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Review

Perspectives on targeting the phosphatidylinositol 3-kinase pathway for personalized medicine in endometrial and ovarian cancers

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ABSTRACT

Endometrial and ovarian cancers show similar genetic and pathological backgrounds. In particular, high frequencies of activating mutations in the phosphatidylinositol 3-kinase (PI3K) pathway, including mutations in *PIK3CA* and *PTEN*, are found in both estrogen-dependent endometrial cancer (type I endometrioid carcinomas) and ovarian clear cell and endometrioid carcinomas. In this review, we focus on the PI3K pathway as a potential molecular target for personalized therapies in endometrial and ovarian cancers. We found that targeting the PI3K/mammalian target of rapamycin (mTOR) pathway produced anti-tumor effects in endometrial cancer upon suppression of the PI3K pathway. The presence of *KRAS* mutations may be a marker for resistance to the inhibition of the PI3K/mTOR pathway. However, the combination of a PI3K/mTOR pathway inhibitor and a MAPK pathway inhibitor, such as a MEK inhibitor, has been shown to suppress cell proliferation synergistically in certain endometrial cancers. In addition, PI3K/mTOR pathway inhibition sensitized endometrial cancer cells to ionizing radiation and produced anti-tumor effects in ovarian clear cell carcinomas in both *in vitro* and *in vivo* studies. Moreover, inhibition reduced the phosphorylation levels of MDM2 (a negative regulator of TP53), stabilized TP53, and induced TP53-mediated apoptosis. The activation of TP53 was associated with increased phosphorylation of TP53 on Ser-46, and its downstream target genes, such as *TP53AIP1*. These findings demonstrate that targeting of the PI3K pathway in both endometrial and ovarian clear cell carcinomas warrants further investigation, including in clinical trials.

1. Introduction

The pathological and genetic similarities between endometrial and ovarian carcinomas have recently received much attention. Endometrial cancer is generally classified into two types: type I includes endometrial endometrioid carcinomas (EC), which are moderately- or well-differentiated and display estrogen dependence; and type II are poorly differentiated (mainly serous or clear cell) carcinomas (or carcinosarcomas) without estrogen dependence [1]. In type I endometrial carcinomas, the phosphatidylinositol 3-kinase (PI3K) pathway is frequently activated by various genetic alterations, including mutations in *PTEN* (34–64% of cancers), *PIK3CA* (25–53%), *AKT1* (2%), and *PIK3R1* (33%) [2–11]. Genetic alterations in the RAS/mitogen-activated protein kinase (MAPK) pathway are also common in type I endometrial carcinomas, including mutations in *KRAS* (20%) and copy number loss of *NF1*, a negative regulator of RAS (13%) [11–15].

Recently, a novel classification for ovarian carcinomas has been proposed, dividing these into type I and type II according to their genetic characteristics [16–18]. High-grade serous carcinoma (HG-SC) is the most common histological type of ovarian carcinomas, with *TP53* mutations in greater than 95% of cases, and it has been proposed that these carcinomas be classified as type II tumors [18–20]. Indeed, the high frequency of *TP53* mutations, *BRCA1*/*BRCA2* mutations (germline or somatic), hypermethylation of *BRCA1* promoter, *RB1* mutations, and chromosomal instability, which characterize HG-SC, are rarely observed in endometrioid (EC), clear cell (CCC), mucinous (MC), and low-grade serous carcinomas (LG-SC). Therefore, it has been proposed that these histological subtypes be classified as type I tumors (Table 1) [18]. Instead, activating mutations in the PI3K pathway and the RAS/MAPK pathway are common in type I ovarian carcinomas (Table 1)

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Table 1
Comparison of a proposed classification between endometrial and ovarian carcinomas.

	Type I		Type II	
	Endometrial	Ovarian	Endometrial	Ovarian
Histological features	Endometiroid carcinoma (Grade 1/2: well-moderate differentiated)	Endometiroid carcinoma (well–moderate differentiated) Clear cell carcinoma Mucinous Carcinoma Low-grade Serous Carcinoma	Seous carcinoma (High-grade) Endometrioid carcinoma (Grade 3:poorly differentiated) Clear cell carcinoma Carcinosarcoma	High-grade Seous carcinoma Endometrioid carcinoma (poorly differentiated) Carcinosarcoma
Genetic features (genes with frequent mutations)	*PTEN* *PIK3CA* *ARID1A* *PIK3R1* *KRAS*	*ARID1A* (E, C) *PIK3CA* (E, C) *KRAS* (M, L-S) *B-RAF* (L-S)	*TP53* Chromosomal Instability	*TP53* *BRCA1* *BRCA2* *RB1* Chromosomal Instability

E: Endometrioid carcinoma, C: Clear cell carcinoma, M: Mucinous carcinoma, L-S: Low-grade Serous carcinoma.

[21–35]. Mutations in *PIK3CA* were found in 40–50% of CCC and 20% of EC, whereas mutations in *KRAS* were found in 60% and 30% of MC and LG-SC, respectively [21]. Mutations in *B-RAF* were found in 14–33% of LG-SC [21,33].

ARID1A mutations were reported to be frequent in both endometrial and ovarian carcinomas with endometrioid histology (type I) (34% and 30%, respectively), as well as in 46–59% of ovarian CCC (type I) [10,11,36–39], supporting the genetic similarity between type I endometrial and type I ovarian carcinomas. Furthermore, *TP53* mutations in both type I endometrial and ovarian tumors generally occur at a low frequency (<20%) [10,11,21,40,41], compared with 80–96% of type II endometrial and ovarian tumors [11,20,21]. Personalized therapies in either endometrial or ovarian cancer should therefore be developed on basis of these gene-based classifications. Here, we review the anti-tumor effects of molecularly targeted therapies for the PI3K/mTOR (mammalian target of rapamycin) pathway, focusing particularly on type I endometrial EC and ovarian CCC tumors. We include findings from our previously published studies, which were approved by the institutional review board (#G0683) and conducted with the informed consent of the patients who provided clinical samples.

2. Targeting the PI3K/mTOR pathway in endometrial EC

In our previous study, we found that *PIK3CA* mutations were observed in endometrial EC at a frequency of 36% [5]. In addition, the coexistence of mutations in *PIK3CA* and *PTEN* and/or *KRAS* is commonly observed, and we found mutations in *PTEN* and *KRAS* in 70% and 30%, respectively, of *PIK3CA* mutant tumors [5,6]. Overall, 80% of the *PIK3CA* mutant endometrial ECs were found to possess mutations in *PTEN* and/or *KRAS* [5,6]. A variety of small molecule inhibitors that target the PI3K/mTOR pathway are available, including dual PI3K/mTOR inhibitors, PI3K inhibitors that target all four catalytic subunits of PI3K (p110) isoforms, PI3K isoform (alpha, beta, gamma, and delta) selective inhibitors, rapalogs that target mTORC1, mTORC1/mTORC2 inhibitors, and AKT inhibitors [42–46]. Of these, the dual PI3K/mTOR inhibitors, the pan-PI3K inhibitors, and the mTOR inhibitors have been administered the most in clinical trials for various types of cancers, including endometrial cancer [47–53]. Thus, targeting the RAS/PI3K pathway may be a promising therapeutic strategy for type I endometrial EC.

In a previous preclinical study, we evaluated the anti-tumor effects of a dual PI3K/mTOR inhibitor, NVP-BEZ235, in comparison with an mTORC1 inhibitor, everolimus, in 13 endometrial EC cell lines [54]. mTOR signaling was evaluated by determining the levels of p-S6 and p-4E-BP1, and PI3K signaling was evaluated by determining the level of p-AKT. Both drugs suppressed p-S6 and p-4E-BP1 at lower doses (<2.5 nM by everolimus treatment and <12.5 nM by BEZ235 treatment), whereas p-AKT was only suppressed by BEZ235 at higher doses (>50–250 nM). In an MTT assay, everolimus treatment was shown to suppress cell proliferation by 20–50% at a dosage of 10 nM and was more effective than BEZ235 treatment, which suppressed cell proliferation by 0–40% at the same dosage. However, BEZ235 treatment induced dose-dependent growth suppression more robustly, when compared with everolimus treatment, in 12 of the 13 cell lines. In addition, cell cycle arrest at the G1 phase was more robustly induced by treatment with BEZ235 than with everolimus. Sensitivity to BEZ235 was higher in EC cells without *KRAS* mutations or amplifications (half-maximal (50%) inhibitory concentration: IC50 < 100 nM), when compared to those with *KRAS* alterations (IC50 > 100–1000 nM) (n = 4). We also confirmed that BEZ235 was able to suppress tumor growth *in vivo*, using mouse xenograft models inoculated with EC cell lines [54]. These results suggest that the dual PI3K/mTOR inhibitor can be a promising molecular targeted therapy in endometrial EC, and the presence of *KRAS* mutations may predict resistance to this inhibitor. The combination of a PI3K pathway inhibitor and a MAPK pathway inhibitor, such as a MEK inhibitor, has been suggested as an effective strategy for cancer cells with alterations in *RAS* [55–59]. To overcome resistance to a dual PI3K/mTOR inhibitor, we treated these endometrial EC cell lines with the PI3K/mTOR inhibitor, SAR245409, and a MEK inhibitor, pimasertib [60]. The combination of SAR245409 (at 1000 nM) and pimasertib (at 30 nM) synergistically suppressed cell proliferation in six of the 12 endometrial EC cells, with an increase in the number of cells at G1 phase [60]. Thus, targeting both the PI3K/mTOR and the MAPK pathways is a promising strategy for the treatment of endometrial EC with *KRAS* alterations. We have previously reported alterations to the RAS/PI3K pathway in grade 3 endometrial EC and these studies included two cell lines (HEC-50B and HEC-108) derived from grade 3 endometrial EC [11]. Therefore, targeting of this pathway may be a therapeutic option even for grade 3 endometrial carcinomas.

Another possible application for PI3K/mTOR inhibition is in combination with another anti-cancer treatment modality, such as ionizing radiation (IR), since radiation therapy is frequently administered to endometrial EC patients who are identified as high-risk due to clinicopathological factors or local recurrence [61]. The PI3K pathway has been shown to be activated by IR in human cancers [62–64]. We demonstrated that both the PI3K pathway (p-AKT) and the MAPK pathway (p-ERK) are hyperactivated in endometrial EC cell lines, all of which possess one or more PI3K