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Review Article

From endometrial glandular dysplasia to endometrial serous carcinoma: insights into underlying biological aspects

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Abstract: Endometrial serous carcinoma (ESC), a clinically aggressive gynecologic cancer, is the prototypical type II endometrial carcinoma. In 2004, we first proposed a model of endometrial serous carcinogenesis that has, with additional evidence, developed into a robust, step-wise model of ESC development based on the progressive accumulation of molecular alterations. In this model, the cancer progresses from a resting endometrium into endometrial glandular dysplasia (EmGD), then serous endometrial intraepithelial carcinoma (SEIC), and eventually advances into ESC. Though various studies have discussed the key molecular alterations involved in ESC development, the pathways and relationships between different players are unclear. In this review, we have summarized the current state of knowledge on key pathways and on relationships between the molecular factors involved in endometrial serous carcinogenesis and discussed potential prevention and therapeutic strategies.

Keywords: Endometrial serous carcinoma, endometrial glandular dysplasia, endometrial intraepithelial carcinoma, p53 mutation, PI3K-AKT-mTOR pathway, targeted therapies

Introduction

Endometrial cancer is the leading gynecological malignancy in the United States. On an upward trend, it is projected to reach an estimated 49,560 new cases and 8,190 deaths in 2013, compared to the estimated 47,130 new cases and 8,010 deaths in 2012 [1-3]. Endometrial carcinoma is divided into two subtypes, Type I and Type II, according to epidemiologic, histologic, clinical, and molecular features [1, 4].

Type I, the prototype of which is endometrial endometrioid carcinoma (EEC), comprises approximately 70-80% of all endometrial carcinomas and has a relatively good prognosis [5]. It typically presents in patients with obesity, hyperlipidemia, or hyperestrogenism and it arises from proliferating or hyperplastic endometrium in younger patients [1, 4-7]. Type 1 cancers are associated with alterations in the *PTEN, PIK3CA, PIK3R1, PIK3R2, LKB1, TSC2,* DNA mismatch repair gene microsatellite instability, β-catenin, and *K-ras* [8-18]. Most Type I carcinomas are diagnosed at an early stage and treated with surgery and radiotherapy. Patients with an International Federation of Gynecology and Obstetrics [FIGO] stage I diagnosis have a 5 year survival rate of 80-90% with recurrence rates of 4-8% after total hysterectomy [19].

In contrast, Type II carcinomas, including endometrial serous carcinoma (ESC) as its prototype, clear cell carcinoma, and carcinosarcoma, are broadly speaking, more clinically aggressive

than Type I cancers. They typically arise in older patients and are less strongly associated with estrogenic risk factors than Type I cancers [1, 6]. Though Type II cancers comprise only roughly 10-15% of all endometrial carcinoma cases, they are responsible for 40% of deaths in uterine cancer [5, 6]. Patients commonly present at an advanced stage and over 50% of patients have lymphovascular spread as well as metastasis to intraperitoneal structures at the time of presentation [6, 17, 20-22]. The 5-year survival rate is approximately 30% for all stages and recurrence ranges from 50-80% after surgical and adjuvant therapies [6, 17]. ESC has been described to be relatively resistant to chemotherapy, While mutations in *K-ras* are rare, ESC is associated with alterations of *TP53,* HER2/neu*, EGFR, PI3K, PTEN, PPP2R1A, CCNE1, FBXW7, BRCA*, E-cadherin, claudins*, CDKN2A,* IMP3*,* Nrf2*,* DLG7*, MELK, IHH, and RORB* [13, 18, 23-37]. ESC has also been found to be associated with breast cancer [38].

Type II endometrial carcinomas and especially ESC, has been extensively studied due to its high morbidity and mortality. In 2004, we first proposed a model of ESC carcinogenesis including the novel precancerous lesion, endometrial glandular dysplasia (EmGD) [39]. Various studies since have discussed molecular players that contribute to ESC advancement. However, the relationships between these biological factors have not been clearly defined. In this review, we will define the pathways and categories of molecular markers and discuss how this knowledge may impact future diagnostic and preventative methods. In addition, we will summarize what is currently known and suggest studies which are still needed for better understanding of this disease.

The model of endometrial serous carcinogenesis

In our previous studies, we have defined a model of endometrial serous carcinogenesis. Originating from resting or atrophic endometrium, the morphologically distinct precancerous lesion, EmGD, develops from various molecular alterations, predominantly the *TP53* gene mutation (resting endometrium with p53 signatures). This precancerous lesion then progresses to a special form of ESC known as serous endometrial intraepithelial carcinoma (SEIC), and eventually transforms into a fully developed ESC [5, 7, 16, 18, 40]. SEIC is not considered to be only a precursor of ESC but instead is now recognized to be a non-invasive, morphologically distinct form of ESC. This is because SEIC contains serous carcinoma cells that are localized within the endometrial cavity but are highly associated with extrauterine disease [16, 26, 39, 41-44].

In contrast to resting endometrium, which typically shows no signs of dysplasia or nuclear atypia, EmGD shows variable loss of cell polarity, nuclear hyperchromasia, a 3-fold nuclear enlargement with appreciable nucleoli, and minimal luminal papillary formation. This precancerous lesion shows less atypia than SEIC and does not contain abnormal mitoses. Compared to the resting endometrium, SEIC cells show a 4-5 fold nuclear enlargement, complete loss of cell polarity, frequent abnormal mitoses, and up to 66% are associated with extrauterine metastasis. The cellular features of SEIC are identical to ESC but without myometrial invasion [39, 45]. From this perspective, SEIC sometimes is referred as stage 1A ESC. Both ESC and SEIC are characterized by either glandular or papillary structures lined by frankly malignant cells with striking nuclear pleomorphism, smudged chromatin, and typically prominent nucleoli. Clinically, both lesions commonly have intraperitoneal metastasis [42]. The representative morphology from resting endometrium to ESC and their corresponding genetic changes are illustrated in the endometrial serous carcinogenesis model (**Figure 1**). The details of the molecular changes within this ESC developmental model are discussed herein.

With the ESC carcinogenesis model, recent studies have attempted to elucidate the various molecular factors associated with each stage of ESC development. p53 has been described to be involved from early stages to cancer and *TP53* gene mutations appear to increase in frequency at advanced stages in the carcinogenesis model. Many of the players of the PI3K-AKT-mTOR pathway are also involved in tumorigenesis. A very recent study by Wild *et al*. illustrated the various stages of ESC development in p53 mutant mice thereby further validating our proposed human model [18]. The authors also described a possible relationship between *TP53* gene mutation and the PI3K-AKT-mTOR pathway. In addition to

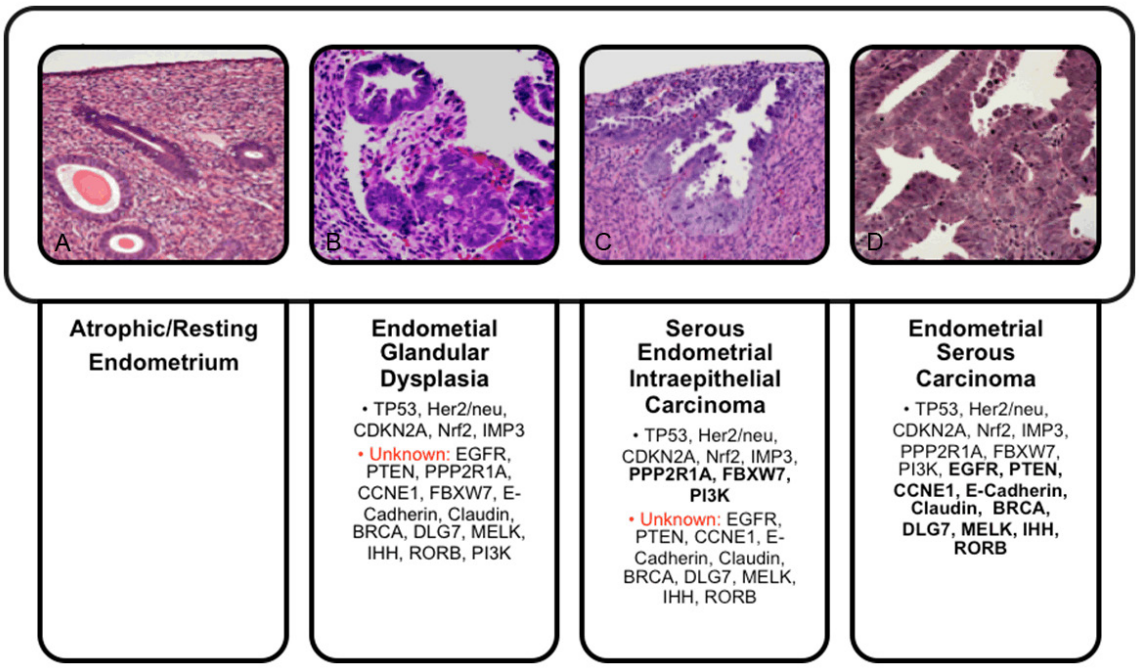

Figure 1. Endometrial Serous Carcinoma Model of Carcinogenesis. Endometrial serous carcinoma progresses from (A) resting endometrium into (B) endometrial glandular dysplasia (EmGD), then into (C) serous endometrial intraepithelial carcinoma (SEIC), and subsequently advances into (D) ESC. Various molecular alterations are present at each stage of carcinogenesis, but further studies are required.

these two pathways, other categories of molecular alterations have been noted. We summarized the current literature of the molecular markers and their proposed molecular pathways for our readers' reference.

p53 gene mutation

p53, encoded by the *TP53* gene, is a tumor suppressor protein designed to induce cell arrest and apoptosis in cells exposed to oncogenic insult [46]. Furthermore, it has a crucial role in cell cycle regulation, DNA repair, and cellular differentiation [26]. It is important for cancer prevention and it is the most commonly mutated gene in human cancers [16, 46]. Previous studies have shown that altered p53 is seen in 10-15% of early stage and up to 40-50% in advanced stage of all endometrial cancers [47]. Among all endometrial cancers, ESC has one of the highest rates of p53 mutation and is associated with poor prognosis [16]. One of the most important players in ESC development, up to 90% of ESC cases show p53 mutation by direct sequencing or single-strand conformational polymorphism and p53 overexpression by immunohistochemical studies [26, 48-50]. Although immunohistochemical overexpression of p53 is not synonymous with its mutation, there is a strong correlation between p53 overexpression and a genetic alteration. Also, mutant p53 overexpression is frequently associated with the deletion of the other allele. As a matter of fact, *TP53* mutation has been shown to be involved in latent precancer p53 signature and EmGD, suggesting that *TP53* gene mutation represents one of the initial molecular events in endometrial serous carcinogenesis [16, 48, 51].

In our previous study, we illustrated that in carcinogenesis progression more p53 mutations were detected. In our immunohistochemical analysis, there were no p53 mutations in resting endometrium [5]. Endometrial cells that developed p53 signatures, or p53 overexpression in benign looking endometrial epithelia, then entered the carcinogenesis progression model [40]. Subsequently, as the cells progressed through EmGD, SEIC, and ESC, p53 mutations were increased in a stepwise fashion and presented in 43%, 72%, and 96% of cases, respectively [5, 51]. Between these ESC lesions and their precursors, there were no differences in terms of the intensity of staining for p53 protein [47]. This "all-or-none" phenome-

non in p53 overexpression represents point and nonsense p53 gene mutations. On the contrary, there is a significant difference from p53 staining in EEC, which has a typical sporadic staining pattern. This suggests that p53 overexpression in these Type I cancers do not contain gene mutations. Instead, the overexpressed proteins are likely to be wild type [47]. From this information, we gather that p53 overexpression in ESC is due a genetic mutation and is an early event in carcinogenesis. Given our proposed model, it is clear that p53 is an initial player in endometrial serous carcinogenesis and that the frequency of p53 alteration increases as the disease advances.

A recent study performed by Wild *et al*. confirmed the causality of p53 and endometrial carcinogenesis. The group used a transgenic mouse model and induced p53 mutations in endometrial epithelium by crossing loxP-flanked $Trp53^{fl/fl}$ mice with those expressing the Ksp1.3-Cre transgene, ultimately resulting in a series of genetic mutations. Many mice developed endometrial serous or clear cell adenocarcinomas that had dysplastic nuclei and papillary growth patterns, histological morphologies nearly identical to its human counterpart. The group generated longitudinal histological sections of uteri from mice that contained varying spectrum of endometrial carcinoma, including EmGD, SEIC, and ESC. They concurred that EmGD represented the earliest identifiable precancerous lesion to ESC as we previously described [7, 16, 39, 48, 51, 52]. The lesions of EmGD and SEIC in mice were morphologically identical to the those in human. The serous carcinoma found in the *TP53* knockout mice model was characterized by papillary projections with fibrovascular cores, which are equivalent to human ESC with papillary structures. The findings from the study by Wild *et al*. accurately reproduced ESC development process as we proposed earlier [18, 39]. More importantly, this study illustrated a causal relationship; the loss of p53 function results in a progressively more advanced ESC.

Apparently, *p53* gene dysfunction is not the only responsible gene for the ESC development.

PI3K-AKT-mTORc1 pathway

In addition to demonstrating ESC development in p53 mutant mice, it seems that PI3K-AKT-mTORc1 pathway (PI3K pathway) also contributes to the ESC and is possibly intrinsically related to the p53 mutation. Dysregulation of the PI3K pathway has been recognized in both type I and type II endometrial carcinomas [18]. The normal activation of this pathway is crucial for cell growth, proliferation, and survival [53, 54]. Activated by tyrosine kinases such as Her2/neu and EGFR, PI3K is a heterodimeric lipid kinase that signals secondary messengers [6, 53, 54]. PI3K is mapped on chromosome 3q26 on the *PIK3CA* gene [12, 53]. Downstream of PI3K, the messengers then activate AKT, a serine/threonine kinase, which then regulates biological processes, including cell growth, proliferation, and survival [54, 55]. Specifically, AKT controls mTOR, a serine/threonine kinase designed to induce protein synthesis and is central to cell growth. On the other hand, PTEN is a lipid phosphatase that inactivates AKT [54]. *PPP2RIA* up-regulation also suppresses AKT activity [56] (**Figure 2**). From published works, we conclude that multiple mutations within the PI3K-AKT-mTOR pathway are involved in endometrial serous carcinogenesis [12, 18, 22, 53, 56-60]. This was further verified by the mouse model study that markers of activation along the PI3K pathway were activated in tumorigenic mice [18]. The alterations of the main molecules in this pathway are summarized as follows.

Her2/neu (erbB2) overexpression: Her2/neu on the *erbB2* gene is an oncogene that belongs to a family of tyrosine kinases [61]. It activates a network of downstream signals to cause a variety of biological processes, including activating PI3K [53, 61]. Over-expression of Her2/neu has been described in up to 50% of type II endometrial carcinoma and is associated with poor prognosis [29, 32, 53, 62, 63]. A recent study by Buza *et al.* suggested that although gene modification and protein overexpression is present, there is a wide heterogeneity in protein expression on immunohistochemical staining [63]. Her2/neu amplification is seen in all developmental stages of endometrial carcinogenesis, but is more pronounced in advanced stages [16]. The mouse model study by Wild *et al.*, however, demonstrated only an amplification of *erbB2* in one case of mouse endometrial clear cell carcinoma and it was not seen in the serous carcinoma [18]. Though Her2/neu alteration is heavily involved in human ESC, its role seems be less prevalent in mouse models.

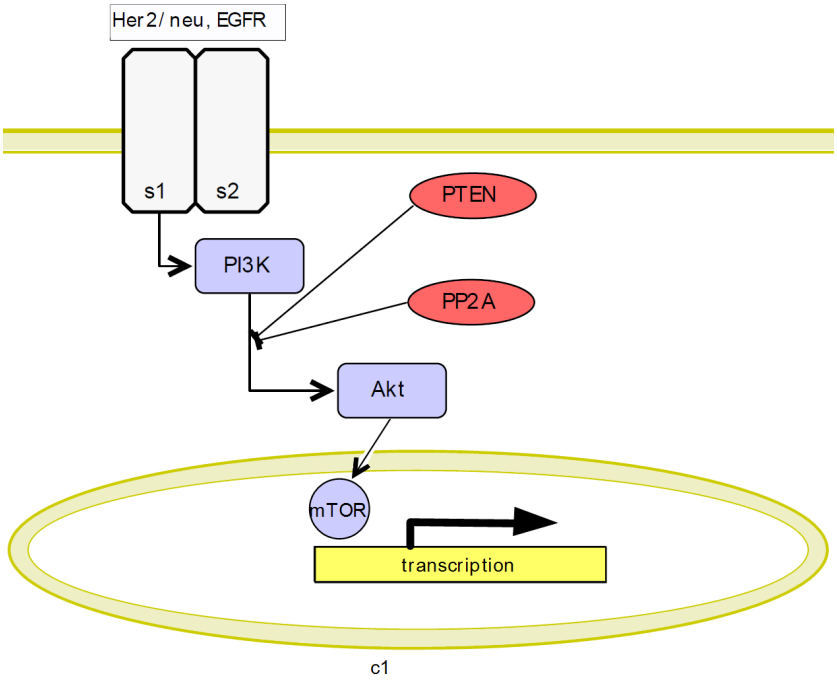

Figure 2. The PI3K-AKT-mTORc1 pathway is extensively involved in endometrial serous carcinoma carcinogenesis. All of the major protein alterations and their respective relationships are highlighted. Abbreviations: HER, human epidermal growth factor receptor; EGFR, epidermal growth factor receptor; PI3K, phosphatidylinositol 3 kinase; PTEN, phosphatase and tensin homolog deleted on chromosome ten; PP2A, protein phosphatase 2, regulatory subunit A, alpha; AKT, v-akt murine thymoma viral oncogene homolog 1; mTORc, mammalian target of rapamycin complex.

Dysregulation of other factors may play a role in ESC carcinogenesis in mice.

EGFR (erbB1) overexpression: EGFR on the *erbB1* gene also belongs to the family of tyrosine kinases. Similar to Her2/neu, it has been implicated in various human cancers, including colorectal, breast, and lung tumors. EGFR upregulation has been shown to have angiogenesis, anti-apoptotic, and metastatic effects. A study performed by Hayes *et al.* demonstrated significant EGFR overexpression in ESC, activating the PI3K-AKT-mTOR pathway [55].

PI3K overexpression: PI3K is an intracellular kinase downstream of the family of tyrosine kinase that signals secondary messengers for cell growth and proliferation. It is encoded by the *PIK3CA* gene, which is frequently mutated in ESC, resulting in an over-activation of the PI3K-AKT-mTOR pathway. Mutations of *PIK3CA* have been described throughout the coding regions and reported in up to 15-21% of type II endometrial carcinomas [12, 57]. Over-activation of the PI3K-AKT-mTOR pathway results in increased cell proliferation and motility, and decreased patient survival [55].

Loss of PTEN: Downstream of PI3K, AKT is a central regulator in the signal cascade to regulate cell growth. Suppressors such as PTEN and PP2A therefore tightly control AKT. PTEN is an intracellular phosphatase that dephosphorylates phosphatidylinositol 3,4,5-trisphosphate to decrease AKT translocation and gene transcription [12]. The loss of PTEN may be caused by gene mutation, promoter methylation or protein degradation [57]. PTEN inactivation is more common in Type I endometrial cancer, however, it can be seen in up to 10% of patients with ESC [53].

Loss of PPP2R1A: Similar to PTEN, PP2A, mapped on the *PPP2R1A* gene, is a serine/threonine phosphatase that inactivates AKT [56] Widely expressed, it is involved in many key tumorigenic pathways and is involved in 17-41% of patients with ESC [22, 58-60]. Kuhn *et al.* concluded that *PPP2R1A* gene mutation is involved in both early and advanced ESC development [22]. Nagendra *et al.* further confirmed the significance of the mutation, stating that it is mutated in 32% of patients [59].

From these various studies, it is apparent that both p53 and the PI3K-AKT-mTORc1 pathway are key players in ESC development. Wild *et al.* proposed a novel relationship between the two separate pathways, linking them by the two-hit mutation hypothesis.

p53 immunoreactivity is associated with poor prognosis, irrespective of ESC tumor stage. On the other hand, the PI3K-AKT-mTO-Rc1 pathway seems to be involved in advancing early form intraepithelial tumors to its more aggressive counterpart. The group suggested that

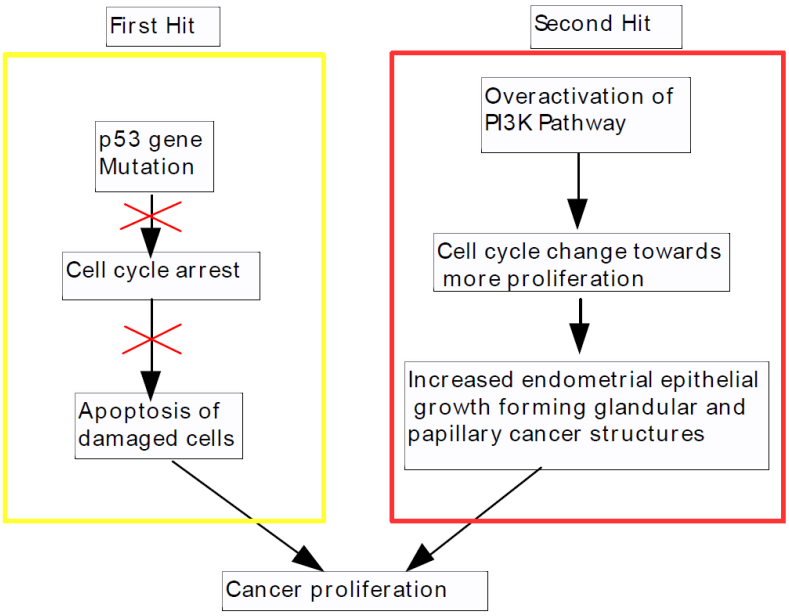

Figure 3. The relationship between p53 gene mutation and PI3K pathway overactivation may be described by the two-hit hypothesis. p53 mutation is the inciting event to ESC carcinogenesis, making endometrial cells more susceptible to further mutations. In contrast, alterations in the PI3K pathway are responsible for promoting ESC carcinogenesis. The combined effect of this two-hit mutation results in active endometrial epithelial cell proliferation.

ESC commonly arises from cells with the first hit mutation, *TP53* gene alterations, making them highly susceptible to a second hit mutation in the PI3K-AKT-mTORc1 pathway [18]. Although from separate pathways, p53 and the molecular players of the PI3K-AKT-mTORc1 pathway combine their effects for added carcinogenic activity. To better understand the interactions between the p53 mutation and the PI3K-AKT-mTOR pathway, we summarized the main relationship among these molecules in **Figure 3**.

Cell cycle genes

Abnormal expression of cell cycle genes are commonly found in cancers because of their functions of regulating cell cycle transitions. Recent studies have suggested that mutations in *CDKN2A* (p16), *CCNE1,* and *FBXW7* are involved in ESC.

CDKN2A (p16) overexpression: CDKN2A, otherwise known as p16, is a tumor suppressor gene. The overexpression of p16 is noted in 92%-100% of ESC cases and is up-regulated in 70% of SEIC cases [34-36, 40, 60]. p16 is known to serve as a surrogate marker for HPV infection [35]. However, since HPV has never been considered as an etiology for ESC development, this finding is suggestive instead of a dysregulation of the p16-INKA/Cyclin D-CDK/pRb-E2F pathway in ESC [64, 65].

CCNE1 overexpression and FBXW7 mutation: CCNE1, mapped on chromosome 19, is a gene that causes transcription of cyclin E1. Cyclin E1 regulates endometrial cell transitioning from the longer G1 phase to the DNA replication S phase. Significant up-regulation of *CCNE1* results in increased levels of cyclin E1, promoting tumorigenesis by accelerating cell division [66]. *CCNE1* overexpression has been described in 16% of endometrial cancers and associated high cyclin E1 levels have been noted in 51-80% of advanced stage of endometrial cancer. Specifically, 25-44% of ESC cases have an overexpression of *CCNE1* [22, 60, 66].

FBXW7 has an antagonistic effect to *CCNE1*; it encodes a ubiquitin-mediated protease that targets cyclin E1 for degradation [60, 66]. A loss-of-function mutation of *FBXW7* also results in an overexpression of cyclin E1 via the lack of cyclin E1 clearance. Studies have shown that 16% of endometrial carcinomas and up to 20% of ESC's display an FBXW7 mutation [22, 60, 67, 68]. In fact, mutations in *CCNE1* and *FBXW7* seem to occur synergistically through separate mechanisms, causing a significant increase in cyclin E1 [22]. There are no studies on the status of *CCNE1* and *FBXW7* in ESC precancers or SEIC. Therefore, although the specific role of these particular genes in the process of endometrial serous carcinogenesis is unknown, the high incidence of their alteration suggests that they are intricately involved in cancer development.

A schematic diagram illustrating the relationships between these various cell cycle proteins

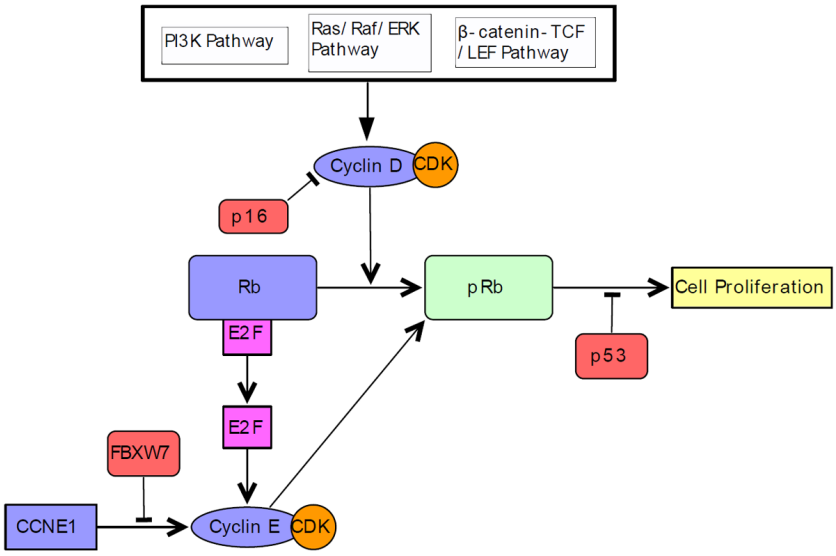

Figure 4. Relationship between cell cycle proteins. Abbreviations: CDK, cyclin-dependent kinase; p16, cyclin-dependent kinase inhibitor 2A; Rb, retinoblastoma protein; E2F, E2 promoter binding factor, FBXW7, F-box/WD repeat-containing protein 7; p53, tumor protein 53.

is illustrated in **Figure 4**. In addition, the above-mentioned molecular players are put into the context of commonly recognized cell regulatory proteins, including cyclin D, retinoblastoma (Rb), and p53 [69, 70].

Cellular adhesion molecules

It is well recognized that the cells of ESC (including its special form SEIC) have a tendency to detach from the main tumor mass within the endometrial cavity and metastasize to extra-uterine locations probably through transtubal pathway, as well as through lymph vascular space invasion [16]. One of the mechanisms of such a unique phenomenon may be related to the changes of adhesion molecules surrounding the serous cancer cells, although it remains in speculation [31, 71]. Among many adhesion molecules, the following have been studied in relation to ESC.

Loss of E-cadherin: E-cadherin is a calcium-dependent, transmembrane protein that allows for cell-to-cell adhesions. The loss of cellular adhesion molecules has been associated with higher rates of cancer progression and invasion [40, 60]. Loss of E-cadherin has been described in many cases of endometrial cancer, with a higher frequency in ESC [31-33, 72]. In fact, loss of E-cadherin was found in up to 62% of ESC [16, 31]. Furthermore, higher expressions of E-cadherin is associated with lower mortality rates and slower cancer progression [60]. While the precise mechanism remains unknown, theories of the loss of E-cadherin include promoter hypermethylation and transcription factor suppressor mutation [60].

Claudin overexpression: Claudins are a family of transmembrane proteins that contribute to tight junction formation. Specifically, claudin-3 and claudin-4 expressions have been associated with cancer progression and invasion [71]. Claudin overexpression is observed in a wide variety of human cancer, as assessed by immunohistochemistry. In ESC, 78% and 56% of cases have been found to be strongly positive for claudin-3 and claudin-4, respectively [71]. The specific mechanisms of cancer induction and involvement are unclear [40].

Transcription factors

Overexpression of Nrf23: Nrf2 is a transcription factor that has a protective mechanism in the body by activating downstream antioxidant and de-toxification enzymes in response to stress [73]. It is associated with tumor aggressiveness and resistance to chemotherapy [16, 74, 75]. Recent studies have shown that Nrf2 is expressed in 40% of EmGD, 75% of EIC and 89% of ESC [16]. Furthermore, a knockdown of Nrf2 significantly decreases the clinical aggressiveness and resistance of ESC cells to chemotherapy.

Overexpression of IMP3: Insulin-like growth factor II mRNA-binding protein 3, or IMP3, is an embryonic protein that is usually absent in adult tissues, but is up regulated in tumor cells. It has been linked to cancer development and invasion and is significantly overexpressed in ESC [40], and therefore it has been described as being a useful diagnostic marker for ESC

Table 1. Molecular Alterations in Endometrial Serous Carcinoma

	AE/RE	EmGD	EIC	ESC
TP53 [5, 26, 47-51]	0	++	+++	++++
Her2/Neu [16, 18, 29, 32, 53, 61-63]	0	+	++	++++
EGFR [53, 55]	0	?	?	+++
PI3K [12, 22, 53, 55, 57, 60]	0	?	*	++
PTEN [53, 60]	0	?	?	+
PPP2R1A [22, 58-60]	0	?	*	++
CCNE1 [22, 60, 66]	0	?	?	++
FBXW7 [22, 60, 67, 68]	0	?	*	+
CDKN2A [16, 36]	0	*	+++	++++
E-Cadherin [16, 31, 53]	0	?	?	++++
Claudin [16, 40, 71]	0	?	?	+++
Nrf2 [16, 73-75]	0	++	+++	++++
IMP3 [16, 37, 40, 52, 76-79]	0	+	++++	++++
BRCA [6, 83, 84]	0	?	?	+
DLG7 [13]	0	?	?	*
MELK [13]	0	?	?	*
IHH [13]	0	?	?	-
RORB [13]	0	?	?	-

AE, atrophic endometrium; RE, resting endometrium. + indicates prevalence between 0-25%, ++ indicates prevalence between 26-50%, +++ indicates prevalence between 56-75%, ++++ indicates prevalence between 76-100%, 0 indicates control, * indicates known presence but unknown prevalence, - indicates down-regulation.

[37]. Specifically, IMP3 expression was found in 14% of EmGD, 89% of EIC and 94% of ESC [16]. This accumulation illustrates the potential for IMP3 to be involved in early ESC development. IMP3 overexpression seems strongly correlated with cancer aggressiveness, demonstrated in ESC and a variety of other tumors [52, 76-79]. Within the endometrium, IMP3 staining varies across the different types of endometrial cancers, making this molecular factor especially sensitive for ESC diagnosis. Specifically, IMP3 staining was prevalent in 0% of mucinous carcinomas, 2.9% of Type I endometrioid carcinomas, 50% of clear cell carcinomas, and 94% in ESC [37]. Although more studies are needed, the overexpression in ESC may contribute to the poor prognosis of ESC.

Other noteworthy molecular factors

BRCA mutations: BRCA genes are tumor suppressor genes that play a role in DNA repair during cellular proliferation [80]. While *BRCA* mutations are widely recognized for their role in development of breast and ovarian cancers, it is also dysregulated in ESC [80, 81]. In fact, 2% of women with ESC were found to have *BRCA* mutations. Furthermore, there appears to be an association between breast, ovarian, and endometrial cancers, and it is thought to be due to the genetic predispositions of *BRCA1* and *BRCA2* mutations [6]. These mutations are thought to result in hereditary breast cancer syndrome, early onset ovarian cancers, and endometrial malignancies [80-83]. Specifically, *BRCA1* has a significant role in ESC development and is present in 2% of patients with ESC [83, 84]. More dramatic evidence of *BRCA1* and *BRCA2* involvement was demonstrated in a group of Ashkenazi Jews, with 35% of ESC patients having a history of breast cancer. In the general population, 9% of ESC patients with a history of breast carcinoma have a *BRCA1* or *BRCA2* mutation [83]. There is a clear relationship between breast, ovarian, and endometrial cancers and their association with *BRCA* mutations [83]. In fact, studies have shown that women with a history of breast cancer are more likely to develop endometrial cancer and tend to develop ESC at a younger age [38, 48, 85]. While studies have shown the mutation involvement in ESC, little is known about its involvement in carcinogenesis or whether there is early involvement [16].

DLG7, MELK, IHH, and RORB dysregulation

Recent studies by Risinger *et al.* showed dysregulation of a variety of genes in ESC. Specifically, the group found that disks large homolog 7 (DLG7) and maternal embryonic leucine zipper kinase (*MELK*) were up-regulated while Indian Hedgehog gene (*IHH*) and Retinoic Acid Related orphan receptor B (*RORB*) were significantly down-regulated in ESC compared to normal endometrium [13]. DLG7 may be involved in cellular change and transformation and the precise function of *MELK* is unknown, though both are stem cell markers. Further studies are required to confirm the regulatory pathways. Controlled by ovarian hormones, IHH is a regulatory protein essential for menstrual

cycle regulation and induces endometrial proliferation. Similarly, RORB is involved in menstrual cycle regulation with the highest expression levels at the secretory phase of endometrial proliferation [13]. It is currently unknown if these hormonally regulated genes involved in ESC may play a partial role in menstrual related hormones contributing to ESC development.

All noteworthy molecular factors mentioned in this review are summarized in **Table 1**. We illustrated the various biological alterations and their prevalence in various stages of ESC carcinogenesis. In the table, there are no mutations listed under atrophic or resting endometrium because we assumed all samples came from control tissues. However, this does not include overall sporadic mutations such as p53 signatures in resting endometrium that are not characterized as dysplastic or neoplastic.

Among the molecular factors studied, few are well known and most are only superficially studied. *TP53,* Her2/neu, Nrf2, and IMP3 are among most critical and well studied. The prevalence of each among every stage of ESC carcinogenesis, including EmGD, is known. Other mentioned molecular factors are only touched upon and require extensive additional studies, including: EGFR, *PI3K, PTEN, PPP2R1A, CCNE1, FBXW7, CDKN2A,* E-cad-herin, Claudin, *BRCA*, DLG7, *MELK, IHH,* and *RORB.* The understanding of these molecular changes is critical in order to develop diagnostic and early preventative strategies.

Potential early diagnostic, preve-ntative, and therapeutic strategies

ESC is associated with a high mortality rate, accounting for roughly 40% of all deaths from carcinomas. Most women present with abnormal vaginal bleeding and up to 46% of patients are then diagnosed with advanced stage disease [6]. Due to the aggressive nature of this cancer, it is critical to explore non-conventional diagnostic and preventative strategies that may reduce the mortality associated with these cancers. Molecular alterations and morphological changes are key aspects to ESC prevention.

TP53 mutation as an inciting event

As previously noted, p53 alterations appear to be one of the earliest molecular changes in ESC, and is apparently the primary inciting event in carcinogenesis. Since there are no well-developed, non-invasive modalities to screen patients for this aggressive malignancy, the high prevalence of such a central molecular event raises the possibility of using serum anti-p53 antibodies for early detection [86]. Serum anti-p53 antibodies have been identified in the subclinical phases of lung and head and neck cancer, and may be worthy of exploration in ESC [87-90]. Assessment of p53 protein level of expression from endometrial brushing samples using the enzyme-linked immunosorbent assay (ELISA) to detect p53 overexpression is another method that may potentially detect ESC at an early or precancerous phase, and our laboratory is currently developing a robust assay for this purpose. Currently, our assay is able to identify as few as 50 cells with p53 protein overexpression in the background of 1000-fold more p53 negative cells. The system is sensitive enough to detect a single endometrial gland either at its precancer or early cancer stage as soon as the cells express p53 protein (both wild-type and mutated) in an aberrant level. It is unclear, however whether elevated p53 levels display the requisite specificity to be deployed in routine practice, or even in the subset of patients at high risk for ESC, including older patients with a family history of ovarian, breast, or uterine cancers. Future studies will hopefully clarify the effectiveness of this method.

Diagnostic immunohistochemical grouping

As discussed above, *TP53* mutation plays a critical role in the development of ESC and its aberrant protein overexpression is found in precancers as well as in full blown ESC. In addition to p53 abnormality, IMP3 is significantly overexpressed early in ESC carcinogenesis. The high level of IMP3 expression and early involvement is unique in ESC [37]. Furthermore, p53 protein overexpression is localized in cancer cell nuclei, while IMP3 is in cytoplasm. We have seen approximately 10% of ESC cases with negative p53 staining, but are strongly stained with IMP3 (data not published). From this perspective, staining with both p53 and IMP3 may have a synergistic and complimentary function to identify lesions of endometrial serous neoplasia. Therefore, we propose to use the combination of immunohistochemical detection of p53 and IMP3 overexpression as an early diag-

nostic tool. Given that p53 alteration is involved in inciting carcinogenesis and IMP3 promotes the aggressiveness of the cancer, the grouping of the two molecular factors to evaluate ESC risk would increase the sensitivity of the early cancer detection. The combination of these biomarkers should be more useful than the existing staining method of using single markers when morphological features of endometrial serous lesions, particularly the precancer EmGD, are not obvious. The overexpression of p53 and IMP3, distinct from resting endometrium, would likely indicate a higher risk of ESC [16]. Early detection of precancers or ESC risk assessment could potentially provide an effective way to prevent this deadly disease for women.

Targeted therapies

Early cancer detection and the identification of various biomarkers serve as the platform for targeted therapy. These drugs leverage the upregulation or activation of specific genes or pathways and have been proven to be efficacious in a variety of cancers, including breast, colon, renal, and blood cancers [53]. In ESC, therapies targeting molecular factors in the PI3K-AKT-mTOR pathway are currently under clinical investigations.

Her2/neu and EGFR stand out as potential targets for directed therapy of ESC. Her2 overexpression has been extensively studied in breast cancer and the success of Her2 inhibitors such as trastuzumab or lapatinib in treatment is widely known [53, 91, 92]. Since approximately 30% of ESC overexpress Her2/neu, presumably these treatments would benefit patients with endometrial cancers as well [40]. However, contrary to this assumption, recent phase II clinical trials of trastuzumab, laptinib, erlotinib and gefitinib showed minimal efficacy, or even detrimental effect, on endometrial cancers in general, which included a small percentage of ESC [53, 62, 91, 93-95]. Though the results are discouraging, the sample sizes of the studies are small and patients are not divided based on cancer subtype or genetic mutation. Furthermore, the recent study of Her2 overexpression heterogeneity on immunohistochemical staining illustrates the variability of protein expression between individuals. Her2 staining also varies depending on the tumor type and the primary site of the tumor. Similar to gastric carcinomas, the staining patterns on ESC were often incomplete, lacking apical membranous staining, or varied in intensity. With the newly described immunohistochemical scoring guidelines, the criticized targeted therapies could be re-evaluated based on the unique biological features of each tumor and could be used to better predict clinical outcomes [63]. These tyrosine kinase inhibitors should not be completely disregarded; further studies should be performed, focusing on patients with ESC including SEIC for those cases with Her2/neu overexpression.

mTOR inhibitors, or rapalogs, are a main focus for ESC therapy and have shown promising apoptotic and anti-tumor results. Phase II clinical trials of everolimus, temsirolimus, and ridaforolimus have shown that up to 69% of patients with endometrial cancers had stable disease for approximately 6 months [96-100]. At the forefront is temsirolimus (currently in phase III trials for renal cell carcinoma), where it probably has a better potential for patients with endometrial cancers including ESC [101]. In addition, studies involving the combination of a rapalog and either a hormonal or chemotherapy agent have shown promising results in endometrial cancers [53, 102-105]. Other factors of interest in the PI3K pathway include AKT and PI3K. Preclinical studies of inhibitors on endometrial cell lines and xenograft mice show encouraging anti-cancer activities [20, 53, 106-108].

Significant improvement has been made in the field through the study of molecular factors and the development of direct therapies. Further phase III clinical trials would evaluate patient survival and the efficacy of the inhibitors on patients with ESC. The success of the therapies targeting the PI3K pathway in phase II trials has led to an optimistic future of ESC treatment. As mentioned earlier, PI3K and its subsequent molecular cascade are upregulated in both EmGD and SEIC. Hopefully, the early diagnosis and application of rapalogs, AKT inhibitors, and PI3K inhibitors may provide an opportunity to prevent pre-cancerous or non-invasive serous cancers from progressing into a full blown ESC. Such clinical trials focusing on the endometrial serous lesions are warranted.

Conclusion

This review summarizes the current knowledge of the primary molecular alterations involved in

endometrial serous carcinogenesis. Molecular genetic alterations involving the p53, PI3K pathways, and cyclin E-FBXW7 appear to represent the major mechanisms in the development of ESC. Of these, p53 has been most extensively studied, and is the central molecular alteration. EmGD is the precancer of ESC, and its role in endometrial serous carcinogenesis is supported by abundant lines of evidence, recently clearly illustrated in a mouse model. Many other molecular alterations that probably play a role, however, have only been superficially studied. The utility of p53 assays, either in samples from serum or endometrial brushes, as a screening tool in high-risk populations, is currently under development. Such novel methods may help to identify precursor or early lesions of ESC. Furthermore, biomarkers directly involved in ESC development are potentially used for targeted therapy.

Disclosure of conflict of interest

None.

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