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Ovarian and endometrial endometrioid carcinomas have distinct *CTNNB1* and *PTEN* mutation profiles

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Abstract

Ovarian endometrioid carcinomas and endometrial endometrioid carcinomas share many histological and molecular alterations. These similarities are likely due to a common endometrial epithelial precursor cell of origin, with most ovarian endometrioid carcinomas arising from endometriosis. To directly compare the mutation profiles of two morphologically similar tumor types, we performed select exon capture sequencing on a panel of genes: *ARID1A, PTEN, PIK3CA, KRAS, CTNNB1, PPP2R1A, TP53.* We found that *PTEN* mutations are more frequent in low-grade endometrial endometrioid carcinomas (67.0%) compared to low-grade ovarian endometrioid carcinomas (16.6%) ($p<0.0001$). In contrast, *CTNNB1* mutations are significantly different in low-grade ovarian endometrioid carcinomas (53.3%) compared to low-grade endometrial endometrioid carcinomas (27.5%) ($p<0.0057$). This difference in *CTNNB1* mutation frequency may be reflective of the distinct microenvironments; the epithelial cells lining an endometriotic cyst within the ovary are exposed to a highly oxidative environment that promotes tumorigenesis. Understanding the distinct mutation patterns found in the PI3K and Wnt pathways of ovarian and endometrial endometrioid carcinomas may provide future opportunities for stratifying patients for targeted therapeutics.

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Disclosure/Conflict of Interest

The authors have no conflict of interest to disclose.

Keywords

Endometrial; ovarian; endometrioid; carcinoma; *PTEN*; *CTNNB1*; mutations

Introduction

Ovarian and endometrial carcinomas comprise the majority of gynecological carcinomas in developed countries [1]. Endometrial carcinoma is the most common with an estimated 287,000 new cases worldwide, however ovarian carcinoma is the most lethal gynecological cancer [2]. Both ovarian and endometrial carcinomas can be subclassified into the histological types; endometrioid, serous, clear cell, mixed, carcinosarcomas and mucinous carcinomas. Endometrial endometrioid carcinomas (EECs) occur most frequently, accounting for 70–80% of cases; these tend to be low-grade with good prognosis and are generally cured by hysterectomy [3, 4]. The remaining 20–30% of endometrial carcinomas are serous, mixed, clear cell carcinomas, and carcinosarcomas. These subtypes are high-risk tumors that tend to have poor outcomes due to metastasis and ineffective treatment options [5]. Endometrial carcinomas have been shown to possess distinct mutations specific to each subtype; EECs are characterized by mutations in *PTEN, ARID1A, PIK3CA, PIK3R1, CTNNB1* and *KRAS* (reviewed in [6]).

Ovarian endometrioid carcinomas (OECs), account for only 10% of ovarian carcinomas. The majority of ovarian carcinomas (70–80%) are high-grade serous carcinomas, which similar to endometrial serous carcinomas are aggressive with poor outcomes. The majority of OECs, like EECs, are also low-grade with good prognosis [7]. Endometrioid carcinomas of the ovary and endometrium are both described to contain *PTEN*, *PIK3CA*, *ARID1A*, *PPP2R1A*, and *CTNNB1* (β-catenin) mutations [8, 9], however the frequency of mutations differs in these tumor types.

There is accumulating evidence that ovarian endometrioid carcinomas arise from transformed endometriosis [10]. Therefore, endometrial and ovarian endometrioid carcinomas evolve from similar precursor endometrial epithelial cells, however the process of molecular pathogenesis is still unclear. The molecular features of OECs and EECs have been characterized in many studies using immunohistochemistry markers and mutational analysis by DNA sequencing (reviewed in [4, 6, 8, 11]). Recently, the ability to sequence multiple genes in the same cases using next-generation sequencing technology has allowed the investigation of the mutational landscapes of these tumor types. There have been studies that infer differences in mutation frequencies in OECs and EECs [12], however to the best of our knowledge, no prior studies have directly compared endometrial and ovarian endometrioid mutation frequencies with a uniform technical approach in a large cohort of tumors. We recently published a study using select exon capture coupled with next-generation sequencing to determine the mutation profiles in the various subtypes of endometrial carcinoma [13]. We have now also performed the same exon capture sequencing method and applied this to a cohort of ovarian endometrioid carcinomas. In this study, we have directly compared the mutation frequencies of seven specific genes in low-grade endometrial endometrioid carcinomas and low-grade ovarian endometrioid carcinomas.

Materials and Methods

Patient Samples

We obtained frozen tumor tissue from 129 endometrial endometrioid carcinomas and 33 ovarian endometrioid carcinomas, for exon capture sequencing, originating from the OvCaRe Tissue Biobank repository, Vancouver, BC, Canada. Patients provided informed

consent, and research ethics was approved, and DNA extracted as previously described [9]. An additional 178 endometrial endometrioid carcinoma tumor DNA samples, with grade information available, were obtained from Washington University, St. Louis, Missouri. All samples from both centers have undergone histological review by gynecological pathologists. All subtype and mutational data for all endometrial carcinomas have been previously reported [13]. An additional 20 ovarian endometrioid carcinoma cases (18 frozen tumors, 2 Formalin-Fixed Paraffin Embedded (FFPE)) were obtained from the OvCaRe Tissue Biobank repository to test *CTNNB1* mutation status, so that a total of 53 OECs were tested for mutation in this gene only, and 33 OEC cases were tested for the other genes in the panel.

Mutation analysis

Genomic DNA (500ng) was used for indexed Illumina library construction, then underwent targeted enrichment using biotinylated RNA capture probes generated from cDNA clones or PCR amplicons representing exons of *ARID1A, PTEN, PIK3CA, KRAS, CTNNB1, PPP2R1A, TP53, BRAF* and *PPP2R5C* and sequenced using the Illumina (GAIIx). All sequencing validation methods and primers used are previously described [13]. The two genes *BRAF* and *PPP2R5C* were excluded from subsequent analysis due to only one mutation found in *BRAF* and no mutations in *PPP2R5C* in the ovarian endometrioid carcinoma cases. To validate the differences in *CTNNB1* mutation frequencies, we re-sequenced the hotspot exon 3 of *CTNNB1* by Sanger sequencing from all 178 low-grade endometrial endometrioid DNA obtained from Washington University. Additional Sanger sequencing validations for the hotspot exon 3 of *CTNNB1* were also performed on 20 low-grade ovarian endometrioid carcinoma cases that were not included in the original select exon capture sequencing set.

Bioinformatics Analysis

Short reads were aligned to the human genome (hg18) using the BWA aligner v0.5.9 [14]. A Random Forest classifier trained on validated SNVs was used to remove false-positive calls [15]. SNVs in the Catalogue of Somatic Mutations in Cancer (COSMIC) [16] were considered to be true positives. All analysis was performed as previously described [13].

Statistical Analysis

Fisher exact tests were used to test the significance of associations between mutations within subtypes. All tests were two-tailed and p-value < 0.05 were considered significant. The Benjamini-Hochberg[17] (B-H) method was used to adjust p-values to account for multiple comparisons (R stats package).

Results

To determine the differences in somatic mutation frequencies between endometrial and ovarian endometrioid carcinomas, we used select gene exon capture sequencing of *ARID1A, PTEN, PIK3CA, KRAS, CTNNB1, PPP2R1A,* and *TP53*. This was performed using 33 cases of ovarian endometrioid and 307 cases of endometrial endometrioid carcinomas (Supplementary Table 1). Comparison of the mutational frequencies of low-grade (grade 1 and 2) endometrial endometrioid carcinomas to low-grade (grade 1 and 2) ovarian endometrioid carcinomas showed a significant difference for *PTEN* (adjusted p<0.0007) and *CTNNB1* (adjusted p=0.02) mutations (Table 1, Figure 1). *PTEN* mutations were found in 67.0% of low-grade EECs, while 16.6% of low-grade OECs harbor *PTEN* mutations. Mutations of *CTNNB1* were identified in 53.3% of OECs, and only 27.5% of EECs. This frequency of *CTNNB1* mutations in EECs is slightly higher than previously published [13] due to additional validations of mutations using Sanger sequencing and select exon capture sequencing. To further verify the high *CTNNB1* mutation frequency found in OECs, we

acquired an additional 20 low-grade OEC cases and tested for hotspot *CTNNB1* mutations using direct Sanger sequencing. We found that 45% (9 of 20) of these cases contained hotspot *CTNNB1* mutations, bringing the overall frequency to 50% (25 of 50) *CTNNB1* mutations in low-grade OECs (Supplementary Table 1). The mutation frequencies of *PIK3CA, ARID1A, PPP2R1A,* and *TP53* are not significantly different between low-grade EECs and OECs. There is a trend towards more *KRAS* mutations in low-grade OEC's (33.3%) compared to low-grade EECs (18.1%), however this is not significant after multiple comparison adjustments (Table 1).

The comparisons of high-grade (grade 3) endometrial endometrioid carcinomas with high-grade ovarian endometrioid carcinomas are limited by the rarity of high-grade OECs (n=3). Each high-grade OEC case harbors different mutations in different genes. Similarly to low-grade EECs, high-grade EECs also have a high frequency of PTEN mutations (87.1%), and a lower frequency of *CTNNB1* mutations (19.4%) (Supplementary Table 2).

Most *CTNNB1* mutations found in both OEC and EEC involve known phospho-acceptor sites. In OECs, 16 of 25 (64%) contain *CTNNB1* mutations that affect serine (S33 and S37) or threonine (T41) amino acid residues, which are phosphorylation targets for glycogen synthase kinase 3-beta (GSK3β). Similarly in low-grade EECs, 43 of 76 (56.6%) of the *CTNNB1* mutations are located at serine (S33, S37, S45) and threonine (T41) residues (Supplementary Table 1). Our analysis of all *CTNNB1* coding sequences also revealed additional somatic mutations outside the hotspot serine/threonine residues.

Discussion

The distinct molecular abnormalities of endometrial and ovarian carcinoma subtypes will be the basis for subtype specific treatment, and may become an essential component of stratified management strategies. Standard treatment options for ovarian and endometrial carcinomas have not yet changed, but a shift towards subtype specific clinical trials highlights the need to better understand the molecular abnormalities and potential therapeutic targets in the different subtypes [18]. The same subtypes at different sites in the gynecological tract, EECs and OECs, have indistinguishable morphology, clinical similarities, and share at least some genetic abnormalities. We have directly compared the mutation frequencies in the same gene set, using the same technology, in low-grade ovarian and endometrial endometrioid carcinomas. This has shown that there are similar mutations patterns but there are also two distinct differences.

Previous literature often refers to endometrial and ovarian endometrioid carcinomas as having similar molecular alterations [19, 20]. The same genes are often mutated, however some studies suggest that OECs and EECs have similar frequencies of *CTNNB1* [12] and *PTEN* mutations [21], but do not directly compare the mutation frequencies of the two tumor types in the same study. In this study, *PTEN* mutations are found at higher frequencies in low-grade EEC compared to low-grade OECs, and *CTNNB1* mutations at higher frequencies in OECs compared to EECs. One limitation of this study, is the small number of low-grade OECs (n=30) analyzed compared to the number of low-grade EECs (n=276), in part a reflection on the relative rarity of OECs. Previous studies have reported low-grade OECs with *CTNNB1* mutation frequencies between 16–54% [22–28], with an average frequency of 40–50%. This range of *CTNNB1* mutation frequency is due to varying methods of detection and limited exon sequencing. *PTEN* mutations are reported in only about 20% of OECs [29, 30]. All these studies also assessed small cohorts of OECs, where the mutation frequencies for both *CTNNB1* and *PTEN* are similar to the frequencies identified in this study.

Recently, our group [13] and others [31–33] have published mutation frequencies in a large number of EECs. *Byron et al.* report a 19% *CTNNB1* mutation frequency from 466 EEC tumors [32]. In this study we report a slightly higher *CTNNB1* mutation frequency of 27.5% from 276 low-grade EECs. This difference is most probably due to sequencing and analysis methods used, as well as the fact that we screened all exons of all genes for mutations. The current study included 25 EEC tumor samples also analyzed by Byron et al.; there were 66 mutations in *CTNNB1* and 46 in *KRAS* reported in the original paper[13], whereas we have now identified 76 *CTNNB1* and 50 *KRAS* mutations, all validated by Sanger sequencing, indicating a low false negative rate in the earlier study. This does not change the conclusions of that study[13] but does indicate that there was a slight underestimation of mutation frequencies. As previously shown *PTEN* mutations are very common in both low-grade (67.0%) and high-grade EECs (87.1%) [13], and is the most frequently mutated gene in this tumor type. In a separate study of 243 EECs, 44% were found to contain *PTEN* mutations, and 16% *CTNNB1* mutations [31], which does confirm the frequency of *CTNNB1* mutations, however the *PTEN* mutation rate is lower than our observations and those reported by others. For example, *Urick et al.* showed that 78% of EEC's have *PTEN* mutations, but did not sequence *CTNNB1* [33]. In conclusion, our study confirms observations from other studies suggesting there are differences in *PTEN* and *CTNNB1* mutation rates in ovarian endometrioid carcinomas and endometrial endometrioid carcinomas. Similarly, ovarian and endometrial serous carcinoma subtypes are morphologically equivalent and were often thought to have similar mutation patterns, with both showing a high frequency of *TP53* mutations. However, more detailed sequencing analyses of these tumor types have revealed mutational profile differences. Mutations of *PPP2R1A* are found at high frequencies in endometrial serous carcinomas but are rarely found in ovarian high-grade serous carcinomas [9, 34]. More recent studies have identified high frequencies of other gene mutations (ie. *FBXW7, CHD4*) [35, 36], in endometrial serous carcinomas but not in ovarian serous carcinomas [37].

The majority of ovarian endometrioid carcinomas are believed to arise from endometriosis [8, 38]. Although ovarian and endometrial endometrioid carcinomas may develop from the same cell type, namely the endometrial epithelial cell, these two tumor types are exposed to different microenvironments that may reflect differences in their mutation spectrums. EECs frequently occur in postmenopausal women with unopposed estrogen [39]; exposure to high estrogen and low progesterone levels have been found to increase proliferation of endometrial cells and thus increases the risk of tumorigenesis [40]. Chromosomal aberrations have been identified at a high frequency in ovarian endometriotic cysts compared to extra-gonadal endometriosis [41]. Endometriosis is thought to occur via retrograde menstruation, where endometrial epithelial cells travel from the uterus through the fallopian tubes and can establish as an endometriotic cyst within the ovary [42]. This creates a unique microenvironment where menstruation-like blood and necrotic tissue is trapped within the cyst, resulting in high concentrations of iron in a confined space [43]. This iron causes oxidative stress, and a hypoxic environment that can in turn lead to DNA damage and mutation accumulation [44, 45]. The mutational analysis of a small number of endometriosis lesions has mostly been confined to a subset of genes, *PTEN*, *CTNNB1* and *KRAS* with only a small number of somatic mutations found in *PTEN* [29], and *KRAS* [46]. It will be important to determine the malignant transformation pathways of endometriosis to OECs, and in so doing, identify the genetic differences between the precursors of endometrial and ovarian endometrioid carcinomas.

Based on the mutation frequencies found in this study, *CTNNB1* mutations in the ovary and *PTEN* mutations in the endometrium are characteristic features of these diseases. Mutations in *CTNNB1* and deregulation of the Wnt pathway are a well-established pathway in cancer signaling first characterized in colorectal cancers [47, 48]. As seen in our study, the majority of

CTNNB1 mutations affect the GSK3β binding sites for β-catenin, which ultimately influences the ability of GSK3β to phosphorylate β-catenin and signal its degradation. Lack of β-catenin phosphorylation results in an accumulation of protein, which then translocates to the nucleus and interacts with the transcription factors TCF/LEF1 (T-cell factor/lymphoid enhancer factor) to induce gene expression of the cell proliferation genes *MYC* and cyclin D1, as well as the inflammatory gene COX2 (reviewed in [49]). β-catenin is also known to play a role in cell adhesion by cytoplasmic interaction with the tumor suppressor E-cadherin at the cell membrane. Ovarian carcinomas with an accumulation of β-catenin in the nucleus, either due to *CTNNB1* mutations or deregulation of other Wnt family member like APC or Axin, is an indicator of good prognosis [23]. Similar to the Wnt pathway, PI3K signaling is also a highly studied cancer pathway, and is one of the most commonly altered pathways [50]. PTEN acts as a negative regulator of the PI3K pathway by dephosphorylating the signaling lipid molecule PIP3 to PIP2; where PIK3CA (p110α) together with PIK3R1 (p85α) acts to phosphorylate PIP2 to PIP3 allowing PI3K signaling to proceed through AKT and mTOR, and thus activation of multiple cell proliferation and cell survival proteins. Mutations in both *PTEN* and *PIK3CA* can act to maintain constitutively activated PI3K signaling (reviewed in [51]). These pathways do not occur linearly, and are interactive within their own signal transduction networks as well as with other pathways, which is shown by convergence on GSK3β in both the PI3K and Wnt pathways (Figure 2). Constitutive activation of AKT by PI3K signaling, by *PTEN* and *PI3K* mutations, leads to the phosphorylation of GSK3β targeting it for degradation, and thus allowing free β-catenin to translocate to the nucleus [52]. Similarly, *CTNNB1* mutations can lead to elevated β-catenin activity by abolishing GSK3β's ability to negatively regulate β-catenin, which accumulates in the nucleus. There are however many other gene regulation events that can result through the activation of PI3K/AKT/mTOR pathway that are not activated through the Wnt pathway. Therefore, in OECs, it may be beneficial to target both the Wnt pathway, to inhibit β-catenin and the PI3K pathway when *PI3KCA* is mutated. In EECs, *PTEN* and *PIK3CA* are both frequently mutated, likely leading to the up-regulation of PI3K signaling, thus targeting the PI3K pathway may be of benefit. Additionally, there is no mutual exclusivity of *CTNNB1* and *PTEN* or *PIK3CA* mutations in EECs and OECs (Figure 1), indicating that they are not functionally equivalent, therefore when both pathways are mutated; simultaneously targeting the PI3K and Wnt pathways may be appropriate. Careful consideration will be needed when deciding which molecules to target in one or multiple pathways, as well as the specific cellular context.

Ovarian and endometrial endometrioid carcinomas share obvious histogenic connections and are morphologically similar, however there are genomic differences, as shown by *CTNNB1* and *PTEN* mutation frequencies. The occurrence of these mutations may reflect different environmental niches during oncogenesis, and ultimately point toward different routes of distinct targeted therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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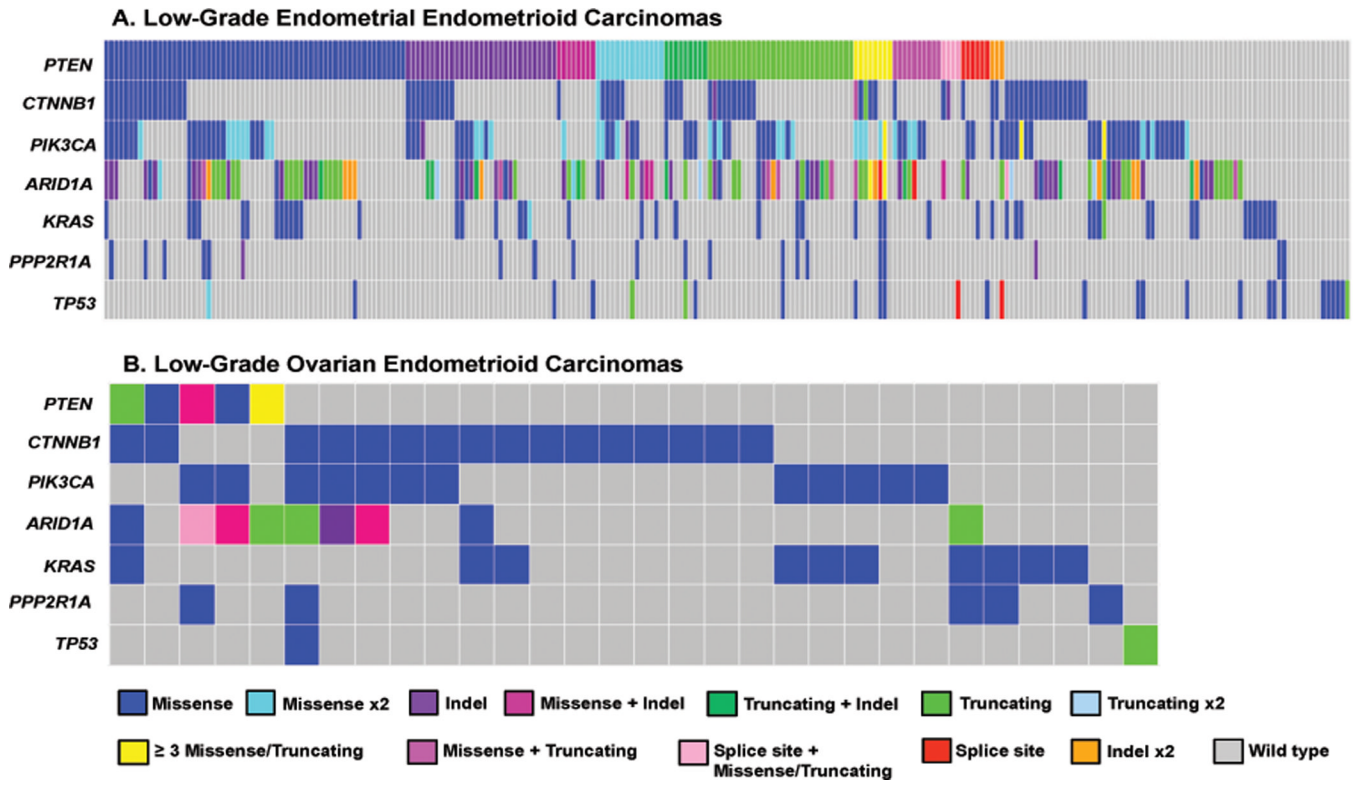

Figure 1. Low-grade ovarian and endometrial endometrioid mutation profiles

A. Low-grade endometrial endometrioid carcinomas (EEC) including grade 1 and 2 (n=276). **B**. Low-grade ovarian endometrioid carcinomas (OEC) including grade 1 and 2 (n=30). Individual columns designate one tumor case, and rows indicate genes. All colored boxes specify a genetic alteration such as missense, truncating, indels, splice site mutations and combinations of these mutations. Grey boxes indicate no mutation were identified by sequencing. These colors are specifically shown in the color legend.

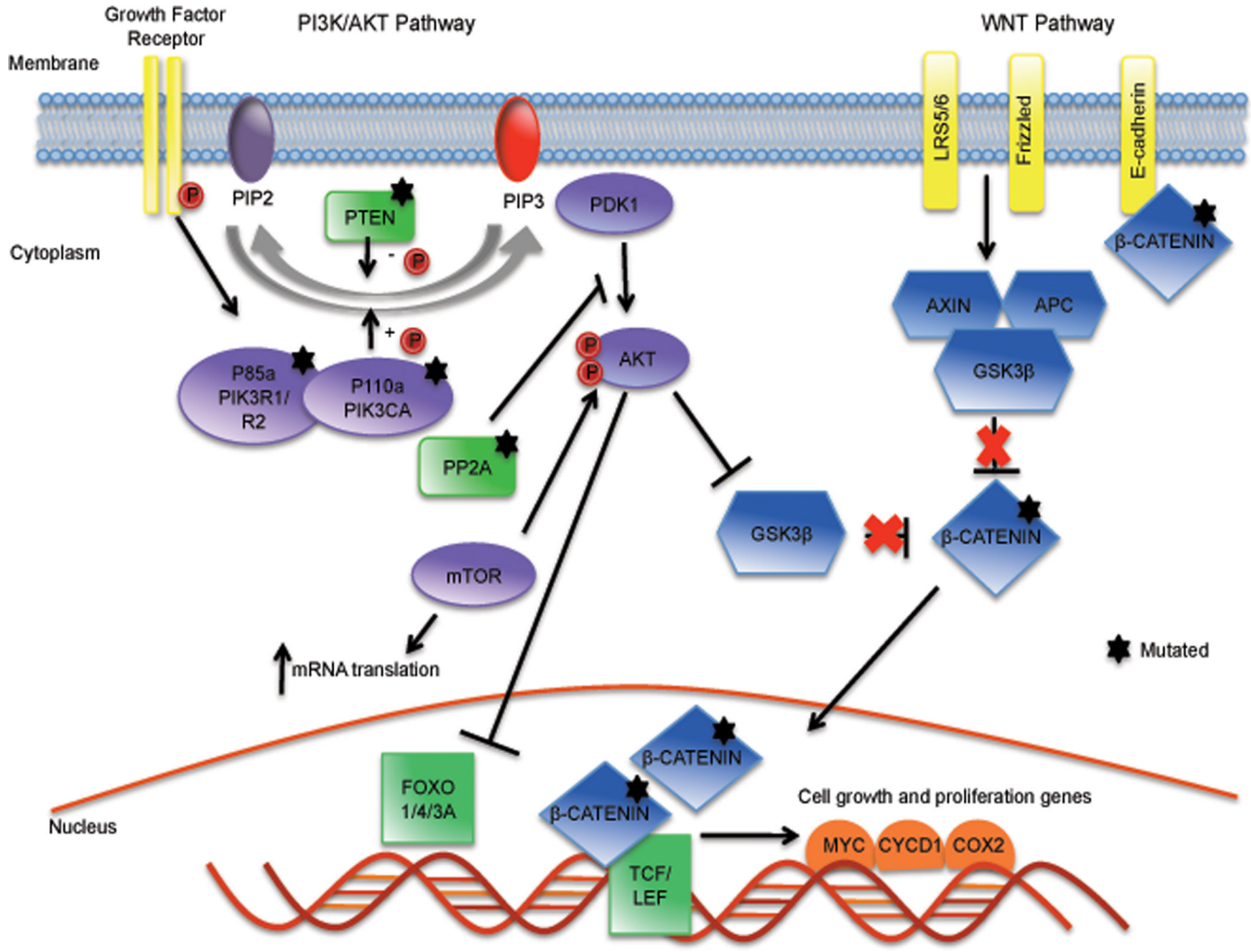

Figure 2. PI3K/AKT and WNT signaling pathways

Theses two signaling pathways show convergence on GSK3β and β-catenin. Genetic alterations caused by mutations in both pathways can result in the transcription of cell growth and proliferation genes. Mutations are indicated by black stars and are found in both ovarian and endometrial endometrioid tumors.

Table 1

Comparison of mutation frequencies in low-grade ovarian endometrioid carcinomas and low-grade endometrial endometrioid carcinomas.

	Low-Grade Ovarian Endometrioid (Grade 1 and 2) (n=30)	Low-Grade Endometrial Endometrioid (Grade 1 and 2) (n=276)	Fisher Exact Test (p-value)	Adjusted p-value*
PTEN	5 (16.6%)	185 (67.0%)	**1.08e-07**	**0.001**
PIK3CA	12 (40.0%)	107 (38.7%)+	1	1
ARID1A	9 (30.0%)	129 (46.7%)	0.086	0.120
KRAS	10 (33.3%)	50 (18.1%)+	0.055	0.120
CTNNB1	16 (53.3%)	76 (27.5%)+	**0.006**	**0.020**
PPP2R1A	5 (16.6%)	19 (6.9%)	0.071	0.120
TP53	2 (6.6%)	28 (10.1%)	1	1

* p-values are adjusted using the B-H method

+ additional mutations have been verified by Sanger sequencing post original publication.