Review Article
Insights into Endometrial Serous Carcinogenesis and Progression

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Abstract: Endometrial serous carcinomas (ESC) constitute only approximately 10% of endometrial cancers, but have a substantially higher case-fatality rate than their more common endometrioid counterparts. The precise composite of factors driving endometrial serous carcinogenesis and progression remain largely unknown, but we attempt to review the current state of knowledge in this report. ESC probably do not evolve through a single pathway, and their underlying molecular events probably occur early in their evolution. *TP53* gene mutations occur in 22.7 to 96% of cases, and p53 protein overexpression is seen in approximately 76%. By gene expression profiling, p16 is upregulated in ESC significantly above both normal endometrial cells and endometrioid carcinomas, and 92-100% of cases display diffuse expression of the p16 protein by immunohistochemistry (IHC). Together, these findings suggest dysregulation of both the p16^{INKA}/Cyclin D-CDK/pRb-E2F and the ARF-MDM2-p53 cell cycle pathways in ESC. By IHC, HER2/neu is overexpressed (2+ or 3+) in approximately 32.1% of ESC, and approximately 54.5% of cases scored as 2+ or 3+ by IHC display *c-erbB2* gene amplification as assessed by fluorescent in situ hybridization. Genetic instability, typically manifested as loss of heterozygosity in multiple chromosomes, is a common feature of ESC, and one study found loss of heterozygosity at 1p32-33 in 63% of cases. A subset of ESC display protein expression patterns that are characteristic of high grade endometrial carcinomas, including loss of the metastasis suppressor CD82 (KAI-1) and epithelial-to-mesenchymal transformation, the latter manifested as E-cadherin downregulation, P-cadherin upregulation, and expression of epithelial-to-mesenchymal transformation-related molecules such as zinc-finger E-box-binding homeobox 1 (ZEB1) and focal adhesion kinase. Preliminary data suggests differential patterns of expression in ESC of some isoforms of claudins, proteases, the tumor invasiveness and progression-associated oncofetal protein insulin-like growth factor II mRNA-binding protein 3 (IMP3), as well as a variety of other molecules. At the morphologic level, evidence that indicates that endometrial glandular dysplasia (EmGD) is the most likely morphologically recognizable precursor lesion to ESC is presented. We advocate use of the term endometrial intraepithelial carcinoma (EIC, or its other appellations) only as a morphologic descriptor and never as a diagnostic/pathologic statement of biologic potential. Given its potential for extrauterine extension, we consider the lesions described as EIC, when present in isolation, as examples of localized ESC, and patients should be managed as such. Morphologically normal, p53 immunoreactive endometrial cells (the so-called "p53 signatures"), show a statistically significant association with ESC, display p53 mutations in a significant subset, and form the start of a progression model, outlined herein, from p53 signatures to EmGD to localized ESC to the more conventionally invasive neoplasm. The identification of a morphologically-recognizable precursor holds the promise of early detection of ESC, with the attendant reduction in its overall associated mortality rate. Deciphering the molecular basis for endometrial serous carcinogenesis should uncover potential targets for diagnosis, therapy, and/or disease surveillance.

Key Words: Endometrial serous carcinoma, endometrial glandular dysplasia, endometrial intraepithelial carcinoma, p53, cadherins, claudins, CDKs, MDM2 and HER2/neu (erb-B2)

Introduction

Sporadic reports of variably papillary endometrial carcinomas with psammoma bodies have appeared in the literature since at least 1963 [1-5]. However, "serous" differentiation comparable to their ovarian counterparts, as well as the comparatively

aggressive behavior of these neoplasms, were concepts that were first emphasized in a textbook authored by Hendrickson and Kempson in 1980 [6]. In 1981, Lauchlan reported a series of 8 endometrial carcinomas, 5 of which were morphologically pure, which the author designated "tubal (serous) carcinoma" [7]. The author noted that the prognosis for this carcinoma, as compared with their endometrioid counterparts, was "strikingly worse", and that "many of the patients died of widespread metastases, with no or only minimal myometrial invasion" [7]. A series of studies published shortly thereafter in 1982 firmly established endometrial serous carcinoma (also known as uterine papillary serous carcinoma) as a distinct clinicopathologic entity [8-11].

Endometrial serous carcinomas (ESC), the prototypical type II carcinoma under the dualistic model of endometrial carcinogenesis [12, 13], constitute 8.75 [14] to 10.16% [8] of endometrial carcinomas, and as such, represent the most frequently encountered non-endometrioid carcinoma of the corpus uteri. ESC are morphologically characterized by papillae, glands or solid sheets of cells with grade 3 cytologic pleomorphism, frequent lymphovascular and/or myometrial invasion, and frequent, albeit not invariably, psammomatous calcifications [6-11, 15]. As compared with their endometrioid counterparts, ESC occur in a significantly older age group [16-18] and more frequently in a background of atrophic or resting endometrium [18], and are over-represented amongst endometrial carcinomas arising in African Americans [19] and are notably chemoresistant. Although the question of whether ESC are prognostically distinct from stage matched high grade endometrial endometrioid carcinoma remains unresolved [17, 20-22], there is an established consensus that the prognosis of ESC is highly dependent on surgical stage after initial evaluation [16, 18, 23, 24]. Unfortunately, at least half of patients with ESC have extrauterine disease at presentation [16], and 33-50% of patients with non-myoinvasive tumors show extrauterine disease after comprehensive surgical staging

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[16, 25, 26]. With contemporary approaches to the management of this tumor, including complete surgical staging and aggressive adjuvant treatments, the prognosis for patients with truly corpus-confined, non-myoinvasive tumors (i.e. International Federation of Gynecology and Obstetrics [FIGO] stage 1a) has improved significantly, with overall survival rates ranging from 83-100% in some studies [25-29]. These factors highlight the critically important role of early tumor detection, whether of the neoplasm in its localized form or of its precancerous forms, in improving the prognostic outlook for afflicted patients. Given that the vast majority of endometrial carcinomas are of the endometrioid histotype [30], most of the investigative endeavors have been focused on this histotype, such that there is now a relatively robust model of etiopathogenesis and progression, which combines risk factors such as unopposed estrogen exposure, morphologically recognizable precancerous changes such as atypical endometrial hyperplasia [31] and endometrial intraepithelial neoplasia [32], and genetic alterations such as microsatellite instability and K-ras, beta-catenin and PTEN mutations [33]. For ESC, however, there are simply no comparable levels of data, and the precise composite of factors driving endometrial serous carcinogenesis remain largely unknown. Nonetheless, in recent years, there have been significant investigative efforts aimed at uncovering a morphologically recognizable precursor lesion for ESC as well as some of the subcellular derangements that underlie its development and progression. These findings are summarized in this commentary, presented in a retrogressive fashion from ESC, its putative precursors and subcellular events that based on current data, contribute to its genesis and progression.

Endometrial Intraepithelial Carcinoma (EIC)

In the decade following the original description of ESC (1980-1990), the concept that a distinction can be made between non-myoinvasive serous carcinomas that are stroma invasive and those that not stroma invasive was simply not well recognized. In 1991, Lee and Belinson reported that out of the 28 recurrences in a series of 227 consecutive operable clinical stage I endometrial carcinomas, 7 were non-invasive. Five of these 7 cases displayed serous

differentiation and notably did not necessarily show well-formed papillae [34]. In 1992, Sherman *et al* [35] reported their experience with 13 pure ESC and 19 ESC admixed with other histotypes. The authors noted that in 89% of these cases, the surface endometrium adjacent to the associated malignancies was lined by malignant cells that ranged from 1 to 5 cells thick and which often formed micropapillae. The process often multifocally involved the adjacent atrophic glands and was often several millimeters away from the invasive tumor. The authors designated this lesion "intraepithelial carcinoma", and interpreted them as being morphologic manifestations of multifocal carcinogenesis [35]. The next report on the subject was by Spiegel [36]. The author identified in 15.4% of hysterectomy specimens (518 endometrial carcinomas and 39 carcinosarcomas) "microscopic foci of malignant epithelium that failed to alter the architecture of an otherwise thin atrophic or weakly proliferative endometrium or endometrial polyp" [36]. These changes were strongly associated with ESC and were designated "endometrial carcinoma in situ", with a distinction being made between "thin carcinoma" (papillations or epithelial bridging, little or no stromal reaction, no endometrial architectural distortion, non-myoinvasiveness) and the other cases in which papillations, stromal reaction or epithelial bridging were absent [36]. The author also proposed that the lesions were precancerous in nature, especially to ESC [36]. These findings were largely confirmed by Ambros *et al* [37], who reported an even stronger association between "endometrial intraepithelial carcinoma" (EIC, so-designated for the first time in this report) and carcinomas with serous differentiation. The authors reported that EIC was identified in 89%, 6%, and 56% of uteri with ESC, endometrioid carcinoma and malignant mixed mesodermal tumors with a serous component respectively [37]. Based on its frequent presence adjacent to the main invasive mass and the frequent maintenance of glandular architecture in involved glands, the authors hypothesized that EIC is a "noninvasive form of carcinoma" that appears "to spread centrifugally outward from the main mass along the endometrial surface" [37]. The authors also asserted that EIC is a likely precursor to ESC based on its frequently abrupt transition with normal endometrium, as is occasionally seen in cervical adenocarcinoma in situ, its frequent presence

away from the main tumoral mass, its presence in association with small volume, non-myoinvasive ESC, and their own experience in seeing EIC in its apparent pure form unassociated with an invasive malignancy [37]. The term "uterine surface carcinoma" was proposed Zheng *et al* in 1998 as a more accurate descriptor for EIC, given its potential for extrauterine spread, as described below [38]. The concept of "minimal uterine serous carcinoma" (MUSC) was formalized in a report by Wheeler *et al* [29]. These authors attempted to make a distinction between EIC (as defined above and without myometrial invasion or lymphovascular space invasion) and stroma-invasive ESC (superficial ESC), which the authors defined as lesions with "confluent glands or infiltrative growth associated with desmoplasia but without myometrial or lymphovascular space invasion". Although their analysis was limited by small numbers, the presence or absence of extrauterine disease significantly trumped any prognostic differences between these 2 groups, if any [29]. Furthermore, since their morphologic distinguishability was noted to be problematic, the authors proposed the umbrella term MUSC to encompass EIC and/or superficial ESC that measure less than 1cm. This term was also used by Hui *et al*, who eschewed the term EIC and referred to their apparently non-invasive cases as "intraepithelial serous carcinoma" [28]. The latter study is also remarkable for the frequency – 88% - with which serous carcinomas arose in or were associated with an endometrial polyp [28]. Similar observations, albeit at lesser frequencies, had previously been reported by others [29, 35, 39, 40].

As noted previously, non-myoinvasive ESC are well-known to potentially display extrauterine disease in 17-67% of cases [25-29]. However, documented examples of patients who were diagnosed with pure, corpus-confined EIC (referred to henceforth as serous EIC) after surgical staging, and who subsequently developed vaginal or peritoneal recurrence of disease are extremely rare. The proffered, anecdotal-type evidence of serous EIC displaying extrauterine extension typically described patients with serous EIC (without invasion in the opinion of the authors), who also had synchronous peritoneal deposits of serous carcinoma at initial staging. In one study of 40 cases of MUSC, 2 cases showed

"intraepithelial serous carcinoma" as the only endometrial serous neoplasm but displayed extrauterine disease [28]. Soslow *et al* described 3 cases of serous EIC associated with peritoneal carcinomatosis [41], and Wheeler *et al* described 2 cases associated with disease in the ovaries [29]. If one takes the position that serous EIC is indeed a non-invasive process, these findings may be explained by the possibility that 1) the peritoneal and endometrial lesions were unrelated, or 2) the endometrial lesions are metastatic deposits from peritoneal serous carcinomas. The first possibility is refuted by the finding of an identical p53 mutation in a case of serous EIC and its associated extrauterine deposit, which is strongly suggestive of a clonal relationship [42]. Differential patterns of WT-1 expression between ESC and peritoneal serous carcinomas argue against the second possibility, although this question has not been specifically addressed in the context of *pure* EIC to our knowledge [43, 44].

It is our opinion that the lesion commonly referred to as serous EIC (or its other appellations) is simply a morphologic manifestation of localized ESC and/or one of ESC's growth patterns whether or not it is localized [45]. We speculate that when serous EIC is identified adjacent to the clearly invasive tumor, it probably represents an outward or centripedal growth of the latter, even if a morphologic connection between them cannot be demonstrated. When even a single endometrial gland (or surface epithelium) is lined by frankly malignant, serous-type cells (which will frequently also overexpress the p53 protein and have a high proliferative index), this is an example of ESC, irrespective of whether the gland is distorted or smooth and whether or not there are papillae, bridging or an associated desmoplastic reaction. While we readily acknowledge that some ESC may have areas that *appear* non-invasive, that ESC may grow in a non-invasive pattern, and that ESC may display this growth pattern at a very early phase (or indeed at *any* phase) in their evolution, we recognize neither the validity nor necessity of specifically asserting, for routine diagnostic purposes, that such areas are intraepithelial and non-invasive, given that endometrial glands typically constitute a complex labyrinth, unlike squamous epithelia, for example. Rather, such cases should simply be designated as ESC, with a comment about

their size and location, as others have also advocated [46]. Every line of evidence that supports the concept of serous EIC as a precursor lesion would be *expected* to be true if these lesions are simply considered as foci of ESC. However, the potential for extrauterine extension, as has been reported in some pure serous EIC, effectively negates the possibility that they are all intraepithelial. As such, although the term "serous EIC" is useful as a morphologic descriptor, it should be discontinued for use as a pathologic statement on a given lesion's biologic potential, which is implied with use of the word "intraepithelial" [45]. Expressed differently, ESC may have areas of *apparently* non-invasive growth. These areas may be adjacent to a conventionally invasive main tumoral mass or may be in isolation. Irrespective of the growth pattern, however, these areas have the same malignant potential of stage-matched (i.e. FIGO stage 1a, conventionally invasive) ESC. Patients who are diagnosed with "serous EIC" in an endometrial biopsy or curettage should receive the same level of initial management afforded to those diagnosed with ESC in the same setting [47, 48].

Endometrial Glandular Dysplasia (EmGD)

Endometrial glandular dysplasia (EmGD), which is a distinctive atypical change of the endometrium, has been proffered as the earliest morphologically recognizable precancer to ESC [49-52]. EmGD was originally described in 2004, the product of a hypothesis by Zheng *et al* that there is a morphologically recognizable lesion that bridges the gap between benign endometrium and the so-called serous EIC, and that this lesion is a more probable candidate precursor lesion to ESC [49]. Retrospectively, the endometria adjacent to the invasive carcinoma in 108 hysterectomy specimens were carefully re-evaluated. These 108 cases included 32 ESC, 16 serous EIC as previously defined up to that point, and 60 endometrioid carcinomas (EEC). Distinctive lesions that were morphologically and immunophenotypically separable from serous EIC, and which were designated EmGD, were identified in 53% of the ESC uteri but in only 1.7% of the EEC uteri (p=0.001). The serous EIC areas were definitionally lined by frankly malignant cells whose levels of atypia were comparable to those of traditional ESC, which were frequently adjacent to them. In contrast, the cells that constituted the

epithelial lining of EmGD foci displayed atypia but not anaplasia. Foci of EmGD, which were grossly inapparent in all cases and microscopically multifocal in 86% of cases, were comprised of glands and surface epithelium whose cells displayed nucleomegaly (2-3 times the nuclear size of adjacent resting endometrium cells as compared with 4-5 in serous EIC), nuclear hyperchromasia or vesicularity, appreciable but non-prominent nucleoli, and in a minority of cases (24%), loss of cellular polarity. Occasional mitotic figures were present but there were no atypical forms. The typical EmGD focus was less than 1mm in maximal dimension, and was comprised of simple glands whose epithelium displayed only minimal stratification (1-2 layers thick) and rare papillae formation [49]. Forty-seven percent of evaluated ESC hysterectomies displayed concurrent EmGD and serous EIC foci. Transitional areas between serous EIC and ESC or between EmGD and serous EIC were readily apparent in 25% of the 32 cases. However, there were no transitional areas noted between EmGD and ESC [49]. The proliferative index and p53 immunostaining index of EmGD was noted to be clearly above those of benign endometrium but clearly below those of serous EIC. In a subsequent molecular study, it was demonstrated that the frequency of loss of heterozygosity (LOH) at the *TP53* locus in microdissected EmGD was significantly higher than in benign endometrium but significantly lower than in serous EIC and ESC, which were comparable [50]. The differences in the LOH frequencies using other microsatellite polymorphic DNA markers between the aforementioned areas were not as clear [50]. Notably, 2 of 18 cases showed LOH (at 17q21, D17S1323, and at 5q, D5S346) in the EmGD lesions but not in the corresponding serous EIC or ESC. One-third of the informative cases, however, showed the reverse (LOH for at least 1 marker in either ESC or serous EIC and lack thereof in the corresponding EmGD). Mutations at *TP53* have been identified in 0%, 43%, 72%, and 96% of benign endometrium, EmGD, serous EIC, and ESC, respectively, and at least half the uteri harboring the latter 3 lesions display one or more identical mutations at *TP53* among the 3 lesions [51]. We also evaluated the possibility that EmGD can be identified in endometrial biopsies that preceded ESC diagnoses, which would bolster their status as a possible precancer and possibly provide a

timeframe for their evolution to invasive disease [52]. Out of 250 patients whose endometrial carcinomas had a ≥50% serous component, 27 had preceding pathologic material that were obtained 3 months or later before the hysterectomies and which were also available for evaluation. A reevaluation of those 27 biopsies showed EmGD in 9 cases [52]. The average duration between the biopsy in which the EmGD was identified and the hysterectomy in which the serous malignancy was identified was 33 months (range 16-98 months). In a control group of 258 hysterectomies that were performed for benign or non-neoplastic indications, there were 71 preoperative samples, and only 1 case of EmGD was identified based on morphologic criteria [52]. The aforementioned studies support the concepts that 1) EmGD is distinct from its background endometrium and is morphologically and immunophenotypically recognizable as such; 2) EmGD is a neoplastic lesion rather than a reactive or metaplastic change; 3) EmGD is specifically associated with ESC and endometrial malignancies with a serous carcinomatous component; 4) EmGD is morphologically and immunophenotypically distinct from EIC/ESC; 5) EIC/ESC probably evolve from EmGD based on identical mutations at the *TP53* locus between them in cases in which both lesions are present; and finally 6) EmGD may represent a marker of increased risk for ESC, based on their identification in endometrial biopsies that presumably predated the development of the ESC, although questions about the possible reversibility of EmGD remain. In these respects, EmGD is, in our opinion [44, 53], the earliest morphologically recognizable precursor lesion for ESC, as it fulfils most of the National Cancer Institute's criteria for a precancer [54]. It is also noteworthy that, unlike serous EIC, we have not encountered a case of peritoneal serous carcinomatosis in which the only endometrial lesion was an EmGD.

The p53 Family

The p53 gene (*TP53*), located on chromosome 17p13.1, is the most commonly mutated gene in human cancers [55]. Activated p53 normally functions as an "emergency monitor" of the cell-cycle, such that cells that undergo DNA damage due to ionizing radiation, carcinogens, mutagens and other unknown factors undergo cell-cycle arrest (to permit repair) or apoptosis

[55]. Mutations of *TP53* hampers or precludes this function via a myriad of pathways, thereby allowing the continued growth of the mutated cells, which may set the stage for additional mutations, their accumulation, and the possibility of the clonal expansion of a neoplastic nidus [55].

At present, p53 mutations have been the most frequently identified genetic alteration in serous neoplasia of the endometrium. In ESC, mutations at exons 5-8 or 4-10, the mutational hotspots for this gene [56], have been identified in 22.7-96% of cases [51, 57-60]. The width of this range may be related to methodological and interpretive differences between studies, and more recent analyses have uniformly found mutations in more than 90% of cases [51, 60]. This is one of the highest rates of p53 mutations among all human malignancies, and it highlights the central role that p53 alterations play in endometrial serous carcinogenesis. The most common type of mutation is missense [51, 57], and in a study by Jia *et al* [51], mutations at codon 248 from CGG to TGG (Arg→Trp) or CAG (Arg→Gln) were the most frequently identified. These mutations result in a transcriptional inactivation of p53, as demonstrated with yeast p53 functional assays [61], or as deduced from the absence of expression in these cases of the Waf-1 product (p21), which is a surrogate indicator of p53 functionality [59].

Overexpression of the p53 protein has been identified by immunohistochemistry in approximately 76% of ESC (range 47.8-100) [28, 57-60, 62-72]. Immunoreactivity is typically, although not invariably, diffuse and is retained at extrauterine sites [51, 64]. Although there are some reports to the contrary [58, 59], recent analyses have found a significant concordance between p53 gene mutation and their protein overexpression [51, 57, 60]. As surmised from the data reported by Lax *et al* [60], approximately 84% of ESC cases with p53 mutations showed significant protein overexpression [60]. These findings are largely similar to those recently reported by Jia *et al* [51]. p53 mutation without protein overexpression may be related to an absent or unstable protein product of the mutant gene [51, 57]. In one such case from the aforementioned Jia *et al* [51] study, for example, the p53 mutation was nonsense mutation at codon 176 (TGC to TGA), which is predicted to result in a truncated p53 protein. The reverse corollary is also true that although p53 protein overexpression is generally due to the p53 gene mutation, there are probably other modes of functional p53 inactivation. In the study of Kovalev *et al* [59], for example, 47% of 15 ESC cases did not display p53 mutations, and 53% of these mutation-negative cases showed p53 protein overexpression but not Waf-1 expression. Since, as previously noted, Waf-1 expression may be considered a surrogate marker of p53 functionality, the authors postulated the presence of mutation-independent modes of p53 inactivation [59]. MDM2 expression, which is closely linked to p53 overexpression in endometrioid carcinomas, is so linked in only a small subset of ESC [73, 74]. These rare cases may exemplify other modes of p53 functional inactivation and protein overexpression in the absence of direct mutations. In support of this possibility is the significantly lower correlation between p53 mutation and protein overexpression in endometrioid adenocarcinomas [75]. The frequency of p53 alterations including protein overexpression and gene mutations in ESC is summarized in **Table 1**.

In addition to their diagnostic [28, 38, 57-60, 62-72] and prognostic uses [76, 77], p53 alterations provide a valuable framework to study the genesis of ESC, since p53 alterations appear to occur at the earliest phases of endometrial serous carcinogenesis and is probably involved in its evolution. In studies of putative precursor lesions of ESC, foci classified as serous EIC (or its other appellations) have been found to show marked immunoreactivity for p53 in 79-100% of cases [41, 49, 57, 62, 69]. For cases in which serous EIC and ESC were present in the same specimen, there has generally been an approximate 100% congruence in their frequency, and to a large extent, pattern of staining in these lesions [57, 62, 69]. Indeed, we are unaware of any examples of serous EIC showing diffuse p53 overexpression and whose synchronous ESC displayed complete immunonegativity, or vice-versa. This highlights the close kinship, at least at the cellular level, between lesions classified as ESC and those classified as serous EIC. Similar to ESC, cases classified as serous EIC have displayed a high frequency of mutations of the p53 gene. Tashiro *et al* identified p53 mutations in 78% of their serous EIC cases,

Table 1 Frequency of p53 alterations in endometrial serous carcinoma

References	p53 overexpression by IHC*	TP53 mutation
Moll et al [64]	85%	NP
Kovalev et al [59]	78%	53%
Bacher-Todesca et al [65]	47.8%	NP
Kounelis et al [66]	76.2%	NP
Busmanis et al [68]	76%	NP
Demopoulos et al [67]	75%	NP
Prat et al [63]	60%	NP
King et al [58]	73%	22.7%
Hui et al [28]	68%	NP
Zheng et al [70]	71.4%	NP
Lax et al [69]	86%	NP
Tashiro et al# [57]	76%	90%
Lax et al# [60]	77%	93%
Erkanli et al [71]	75%	NP
Halperin et al [72]	81.8%	NP
Jia et al [51]	100%	96%
Sherman et al [62]	86%	NP
Mean	76%	51.7%
Range	47.8-100%	22.7-96%

*High or maximal staining for each study; #overlap in cases; NP, not performed

including 3 cases that were unaccompanied by conventionally invasive ESC [57]. Parallel values in another study for serous EIC and ESC were 72.2% and 96% respectively, a difference that approached but did not attain statistical significance [51].

p53 mutational and protein overexpression analysis of EmGD lesions support the notion that these lesions are related to ESC and are probably precancerous. When EmGD is compared to serous EIC or ESC, there are progressive increases in genetic alterations. In our study of 14 uteri harboring EmGD as well as ESC or EIC/ESC, 11 cases displayed multiple p53 mutations. 6 of these 11 cases displayed at least 1 identical p53 mutation in the EmGD lesion as well as the synchronous ESC or serous EIC. In the remaining 5 cases, the mutations were restricted to the EIC or ESC and were not identified in the synchronous EmGD [51]. The high frequency of an identical p53 mutation in EmGD and EIC/ESC in such a small dataset indicates that this finding is highly unlikely to be fortuitous and suggests that EmGD is the likely precancer to at least a subset of ESC. The 5 cases in which the p53 mutation was absent in the EmGD and present in the EIC/ESC maybe related to the divergent foci of clonal expansion. For example, a single uterus may have multiple EmGD lesions [49, 50], not all of which will display p53 mutations [51]. It may be speculated that those EmGD lesions with this mutation will have a selective advantage and will emerge as the ESC. Analysis of an EmGD lesion adjacent to such a p53-mutated ESC may therefore lack this mutation. It is also noteworthy that only 31% of EmGD display marked intensity of p53 overexpression, as compared with most of their EIC/ESC counterparts [49].

Genetic instability, whether manifested as LOH at multiple chromosomes or aneuploidy, is a common feature of ESC [60, 63, 78-81]. As previously noted, only a small subset (11%) of EmGD lesions show LOH patterns that are absent in the synchronous EIC/ESC, as compared with the 33% of cases in which LOH is present in EIC/ESC but is absent in the synchronous EmGD. These findings provide strong evidence of an increased level of genetic instability in EIC/ESC as compared with EmGD. Notably, LOH at around the p53 gene is significantly less in EmGD as compared to EIC/ESC, perhaps for the aforementioned reasons [50]. Parenthetically, a high frequency of LOH at 1p32-33, identified by some authors in 63% of ESC, is probably indicative of a tumor suppressor gene at that locus and requires further study [82].

In our various studies of the immunoreactive patterns of EmGD, we noted that some morphologically normal endometrial cells were strongly p53-immunoreactive, irrespective of whether the PAB1801 (Oncogene Science, Manhassett, NY) or the D07 (Dako,
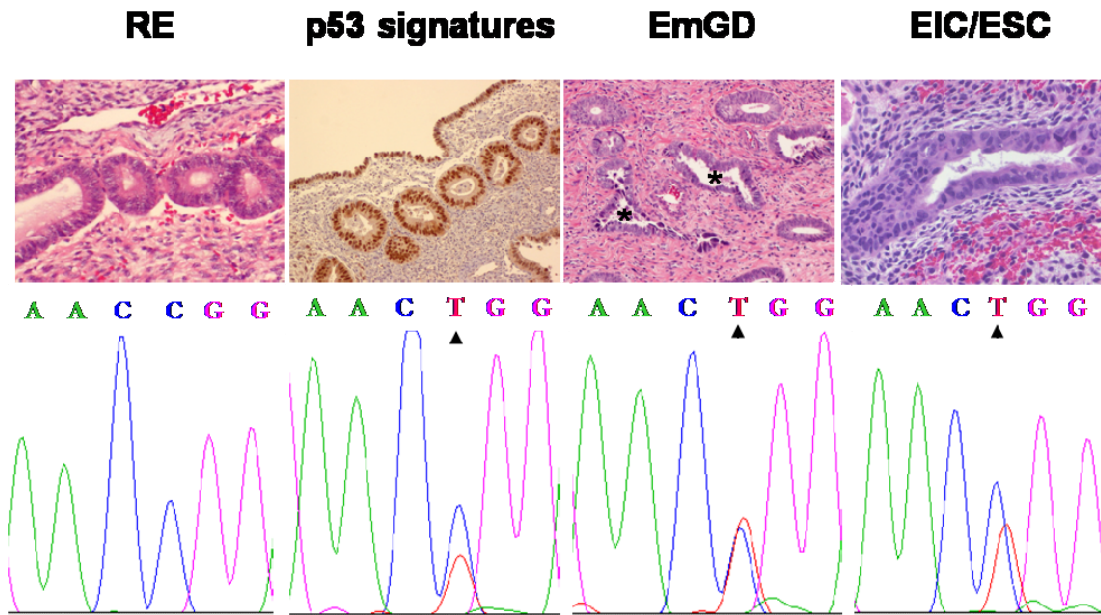
Figure 1 *TP53* gene sequencing results from laser capture microdissected (LCM) samples. Top row showed representative images of H&E staining of resting endometrium (RE), endometrial glandular dysplasia (EmGD), and ESC with an apparently non-invasive growth pattern (EIC/ESC) and p53 immunohistochemical staining of morphologically normal endometrial cells (p53 signatures). (original magnifications: 200 x). The degree of nuclear atypia in EmGD (the glands with *) clearly exceeds that of the RE but falls short of EIC/ESC. Samples of p53 signatures, EmGD, and EIC/ESC from DNA sequence analyses showed identical p53 gene mutations of exon 7 at codon 248 from CGG to TGG (Arg to Trp), while no mutation was found in the corresponding RE sample. Identical mutation was also observed in area of invasive ESC (not shown) in the same uterus. These samples were obtained from one of the cases previously studied in Zheng laboratory.

Carpinteria, CA) monoclonal antibody is used. The intensity of staining of these foci was significantly above the weak and patchy staining that may occasionally be seen in reactive or metaplastic endometrial cells [83]. Similar findings in the fallopian tube have been reported by the Crum group in the setting of pelvic serous carcinogenesis [84-87]. The authors named these foci "p53 signatures" [84-87], which is a term we maintained in our analysis of their endometrial correlates [88]. p53 immunostains were performed on the non-cancerous endometrium in 182 hysterectomy specimens, including 62 harboring ESC/EIC, 60 harboring EEC, and 60 with no neoplastic epithelial process. At least 1 p53 signature was identified in 1.7%, 3.3%, 38.7% of benign, EEC, and EIC/ESC harboring uteri, respectively, indicative of a remarkably strong association with the serous histotype in this analysis. p53 signatures were typically less than 1mm and multifocal. The p53 signatures were laser capture microdissected and subjected to p53 mutational analysis at exons 5-8. First, the most common mutations

seen in ESC (missense mutations, most frequently codon 248 from CGG to TGG (Arg→Trp) or CAG (Arg→Gln) in exon 7) [51], were also seen in these p53 signatures, and 42% of p53 signatures displayed at least 1 mutation. Second, p53 mutations were identified only in the p53 signatures associated with EIC/ESC, and not in those associated with EEC. Third, 4 (50%) of 8 uteri with p53 signatures and synchronous EmGD, and EIC/ESC showed at least 1 identical p53 mutation in all 3 lesions. 2 others showed at least one concordant mutation between the p53 signatures and either the EmGD or EIC/ESC. The final 2 showed discordant mutations [88]. Rare cases displayed discordant p53 mutations in different p53 signatures from the same uterus [88]. Representative pictures of p53 signature and corresponding endometrial serous lesions from precancer EmGD to EIC/ESC are presented in **Figure 1**.

Based on the findings outlined above, the following conclusions can be drawn. 1) p53-

immunoreactive, morphologically normal cells (p53 signatures) are specifically associated with the serous histotype, at least relative to their endometrioid counterparts. 2) These lesions are often multifocal but only a subset (42%) of them display p53 mutations. 3) EmGD are frequently multifocal and display p53 mutations in a similar proportion of cases (43%). 4) EIC/ESC display a significantly higher frequency of diffuse p53 protein overexpression, and presumably functional p53 inactivation, than EmGD. 5) EIC/ESC display significantly higher levels of genetic instability and frequency of p53 mutation than EmGD. We therefore propose a progression model from p53 signatures to EmGD to localized ESC (serous EIC) to ESC, with p53 mutations at each stage conferring a selective advantage that promotes progression to the next stage. Although this is p53-centric model, it acknowledges the probable presence of an unclear myriad of other pathways and factors that contribute to each step, including the cause(s) of the original p53 signature-associated mutations. Critical telomere shortening, which may contribute to genomic instability [89], has been identified in the normal endometrium adjacent to Type II but not Type I endometrial cancers, and the presence of such critical shortening in animal models has been associated with lesions with the growth pattern of serous EIC [90]. Finally, it is possible that a small subset of ESC evolve either through pathways more characteristic of EEC or devolve from EEC itself. Such phenomena may explain, at least in part, ESC with *k-ras* or *PTEN* mutations [60], ESC arising in younger patients or in a background of hyperplastic endometrium [35, 91], microsatellite unstable ESC [92], p53 immunonegative and/or mutation-negative ESC, and mixed EEC/ESC.

p63 is a homologue of p53 that is thought to be necessary for epithelial differentiation of the lower gynecologic tract [93]. In one study of p63 expression in the endometrium, the authors found intense staining in metaplastic cells, a basal cell-like staining distribution that was most evident in fetal endometrium, and patchy staining in other endometria [94]. ESC cells were found to have scattered positive cells [94]. Idrees *et al* [95], however, reported that 57% and 75% of their ESC and serous EIC cases were respectively positive for p63, and a generally strong correlation between p53 and p63 expression was found. No other studies have been reported on the subject to our knowledge. Hence, the role of p63, as well as the third related molecule, p73, in serous carcinogenesis is presently unknown. Other cell cycle-related proteins are discussed separately below.

HER2/neu (c-erbB2)

c-erbB2, located on chromosome 17q11.2-q12, is a proto-oncogene that encodes the human epidermal growth factor receptor 2 (HER2/neu, c-erbB2), a 185-kDa, tri-domain receptor that is comprised of an extracellular ligand-binding domain, a transmembrane region and a cytoplasmic domain with tyrosine kinase activity [96]. Although currently considered an "orphan" receptor, HER2/neu participates in the complex signaling pathways that regulate cellular differentiation and growth because it is the preferred heterodimerization partner for the other receptors in the erbB family (HER1, HER3, HER4) [97]. Amplification of the *c-erbB2* gene results in a massive numerical increase in the cellular HER2/neu protein molecules in a given cell, possibly leading to constitutional activation of the tyrosine kinase and eventuating in cellular proliferation [98]. HER2/neu protein overexpression and/or gene amplification have been described in a number of human tumors [99]. In breast cancers, where HER2/neu has been most

Table 2 Her2/neu status in endometrial serous carcinoma, as determined by immunohistochemistry

References	Her2/neu status			
	Number	0-1+	2+	3+
Slomovitz *et al* [107]	68	56	10	2
Santin *et al* [106]	26	10	7	9
Villella *et al* [105]	17	12	0	5
Odicino *et al* [108]	12	10	0	2
Singh *et al* [109]	45	26	13	6
Total	168	114 (67.9%)	30 (17.8%)	24 (14.3%)

extensively studied, HER2/neu overexpression/amplification has been found to be both an adverse prognostic factor and a predictive factor for response to the humanized monoclonal antibody trastuzumab (Herceptin®, Genetech, San Francisco, CA, USA), as well as anthracycline and taxane-based adjuvant chemotherapeutic regimens, and possibly hormonal therapies [100, 101].

In 2002, Santin et al reported that 8 of 10 ESC expressed HER2/neu by IHC at the 2+ or 3+ levels [102]. Furthermore, the authors demonstrated that in vitro, ESC cells were sensitive to herceptin-mediated antibody-dependent cellular cytotoxicity, even though the cells were chemoresistant in vivo [102]. This raised the possibility of the use of trastuzumab in patients with ESC, especially in the setting of chemoresistance. The authors subsequently reported that HER2/neu overexpression and/or gene amplification is an adverse prognostic factor in patients with ESC, and that HER2/neu tended to show higher levels of expression in ESC from African Americans [103, 104], which is a possible explanation for the racial disparities in overall survival that has been observed in this neoplasm [19]. Combined data from other studies [105-109] indicates that 1) HER2/neu is overexpressed (2+ or 3+) in 32.1% of ESC by IHC, and 2) approximately 54.5% of cases scored as 2+ or 3+ by IHC display c-erbB2 gene amplification by fluorescent in situ hybridization, (**Tables 2-4**). Another study in which scores were not specifically outlined reported a 18.9% rate of HER2/neu immunopositivity in ESC [110]. Finally, Morrison et al [111] reported HER2/neu overexpression and gene amplification rates of 43% and 29% respectively, which were significantly above what was found for their endometrioid cancers.

The adverse prognosis associated with HER2/neu overexpression by IHC, especially in the presence of concurrent gene amplification, was confirmed in additional studies [107, 108, 111]. Singh et al, however, could not identify independent outcome differences between the HER2/neu positive and HER2/neu negative ESC patients, which the authors attributed to their small sample size [109]. Our review of the data reported in the aforementioned studies do not show a clearly increased propensity for HER2/neu overexpression in advanced stage ESC as compared to localized disease, and HER2/neu expression has not been specifically evaluated in the precancerous lesions of ESC to our knowledge. Nonetheless, the frequency of c-erbB2 alterations in ESC suggests that it plays a significant role in its evolution and/or development. The fact that HER2/neu is overexpressed and/or amplified in only a subset of ESC bolsters the aforementioned possibility that ESC evolve through more than one pathway. Since HER/neu is also overexpressed in subsets of some of the other histotypes [112, 113], HER2/neu positive ESC may fall into a larger group of endometrial carcinomas that are not clearly definable by morphologic features, perhaps akin to breast cancers, wherein gene expression profiling has clearly shown that HER2/neu overexpressing cases are a distinct molecular subclass [114, 115].

Intercellular Molecules

The precise events that initiate or contribute to tumor progression in ESC are not clear. The propensity for ESC to metastasize to the abdominopelvic organs, even when they are small and localized in the endometrium, and do not display lymphovascular invasion, is well-known. Qualitative and/or quantitative perturbations in intercellular molecules probably contribute to this propensity for invasiveness and migration. Two of the most widely studied, cadherins and claudins, are discussed below.

Table 3 Comparison of HER2/neu status in ESC as determined by IHC and fluorescent in situ hybridization

References	Santin et al [106]			Odicino et al [108]			Slomovitz et al [107]			Villella et al [105]		
IHC score	0-1+	2+	3+	0-1+	2+	3+	0-1+	2+	3+	0-1+	2+	3+
Cases (N)*	13**	7	9	10	0	2	56	10	2	6	0	3
Amplified	0	2	9	0	NA	2	NP	1	1	1	NA	3
Not amplified	13	5	0	10	NA	0	NP	9	1	5	NA	0

*Successful hybridizations; **as outlined in original report; NA, not applicable; NP, FISH not performed; IHC, immunohistochemistry

Table 4 Summary of FISH/IHC correlation data on HER2/neu in endometrial serous carcinoma

IHC score	Number of cases tested	Amplified N(%)	Not amplified N(%)
0-1+	29	1 (3.4)	28 (96.6)
2+	17	3 (17.6)	14 (82.4)
3+	16	15 (93.75)	1 (6.25)

Summarized from references 105-108

E-Cadherins

The classical (type 1) cadherins are transmembrane components of the cellular adherens junctions that mediate predominantly homotypic cell-to-cell adhesions. The cytoplasmic domains of cadherins are connected to the intracellular actin cytoskeletal network through interactions with the catenins, and are thereby involved in a variety of cellular signaling pathways [116]. Epithelial (E) cadherins have generally been considered suppressors of tumor progression and invasiveness [117], and E-cadherin-mediated cell-cell adhesion is inactivated via a variety of mechanisms in many human malignancies [118]. Published studies on E-cadherin expression in ESC have reported somewhat incongruent findings. Two large studies by Mell *et al* [119] and Stefansson *et al* [120] both reported that reduced expression of E-cadherin in endometrial carcinomas correlated significantly with the serous histotype, and histologic grade was included in each analysis. Holcomb *et al* [121] also reported that E-cadherins were significantly less likely to be seen in ESC as compared to endometrioid carcinomas. However, neither Shaco-Levy *et al* [122] nor Demopoulos *et al* [67] could identify statistically significant differences between EEC and ESC regarding their frequencies of E-cadherin expression. In one study that specifically compared high grade EEC and ESC, there were actually more ESC cases displaying moderate or strong E-cadherin staining (41%) than were EEC cases displaying the same (6%) [123]. Furthermore, a recent study by Nofech-Mozes *et al* reported that 100% of 37 cases of ESC showed E-cadherin expression in >50% of cells [110]. These discrepancies may be centered on methodological (differences in IHC assays and techniques) or interpretive (subjective differences in how staining patterns are interpreted) issues. Nonetheless, it can be asserted that there are published data indicating that E-cadherin is inactivated or downregulated in some ESC. The underlying

molecular basis for this downregulation or inactivation is unclear. However, as with many other high-grade carcinomas, an epithelial-to-mesenchymal transition (EMT) is probably involved [124]. Cadherin switch, which commonly accompanies EMT, has been reported in ESC, with upregulation of P-cadherin accompanying the E-cadherin downregulation [120]. Additionally, transcription factors involved in EMT, such as the zinc-finger E-box-binding homeobox 1 (ZEB1), which are expressed in high-grade and Type II endometrial carcinomas, have been shown to suppress E-cadherin expression and to increase migratory and invasive properties of endometrial cancer cell lines [125]. Molecules that have been implicated in EMT (and its concomitant downregulation of E-cadherin) such as focal adhesion kinase [126], are overexpressed in high grade endometrial cancers, including ESC [127]. CD82 (KAI-1), which is thought to be metastases suppressor by, in part, stabilizing E-cadherin-mediated intercellular adhesions [128] is nearly uniformly lost in uterine high-grade cancers, including ESC [129]. Finally, the HER2/neu protein has been shown to directly interact with the cadherin-catenin complex via beta-catenin and plakoglobin [130, 131]. Epidermal growth factor has also been shown to scatter E-cadherin positive cervical cancer cell lines, for example, probably via tyrosine phosphorylation of the beta-catenin and plakoglobin components of the cadherin-catenin complex [132]. Since ESC may be one of the uncommon human malignancies in which HER2/neu overexpression is accompanied by E-cadherin downregulation in a significant subset, the role of the interplay between these 2 molecules deserve further study.

Claudins

Claudins are critically important components of the intercellular tight junctions that regulate paracellular transport [133, 134]. At least 18 isoforms have been identified in human tissues [133, 134]. As expected, claudin

proteins can be detected across a wide spectrum of human neoplasms [135]. In 2003, Rangel et al reported that claudin 3 and claudin 4 are significantly upregulated at the RNA and protein levels in several histotypes of ovarian cancer, and that this upregulation was absent in ovarian cystadenomas [136]. Similar findings claudin 3 and claudin 4 upregulation in ovarian serous carcinomas above normal ovarian surface epithelium were reported shortly thereafter [137]. Engineered expression of claudin 3 and claudin 4 in ovarian surface epithelial cells were subsequently found to be associated with increased motility and invasiveness *in vitro* [138]. Analysis of the gene expression profiles of a small group of ESC showed that both claudin 3 and claudin 4 were significantly upregulated in ESC as compared to normal endometrial cells, findings that were confirmed for claudin 4 at the protein level by IHC [139]. It is unclear, however, if claudin 3 and 4 are significantly more upregulated in ESC than in EEC. Konecny *et al* [140] found 78% of ESC to be strongly positive for claudin 3 by IHC, as compared with 38% for EEC. Parallel values for claudin 4 were 56% versus 9% respectively, differences that were statistically significant for both claudins [140]. Sobel *et al* [141], however, did not identify any significant differences between these histotypes regarding the expression of either claudin 3 or 4. Rather, the authors reported that claudin 1 and 2 significantly differentiated EEC and ESC, with EEC showing a low claudin 1/high claudin 2 immunoprofile, and ESC displaying the reverse [141]. Although additional studies are required to define the specific isoform that is involved in endometrial serous carcinogenesis and progression, the identification of claudins has provided the possibility of another therapeutic target in this aggressive neoplasm [142].

Cell Cycle-associated Proteins

The cell cycle of eukaryotic cells is regulated and controlled, especially at the critical G1→S point, by a complex interplay between cyclins, cyclin-dependent kinases (CDKs), and their inhibitors [143-146]. Cyclins, whose levels are tightly controlled at each level of the cycle, serve to activate CDKs by phosphorylation. Activated CDKs in turn phosphorylate the retinoblastoma protein (pRb), thereby activating it and releasing it from the E2F/pRb complex, where it is normally kept in a

hypophosphorylated state that precludes the transcription of genes necessary for the cell to proceed through S phase of the cell cycle. CDKs are closely regulated by CDK inhibitors (CDKI), which can be classified into 2 main families, an INK4 family (p16^{INKA}, p15^{INK4B}, p18^{INK4C}, p19^{INK4D}) that primarily regulate CDK4 and CDK6, and the CIP/KIP family (p21^{Cip1}, p27^{Kip1}, and p57^{Kip2}) which targets a wider spectrum. Dysregulation of one or more of the cell cycle proteins is characteristic of most human malignancies [145, 146]. Two main cell-cycle pathways are frequently dysregulated in human tumors, and both appear to be involved in endometrial serous carcinogenesis [147].

Several lines of evidence indicate that the pRB pathway (p16^{INKA}/Cyclin D-CDK/pRb-E2F) is dysregulated in ESC. Although p16 is inactivated in many malignancies, the protein may occasionally accumulate in the cell of some neoplastic processes. This may be used to surmise a functional inactivation of the pRb pathway, with p16 accumulating due to the absence a negative feedback that would otherwise be provided by functional pRB. In endometrial cancers, mutations of the p16 gene, although infrequent, is generally accompanied by loss of p16 protein expression [148]. By gene expression profiling, p16 is upregulated significantly in ESC above both normal endometrial cells [139] and endometrioid carcinomas [149]. By IHC, 92-100% of ESC displays diffuse expression of p16, which is significantly above the immunopositivity rates for each of the other histotypes that have been tested [150-152]. A subset of cases display increased expression at their invasive edges [153].

The second pathway that is probably dysregulated in ESC is the ARF-MDM2-p53 pathway. The role of p53 alterations in endometrial serous neoplasia has been previously outlined. MDM2 and p53 are normally components of an autoregulatory negative feedback loop, such that the p53 levels of a cell are kept low by MDM2 [154]. ARF (p14) has been shown to stabilize p53 by promoting the degradation of MDM2 [155]. Mutations at the *INK4A/ARF* locus, which encode both ARF and p16^{INKA} (different promoters and alternative reading frames) therefore dysregulate both pathways [155]. The fact that the *INK4A/ARF* gene is the most highly upregulated (relative to normal

endometrial cells) in ESC, coupled with a similar high expression of both of its transcripts (p16 and p14) has caused some authors to postulate that loss of function of both the pRb and p53 proteins results in a lack of a negative regulatory feedback, and that this may be a consistent feature of ESC [139].

The other cell cycle proteins have shown varying degrees of derangement such that no clear patterns emerge. p27, a CDKI in the CIP/KIP family, was found to be lost or significantly reduced (relative to normal endometrium) in 24 of 32 cases of ESC from 3 series [156-158], including in 63% of stage I cases in one study [157]. However, p27 loss appears to be non-specific to ESC [156], and one study of ESC-derived cell lines found *overexpression* of p27 [159]. Kallakury *et al* [160] reported ESC immunopositivity rates of 24%, 71% and 24% for p34CDC (a cell cycle regulator), cyclin A and cyclin B1 respectively, but there was no correlation with histotype. Schmitz *et al* [157] reported "overexpression" of cyclin D1 (defined as >5% of cells with nuclear staining) was present in 19% of ESC. Horrée *et al* [153] reported that several cell cycle proteins (cyclin E, CDK2, and the aforementioned p16) are significantly more expressed at the invasive fronts of endometrial carcinomas. Cables, a CDK regulator that contributes to the regulation of the G1→S progression in the cell cycle, is lost in ESC, as it is in most endometrial carcinomas in an apparently grade-dependent manner [161].

In summary, although a progressive dysregulation in cell cycle proteins can be documented in EEC [162], no such sequential patterns emerge in ESC. However, alterations involving p53 and p16 indicate that cell cycle dysregulation is integral to endometrial serous carcinogenesis and that it occurs early in its evolution.

Other Molecules

Although a comprehensive cataloguing of the myriad of other proteins and genes that have been noted in various reports to be aberrantly expressed or regulated in ESC is beyond the scope of this communication, we briefly note a few here that are of interest to us. It is unclear if these changes are specifically related to ESC, or whether they simply highlight the state of regulatory disarray and other properties that

typify a high-grade malignancy.

Consistent with its propensity to display invasiveness, ESC show a significant upregulation in a number of proteases, including matrix metalloproteinases and a number of kallikreins [139, 163, 164]. Shaco-Levy *et al* [122] reported higher expression of matrix metalloproteinase (MMP)-2 and tissue inhibitor of metalloproteinases (TIMP)-1 in ESC as compared to low grade EEC. However, when grade 3 EEC is used for the comparison, EEC expressed more MMP-2 (as well as MMP-9) than ESC [165]. Notably, ESC expressed more MMP-2 and MMP-9 at its invasive edge [163]. Insulin-like growth factor II mRNA-binding protein 3 (IMP3), an oncofetal protein that appears to be involved in tumor invasiveness and progression [166], is significantly more expressed in ESC than in the other histotypes of endometrial carcinoma, at both the mRNA [149] and protein levels [167, 168]. Folate receptor alpha, a membrane bound molecule that is upregulated in many high grade carcinomas [169], is similarly upregulated in endometrial carcinomas, but appears to show a significant association with the serous histotype [170, 171]. Urokinase plasminogen activator receptor, a protein that participates in the activation of plasminogen and hence in extracellular matrix degradation and tumor invasiveness, is highly expressed in endometrial cancers in a grade and stage-dependent fashion, and as such is highly expressed in ESC [172].

These findings, in addition to many others in the literature, deserve further exploration not only to help decipher the molecular basis for endometrial serous carcinogenesis, but to uncover potential targets for diagnosis, therapy, and/or disease surveillance.

Conclusions

ESC constitutes only 10% of endometrial cancers, but have a substantially higher case-fatality rate than their more common endometrioid counterparts. This is attributable, at least in part, to the advanced stage at which many patients with ESC present. Early detection of ESC or its precancer(s), for example in endometrial biopsies of postmenopausal women being evaluated for abnormal bleeding, may therefore reduce the morbidity and mortality associated with this aggressive neoplasm. We

have presented evidence that supports the notion that EmGD is the earliest, morphologically recognizable precancer to ESC. We advocate use of the term serous EIC only as a morphologic descriptor and never as a diagnostic/pathologic statement of biologic potential. Given its potential for extrauterine extension, we consider the lesions described as serous EIC, when present in isolation, as examples of localized ESC, and patients should be managed as such. Morphologically normal, p53 immunoreactive endometrial cells (the so-called "p53 signatures") display *TP53* mutations in a substantial subset, and form the start of a progression model, outlined herein, from p53 signatures to EmGD to localized ESC to the more conventionally invasive neoplasm. The molecular events that underlie endometrial serous carcinogenesis and progression remain largely unclear, but the dysregulated pathways provide early insight. Three points deserve emphasis: First, we could uncover no lines of evidence that suggest substantive differences between extrauterine and intrauterine disease ESC. Such differences may have provided insights into tumor progression mechanisms. The molecular events in ESC appear to occur early. Second, there may be some heterogeneity in how ESC develop and evolve. Third, consistent with the dualistic model of endometrial carcinogenesis, most ESC evolve via different pathways from Type I endometrial cancers. p53 gene mutations and/or protein overexpression occurs in the majority of ESC. The p16^{INKA}/Cyclin D-CDK/pRb-E2F and the ARF-MDM2-p53 pathways both seem to be dysregulated in ESC. Although some other cell cycle proteins seem to be abnormally expressed in ESC, the progressive and sequential dysregulation of these proteins that is seen in EEC has not been documented in ESC. C-erbB2 alterations occur in a substantial subset of ESC, as are alterations of adhesion molecules such as claudins. A subset of ESC display protein expression patterns that are characteristic of high grade endometrial carcinomas, including loss of the metastasis suppressor CD82 (KAI-1) and epithelial-to-mesenchymal transformation, the latter manifested as E-cadherin downregulation, P-cadherin upregulation, and expression of epithelial-to-mesenchymal transformation-related molecules such as zinc-finger E-box-binding homeobox 1 and focal adhesion kinase. Preliminary data suggests differential patterns of expression in ESC of some isoforms of claudins, proteases, the tumor invasiveness and progression-associated oncofetal protein Insulin-like growth factor II mRNA-binding protein 3 (IMP3), as well as a variety of other molecules. These findings, in addition to many others outlined above and in the literature, deserve further exploration, not only to help decipher the molecular basis for endometrial serous carcinogenesis, but to uncover potential targets for diagnosis, therapy, and/or disease surveillance.

Acknowledgements

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