to FGFR inhibitors. This mutation causes an increased activation of pro-proliferative TK and downstream ERK and AKT in the cells. However, targeting FGFR2 attenuates the activation of its mutated form, and has often proven beneficial in restricting the endometrial carcinogenesis [63, 64]. It has been reported that 10% of the total endometrial carcinoma cases in humans occur due to missense mutations in the FGFR2 gene [58, 60]. Missense mutations at the third immunoglobulin-like site of FGFR2 trigger a modulation in the ligand receptor interaction. These mutations cause a change in the activity status of FGFR2, which is ligand-dependent and functions via an autocrine/paracrine manner. Alternatively, mutations within the TK site of receptor trigger a higher oncogenicity that appears independent of its ligand [17]. S252W mutation in FGFR stimulates receptor-ligand interaction in the FGF pathway. In addition to receptor activation, an increased expression levels of the FGF ligands, FGF1 and FGF2, is also an important cause of the increased multiplication rate of endometrial cells and endometrial tissue enlargement [65]. The endometrial hyperplasia amplifies with the disease advance-

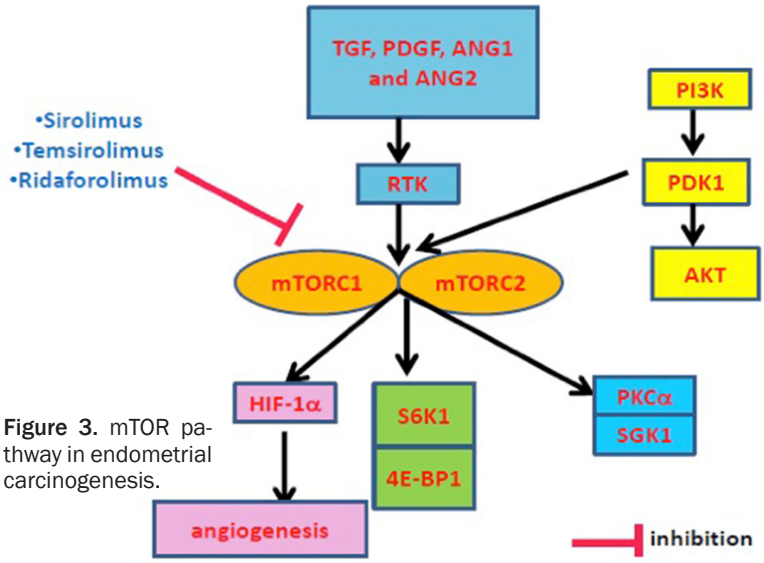

Figure 3. mTOR pathway in endometrial carcinogenesis.

ment, proportionally with the grade and depth of myometrial appropriation [65]. An FGF ligand trap FP-1039 specifically inhibits FGF1 and FGF2 and intervenes in the FGF signalling through FGFR1. This action restricts the uncontrolled cancer cell growth and angiogenesis in the adjacent blood vessels of the affected endometrial tissues [66]. Mutations have been observed at various sites within the FGFR TK-domain. Whereas the mutation N500K has its target site in the constitutive kinase activation domain, the mutations S373C and Y376C in the enzymatic region involve additional cysteine residues and lead to increased intermolecular S-S bonding [67]. Moreover, other mutations, such as I547V, N549K and K659E, were identified at the kinase site of FGFR2 within the endometrium [68].

Targeting FGFR in endometrial cancer

Because of the ubiquitous involvement of up-regulated FGF pathway in endometrial cancer, therapeutics targeting FGFR are being critically studied, and the generation of specific drugs that inhibit FGF-induced carcinogenesis are of particular interest for this disease. Small-molecule inhibitors that specifically target the ATP binding pocket within the active site of FGFR protein kinases have gained recognition as anti-endometrial cancer therapeutics. Thus, high throughput screening is used as a strategy for identifying the ATP mimetics that bind to the ATP-interacting pockets of the targeted protein kinases of FGFR2 [69, 70]. This appears to be a successful endeavour, as evident from the extensive number of protein kinases as drug targets, which is only exceeded by the G protein-coupled receptors that are targeted by around 60% of all drugs currently on the market [71]. The major drugs belonging to the first and second generations of FGFR inhibitors include PD173074, brivanib, dovitinib, intedanib, nintedanib, lenvatinib, ponatinib, Ki23057, E7080, MK-2461, E-3810 and AZD4547-47142 [67]. NP603 and 6b also subsequently appeared on the list of key FGFR inhibitors [72, 73]. Brivanib, dovitinib, intedanib, nintedanib, lenvatinib, ponatinib are effective even at nanomolar concentrations. Their very low IC-50 values are indicative of their strong potential as FGFR inhibitors. Xenograft models, as well as the *in vitro* studies in cancer cells, proved the anti-proliferative, pro-apoptotic, anti-tumorigenic and anti-angiogenic roles of these first-generation FGFR inhibitors, which are currently undergoing the pre-clinical and different phases of clinical trials [61]. The first-generation FGFR inhibitor, PD173074, that bears a pyrido [2, 3-d]pyrimidine core restricts the FGFR1 tyrosine kinase activity, and its IC50 value is around 0.02 μM [74]. Another FGFR inhibitor of the same class, SU5402, that has an indolin-2-one active part, limits the FGFR1 activation at an IC50 of 0.03 μM. The IC50 for the broad-spectrum TK inhibitor AZD4547142 is around 0.026 μM. Despite the advantages, these narrow-spectrum inhibitors cause an increased drug resistance, paving the way for the broad-spectrum compounds, such as AZD4547142, that reduce the chances of reappearance of the drug-resistant endometrial malignant growths [75]. The second-generation FGFR inhibitors, dovitinib and ponatinib, have reached the phase II clinical trials for endometrial cancers. Interestingly, the statistics reveals that a higher number of patients are enrolled in the clinical trials of dovitinib due to its capability of inhibiting the tumor progression, as well as angiogenesis in endometrial cancer [61].

mTOR pathway

mTOR activation and endometrial cancer: The serine/threonine-based mTOR plays a key regulatory role in the cell growth, multiplication, pro-

liferation, differentiation and death. Rapamycin restrains mTOR activation by arresting the cells at G1-phase, and thereby blocks the cell cycle advancement [76]. The endometrial cancer cells undergo a hypoxic phase during the growth, metabolism and neovascularization, at which stage the hypoxia-inducible factor (HIF) has a key function (**Figure 3**). mTOR and its growth factors control cellular hypoxia by altering the HIF translation. mTOR1 and mTOR2 are the two complex forms of mTOR. mTOR1 activates Ribosomal protein S6 kinase beta-1 (S6K1) and Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), promoting protein translation through major contribution of 4E-BP1 [77]. Consequently, the altered activation of mTORC2 results in an increased expression of p-mTOR and over-activation of PI3K/AKT pathway, as well as VEGF-A and phopholipase-D that serve as down-stream mediators of mTOR in the malignant endometrial cells [78]. mTOR1 and mTOR2 together activate PKC-α and SGK1 pathways that deregulate the actin cytoskeletal organization in endometrial cancer. The combined action of growth factors, nutritional sources, stress and amino acids activates mTOR signalling. Then, via the AMP-activated protein kinases (AMPKs) that serve as energy sensors, the normal mTOR signalling undergoes a change in endometrial malignancy. Angiopoietins 1 and 2 (ANG 1, ANG 2), basic fibroblast growth factor, tumor growth factor-β and PDGF are expressed as down-stream products of HIF action and trigger the PI3K/AKT/mTOR signalling [79]. Additionally, mTOR is capable of altering PTEN and P53 signalling pathways, which are associated with increased expression of S6K1 in human endometrial cancer [80]. The molecular profiling study in 373 endometrioid cancer cases showed changes in PI3KCA, PIK3R1, AKT1 and PTEN in 59.7%, 33%, 3.2% and 66% of patients respectively. The tumor suppressor P53 signalling was altered in 92% of the patients, and the mechanism was shown to be markedly related to the mTOR pathway [81].

Anti-tumor effects of mTOR inhibitors in endometrial cancers

mTOR inhibitors that have undergone various phases of clinical studies include sirolimus (a rapamycin mimic) and its three analogues, temsirolimus (CCI779), ridaforolimus (AP2357) and ridaforolimus (AP2357), that function via immune suppression. The inhibitory functions of these macrolides involve reduced phosphorylation and activation of S6K1 and 4EBP1. These proteins suppress cyclin-dependent kinase activation in the cell cycle, thus attenuating cell division and proliferation and promoting an autophagy-dependent programmed cell death in endometrial cancers [82]. A phase II clinical study of orally administered everolimus (10 mg for 28 days/cycle) revealed a significant reduction in PTEN levels and marked clinical responses [83]. A co-administration of everolimus with chemotherapy in recurrent endometrial cancer cases showed a significantly reduced (by 25-50%) progression of cancer cell proliferation. A phase II clinical trial with ridaforolimus demonstrated improvements in about 30% of the cases in one study, while another trial showed an increase in survival by 6-8 months in matured endometrial malignancy [84]. However, the drug had strong toxic side effects, with a drug-linked mortality reported. A phase II study with the third compound, temsirolimus, showed a 69-70% inhibitory response in recurrent endometrial cancer, particularly in combination with chemotherapy. Nonetheless, a mechanistic study revealed that the temsirolimus-mediated action, appears to be independent of PTEN, although it involves mTOR pathway [85].

Studies are also underway to investigate which mTOR inhibitors can be used not only in combination with chemotherapy, but also alongside hormonal treatments, EGFR, VEGF, FGF and PDGF blockers for combating the endometrial cancer progression and subsequent metastases [86]. Currently, combination therapies with diverse mode of action have gained recognition in the attempts to overcome the drug resistance complications. A phase II clinical trial using temsirolimus in combination with bevacizumab showed a good response at the primary recurrent stage, with initial 20% reduction in cell proliferation and a further six months of reduced cell proliferation. The combination of temsirolimus and bevacizumab exhibited a better outcome, causing restricted cell proliferation in recurrent endometrial carcinogenesis in 50% of the patients, at least for a period of around six months. However, several toxic effects such as rectovaginal fistula, gastric ulcers, nasal bleeding, pulmonary embolism

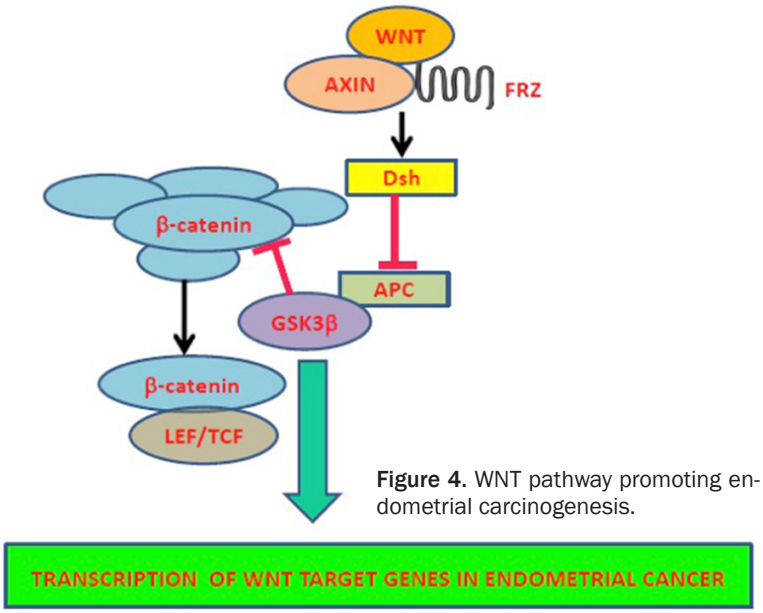

Figure 4. WNT pathway promoting endometrial carcinogenesis.

and others were observed, and there were several reports of mortality linked to the combination therapy [87]. A synergistic effect of rapamycin and paclitaxel (the latter functions via disruption of microtubule polymerization) was reported in pre-clinical studies. The use of this combination showed attenuation of cell growth due to restoration of mTOR signalling and resulted in an increased cell death in endometrial cancer cell lines [88]. In fact, a combination of antiangiogenic therapeutics like bevacizumab with paclitaxel also proved quite effective. However, the administration of temsirolimus instead of bevacizumab appeared less potent. A distinct link between an inhibited mTOR signalling and altered DNA repair (particularly a modulated homologous recombination) that results in increased apoptosis has been reported. Additionally, a direct correlation between altered immune responses and mTOR pathway inhibition has been demonstrated [81].

Wnt pathway

Wnt pathway deregulation in endometrial cancer: Under normal conditions, WNT4 gene expression is prominent in the endometrial cells [12]. However, an altered expression and changed ratio among different Wnt proteins have been reported in endometrial cancer. While the WNT4 gene showed a reduced expression, WNT2, WNT3 and WNT5A mRNA exhibited an increase in endometrial carcinoma compared to the normal cells. A marked increase in the WNT7A gene was widely reported in endometrial cancers [89]. Nonetheless, a different situation emerges in the presence of estrogen receptors, where the receptors suppress WNT7A expression [90]. Changes in WNT10A and WNT10B proteins have also been reported, predominantly in the estrogen-dependent endometrial cancers, where WNT10B undergoes an up-regulation at the initial stages of tumorigenesis and reduction at the late stages of malignancy and in metastases [91]. Indeed, an enhanced estrogen receptor signalling plays a contributory role in augmenting the Wnt signalling in malignant transformation of endometrial cells causing endometrial hyperplasia. The Wnt signalling pathway undergoes prominent modulation in endometrial cancer, and the genes SFRP$ (secreted frizzled-related protein 4) and SFRP1 show a significantly down-regulated expression. This results in the changed activation status of dishevelled protein (Dsh) and glycogen synthase kinase-3b (GSK3b), as well as phosphorylation of β-catenin in the endometrial cells (**Figure 4**). Generally, a reduced phosphorylation of β-catenin has been reported during the unrestricted endometrial cell growth and proliferation, causing an enhanced nuclear localization of non-phospho β-catenin, its enhanced complexation with T-cell factor/lymphoid enhancing factor (TCF/LEF) and transcription of the downstream genes, such as the cellular proliferation marker Ki67 and cyclin D1 that are required for sustaining the G1 phase within the cell cycle [92, 93]. Missense mutations or mutations in exon 3 of the catenin beta 1 (CTNNB1) gene are also evident in endometrial cancers, causing increased accumulation of β-catenin in the nucleus [51]. Mutations in the Adenomatous polyposis coli (APC) gene confer a reduced phosphorylation of β-catenin, and oncogenic K-ras gene KRAS, triggers an aberrant regulation of the Wnt/β-catenin signalling [94]. The proliferation, differentiation, migration and

apoptosis of the tumorous endometrial cells experience regulation by the WNT signalling pathway through the gain-of-function or loss-of-function, respectively, in the CTNNB1 and APC genes. A shift in the expression of Wnt inhibitor, Dickkopf-related protein 1 (DKK1), from its increased level to a decrease in non-malignant and malignant endometrial cells, respectively, is highly evident, and induction of DKK1 demonstrates a marked inhibition of the nuclear levels of β-catenin and hence Wnt pathway activation. This strongly significant reduction in DKK3 demonstrates an important link with the matured stages of endometrial cancer [95]. Somatic changes through the loss or gain-of-functions in the Ring Finger Protein 43, R-Spondin 2 (RSPO2) and RSPO3 genes are also important triggers of endometrial cancer [96].

Targeting WNT in endometrial cancers

Inhibition of the canonical and non-canonical WNT signalling pathways is a key process of restricting the endometrial carcinogenesis, and gynaecological oncologists have been successful in this endeavour. The potent possible inhibitors of Porcupine O-Acyltransferase and activators of NOTUM that stimulate and supress the WNT pathway, correspondingly, are important targets in endometrial cancer [97, 98]. Different family members of the WNT pathway in humans, such as WNT2B, WNT3A, FZD1 and others. have also been recommended as the targets for reducing the progression of endometrial cancer [96]. The therapeutic role of these targets is currently under verification using knock-out mouse models and Si-RNA, ShRNA or anti-specific antibodies in cells. A few examples of the frizzled receptor binding antibodies include vantictumab, OTSA101-DPTA-90Y and ipafricept (OMP-54F28), which are currently under clinical trials [99]. The receptor TKs, such as ROR1, NTRK, DDR1 and DDR2, have also been postulated as probable targets linked to cytotoxicity and ultimate cell death. Tankyrase inhibitors that degrade AXIN have been considered as therapeutics for the WNT pathway-induced endometrial carcinogenesis. However, a relatively low specificity of these inhibitors appears to be a reason for their restricted use [100]. In addition, β-catenin inhibitors (BC2059, PRI-724, ICG-001, etc.) that suppress TCF/LEF-mediated expression of pro-proliferative and anti-apoptotic genes have been successful in animal models [101]. Because of an intrinsic linkage between WNT signalling and the immune system, particularly in relation to the chemokine expression in dendritic cells and T lymphocytes and the functioning of the regulatory T cells, cautious usage of WNT pathway inhibitors is essential in the treatment of endometrial cancer and associated diseases [102].

Conclusions and future directions

Endometrial cancer becomes extremely aggressive at the advanced stages, and only modest recovery can be achieved with the currently available therapies. Multi-targeted PI3K, mTOR, FGF and WNT pathway inhibitors, having anti-tumorigenic and anti-angiogenic activities, were proposed as possible therapies for this gynaecological condition. However, a significant gap lies between the pre-clinical and systematic clinical studies when it comes to these inhibitors, thus restricting their full-fledged usage. Consequently, chemotherapy and hormone therapy are still considered the most appropriate treatments for endometrial cancer. Although the early stages endometrial cancer can be treated successfully, the recurrent and relapse cases fail to respond to these standard approaches. Moreover, the non-specific loss in viability is another major drawback of chemotherapy. Hence, site specific targeting and drug delivery become essential. Selective biological targeting of estrogen receptors appears advantageous in this context, as it has a better target specificity compared to chemotherapy.

Chemoresistance is another major cause of therapeutic failures in endometrial cancer. Chemoresistance is associated with perennially activated PI3K/AKT and anti-apoptotic genes (Fas/FasL and Bcl-2) and the loss in P53 activity. A close link between tumor heterogeneity, genetic structure and chemoresistance has been observed. Resistance to therapy ultimately leads to metastasis and resurgence of endometrial malignancy. However, serious efforts are being made to identify novel biomarkers for early disease diagnosis and use them as specific targets for endometrial cancer. Taking into account the cross-talk between diverse signalling pathways in the endometrial carcinogenesis, a combination therapy may prove effective in this context, and the recent clinical trials support this view. A combination of MAPK

and PI3K inhibitors, Everolimus (that targets mTORC1) along with Letrozole (that targets aromatase) shows greater efficacy compared to the effects of individual drugs. Early trials with FGFR antagonists and mTOR inhibitors have demonstrated promising results in endometrial cancer, and their use in combination with chemotherapy, estrogen therapy and PI3K inhibitors appears encouraging. Although phase I-II clinical studies have been carried out to investigate the targeting of PI3K/AKT/mTOR and FGF pathways, phase II trials and beyond are still very rare. Moreover, a more comprehensive knowledge of molecular pathophysiology of malignant endometrial tumors will be useful for a better bench-to-bedside translation of targeted therapeutics.

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