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Molecular Pathology and Cytogenetics of Endometrial Carcinoma, Carcinosarcoma, and Uterine Sarcomas

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Abstract

Molecular pathology and genetics are the subjects of increasing focus since they are providing a link between etiologic factors and the heterogeneity of clinicopathologic manifestations that have been covered in the preceding chapters. In endometrial cancer, two divergent pathways have been delineated that may be thought as analogous to the hormone-dependent and -independent subtypes in cancers of breast and prostate. Most hormone dependent EC are EEC, which from a molecular point of view can be classified into different subgroups: (a) ultramutated, due to POLE mutations; (b) hypermutated tumors with MSI, most frequently due to MLH1 promoter, but also seen in Lynch syndrome; and (c) MSS EC with low mutation rate, the most frequent subgroup of EEC. Hormone-independent tumors are represented by serous carcinomas, characterized by a high rate of mutations in p53 that produce genomic instability with extensive somatic copy number alterations. Knowledge on alterations in sarcomas will hopefully lead to advances in diagnosis and therapy that are urgently needed in women where spread beyond the uterus has occurred.

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Endometrial Carcinoma

Molecular Abnormalities

During the last few years, it has been demonstrated that endometrioid (EEC) (type I) and non-endometrioid (type II) endometrial carcinomas (NEEC) not only differed from epidemiologic, clinical, and morphologic viewpoints but also regarding molecular alterations implicated in their initiation and progression. Several different molecular pathways are involved in EEC development, including DNA mismatch repair (MMR), phosphoinositide 3-kinase (PI3K)/Akt, RAS-RAF-MEK-ERK, fibroblast growth factor (FGF), and WNT pathways. Alterations in some of these pathways have also been found in atypical endometrial hyperplasia, indicating their role in tumor initiation, but they are infrequent in NECC. In contrast, *TP53* mutations occur in a high percentage of NEEC, mainly in serous carcinomas and in its precursor lesion, endometrial intraepithelial carcinoma, but are detected only in a subset of grade 3 EECs. In addition, it has been suggested that *TP53* inactivation may be implicated in the phenotypic change from EEC to NEEC as observed in some mixed carcinomas [1, 2] (Table 1).

Recently, the Cancer Genome Atlas Research Network (TCGA) [2] proposed a new molecular classification of endometrial cancer (EC). Based on a combination of somatic mutations, microsatellite instability (MSI), and somatic copy number variations, the endometrial tumors were classified into four groups: (1) an ultra-mutated group with unusually high mutation rates; (2) a hypermutated group with microsatellite instability (MSI), most with MLH1 promoter methylation; (3) a group with lower mutation frequency and most of the microsatellite stable (MSS) endometrioid cancers; and (4) a group that consists primarily of serous-like cancers with extensive somatic copy number alterations and a low mutation rate. Groups 1, 2, and 3 included predominantly endometrioid carcinomas, whereas group 4 included serous carcinomas and some grade 3 endometrioid carcinomas.

POLE Mutations

The ultra-mutated group of EC is characterized by mutations in the exonuclease domain of POLE, which is a catalytic subunit of DNA polymerase epsilon involved in nuclear DNA replication and repair [3]. Seventy five percent of mutations are located at hot-spots P286R and V411L. Ultra-mutated tumors represented 7 %

Table 1 Most frequently mutated genes in histological types of endometrial cancer

GENE	Endometrioid carcinoma (%)	Serous carcinoma (%)	Carcinosarcoma (%)
PTEN	52	<5	19
PIK3CA	35	36	35
PIK3R1	25	<5	10
CTNNB1	24	<5	<5
ARID1A	25	7	14
KRAS	17	<5	12
CTCF	14	<5	5
FGFR2	9	7	<5
TP53	9	74	91
FBXW7	7	26	38
PPP2R1A	<5	23	28
CHD4	<5	13	17

of EC in the TCGA series and showed an increased C → A transversion frequency [2]. The majority demonstrated defining morphological features of endometrioid differentiation, they were frequently high grade (60 %) and rich in tumor-infiltrating lymphocytes and/or peritumoral lymphocytes (84 %); many tumors showed morphological heterogeneity (52 %) and ambiguity (16 %). Foci demonstrating severe nuclear atypia led to concern for serous carcinoma in 28 % of the tumors [4].

At the molecular level, the majority of the TCGA *POLE*-mutated tumors were microsatellite stable (65 %), and *TP53* mutations were present in 35 % of them. They also harbored mutations in *PTEN* (94 %), *FBXW7* (82 %), *ARID1A* (76 %), and *PIK3CA* (71 %). Since all patients in TCGA and other cohorts [4, 5] were alive without disease, it has been suggested that ultra-mutated tumors have an excellent prognosis despite of adverse molecular and pathological features. However, other authors have not found *POLE* mutations as prognostic factor in EC [6]. Some studies have demonstrated that *POLE* mutations may induce MSI by generating somatic mutations in DNA mismatch repair genes, most frequently in *MSH6*, in a subset of tumors. Thus, *POLE* testing in MSI ECs could serve as a marker of somatic disease origin and therefore, may be a valuable exclusionary criterion for Lynch syndrome gene testing [6, 7].

DNA Mismatch Repair Deficiency

Microsatellite instability represents a pattern of mutations in cells with a replication error phenotype due to deficient DNA MMR. Microsatellite loci contain repetitive elements of 1–6 nucleotides in length and are most commonly (CA) or poly A/T sequences. MSI status can be detected by using a standard panel of five microsatellite markers. When at least two of the five markers show MSI, tumors are classified as MSI-high (MSI-H). In contrast, tumors without size alteration in microsatellites or those with only one altered marker are classified as microsatellite stable (MSS) and MSI-low (MSI-L), respectively. From a clinicopathologic point of view, MSI-L tumors should be included with MSS tumors [8]. Microsatellite instability was first reported in colorectal adenocarcinomas of patients with Lynch syndrome (hereditary nonpolyposis colorectal cancer, HNPCC). This status of high-frequency mutagenesis is caused by mutations in the main DNA MMR genes, such as *hMLH1* and *hMSH2* and less frequently *hMSH6*, *hPMS1*, and *hPMS2*. MSI is also seen in approximately 15 % of sporadic colorectal carcinomas, usually reflecting loss of expression of hMLH1 associated with gene silencing by *hMLH1* promoter methylation [9].

Available data indicate that EC is the most common extracolonic tumor in Lynch syndrome, with lifetime risk estimates ranging from 40 to 60 % in female mutation carriers [10]. As a result, the original Amsterdam criteria for Lynch syndrome were revised in 1999 to include EC among the diagnostic criteria [11]. It has been suggested that EC is the most common malignancy among women carrying hMSH6 germ line mutations [12].

MSI is seen in approximately 15–45 % of sporadic EEC [13], usually reflecting loss of expression of hMLH1 associated with gene silencing by *hMLH1* promoter methylation. This change has been reported in 69–92 % of EC with MSI [14, 15]. In addition, it has been shown that the *hMLH1* promoter is frequently methylated in the histologically normal endometrium [15] and atypical endometrial hyperplasia [14] of patients with ECs and that the methylation status is similar to that in the carcinoma. These findings support the notion that, in a subset of tumors, epigenetic changes in DNA MMR genes might be the initial events that trigger the genetic alterations involved in endometrial carcinogenesis.

Immunohistochemistry can be used to explore MMR gene inactivation in EC. Currently, there are antibodies available to study the expression of the most important MMR proteins, such as hMLH1, hMSH2, hMSH6, and hPMS2. In colon cancer, large studies comparing immunohistochemistry and MSI genotyping have demonstrated a 93–100 % sensitivity to detect MSI by immunohistochemistry analysis. Although there are not such large series in EC, different

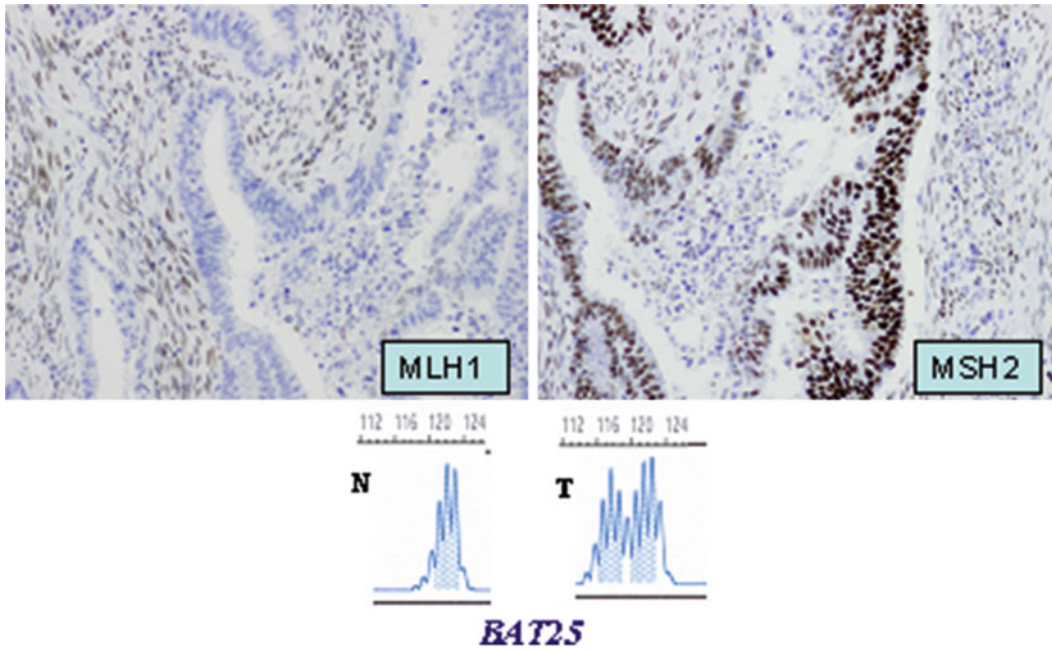

Fig. 1 Absence of MLH1 expression and preserved MSH2 expression in an EEC with microsatellite instability. Note abnormal size of BAT25 microsatellite in tumor tissue (T) with respect to normal tissue (N)

studies have reported a 70–100 % sensitivity when using immunohistochemistry (Fig. 1) [16, 17].

MMR deficiency in cancer produces instability not only in microsatellites that are located in noncoding sequences, such as those used for MSI genotyping, but also in mononucleotide tract repeats located in coding sequences of different genes. The proteins encoded by these genes participate in a variety of essential cellular processes like signal transduction (TGFβRII, IGFIIR, PTEN), apoptosis (BAX), DNA repair (hMSH3, hMSH6, MBD4), transcriptional regulation (TCF-4), protein translocation and modification (SEC63, OGT), or immune surveillance (β2M). It is generally believed that this subset of critical targets specifically promotes MSI carcinogenesis in a large proportion of tumors. Moreover, several studies have demonstrated that selection of target gene mutations in MSI cancers is a tissue-specific process. Whereas some of the genes were proposed to be real target genes for mutation in the most common types of cancers with MSI (colon, gastric, and endometrial cancer) (*TGFβRII*, *BAX*, *IGFIIR*, *MSH3*, *MSH6*, and *GRB14*), selection of other genes for mutation appeared to be dependent on the primary site of the tumor. ECs with MSI accumulate significantly fewer mutations at coding repeats compared to gastrointestinal MSI tumors. For example, the almost systematic *TGFβRII* gene mutation in MSI gastrointestinal tumors was observed in only 0–10 % of the MSI EC in different series [18–20].

Although MSI occurs in a substantial fraction of sporadic EC, data on whether these endometrial tumors differ from their MSI-negative counterparts in clinical characteristics, pathologic features, and survival is controversial; although some studies have reported favorable survival associated with MSI EEC, other series did not find differences in grade, recurrence rate, and survival between MSI-positive and -negative EC [13].

Several studies have analyzed the morphological features associated with MSI, irrespective of the sporadic or hereditary nature of the tumors. MSI EEC tumors frequently have peritumoral lymphocytic infiltration and tumor-infiltrating

lymphocytes (40/10 high-power fields), and some MSI ECs exhibit areas of dedifferentiation [21].

Alterations in the Phosphoinositide 3-Kinase (PI3K)/Akt Pathway

In EEC, the constitutive PI3K-AKT pathway is frequently activated in response to alterations of certain genes, such as those inactivating *PTEN*, mutations or amplifications of *PIK3CA* and somatic missense mutations within AKT kinases.

PTEN gene is located in 10q23, a region undergoing frequent somatic deletion in tumors. It encodes a 403-amino acid dual-specificity phosphatase containing a region of homology to tensin and auxilin, which are two cytoskeletal proteins. Among other activities, PTEN antagonizes the PI3K/AKT pathway, which results in downregulation of AKT phosphorylation activation. Thus, decreased expression of PTEN leads to increased levels of phospho-AKT, which results in both suppression of apoptosis and induction of cell cycle. *PTEN* is mutated in the germ line of patients with Cowden's disease, a rare autosomal dominant cancer syndrome, which occasionally may be associated with EC. However, *PTEN* is also frequently somatically mutated in tumors from various tissues. *PTEN* may be also inactivated by deletion, as shown by the elevated frequency of loss of heterozygosity in different tumor types. Finally, a third proposed mechanism for *PTEN* inactivation is promoter hypermethylation. However, the true significance of *PTEN* promoter methylation is still under discussion.

Loss of heterozygosity at chromosome 10q23 occurs in 40 % of EECs [22]. Moreover, *PTEN* is the most frequently mutated gene in EEC (Fig. 2). The frequency of *PTEN* mutations in EEC varies between 24 and 50 % [2, 23–25] in different series, although one study has reported an incidence as high as 83 % [26]. In addition, *PTEN* silencing may occur not only in EEC and endometrial hyperplasia [25–28] but also in isolated glands in up to 40 % of premenopausal women [29], indicating a major role of this alteration in the initiation of some EEC.

PTEN mutations may occur throughout the entire coding region, but are more frequent in exons 5, 7, and 8. A high percentage of mutations in exon 5 (around 60 %) are single base substitution, being more common in codon 130 (Fig. 2).

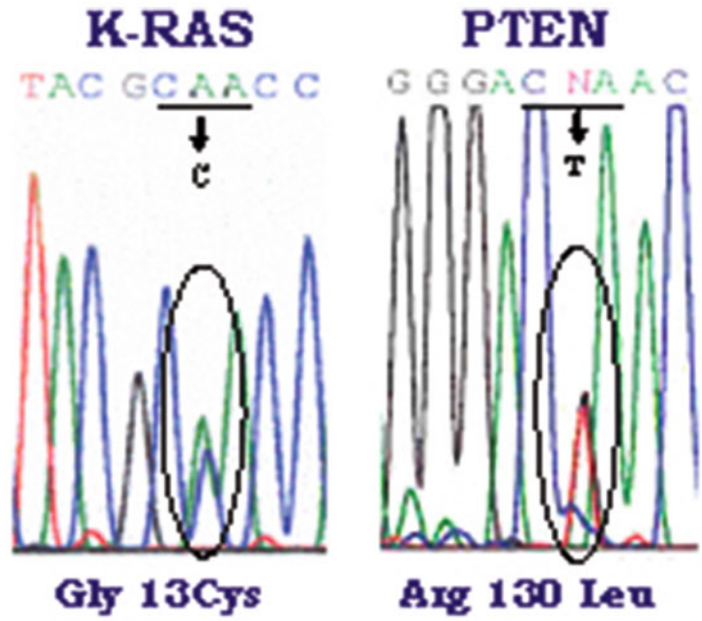

Fig. 2 Common single point mutations in *PTEN* and *K-RAS* genes

In contrast, frameshift mutations are more frequent in exons 7 and 8, where two hot spot deletions or insertions have been identified: two (A)6 sequences in codons 265–267 and codons 321–323. Mutations in those sites are characteristic of MSI tumors and suggest that some mutations in the *PTEN* gene are consequence of loss of DNA repair mechanism. Opinions differ, however, on the relationship between occurrence of *PTEN* gene mutations and the presence of MSI in EC. Thus, most series [24, 30, 31] have demonstrated that *PTEN* gene mutations occur more frequently in EC with MSI (65–86 %) than in those without it (20–36 %). However, other authors failed to find any relationship between high frequency of *PTEN* gene mutations and MSI in EC [26].

PTEN mutations have been detected more frequently in Caucasians relative to African-Americans, and have been correlated with young age, low FIGO-stage, low grade, and favorable prognosis in some studies [32–34]. However, other series have reported higher incidences of *PTEN* in advanced tumors (72 % of PTEN mutations in FIGO stage Ic as opposed to 56 % in FIGO stage Ia), as well as in less differentiated versus well-differentiated carcinomas (81 % in G2 vs. 44 % in G1 ECs) [35].

It has been suggested that PTEN immunostaining may be an effective method to screen for abnormal PTEN expression in tumors and premalignant lesions. However, some variability has been observed with different antibodies and techniques, particularly when correlating the immunohistochemical results with the presence of molecular alterations. Some studies have suggested that the monoclonal antibody 6.H2.1 is the only antibody that recognizes a pattern of PTEN expression that correlates with the presence of molecular alterations in *PTEN* (mutations, deletions, or promoter hypermethylation) [36, 37].

The PI3K pathway can be activated in EC not only by PTEN inactivating mutations but also by mutations in other genes. PI3K is a heterodimer composed of a catalytic subunit (p110α) encoded by *PIK3CA*, which is located at chromosome 3q26.32, and a regulatory subunit (p85α) encoded by *PIK3R1*. A high prevalence of mutations in the *PIK3CA* gene has been reported in EECs (up to 36 %) [2, 38–43], with most studies focusing on exons 9 and 20, as these two exons account for >80 % of mutations in other tumor types, and they encode the C-terminal helical and kinase domains of p110α [41, 42]. A significant association between *PIK3CA* and *PTEN* mutations has also been observed, suggesting an additive effect of these alterations in the activation of the PI3K/AKT pathway [41–43]. *PIK3CA* and *KRAS* mutations appear to be mutually exclusive [40, 43, 44]. However, their association with other genetic defects, such as *CTNNB1* mutations or MSI, remains to be established [41, 42]. A link between *PIK3CA* mutations and adverse clinicopathologic parameters such as grade and stage has been described in some studies [42, 43]. Moreover, mutations in exon 20 are observed more frequently in high-grade than low-grade EECs (67 % vs. 33 %), while grade 1 ECCs are more frequently associated with exon 9 mutations (up to 57 %) [41]. *PI3KCA* amplification has also been reported in 12 % of EECs, occurring independently of mutational events at the same locus, and they are strongly associated with age, suggesting a role of *PIK3CA* amplification in the initiation and progress of ECs in older women [43].

More recently, mutations within the *PI3K* regulatory subunit (*PIK3R1)* have been reported in up to 43 % of EECs, preferentially localized in the p85α-iSH2 domain that mediates binding to p110α [2, 44]. These mutations are mutually exclusive with those affecting *PIK3CA*.

The AKT serine/threonine kinases regulate diverse cellular processes (survival, proliferation, invasion, and metabolism) and they are activated by direct recruitment to the plasma membrane via the pleckstrin homology (PH) domain. A missense mutation in the PH domain of *AKT1* (E17K) previously described in other tumors [45], was demonstrated in 2 % of EECs [46]. Interestingly, the two tumors that displayed *AKT1* mutations did not exhibit any mutations or LOH in *PTEN*, nor mutations in *PIK3CA* or *KRAS*. Subsequently, *AKT1* mutations were demonstrated in 4–12 % of EECs [47, 48], while additional mutations in

other AKT family members (*AKT2* and *AKT3*) have been also described.

Alterations in the WNT Signaling Pathway

The Wnt signaling pathway plays an important role in normal and tumor cells. In the absence of an extracellular Wnt signal in normal cells, the free (cytoplasmic) β-catenin (coded by *CTNNB1*) level is low since the protein is targeted for destruction in the ubiquitin–proteasome system after phosphorylation by glycogen synthase kinase-3β(GSK-3β). The latter forms a complex with the adenomatous polyposis coli (APC) protein and other proteins, such as AXIN1, AXIN2, and protein phosphatase 2A. The most common molecular alterations in tumor cells leading to disruption of β-catenin degradation are mutations that inactivate APC or activate β-catenin itself. These alterations produce an accumulation of cytoplasmic β-catenin that translocates into the nucleus and, interacting with members of the lymphoid enhancer factor-1/T-cell factor (Lef-1/Tcf), activates transcription of various genes, such as *CNDD1* and *MYC*.

Regarding EC, the Wnt signaling pathway is altered only in EEC. In these tumors, mutations of APC have not been detected [49, 50], but *CTNNB1* mutations occurred in approximately 15–36 % of EEC (Fig. 3) [2, 49–53], and in 14 % of endometrial atypical hyperplasias [24]. Most mutations affect the aminoacids implicated in the downregulation of β-catenin through phosphorylation by this serine/threonine kinase (serine 33, serine 37, threonine 41, and serine 45) and two adjacent residues. Mutations in these residues render a fraction of cellular β-catenin insensitive to APC-mediated downregulation and are responsible for upregulation of cytoplasmic β-catenin and its accumulation in the nuclei of tumor cells, which can be detected by immunohistochemistry.

From a morphologic point of view, several studies have stressed the association between nuclear β-catenin accumulation and squamous metaplasia in EEC. Although nuclear β-catenin may be associated with usual squamous metaplasia, it is more characteristically associated with morular metaplasia and *CTNNB1* mutations are found in 50 % of atypical endometrial hyperplasias with squamous morules [28] (Fig. 3).

Some series have not found significant relationship between *CTNNB1* gene mutations and clinicopathologic features, such as age, tumor grade, and stage. However, in the TCGA series, *CTNNB1* mutations were observed in 47 %, 36 %, and 17 % of grade 1, 2, and 3 EECs, respectively [2]. One study has shown an association with low-grade tumors and absence of lymph node metastases [53], suggesting that *CTNNB1* mutations might occur in a subset of less aggressive ECs. In contrast, a recent study has found that *CTNNB1* exon 3 mutations characterize an aggressive subset of low-grade and low-stage EEC occurring in younger women [52].

Mutations in *SOX17* gene, which mediates proteasomal degradation of β-catenin, occur in 8 % EEC without MSI at recurrent positions

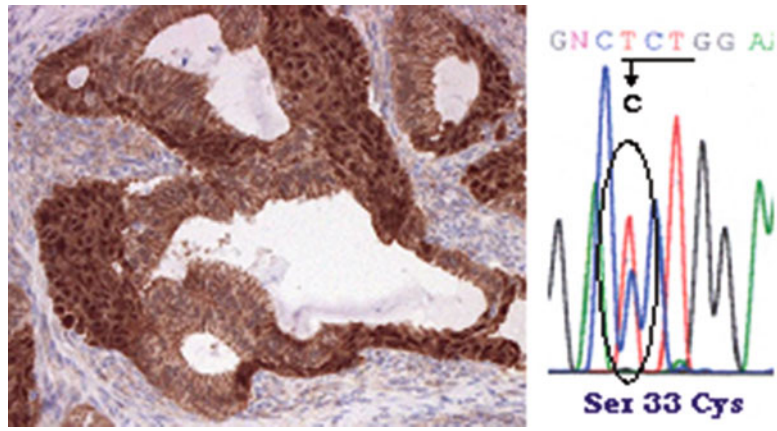

Fig. 3 β-catenin nuclear accumulation in areas of squamous metaplasia in an EEC, which carry a single point mutation in codon 33

(A96G and S403I) and are mutually exclusive with *CTNNB1* mutations [2].

Alterations in the RAS-RAF-MEK-ERK Signaling Pathway

The RAS-RAF-MEK-ERK signaling pathway plays an important role in the development and progression of ECs. The *RAS* gene family consists of three closely related genes (*KRAS*, *NRAS*, and *HRAS*) that encode proteins with GTPase activity, which are localized at the inner plasma cellular membrane and involved in several signal transduction pathways.

KRAS mutations in codons 12 and 13 have been identified in 10–30 % of ECs (Fig. 2) [2, 50, 54–56]. Although some authors have failed to demonstrate a correlation between *KRAS* mutations and stage, grade, depth of invasion, age, or clinical outcome in EC, others have reported associations between *KRAS* mutations and presence of coexistent endometrial atypical hyperplasia, lymph node metastases, and clinical outcome in postmenopausal patients above 60 years [57]. An association between *KRAS* mutations and mucinous differentiation has also been reported [56, 58]. Several studies have tried to correlate *KRAS* mutations and MSI in EC, but results are contradictory.

Other *RAS* genes are infrequently mutated in EC. In the TCGA series, about 3 % of EECs carried point mutations at *NRAS* [2].

BRAF, which encodes a *RAF* family member that functions downstream of RAS, has been reported to be somatically mutated in a number of human cancers. Activating mutations of *BRAF* have been frequently observed in MSI colorectal carcinomas, in which mutations of *BRAF* and *KRAS* have been reported to be mutually exclusive [59]. Several series have analyzed the frequency of *BRAF* mutations in EC. Although one of these studies reported a 21 % incidence of *BRAF* mutations in EEC suggesting an association with MSI status [60], and another study reported 10 % of *BRAF* mutations in EEC [61], most studies have found a very low incidence of *BRAF* alterations [2, 62, 63], indicating a minor role of this gene in endometrial carcinogenesis.

In 10–12 % of EECs, somatic mutations in the tyrosine kinase receptor *FGFR2* have been reported that are identical to the germline mutations associated with craniosynostosis and skeletal dysplasia syndromes [2, 64–66], the most common being S252W and N549K. *FGFR2* mutations are associated with enhanced FGF signaling and downstream activity, predominantly through the RAS-MAPK pathway. Interestingly, while mutations in *KRAS* and *FGFR2* are mutually exclusive events, *FGFR2* and *PTEN* mutations frequently coexist [67].

ARID1A Gene Alterations

ARID1A is a recently identified tumor suppressor gene located at chromosome 1p36 that encodes a large nuclear protein (BAF 250A). This protein is a key component of the multi-protein SWI/SNF complex involved in chromatin remodeling that plays an integral role in controlling gene expression and regulating widely diverse cellular processes, from differentiation during development and proliferation, to DNA repair and tumor suppression [68, 69].

ARID1A mutations were recently described in ovarian clear cell carcinomas, 30 % of ovarian low-grade endometrioid carcinomas and in some cases of atypical endometriosis, a putative precursor of ovarian clear cell and endometrioid carcinomas, suggesting that *ARID1A* loss is a relatively specific event in the genesis of these tumors [70, 71]. Interestingly, most *ARID1A* mutations are insertion/deletion mutations, leading to generation of premature stop codons due to a frameshift, and giving rise to truncated proteins prone to degradation.

A number of studies have demonstrated that the loss of BAF250A protein is correlated with *ARID1A* mutation status [71, 72] (Fig. 4) and a high incidence of *ARID1A* mutations has been reported in both low-grade (up to 40 %) and high-grade (up to 60 %) EECs [73, 74]. Interestingly, in both grade 1 and grade 3 EECs, *ARID1A* mutations are significantly associated with concurrent mutations in *PTEN* and *PIK3CA*, suggesting a cooperative role of these pathways in EEC tumorigenesis [75]. In addition, *ARID1A* mutations seem to be mutually exclusive with

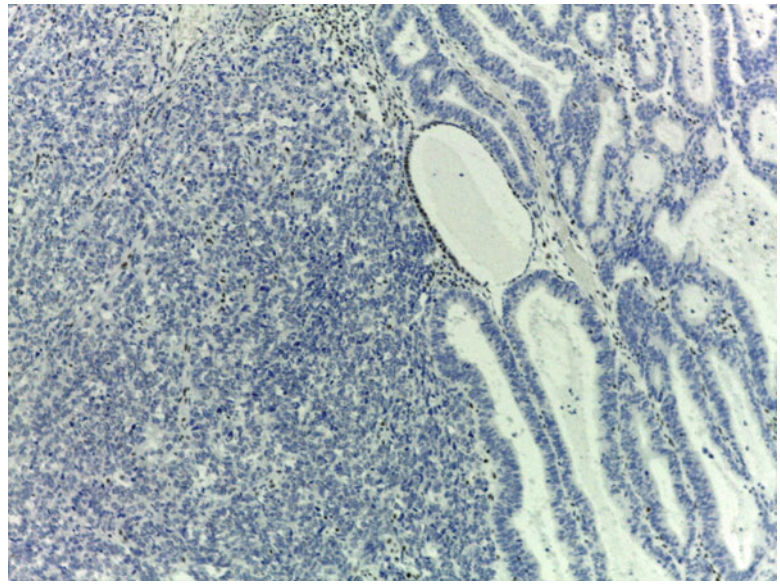

Fig. 4 Endometrioid carcinoma showing loss of ARID-1A expression in both components (dedifferentiated (left) and well-differentiated (right) endometrioid carcinoma). Notice preserved expression in preexistent normal endometrial gland

TP53 mutations, but are associated with MSI [76, 77]. Interestingly, whereas near 75 % of sporadic EECs with MSI also carried *ARID1A* mutations, only 15 % of Lynch-associated EECs did, suggesting that *ARIDIA* is a causative gene instead of a target gene of MSI [77].

TP53 Gene Alterations

The *TP53* tumor suppressor gene was initially identified as being essential for DNA damage checkpoint, but it was subsequently found to have a broader function after cellular stress, such as oncogene activation or hypoxia. The p53 protein is found at very low levels in normal cells. After stress, different pathways lead to posttranslational modification of the protein and its stabilization. This accumulation activates the transcription of a wide range of genes involved in various activities, including cell cycle inhibition and apoptosis depending on cellular context, extent of damage, or other unknown parameters.

Inactivation of *TP53* is essentially due to small mutations (missense and nonsense mutations or insertions/deletions of several nucleotides), which lead to either expression of (90 %) or absence of expression (10 %) of the mutant protein. Thus, there is no a complete concordance between genotyping and immunohistochemistry in tumors with *TP53* mutations. No inactivation of *p53* gene expression by hypermethylation of transcription promoters has been demonstrated. In many instances, these mutations are associated with loss of the wild-type allele of the *TP53* gene located on the short arm of chromosome 17.

TP53 mutations have been detected in approximately 10 % of EECs, being more frequent among grade 3 or advanced stage EECs [2, 78–82]. In contrast, 50–80 % of serous carcinomas carry *TP53* mutations, more frequently associated with protein overexpression (Fig. 5) [2, 83, 84]. For this reason, p53 immunohistochemistry may help in the differential diagnosis of uterine serous carcinoma when it exhibits glands without papillary architecture from EEC [85] although it is important to note that EEC may have *TP53* mutations.

TP53 mutation and expression have been reported to be an adverse prognostic factor in EC in some studies, but not in others. It has been proposed that the functional activity of mutant p53 protein is a strong predictor of survival in these patients [82]. Thus, the presence of dominant-negative *p53* mutations, those that produce mutated proteins that complex with and inactivate wild-type protein, are associated with poor prognosis in advanced EEC.

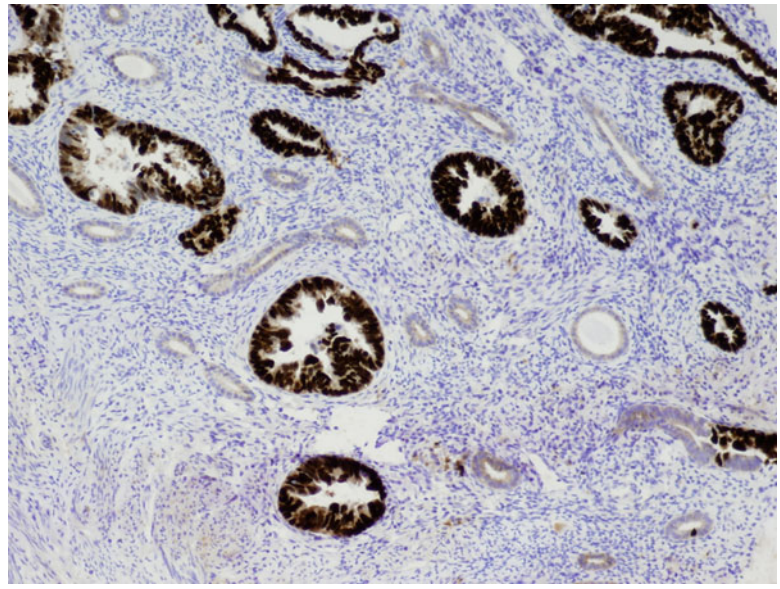

Fig. 5 p53 positive immunostaining in endometrial intraepithelial carcinoma. Note the admixture of atrophic (p53-wild-type) and neoplastic (p53-diffusely and strongly positive) glands

Table 2 Most frequent amplified genes in histological types of endometrial cancer

GENE	Endometrioid carcinoma (%)	Serous carcinoma (%)	Carcinosarcoma (%)
MECOM	4	28	21
CCNE1	1	26	42
ERBB2	1	26	10
PIK3CA	3	22	14
MYC	5	22	21

One of the principal features of tumors with *TP53* mutations is the high level of chromosomal instability that produces losses and gains that involve large chromosomal regions and specific genes. For this reason, serous carcinomas frequently carry amplification of genes like *CCNE1*, *HER2*, *MYC*, and *PIK3CA* [86, 87] (Table 2). Regarding *HER2*, although previous studies found inconsistencies regarding HER2 overexpression and amplification, the Gynaecological Oncology Group (GOG) phase II trial of trastuzumab in advanced and recurrent EC found that *HER2* was amplified in 28 % of serous carcinomas as opposed to 7 % of EECs, demonstrating a correlation between HER2 overexpression and *HER2* amplification [88]. However, no objective responses to trastuzumab therapy alone were reported in tumors displaying either *HER2* overexpression or amplification. Marked heterogeneity of *HER2* gene amplification has been described in endometrial serous carcinoma [89].

Cytogenetic Abnormalities

Cytogenetic studies have shown that most ECs have hyperdiploid karyotypes with relatively simple abnormalities, both numerical and structural, although cases also exist with complex chromosomal rearrangements [90]. Although aberrations of chromosome 1 leading to trisomy/tetrasomy 1q are the most frequent abnormalities reported, no specific karyotypic changes have been detected. A recent comparative genomic hybridization (CGH) study revealed more complex chromosomal imbalances in hormone-independent, type II ECs than in hormone-related, type I carcinomas. Moreover, the same

study showed increased karyotypic complexity in relation to tumor grade in type I ECs, supporting the idea that tumor-phenotype is altered with accumulation of genomic imbalances [91]. Recently the same group compared DNA ploidy status with karyotypic and comparative genomic hybridization data on 51 ECs [92]. They found that gains of material from chromosomes 8 and 7 might be specifically correlated with DNA aneuploidy in ECs, The most frequent CGH findings in the DNA diploid tumors were gains of 1q and of parts of chromosome 10, suggesting that such gains could be an early event in ECs. In contrast, aberrations on chromosome 7 and 8 were rare in DNA diploid tumors but frequent in DNA aneuploid tumors. Of interest, none of the typical genes known to be altered in ECs, like *PTEN*, *KRAS*, and *CTNNB1*, are located on chromosomes 7 and 8.

Carcinosarcomas (Malignant Mixed Müllerian Tumors)

Molecular Abnormalities

A number of immunohistochemical and molecular studies support the monoclonal nature of uterine carcinosarcomas (CSs) [93]. For example, immunohistochemical studies have documented the expression of epithelial markers in the sarcomatous components of a large proportion of tumors. Moreover, X-chromosomal inactivation assays, mutational analyses, and LOH studies have all shown the carcinomatous and sarcomatous elements to share common genetic alterations [94, 95]. Provisional TCGA data (Tables 1 and 2) demonstrated a molecular profile more similar to serous than endometrioid carcinomas. However, a recent study including 17 uterine and 5 ovarian carcinosarcomas demonstrated that molecular alterations typical of EEC are also found in CSs. Thus, 40 % and 32 % of these tumors carried *PTEN* and *ARID1A* mutations respectively [96]. Mutations in *PIK3CA* are also frequent in uterine carcinosarcoma [96, 97]. More than 70 % of uterine CSs overexpressed EGFR, mainly in the sarcomatous component, but only about 20 % of them also carried *EGFR* amplification [97].

Uterine carcinosarcomas differ in their mutational profile from Müllerian adenosarcomas. These mixed tumors with a benign epithelial component frequently carry alterations of the PIK3CA/AKT/PTEN pathway (72 %), but infrequent *TP53* mutations (17 %). In addition, the most frequent amplified genes in Müllerian adenosarcomas are *CDK4* and *MDM2* (28 %), and *MYBL1* (22 %) if sarcomatous overgrowth is present [98].

Cytogenetic Abnormalities

It has been reported that karyotypes and CGH profiles of CSs are very similar to uterine carcinomas and different from sarcomas. Genetic imbalance profiles of CSs frequently mirror those of the epithelial component present in the tumor [91].

Uterine Sarcomas

Leiomyosarcoma

Molecular Abnormalities

Several series, including a relatively low number of tumors, have reported a 13–37 % frequency of *TP53* mutations in these tumors [99–101]. *PTEN* mutational status has been studied in uterine sarcomas since these tumors frequently show loss of heterozygosity of 10q23.3 [102]; however, the incidence of *PTEN* mutations seems to be very low since only one mutation has been detected among 33 leiomyosarcomas analyzed in two different series [103, 104].

MED12 exon 2 mutations are frequently identified in uterine leiomyomas [105] but are mutually exclusive with uterine leiomyomas carrying a 12q14-15 (*HMGA2*) rearrangement [106]. However, *MED12* mutation is a less frequently oncogenetic mechanism in uterine leiomyosarcoma and in extrauterine leiomyomas [107–109].

Table 3 Most frequent/characteristic cytogenetic abnormalities in mesenchymal uterine tumors

Tumor type	Characteristic cytogenetic abnormality	Molecular event
Endometrial stromal tumor	*t*(7;17)(p15;q21)	*JAZF1–JJAZ1* fusion
	t(6;7)(p21;p15)	*JAZF1–PHF1* fusion
	6p21 translocations	*PHF1* rearrangement
	t(10;17)(q22;p13)	*YWHAE- NUTM2AB* fusion
	t(X;22) (p11;q13)	*ZC3H7B- BCOR* fusion
	t(X ;17) (p11.2;q21.33)	*MBTD1-CXorf67* fusion
Intravenous leiomyomatosis	der(14)*t*(12;14)(q15;q24)	*HMGA2* rearrangement
		22q deletion
Leiomyosarcoma	complex karyotype	

Cytogenetic Abnormalities

Most reported karyotypes in uterine leiomyosarcomas are complex without consistent numerical and structural aberrations (Table 3). In addition, CGH studies have confirmed a high frequency of gains and losses of several chromosomal regions [110]. This large number of nonrandom aberrations suggests that increased genetic instability plays a role in the origin of these tumors. The majority of molecular and cytogenetic data do not support an origin of leiomyosarcoma from its benign counterpart. A study of a series of smooth muscle tumors showed different gene expression profiles for leiomyosarcoma and leiomyoma [111]. However, MED12 mutation has been recently detected in a small subgroup of uterine leiomyosarcomas and in extrauterine leiomyomas [108, 109].

The transcriptional profile of a small group of cellular leiomyomas with a specific chromosome abnormality, e.g., del(1)(p11p36), is more similar to that seen in leiomyosarcoma than to profiles of normal myometrium and conventional leiomyoma [112]. A recent study demonstrated that 1p deletion occurs in approximately 25 % of cellular leiomyomas potentially associated with clinicopathologic features that are present with uterine sarcomas [113].

Several uterine smooth muscle proliferations, i.e., intravenous leiomyomatosis (IVL), disseminated peritoneal leiomyomatosis (DPL), and benign metastasizing leiomyoma (BML) are unusual because of their "aggressive" clinical behavior but they do not belong to the malignant category of smooth muscle tumors. However, several cytogenetic alterations have been detected that are worth discussing. A nonrandom pathogenetic event in IVL is the finding of a karyotype showing a der(14)*t*(12;14)(q15;q24) in addition to two normal copies of chromosome 12 (Table 3). The presence of *t*(12;14) in IVL, which is the most frequent abnormality in conventional leiomyomas, suggests a pathogenetic relationship between these two smooth muscle proliferations [114]. Recently an aCGH study in 9 IVL, reveled several losses and gains, including large deletions of 22q chromosome region in 6 [115]. Deletion at 22q is also a frequent aberration observed in BML by karyotyping [116] and aCGH [117]. Finally, DPL, a rare condition presenting with multiple benign smooth muscle proliferations throughout omental and peritoneal surfaces, has been suggested to have a common pathogenesis with conventional leiomyoma because of similar chromosome aberrations involving chromosomes 1, 3, 7, and 12 [118, 119].

Low-Grade Endometrial Stromal Sarcoma

Molecular Abnormalities

No mutations in *TP53*, *PTEN*, *KRAS*, or *CTNNB1* have been described in low-grade endometrial stromal sarcomas (LG-ESS); however, nuclear β-catenin expression is seen in up to 40 % of these tumors [120]. This immunohistochemical pattern might be related to the downregulation of SFRP4, a negative modulator of the Wnt pathway [121].

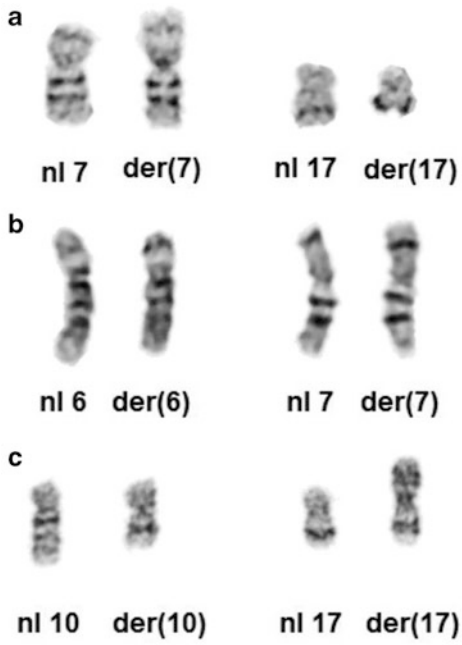

Fig. 6 Partial GTG-banding karyotype showing the most frequent translocations seen in a low-grade ESS: *t*(7;17)(p15;q21) (**a**) and *t*(6;7)(p21;p15) (**b**), and in high-grade ESS *t*(10;17)(q22;p13) (**c**)

Cytogenetic Abnormalities

Cytogenetic abnormalities reported in LG-ESSs demonstrate wide karyotypic heterogeneity. The most common abnormality is a *t*(7;17)(p15;q21) (Fig. 6a) resulting in the fusion of *JAZF1* and *SUZ12(JJAZ1)* genes at 7p15 and 17q21, respectively [122]. *JAZF1-SUZ12* fusion has been detected mostly in endometrial stromal nodules (~65 %), in ~48 % of low-grade (LG)-ESS and in ~12 % of undifferentiated ESSs [123, 124].

The second most frequent abnormality in these tumors is a *t*(6;7) (p21;p15) (Fig. 6b), a so-called variant translocation of the *t*(7;17), because of the involvement of 7p15 and 6p21 instead of the 17q21. [125]. At molecular level, this translocation resulted in a fusion gene between the *PHD* finger protein 1 (*PHF1*) gene, located in chromosome 6, band p21 and the JAZF1 at 7p15. Recently, the same authors expanded our knowledge of the 6p21 rearrangements in ESS. The *PHF1* gene can fuse with *JAZF1* at 7p15, with *EPC1* at 10p11 and *MEAF6* at 1p34 [126]. Moreover, it seems that there is a correlation in ESSs showing sex cord-like differentiation having *PHF1* genetic rearrangement [127].

Two additional translocations have been described in ESSs, a *t*(X;22) (p11;q13) and *t*(X;17) (p11.2;q21.33) associated with a *ZC3H7B-BCOR* fusion and *MBTD1-CXorf67* fusion, respectively [128, 129]. Gene expression profile showed that the *t*(X;17)/*ZC3H7B-BCOR* fusion clustered together with the *t*(7;17)/JAZF1-SUZ12.

Although endometrial stromal tumors are genetically heterogeneous, the different genes involved in stromal nodules and low-grade ESS are functionally related (*PHF1, SUZ12*, *EPC1*, *MBTD1*), being members of the polycomb gene family. Of interest, *ZC3H7B-BCOR*, *MEAF6-PHF1*, and *EPC1-PHF1* fusions were also identify in ossifying fibromyxoid tumors [130] and *JAZ1-PFH1* in an ossifying sarcoma of the heart [131].

High-Grade Endometrial Stromal Sarcomas

Cytogenetic Abnormalities

The most common cytogenetic alteration reported in high-grade ESS is a *t*(10;17)(q22;p13) associated with a *YWHAE-NUTM2AB* (aka FAM22A/B) fusion [132]. Tumors with *YWHAE-NUTM2AB* rearrangements constitute a distinct group of ESS, which is associated with small epithelioid cells, frequent necrosis, and more aggressive clinical behavior compared to JAZF1-LG-ESS but less aggressive than undifferentiated uterine sarcoma [133] (Fig. 6c). Thus, their distinction from undifferentiated uterine sarcoma is important for prognostic and therapeutic purposes, and standardized FISH analysis may be used in this setting [134, 135]. HG-ESSs with *t*(10;17) typically show strong and diffuse nuclear positivity for cyclinD1, Therefore, this can be used as a surrogate screening marker for these tumors [136]. Of interest, the same *t*(10;17)/YWHAE-NUTM2AB has been also reported in clear cell sarcoma, a subgroup of childhood renal tumors [137].

Other Sarcomas

Other sarcomas rarely occur in the uterus, e.g., embryonal rhabdomyosarcoma, primitive neuroectodermal tumor, or liposarcoma among others [138]. Inflammatory myofibroblastic tumors of the female genital tract are rare but characteristically show *ALK* rearrangement [139].

Conclusions

- From a molecular point of view, endometrial cancer is classified into four groups: ultra-mutated, hypermutated, with low mutation frequency and microsatellite stable, and serous-like.
- Ultra-mutated endometrial carcinoma is characterized by mutations in the exonuclease domain of POLE that produces an unusually high mutation rate.
- Tumors with POLE mutations seem to have an excellent prognosis in spite of adverse molecular and pathological features.
- The hypermutated endometrial carcinomas are tumors with microsatellite instability (MSI), most with MLH1 promoter methylation. Immunohistochemistry is a sensitive tool to detect MSI.
- EC is the most common extracolonic tumor in patients with Lynch syndrome.
- There are no differences in grade, recurrence rate, and survival between MSI-positive and -negative EC in most studies.
- Most EECs are MSS EC with low mutation rate. In this group, the most frequently mutated genes are in the PI3K-AKT pathway (*PTEN, PIK3CA, PIK3R1*).
- *CTNNB1* mutations occur more frequently in grade 1 EEC and correlate with immunohistochemical nuclear expression of b-catenin. From a morphologic point of view, nuclear b-catenin accumulation is frequently seen in association with squamous morular metaplasia in EECs.
- *ARID1A* mutations occur in 20–40 % of EEC depending on grade, are more frequent in MSI tumors, and are associated with BAF250A protein expression loss.
- 90 % of EC with extensive somatic copy number alterations and low mutation rates are serous carcinomas, although 10 % of high-grade EEC may have this molecular signature.
- Genomic instability in serous carcinoma is secondary to p53 mutations.
- HER-2 amplification/overexpression is more characteristic of serous carcinomas. However, overexpression of HER-2-neu is not a well-established prognostic marker in EC.
- Molecular-genetic studies support the monoclonal nature of CSs, as they have shown that the carcinomatous and sarcomatous elements share common genetic alterations.
- CSs more frequently have a molecular profile similar to serous carcinomas (TP53 mutations); however, up to 30–40 % have molecular alterations that are more typical of EEC (*PTEN*, *ARID1A*).
- The most common chromosome translocations observed in LG-ESS are *t*(7;17)(p15;q21) associated with *JAZF1-SUZ12* fusion, and translocation involving *PHF1* gene at 6p21, which can frequently fuse with *JAZF1* at 7p15, with *EPC1* at 10p11 and *MEAF6* at 1p34. Rarely, *t*(X;22) (p11;q13) and *t*(X;17) (p11.2;q21.33) associated with a *ZC3H7B-BCOR* fusion and *MBTD1-CXorf67* fusion, respectively, can be also observed.
- High-grade endometrial stromal sarcomas are characterized by the *t*(10;17)(q22;p13) associated with *YWHAE-NUTM2AB*.

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