Chapter 4
Molecular Pathology of Endometrioid Adenocarcinoma

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Introduction

The most common subtype of endometrial adenocarcinoma is endometrioid adenocarcinoma, with prevalence rates of around 80% [1, 2]. According to the Bokhman classification [3], these tumors are generally classified as Type I and tend to be associated with a better prognosis than Type II tumors [1, 2]. Endometrioid endometrial adenocarcinomas generally present at an earlier stage than non-endometrioid tumors and often have lower rates of recurrence [2]. Despite these less aggressive clinical characteristics, a subset of endometrioid carcinomas does behave more aggressively, and recent research has focused on characterizing the genotypic differences that may account for this. Molecular characterization of endometrioid adenocarcinoma can also provide potential therapeutic targets for

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matched targeted therapy trials, as current chemotherapy and radiation therapy approaches to the treatment of advanced/recurrent endometrioid-type endometrial cancer are not optimal.

PI3K/AKT Pathway

Activation of the PI3K/AKT signaling pathway is common in endometrial cancer, with pathway alterations reported to occur in over 80% of endometrioid endometrial cancers [4–6]. *PTEN* alteration is the most common, but other genes in this pathway have been found to be mutated in endometrial cancer as well, including *PIK3CA*, *PIK3R1*, and *PIK3R2* [4, 6, 7]. Additionally, mutations in multiple genes comprising this pathway have been shown to occur concomitantly [4, 8–10]. Survival outcomes have been mixed, but the literature suggests that PI3K pathway mutations may be associated with worse clinical outcomes [8, 11, 12]. Further, a study by Nout et al. showed a worse disease-free survival in endometrioid endometrial carcinomas when mutations within multiple signaling pathways, including the PI3K/AKT pathway, co-occur [8].

PTEN

The phosphatase and tensin homolog (*PTEN*) gene encodes a protein which functions as a tumor suppressor within the PI3K/AKT pathway [13, 14]. Inactivation of the *PTEN* gene is one of the most frequent mutations within this pathway and within endometrioid endometrial cancer in general, with described prevalence rates ranging from 15 to 80% [4, 13, 15–20].

PTEN mutations have been identified in both endometrial hyperplasia and in endometrial cancer and are thought to be an early event in tumorigenesis [21–23]. These mutations have been seen in both sporadic tumors and, to a lesser extent, in tumors associated with Lynch Syndrome [24]. *PTEN* mutations are more common in endometrioid endometrial cancer than in mixed or serous tumors [14, 16–18, 25]. Data regarding the relationship between *PTEN* mutations and microsatellite instability (MSI) status are mixed, with some studies showing higher rates of MSI-high in tumors with *PTEN* mutations, while others show no relationship [5, 25, 26].

However, Djordjevic et al. recently demonstrated that intact PTEN protein expression (and the presence of *PTEN* wild-type gene) was associated with microsatellite-stable (MSS) non-endometrioid endometrial carcinomas, while no such relationship existed in endometrioid endometrial tumors [27]. Approximately 90% of deleterious *PTEN* mutations are associated with immunohistochemical (IHC) loss of PTEN protein [14] (Fig. 4.1). Interestingly, in approximately 40% of endometrial carcinomas, IHC loss of PTEN protein expression is associated with no gene sequence abnormality [14]. This is likely due to the fact that PTEN protein and

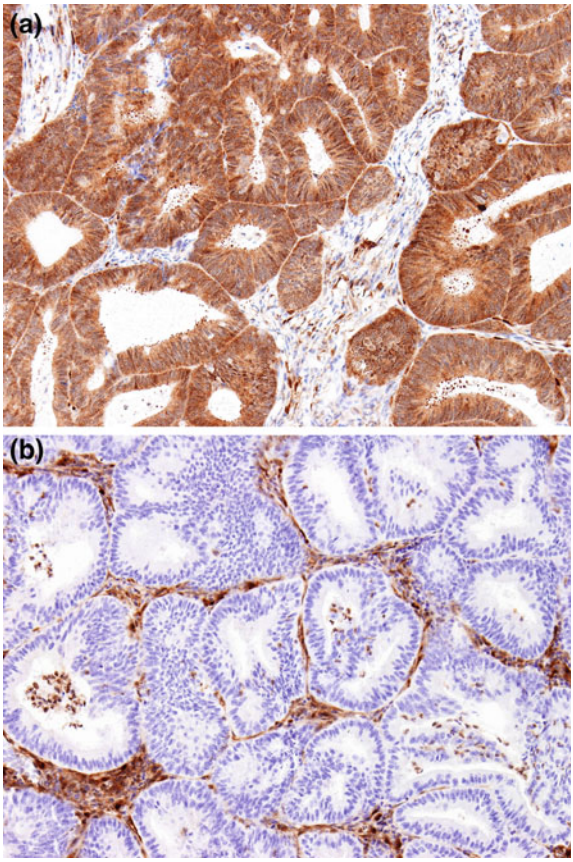


Fig. 4.1 PTEN immunohistochemistry. **a** Endometrial carcinoma with intact positive protein expression of PTEN. No *PTEN* gene mutation was detected by next-generation sequencing. **b** Endometrial carcinoma with *PTEN* gene mutation and associated loss of PTEN protein expression. Note intact expression of PTEN protein in adjacent stromal cells, which acts as an internal positive control

mRNA can be regulated by a variety of different mechanisms independent of gene mutation [28]. Therefore, for clinical purposes, immunohistochemistry may be a preferable method of detecting endometrial carcinomas with loss of PTEN function.

Multiple studies have attempted to characterize the relationship between *PTEN* endometrial cancer mutations and survival outcomes. In a single institution study of 221 endometrial cancer patients, Akiyama-Abe et al. performed IHC staining for PTEN and found loss of protein expression in 25% of tumors. In those with loss of PTEN expression, the authors found a significant association with endometrioid histology and decreased lymphatic–vascular invasion, as well as a significant improvement in overall survival [16]. Interestingly, they did not find any differences in rates of advanced stage at presentation or early grade tumors. Improved outcomes including survival and recurrence rates with *PTEN* mutations have similarly been shown in some, but not all, prior studies [25, 29, 30]. In contrast, a recent study of 187 endometrioid endometrial cancer patients by Westin et al. found that, in aggregate, there was no difference in progression-free survival of patients with IHC-determined loss of PTEN function compared with those tumors that

retained PTEN function. However, on a sub-analysis of stratification by body mass index (BMI), loss of PTEN function in the presence of obesity (BMI ≥ 30) was associated with significantly improved progression-free survival, whereas non-obese patients (BMI <30) were found to have significantly worse progression-free survival in the setting of PTEN loss [31].

PIK3CA

The *PIK3CA* gene encodes the p110-alpha subunit of PI3K, which functions as the catalytic subunit of the protein complex [6, 32]. Mutation prevalence for endometrial cancer has been reported to be between 20 and 36% [4, 10, 11, 32–35], with mutations being more frequent in endometrioid than non-endometrioid tumors [4, 32]. Concurrent *PIK3CA* and *PTEN* mutations in endometrial carcinomas have been found in multiple studies [4, 10, 11]. There are also some data to support higher rates of MSI-high in endometrial tumors with *PIK3CA* mutations [36, 37], though not all studies have found this to be the case [5].

In general, endometrial tumors with *PIK3CA* mutation appear to be more aggressive than those without, with trends toward worse survival outcomes [12, 17, 36]. McIntyre et al. [36] found that *PIK3CA* mutations were associated with worse disease-specific survival in grade 3 endometrioid tumors, but this association did not persist on multivariate analysis and, interestingly, was not present for serous tumors harboring *PIK3CA* mutations. Catasus and colleagues similarly investigated 109 predominantly endometrioid endometrial carcinomas and found increased rates of myometrial invasion and lymphatic–vascular space invasion in association with *PIK3CA* mutations. Interestingly, they showed higher rates of grade 3 tumors as well as increased myometrial invasion or cervical involvement when mutations occurred in exon 20, compared with mutations on exon 9 which were more often associated with early grade tumors and invasion of less than half of the myometrium [11]. These data suggested that, in addition to *PIK3CA* mutations being important for survival outcomes, some *PIK3CA* mutations may be more relevant than others. This mutational diversity phenomenon, the overall tendency toward worse prognosis associated with *PIK3CA* mutations, and the complex nature of the PI3K/AKT pathway may account for some of the reasons why, despite the availability of multiple PI3K/AKT pathway inhibitors, clinical trials have failed to show consistent benefit with the use of PI3K/AKT targeted therapy in endometrial cancer [38].

PIK3R1 and PIK3R2

The *PIK3R1* and *PIK3R2* genes encode the p85-alpha and p85-beta regulatory subunits of PI3K [4, 6], which form a dimer that assists in stabilization of PTEN. A 2011 study by Cheung et al. further characterized the role of *PIK3R1* in

endometrial tumors and described largely for the first time the presence of *PIK3R2* mutation in endometrial cancer [4, 39]. Mutation rates in endometrial carcinoma are 20–43% for *PIK3R1* [4, 40], and 5% for *PIK3R2* [4]. Findings from these studies suggest that *PIK3R1* and *PIK3R2* mutations may lead to activation of the PI3K/AKT pathway and thereby contribute to endometrial cancer tumorigenesis.

ARID1A

The *ARID1A* gene encodes a non-catalytic subunit of the SWI/SNF complex, which aids in chromatin remodeling [41, 42]. Bosse et al. [42] found that 27% of endometrioid endometrial cancers had *ARID1A* mutation and that these mutations were commonly associated with PI3K/AKT pathway mutations. *ARID1A* mutations appear to be more common with MSI-high tumors [37, 42–44], and it has been suggested that *ARID1A* may play a role in epigenetic silencing of *MLH1* [42]. An interesting study by Mao et al. analyzed *ARID1A* mutations in 246 cases ranging from normal endometrium to high-grade endometrial cancer. They found no mutations in normal tissue, areas of clonal but not complete loss within 16% of complex atypical hyperplasia cases, complete loss in 25% of low-grade endometrioid endometrial cancers, and complete loss in 44% of high-grade endometrioid tumors [45]. These results were notable, as they suggested a possible role in tumor progression for *ARID1A* mutations which had not previously been well described. As data are still limited regarding *ARID1A* mutations in endometrial cancer, little is available regarding survival outcomes. While Allo and colleagues found that *ARID1A* mutations do appear to be present within high-grade endometrioid tumors, they were unable to find a difference in progression-free survival within the endometrioid endometrial cancer group [43].

KRAS

The *KRAS* gene encodes the K-Ras protein, which functions along the RAS/MAPK pathway and helps regulate cell division [46]. Prevalence rates of *KRAS* mutation in endometrial cancers have been reported to be between 10 and 30% [4, 47, 48]. Several studies have found similar rates of *KRAS* mutation in endometrial hyperplasias and endometrial cancers, suggesting that *KRAS* mutation may represent an early event during tumorigenesis [47, 49].

KRAS mutations are more frequent among endometrioid and mixed endometrioid histologies, compared to non-endometrioid endometrial cancers [18, 50, 51]. Furthermore, *KRAS* mutation rates are higher in endometrioid tumors showing increasing amounts of mucinous differentiation [52], which may be clinically significant since mucinous differentiation has been associated with lymph node involvement [53]. Some studies have suggested that endometrial cancers with

KRAS mutation tend to be associated with lower endometrioid grade, though others have found no association with grade [50, 51, 54]. Like many other mutations in endometrioid endometrial cancer, *KRAS* mutations are more frequently found in MSI-high tumors than MSS tumors [44]. Interestingly, atypical endometrial hyperplasia with MSI-high exhibits wild-type *KRAS*, suggesting that defects in DNA mismatch repair precede *KRAS* mutation [24, 55].

There are limited data regarding clinical outcomes in endometrioid endometrial cancers with *KRAS* mutation. Birkeland et al. analyzed *KRAS* mutations from 264 primary and 22 metastatic endometrial carcinomas. They found *KRAS* mutations to be more prevalent among grade 1 and 2 tumors, in those with endometrioid histology, and in obese women. There was no association with prognosis, and there were no differences in mutation rates among the primary and metastatic tumors [56]. In contrast, Ito et al. showed that in a cohort of 221 endometrioid endometrial cancers, there was a higher prevalence of *KRAS* mutation among patients older than 60 years of age who had recurrence of their disease or died due to disease [54].

Several studies have also examined for a possible association between tamoxifen use and *KRAS* mutation within the endometrium [46, 57, 58]. A small retrospective study by Turbiner et al. found that women with endometrial cancer who were taking tamoxifen for breast cancer had a higher incidence of *KRAS* mutations. Within the tamoxifen cohort, 16 of the 18 tumors were endometrioid, one was of mixed histology, and one was a clear cell carcinoma [46]. Interestingly, a subsequent study by Tsujioka et al. similarly saw increased *KRAS* mutations in benign polyps within the endometrium of women taking tamoxifen, but found that after cessation of tamoxifen use the *KRAS* mutations were no longer identified [58].

Several studies have suggested that the presence of a *KRAS* mutation may correlate with poorer responses to several targeted therapies, especially those targeting the PI3K/AKT pathways such as mTOR inhibitors [6, 59]. A small in vitro study by Weigelt et al. found an increased resistance to mTOR inhibitors in endometrial cancer cell lines harboring *PIK3CA* and/or *PTEN* mutations with a coexisting *KRAS* mutation, though it did show that a subset of these cells still retained some sensitivity to other forms of PI3K pathway modulation [60]. A recent phase II trial of everolimus in 35 patients with recurrent endometrial cancer showed that none of the patients with a *KRAS* mutation and positive staining for pS6 (a marker of downstream activation of the PI3K/AKT/mTOR pathway) had a prolonged response to the mTOR inhibitor [61]. In contrast, an in vitro and in vivo study of the effects of metformin on endometrial cancer cell lines by Iglesias et al. found increased apoptosis in cells with *KRAS* mutation, as well as lower mean tumor weights. Interestingly, the presence of a *PTEN* mutation had no effect on tumor response to metformin in these cell lines. Metformin's mechanism of action as a potential cancer therapeutic is thought to involve a decrease of tumor growth, and based on these data, it appears that this effect is potentiated in *KRAS* mutant cells. The authors therefore suggested that this may be due to phosphorylation of the activated K-Ras protein by Protein Kinase C, which subsequently leads to its removal from the plasma membrane and, ultimately, to apoptosis of the tumor cell [62].

CTNNB1

The *CTNNB1* gene encodes the protein β-catenin, which functions as a member of the canonical Wnt pathway. In normal endometrium, β-catenin is expressed primarily at the cell membrane of glandular epithelial cells. *CTNNB1* mutation leads to less degradation of β-catenin protein, causing the protein to accumulate in the cytoplasm or translocate to the nucleus (Fig. 4.2), where it subsequently serves as a transcription factor for Myc, cyclin D1, and E-cadherin [63–65]. *CTNNB1* mutations have been discovered in up to 45% of endometrioid endometrial cancers [20, 65–69]. In 2013, The Cancer Genome Atlas (TCGA) reported on a genomic investigation of 373 endometrial carcinomas, which identified frequent mutations in the *CTNNB1* gene, specifically in the subset of endometrioid carcinomas [44]. Interestingly, in this analysis, 52% of the microsatellite-stable (MSS) tumors tested had a mutation in *CTNNB1*. In contrast, tumors with high microsatellite instability showed infrequent *CTNNB1* mutations [44].

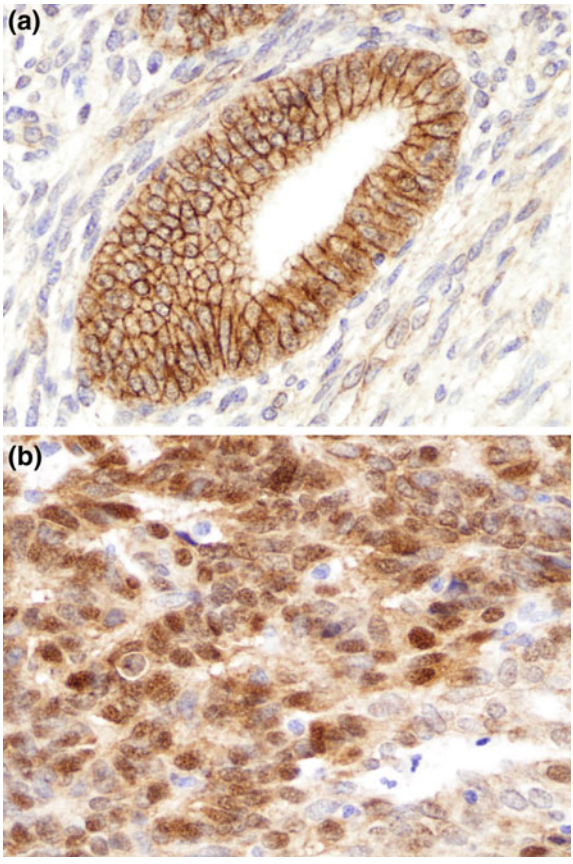


Fig. 4.2 β-catenin immunohistochemistry in normal endometrium (**a**) and endometrial carcinoma with *CTNNB1* gene mutation (**b**). In normal endometrial epithelium, β-catenin protein shows strong, membranous expression, with little-to-no cytoplasmic or nuclear expression. In endometrial carcinomas with *CTNNB1* (encodes β-catenin) mutation, β-catenin protein is inhibited from degradation, allowing translocation from the membrane to the cytoplasm and nucleus. Nuclear expression helps to drive activation of the WNT signaling pathway

Several earlier studies have suggested an association of *CTNNB1* mutations with lower grade and earlier stage endometrial cancers, as well as with endometrioid histology [63, 66, 70–72]. Moreno-Bueno et al. investigated 128 endometrial cancers, including 95 with endometrioid and 33 with non-endometrioid histology. *CTNNB1* mutations were detected in 14.9% of the endometrioid tumors, but in none of the non-endometrioid tumors [66]. Fukuchi et al. analyzed 76 endometrial tumors and found that of the 10 tumors with *CTNNB1* mutations, all except one were well- or moderately differentiated endometrioid carcinomas. Among these tumors, all except one were stage 1 or 2 at the time of diagnosis [63]. Similarly, findings of a predominance of grade 1 or 2 tumors have been reported by several other studies [70–72].

Recent findings suggest that endometrial cancers with beta-catenin mutations may represent a more aggressive subset of early endometrioid endometrial cancers [72–75]. Liu et al. performed consensus clustering of 271 of the endometrioid endometrial cancers used in TCGA, which revealed four distinct clusters of gene signatures. The group designated Cluster 2 represented a subset of low-grade, low-stage tumors with significantly higher frequencies of *CTNNB1* mutations and evidence for activation of the WNT/β-catenin signaling pathway. This group exhibited lower overall survival than even the higher grade and higher stage clusters, and was comprised of a younger, more obese subset of patients [73]. Similarly, Myers et al. performed a case-control analysis of 50 patients with low-grade, stage IA endometrioid endometrial carcinomas in order to further characterize those patients who had a recurrence of their early disease [74]. This study investigated the frequency of three commonly mutated genes in endometrial cancer, including *PIK3CA*, *CTNNB1*, and *KRAS*. They found that *CTNNB1* mutations were more frequent among the 12 patients with recurrent disease than among the 38 patients who did not recur and that there were no differences in rates of *PIK3CA* or *KRAS* mutations. In contrast to Liu et al., however, Myers et al. found the subset of patients with a recurrence to have a lower body mass index (BMI) than those without a recurrence of their disease.

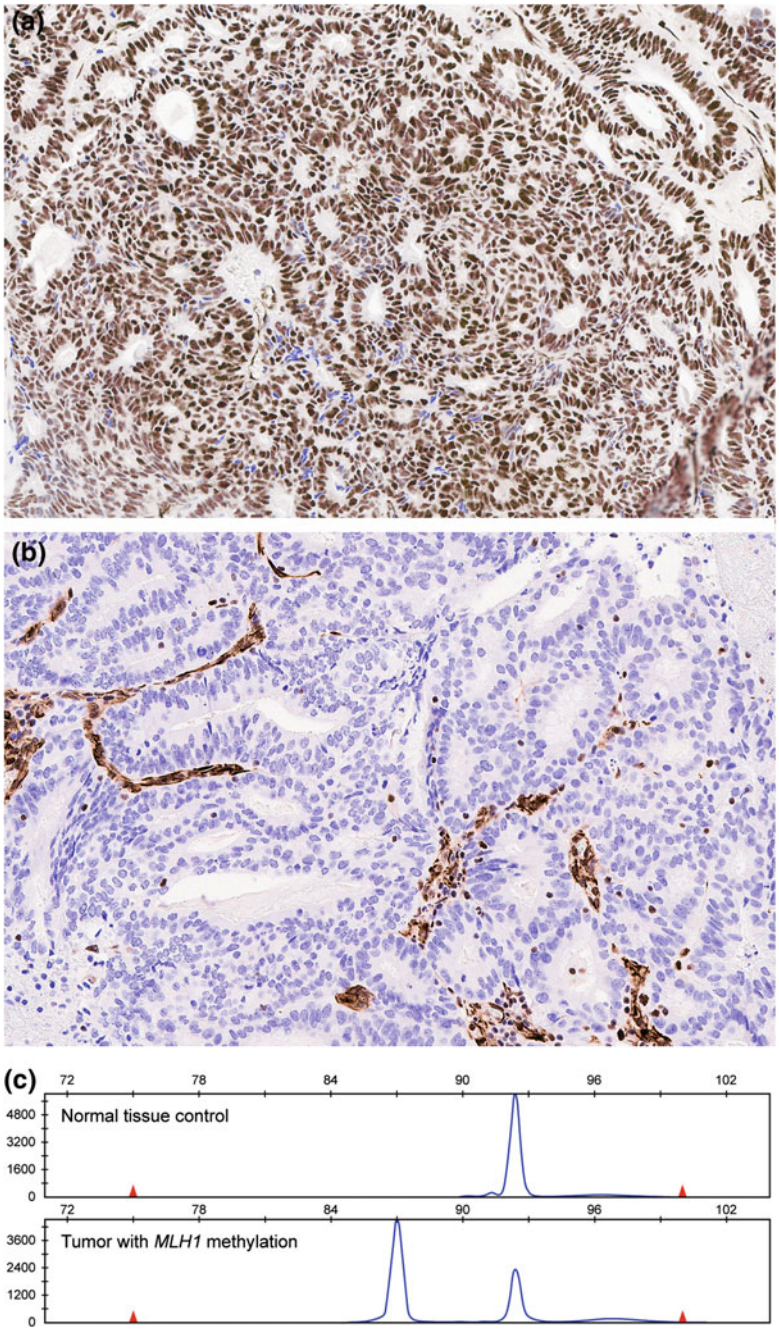
TP53

The *TP53* gene encodes the p53 protein which assists in cell cycle arrest and apoptosis, and its mutation is frequent in numerous cancer types, including endometrial cancer [76, 77]. While prevalence rates are much higher in non-endometrioid than endometrioid endometrial carcinomas [18, 78], the majority of publications evaluating *TP53* mutations in endometrial cancer were done in predominantly endometrioid tumors, and rates of *TP53* mutation have still been reported to be 10–35% [44, 69, 77–83]. Lower grade endometrioid carcinomas may have higher frequencies of concurrent *TP53* and *PTEN* mutations compared with serous carcinomas and grade 3 endometrioid tumors, suggesting that the mechanism for p53-related tumorigenesis is different in endometrioid versus non-endometrioid

tumors [44]. In support of this idea, Kaku et al. found a higher rate of *TP53* mutations in endometrial carcinomas without associated hyperplasia than in those with hyperplasia [84].

As discussed above, data from TCGA suggested that *TP53* mutations tended to cluster within the endometrial tumors showing serous histology and grade 3 endometrioid histology [44]. Other authors have found a similar association between grade 3 tumors and *TP53* mutation [78, 80, 85, 86]. Interestingly, a study by Kuhn et al. found prevalence rates of 30% within a sample of 20 undifferentiated endometrial tumors, 12 of which had both an endometrioid and undifferentiated component. When present, the *TP53* mutations were seen in both the undifferentiated and its corresponding endometrioid components, with the exception of one tumor which only showed a *TP53* mutation in the undifferentiated aspect of the tumor, suggesting a possible role of p53 in tumor progression (Kuhn). While several studies also suggest an association between *TP53* mutation and advanced stage, not all studies have found this to be the case [78, 82, 85, 87]. Similarly, no consensus findings of a relationship between *TP53* mutation and depth of invasion, lymphatic–vascular space invasion, or metastatic disease have been demonstrated [78, 82, 85, 87].

In general, clinical outcomes in patients with endometrioid endometrial cancer harboring *TP53* mutations appear to be worse than in those without *TP53* mutations. Lee et al. examined 131 patients with predominantly endometrioid endometrial cancer and found *TP53* mutation to be an independent prognostic indicator of poor overall survival and disease-free survival [82]. Other studies have shown a similar association with poor overall survival or disease-free survival, though many studies were unable to demonstrate a statistically significant difference in multivariate analysis [78, 80, 85, 88–93]. Reasons for the somewhat heterogeneous findings of these studies may include the wide range of numbers of patients, differences in histologic representation, and variation in methodologies for evaluation of *TP53* mutational status. Several studies have also looked at the effect of *TP53* mutations on outcomes in important subpopulations. For example, a study of 136 endometrial cancer patients by Oreskovic and colleagues found worse overall survival on multivariate analysis in those patients with grade 1 and grade 2, but not grade 3, tumors [94]. There is some evidence that the presence of *TP53* mutation can help impact therapeutic approaches to patients with endometrioid endometrial carcinoma. Saffari et al. found that, in a group of 53 endometrioid endometrial carcinoma patients, *TP53* mutation was associated with worse overall survival on multivariate analysis. In those women with *TP53* mutations who received adjuvant radiation therapy, survival outcomes were similar to wild-type patients with and without radiation treatment, and all three of these subgroups demonstrated better survival than patients with *TP53* mutation-containing tumors who did not receive adjuvant radiotherapy [93].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1200
0
Tumor with MLH1 methylation

◂**Fig. 4.3** *MLH1* methylation associated with MLH1 protein loss by immunohistochemistry. **a** Endometrial carcinoma with retained nuclear expression of MLH1 protein. **b** Endometrial carcinoma with loss of MLH1 protein. Note retained positive expression of MLH1 in adjacent stromal cells. **c** PCR-based *MLH1* promoter methylation analysis. Tumor DNA is analyzed concurrently with DNA from normal tissue control from the same patient. Top tracing, normal tissue with no *MLH1* methylation; bottom tracing, tumor with presence of *MLH1* methylation

Microsatellite Instability

DNA mismatch repair (MMR) is controlled by a family of nuclear proteins, including MLH1, MSH2, MSH6, and PMS2. Defects in MMR can result from germline mutations in the genes encoding these proteins (Lynch Syndrome) or, in sporadic endometrial and colorectal carcinoma, from hypermethylation of the *MLH1* gene promoter. MMR defects are manifested as high levels of microsatellite instability (MSI-high, assessed clinically via a PCR-based assay) and by loss of mismatch repair protein expression in immunohistochemistry-based assays as demonstrated in Fig. 4.3 [95]. Prevalence of MSI-high in endometrial cancer has been reported to be around 15–40% [26, 50, 96–102], with 15–25% being the most common. In most published studies, no distinction is made between germline versus sporadic MMR loss, although it can be inferred that the vast majority of endometrial cancers with defective MMR are sporadic cancers with MLH1 protein loss due to *MLH1* gene methylation.

MMR loss and *MLH1* hypermethylation are thought to be early events during tumorigenesis in endometrial cancer, as hypermethylation patterns have been observed in endometrial hyperplasias [47, 103]. MSI-high is more common among endometrioid carcinomas compared to non-endometrioid tumors, including serous and clear cell carcinomas [27, 50, 101, 104, 105]. The relationship between tumor grade and MSI status is somewhat unclear, as some studies show an association with increasing grade in MSI-high tumors, while others show no association [104, 106–108]. Similarly, evaluating the relationship between MSI status and clinical stage has led to conflicting results, with several studies showing an association of MSI-high tumors with more advanced stage disease, others showing an association with earlier stages, and some studies showing no association with stage [101, 104, 106, 107, 109]. MSI-high tumors have been reported to have an increased risk of lymphatic–vascular space invasion [102, 104], but their relationship with depth of myometrial invasion is not clear [101, 104, 106]. MMR deficiency, particularly MLH1 protein loss and *MLH1* methylation, has been associated with a subset of undifferentiated endometrial carcinoma [110–113]. It is uncertain whether undifferentiated endometrial carcinoma should be considered a subtype of grade 3 endometrioid adenocarcinoma or a non-endometrioid carcinoma. Compared to grade 3 endometrioid adenocarcinoma, undifferentiated carcinomas typically have lower hormone receptor and cytokeratin expression [110] and may have a more aggressive disease course [111, 114, 115].

The impact of MSI-high on survival outcomes in endometrial cancer is similarly unclear, despite a number of different publications examining this issue. Details of several of the larger studies evaluating MMR status and survival outcomes are summarized in Table 4.1. Some authors have identified improved outcomes in MSI-high tumors [97, 98, 101, 102], others found worse outcomes [116, 117], and some have found no association [100, 105, 106, 118]. One large study by Zighelboim et al. analyzed 446 prospectively collected endometrioid endometrial carcinomas [100]. MSI status was determined by PCR, as was *MLH1* methylation status. No differences in overall survival or disease-free survival were observed between MSI-high and microsatellite-stable groups. Similarly, *MLH1* methylation status had no impact on overall or progression-free survival. One of the more recent larger analyses was performed by Ruiz and colleagues, who evaluated 212 endometrioid endometrial tumors. MSI status was evaluated by IHC. They evaluated OS and PFS both within early stage (I and II) and advanced stage (III and IV) and found no differences in survival measures within either subgroup [106]. The reasons for conflicting results between these various publications are unclear. As noted in Table 4.1, MMR deficiency has been measured in a variety of different ways in these studies, which could impact results. Endometrioid and non-endometrioid carcinomas have very different clinical courses and survival outcomes; an impact of MMR on survival may be missed in studies that include both these histologies. Lower grade, early-stage endometrioid carcinomas can recur five or more years following hysterectomy, so studies with shorter follow-up intervals may miss an association with MSI-high. It is also possible that these differences may be due at least in part to underlying differences in other concurrent gene mutations not fully evaluated in these studies.

POLE

As discussed previously, based on the molecular analysis of 373 endometrial carcinomas, TCGA [44] proposed a genomic categorization of endometrial cancer into four groups. “Ultramutated tumors” represent the first category in this classification and consist of tumors with very high mutations rates. All of these tumors harbor mutations in the *POLE* gene, which encodes the catalytic subunit of the DNA polymerase epsilon, which synthesizes the leading strand during DNA replication and also plays a role in the recognition and removal of mispaired nucleotides [119, 120]. Tumors with *POLE* mutations may have as many as a million base substitutions per tumor, particularly of the G:C>T:A form [121]. It has recently been shown that germline exonuclease domain mutations of *POLE* and *POLD1* genes confer a high risk of multiple colorectal adenomas and carcinomas [122]. In addition to endometrial and colorectal cancer, *POLE* mutations have also been reported in lung cancer and melanoma [123, 124]. Their inheritance is dominant, and they have a high penetrance with a variable phenotype.

Table 4.1 Selected studies evaluating survival outcomes in MMR deficient/MSI-high endometrial carcinomas

Study	Tumor histology	Tumor stage	Number of patients	MSI assessment method	Survival result
Maxwell et al., Obstet Gynecol [97]	Endometrioid	All stages	131	PCR (MSI-high when $\geq$2/3 markers were abnormal)	MSI-high had improved OS
Cohn et al., Obstet Gynecol [102]	Endometrioid and non-endometrioid	All stages	294	IHC (MMR deficiency defined as loss of any of the 4 MMR proteins)	MMR loss had worse PFS, no difference in OS
Black et al., J Clin Oncol [101]	Endometrioid and non-endometrioid	All stages	473	PCR (MSI-high if $\geq$2/5 markers with allelic shift)	MSI-high had improved RFS and OS
Zighelboim et al., J Clin Oncol [100]	Endometrioid	All stages	446	PCR (MSI-high if $\geq$2/5 were abnormal; MSI-low if 1/5 abnormal)	MSI-high showed no difference in RFS and OS, also no difference when comparing methylation status
Cote et al., Int J Gynecol Pathol [118]	Endometrioid and non-endometrioid	All stages	76	PCR (MSI-high if $\geq$30% of markers showed instability, MSI-low if <30%)	MSI status not predictive of OS
Nelson et al., Gynecol Oncol [105]	Grade 3 or dedifferentiated endometrioid	All stages (then subanalyses)	102 (64 endometrioid)	IHC (MMR deficiency defined as loss of at least 1 MMR protein)	No difference when all or early stage only included; MMR loss associated with increased risk of disease-specific death in advanced stage endometrial cancer in univariate but not multivariate analysis
Ruiz et al., Gynecol Oncol [106]	Endometrioid	All stages (then subanalyses)	212	IHC (MMR deficiency defined as loss of at least 1 MMR protein)	In both early stage and advanced stage, no association seen between MSI and OS or PFS

The majority of *POLE* mutations in endometrial cancer are sporadic and have been reported to represent 5–7% [44, 121, 125] of endometrial cancers. In endometrial carcinoma, most *POLE* mutations tend to cluster in two hot spots, in exons 9 and 13 [126, 127]. Paradoxically, despite being "ultramutated," these tumors have been associated with a favorable prognosis [44, 126, 128]. This observation has recently been corroborated by a large study, which reported the *POLE* mutant tumors as having approximately one-third the risk of recurrence as that of *POLE* wild-type (predominantly endometrioid in this study) endometrial cancers, and an even lower risk of death [125]. It has been hypothesized that improved prognostic outcome in patients with these tumors may be attributable to the fact that the marked number of base substitutions leads to too many gene alterations, which hinder tumor cell growth and survival.

Endometrial *POLE* mutant tumors have characterized by pure endometrioid histology, mixed histology with endometrioid components, or ambiguous histology [121, 126, 128]. Several studies also reported small numbers of serous endometrial carcinoma with *POLE* mutations, but it is not certain whether the cases underwent a centralized review [121, 129–131]. The majority of endometrioid tumors are of high cytological grade; as many as 84% have been described to have tumor infiltrating lymphocytes [128].

Similar to *POLE* wild-type endometrioid tumors, *POLE* mutants frequently carry *PTEN* (94%), *PIK3CA* (71%), and *ARID1A* (76%) mutations; however, unlike most *POLE* wild-type tumors, the majority of *POLE* mutants are microsatellite stable (65–100%) [44, 121, 126, 128]. It has been suggested that in cases where microsatellite instability and *POLE* mutations coexist, the latter is likely a secondary event [132]. Furthermore, while all eight TCGA *POLE* mutant cases were found to have mutations in at least one mismatch repair gene, only two of these cases were microsatellite instability high, suggesting that some of the mutations are "functionally suboptimal" with respect to their classical mismatch repair gene mutant counterparts [44, 126, 133].

Approximately one-third (35%) of *POLE* mutant endometrial tumors also have *TP53* mutations [121, 126, 128]. Given the good prognostic outcome of the *POLE* mutant group, the clinical significance of these *TP53* mutations is likely different than that of the *TP53* mutations in serous carcinoma/copy number high (as per TCGA classification) tumors. The presence of *TP53* mutation in some *POLE* mutants with histological features other than those of clear-cut endometrioid adenocarcinoma is important to note, as the use of p53 immunohistochemistry may lead to misclassification of these tumors as serous carcinomas.

POLE mutations may be a useful biomarker in order to spare patients with high-grade endometrioid tumors from aggressive post-adjuvant treatments, as the tumors appear to have an indolent course. Currently, the only way to detect *POLE* mutations is by sequencing the *POLE* gene. Therefore, development of surrogate markers to enable their detection would be very important.

The Cancer Genome Atlas

The Cancer Genome Atlas (TCGA) is a National Cancer Institute-funded effort to comprehensively classify, at a genomic level, various types of cancer. Genomic characterization included next-generation sequencing of the whole exome, methylation profiles, miRNA profiling, gene expression analysis, and reverse phase protein lysate array. These data are publicly available for individual investigator analysis.

Endometrial cancer, both serous carcinoma and endometrioid carcinoma, has been characterized by TCGA [44]. These data reaffirmed high rates of PI3K/AKT pathway mutations within the endometrioid subtype and showed significant rates of *CTNNB1*, *KRAS*, and *POLE* mutation as well. Additionally, TCGA described a subset of endometrioid tumors which molecularly appeared to be more similar to type 2 tumors, and the authors therefore postulated that treatment approaches mirroring those used in uterine serous carcinomas may be beneficial in this group.

Re-analysis of the endometrioid group only (271 patients) revealed extraordinary heterogeneity in these tumors [73]. Four transcriptome clusters of endometrioid endometrial carcinoma were identified, as highlighted in Fig. 4.4. Clusters 1 and 2 each consisted mainly of patients with early-stage and grade 1 or 2 tumors. Clusters 3 and 4 primarily comprised patients with grade 3 tumors presenting with stage III or IV disease at the time of hysterectomy. At the transcriptome level, Cluster 1 is the "classic" endometrial cancer, with high expression of *ESR1* and *PGR* (genes encoding estrogen receptor and progesterone receptor). Remarkably, Cluster 2, which had a similar patient profile as Cluster 1, had significantly lower expression

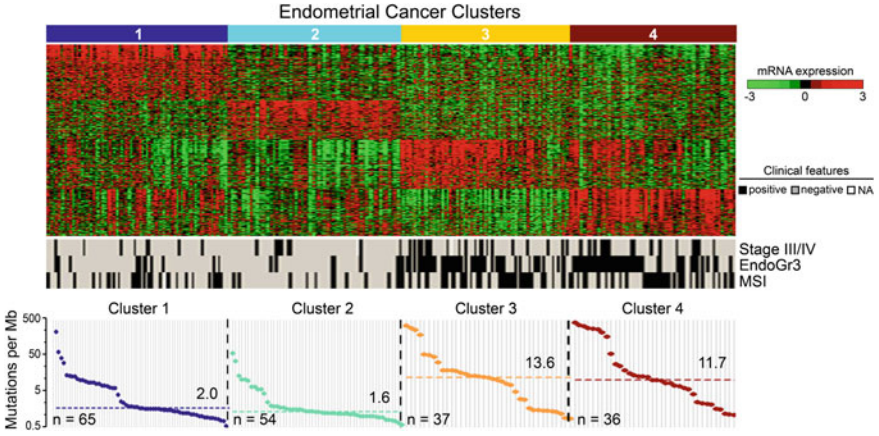

Fig. 4.4 Summary of The Cancer Genome Atlas (*TCGA*) analysis of 271 endometrioid-type endometrial carcinomas. Transcriptome Clusters 1 and 2 are primarily composed of patients with low-grade, early-stage disease, while Clusters 3 and 4 are dominated by patients with grade 3 endometrioid tumors, stages III or IV at the time of diagnosis. Clusters 3 and 4 also had significantly more mutations than tumors in Clusters 1 and 2

of these hormone receptors but higher expression of *WNT5A* and *WNT5B*, genes activated by WNT/β-catenin signaling. Cluster 2 patients were also significantly younger and more obese than patients in the other clusters, including Cluster 1. Unexpectedly, Cluster 2 patients had significantly worse survival than those in Cluster 1. Clusters 3 and 4 displayed similar transcriptome heterogeneity, with Cluster 3 characterized by higher expression of genes associated with cell cycle progression, such as *FOXM1*, *CCNB1*, and *CDC20*. This cluster had the worst survival of the 4 clusters. Cluster 4 had higher expression of genes associated with activation of the immune response, such as *STAT1*, *LCK*, *GIMAP5*, and *GIMAP7*. Although Cluster 4 was mainly composed of patients with high-grade, late-stage disease, these patients had better overall survival than the patients in Cluster 2. Cluster 3 patients had the worst overall survival.

The four clusters also had distinctive mutation spectra. *PTEN* and *PIK3CA* mutations were common in all four clusters. *KRAS* mutations were common in Clusters 1, 3, and 4, but infrequent in Cluster 2. *CTNNB1* mutations were most common in Cluster 2. Clusters 3 and 4 had the majority of the *TP53* mutations. Clusters 3 and 4 had the highest mutations per megabase, significantly higher than the mutational load in Clusters 1 and 2.

The TCGA data highlight the genetic and clinical diversity of the endometrioid histotype. These data also help to refute "dogma" that is commonly taught regarding endometrioid-type endometrial cancer. For example, conventional wisdom holds that young, obese women with endometrial cancer have good prognosis disease that is hormone driven. While certainly their prognosis is better than that for patients diagnosed with endometrial serous carcinoma, the TCGA data highlight above that a substantial subset of patients actually has endometrial cancers driven not by hormones but rather by activation of the WNT/β-catenin signaling pathway. Similarly, the higher grade and advanced stage endometrioid cancers are also heterogeneous. The subset of grade 3 endometrioid tumors with a more "immune-driven" genotype has better outcomes. The challenge to young investigators caring for endometrial cancer patients will be to productively incorporate this substantial TCGA data into rational clinical trials and, ultimately, into routine clinical practice.

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