PTEN Mutations as Predictive Marker for the High-Grade Endometrial Cancer Development in Slovak Women

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Summary

Endometrial carcinoma (ECa) is one of the most common neoplasia of the female genital tract. The phosphatase and tensin *(PTEN)* homolog is the most frequently mutated tumor suppressor gene in endometrial carcinoma. *PTEN* encodes a phosphatase, a key regulatory enzyme involved in a signal transduction pathway that regulates cell growth, migration and apoptosis. The study evaluates an association between the morphological appearance of endometrial hyperplasia and ECa, and the presence of *PTEN* variations, PTEN protein level and intracellular localization. A total of 67 archived formalin-fixed and paraffin-embedded human biopsy tissue specimens with normal proliferative and secretory endometrium, endometrial hyperplasia without atypia and endometrial atypical hyperplasia, endometrioid the grade G1 and G3 and serous subtype of ECa were evaluated by sequencing for the presence of mutations in coding regions of *PTEN* gene of endometrial epithelial cells. The *PTEN* gene expression and intercellular localization of PTEN protein were evaluated immunohistochemically by immunoreactive score (IRS). *PTEN* mutation spectrum in endometrial carcinoma was identified for Slovak population. Twenty-eight non-silent mutations were identified in *PTEN*, twelve of them being novel, not annotated in Catalogue of Somatic Mutations in Cancer. Higher frequency of *PTEN* mutations was observed in serous carcinoma compared to global average. No correlation was observed between samples IRS, PTEN cellular localization and identified mutations. *PTEN* sequencing can be beneficial for patients considering prognosis of disease and sensitivity to treatment.

Keywords

Endometrial hyperplasia • Endometrial carcinoma • *PTEN* • Sequencing • Immunohistochemistry

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Introduction

Endometrial carcinoma (ECa) is one of the most common gynecologic malignancy worldwide [1,2]. Various histological subtypes of ECa (endometrioid, serous, etc.), with divergent tumor genesis pathways, have been identified to date [3–6]. There are two main histological types of endometrial carcinoma that can be distinguished, type I (endometrioid) (arises from hyperplastic endometrium, is estrogen related, less aggressive) and type II (non-endometrioid) (from atrophic endometrium, is unrelated to estrogen, with aggressive phenotype) [3,7].

During the past decade, a number of cancer-causing genes have been analyzed in ECa what led to shifting from the traditional dualistic model (type 1 versus type 2) of ECa to classification based on molecular characteristics. The WHO classification (2020) [8]

subclassified the ECa into four molecular categories: a) those with mutations in the gene encoding the exonuclease domain of the enzyme DNA polymerase epsilon (POLEmut); b) those that are mismatch repair deficient (MMRd); c) those with mutations in the oncogene *TP53* (p53abn); d) those having none of these 3 molecular defects (No Specific Molecular Profile (NSMP)) [8]. *PTEN*, *CTNNB1*, *PIK3CA*, *ARID1A* and *KRAS* mutations have been identified in NSMP ECa. Characterization based on these molecular profiles should lead to better clinical outcomes a prognostication. WHO continues with classification of tumors based on morphology and immunochemical methods as some molecular techniques remain unavailable globally [9].

Decreased expression of *PTEN* (phosphate and tensin homolog) gene has been indicated in various types of human cancer, including glioblastoma, melanoma, prostate cancer, breast cancer, lung cancer, choriocarcinoma, ovary cancer and endometrial cancer [10]. *PTEN* is tumor suppressor gene located on chromosome 10 (10q23). *PTEN* is mutated in about 50 % of ECa cases [11], most frequently in endometrioid ECa. Alterations of *TP53* have been found in > 50 % of all human tumors [12,13], and it is the most frequent genetic alternation in serous endometrial carcinomas [14].

PTEN encodes a phosphatase (PTEN), 47 kDa, a key regulatory enzyme of cellular biological processes [15]. PTEN consists of an N-terminal phosphatase domain and a C2 domain: the phosphatase domain contains the active site, responsible for the enzymatic function of the protein, while the C2 domain binds the membrane phospholipids [16]. The primary target is the lipid second messenger phosphatidylinositol 3,4,5-triphosphate that is involved in the PI3K/Akt/mTOR signaling pathway that regulates cell growth, migration and apoptosis, therefore PTEN is the main negative regulator of this pathway [17]. A different *PTEN* status may affect cancer progression, disease prognosis, and treatment strategies, and result in natural resistance or sensitivity to treatment measures in some patients [18]. In general, it seems that patients with the *PTEN* mutation have a significantly poorer prognosis in survival and disease recurrence, they are prone to distant metastasis [18,19]. The identification of *PTEN* mutations can increase the sensitivity of tumor cells to some of the inhibitors of the PI3K/Akt/mTOR signaling pathway [20].

Another important reason for characterization of *PTEN* mutation status is interaction between PTEN and p53. Many drugs used to treat cancer directly or indirectly damage DNA, an event that triggers p53 activation. The absence or mutation of *PTEN* leads to subsequent loss of the p53 protein and an inability of cells to respond to DNA-damaging agents with an apoptotic response [21]. Hence it could be beneficial for therapeutic interest to also consider mutation status of *PTEN* even if the *TP53* was sequenced in patient.

Conservative therapy by oral progestin can be selected for patients with endometrial atypical hyperplasia or endometrioid carcinoma if they desire to preserve fertility. However, patients with *PTEN* null tumor cells have still significantly higher risk of hysterectomy. These findings highlight the possible importance of *PTEN* analysis, maybe even more helpful than *TP53* sequencing.

The current study follows our previously published work [22], including further samples, mainly normal proliferative, secretory and atrophic endometrium, endometrial hyperplasia without atypia, serous carcinoma and immunohistochemistry analysis of samples. The aim of the study is to evaluate an association between the morphological appearance of endometrial hyperplasia and ECa, and the presence of *PTEN* alterations, quantity of PTEN protein and its intracellular localization. The goal of this study is to help elucidate the importance of *PTEN* in diagnostic and management of ECa.

Material and Methods

Collection and histopathological characterization of specimens

The archived endometrial samples were obtained from the Institute of Pathological Anatomy, Faculty of Medicine Comenius University in Bratislava, University Hospital Bratislava. Formalin-fixed and paraffin-embedded human biopsy hysterectomy and curettage tissue specimens were obtained from uterus of Slovak women.

The specimens were collected between 1997 and 2011. A total of 67 samples were classified by light microscope (NikonEclipse E 400, optical microscope, Tokyo, Japan). Microscopical objects were captured microphotografically (NikonCoolpix 990, digital camera, Tokyo, Japan). Findings were evaluated semi-quantitatively as follow. The result of the basic dying with hematoxylin and eosine were considered as positive, if the nucleus of the cells dyed blue, cytoplasm and connective tissue dyed pink and muscles dyed red.

The procedures of the study received ethics approval from the Ethics Committee of Faculty of Medicine, Comenius University in Bratislava, Slovak

Republic, responsible for the human experimentation. Date of approval is July 9, 2007.

Immunohistochemical analysis

For the immunohistochemical staining Dako EnVision™ FLEX system K8010 (Dako, Glostrup, Denmark) and Dako Autostainer Plus S3400 (Dako, Glostrup, Denmark) were used according to the manufacturer instructions. Monoclonal Mouse Anti Human PTEN M362729 (Agilent, CA, USA) antibody specifically binding to the last 100 C-terminal amino acids of PTEN was used for detection of PTEN protein in the samples.

The evaluation of the intensity of dyeing was performed as follow: 0 (negative), 1 (weakly positive), 2 (mediumly positive), 3 (strongly positive). The quantity of stained cells was rated on a scale: 0 (no stained cells), 1 (1-10 %), 2 (11-50 %), 3 (51-80 %), 4 (>80 %).

When multiple sections of the same sample were examined, each histological preparation was evaluated separately and then averaged to a single value. Immunoreactive scores (IRS) were processed as the result of the values of intensity and the quantity of dyeing. A total score of 8-12 was considered as strong immunoreactivity, 4-7 was moderate, 1-3 weak, and 0 negative.

PTEN sequence analysis

Genomic DNA from all samples was isolated from microdissected sections of biopsy tissue specimens by QIAampR Micro Kit (Qiagen Manchester Ltd., Manchester, UK). All nine exons of *PTEN* were amplified separately. The sequences of primers used for the amplification of exons are listed in supplement 1.

PCR amplifications were performed in 50-µl reaction volumes containing 150-200 ng of genomic DNA, 25 mM $MgCl_2$ (Roche, Germany), 10mM each of dGTP, dATP, dTTP a dCTP), 0.5 µM of each primer (Sigma-Genosys, Lamda Life, Slovakia), and 5 unit of FastStar Taq DNA Polymerase (Roche, Germany), 2.5 µl of buffer without Mg^{2+} for Taq DNA polymerase (Roche, Germany) and nuclease free water to a total volume of 50 µl.

After the denaturing step at 95 °C for 10 minutes, 40 cycles of denaturation at 94 °C for 15 s, annealing for 20 s, and elongation at 72 °C for 30 s were performed, followed by final elongation at 72 °C for 10 minutes. Annealing temperatures were as follows: 65.1 °C for exon 1, 45.3 °C for exon 2, 50.8 °C for exons 3, 5, 7, and 9, 44.9 °C for exon 4, 56.1 °C for exon 6, and 53.4 °C for exon 8. In the case of samples with highly damaged DNA (where the standard protocol did not work) we used GoTaq G2 Hot Start Colorless Master Mix (Promega, Madison, USA) and decreased temperatures of annealing.

PCR fragments were purified by ExoSAP-It PCR Product Clean Up (Affymetrix, California, USA) as described by the manufacturer and prepared for automated sequencing analysis using BigDye Terminator v. 1.1 Cycle Sequencing Kit (Applied Biosystems, California, USA). Before analyses of sequencing products by ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, California, USA) samples were purified by ExTerminator kit (Ecoli, Bratislava, Slovakia) as described by the manufacturer. The individual sequences were compared against the reported genomic sequence of PTEN using Chromas ver. 2.33. (Technelysium Pty Ltd.).

Statistical analysis

The statistical analysis of the results was carried out using Fisher's exact test and χ^2 test of independence performed using IBM SPSS Statistics software ver.25. *P* values < 0.05 were considered statistically significant. Furthermore, logistic regression was used to calculate the odds ratio.

Results

Histopathological characterization of specimens

A total of 67 samples were classified by light microscope. The physiological samples included endometrium in proliferative phase (PE, 5x) and secretory phase (SE, 5x); atrophic endometrium (AE, 5x), the pathological samples included endometrial hyperplasia without atypia (EH, 10x), endometrial atypical hyperplasia (EAH, 7x), endometrioid ECa with histological differentiation grade G1 (ECG1, 20x) and G3 (ECG3, 5x); and serous ECa (SC, 10x).

PTEN sequence analysis evaluation

Overall summary of *PTEN* mutation analysis with respect to severity of disease is presented on Figure 1a, whereas only non-silent mutations were included in statistical evaluation. Interestingly, the proportion of patients with *PTEN* mutations in N-domain increases with severity of diagnosis in both branches (PE / SE - EH / EAH - ECG ($p<0.05$) and PE / SE – SC (statistically nonsignificant)), in samples from healthy patients, there is no case of an N-domain mutation (Fig. 1b).

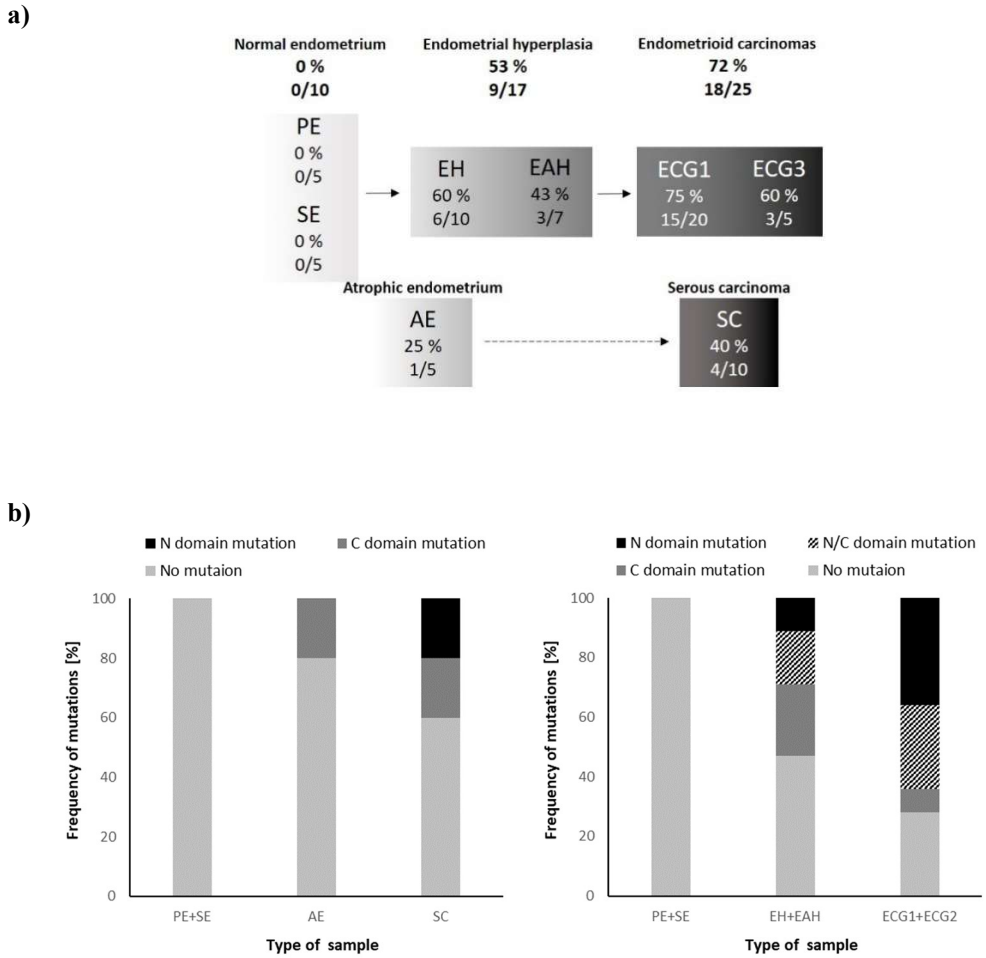

Fig. 1. *PTEN* mutation occurrence in endometrial samples of Slovak women (**a**) and domain localization of mutation in regard to severity of diagnosis (**b**). PE – proliferative endometrium, SE – secretory endometrium, AE – atrophic endometrium, EH – endometrial hyperplasia without atypia, EAH –endometrial atypical hyperplasia, ECG1/3 – endometrioid carcinoma, grade 1 or 3, SC – serous carcinoma

Of the 50 mutations, 32 (64.0 %) were clustered in exon 5 and 8; 17 (34.0 %) mutations were in exon 5 and 15 (30.0 %) in exon 8. Of the 50 mutations, 26 (52.0 %) were frameshift mutations, and the remaining 24 (48.0 %) were single base substitutions. All 26 frameshift mutations were predicted to create new stop codons and produce truncated protein products. Of the 24 single base substitution mutations, 4 (16.7 %) were nonsense mutations resulting in new stop codons, 20 (83.0 %) were missense mutations resulting in single amino acid substitution. Of the 15 samples of grade G1 endometrioid carcinoma which contained mutations, 7 cases harbored more than one mutation (one specimen harbored 2 mutations in one exon). Of the 5 samples of grade G3 endometrioid carcinoma, 3 samples contained mutation, 1 case harbored more than one mutation.

We identified three hotspots, codon 323, 130 and 117. Frequencies of mutations in different histologic diagnoses are presented in Table 1.

In summary, we detected 37 different mutations, nine of them were silent (supplement 2). Seventeen of them were annotated in COSMIC (Catalogue of Somatic Mutations in Cancer) as pathogenic variants, except two with unknown impact. Twelve non-silent mutations were not annotated in COSMIC.

Consequences of PTEN mutation on PTEN localization

Immunohistochemical analysis was performed only on 4 x PE, 4 x SE, 4 x AE, 6 x EH, 6 x EAH, 11 x ECG1, 2 x ECG3, and 6 x SC samples. The rest samples were excluded from the evaluation, as the dyeing of their stroma, which serves as the positive control of correct staining method, was not successful. Unsuccessful stroma staining was a purely methodological problem as samples with unstained stroma occurred among the all histological categories (χ^2 test).

Table 1. The most common hotspots in in endometrial samples of Slovak women

	Frequency				
	Hyperplasia	**ECG1**	**ECG3**	**SC**	**Total**
Deletion in codon 323 with stop effect	3/17 (17.6 %)	8/20 (40.0 %)	1/5 (20.0 %)	0/10 (0.0 %)	12/50 (24.0 %)
*Mutations in codon 130**	1/17 (5.9 %)	6/20 (30.0 %)	1/5 (20.0 %)	1/10 (10.0 %)	9/50 (18.0 %)
Insertion in codon 117 with stop effect	1/17 (5.9 %)	3/20 (15.0 %)	0/5 (0.0 %)	0/10 (0.0 %)	4/50 (8.0 %)

ECG1/3 – endometrioid carcinoma, grade 1 or 3, SC – serous carcinoma. *Arg to Leu (4x), Arg to Gly, Arg to Pro, Arg to Gln, Del

As the used monoclonal human anti PTEN antibody binds specifically to last 100 C-end amino acids (Figure 2). Therefore, it was studied if non-silent mutations in mentioned domain (codons 303 - 403) or mutations wherever before the mentioned domain leading to premature stop codon formation have effect on PTEN binding ability of antibody. The studied effect was not statistically significant (Fischer exact test, p-value 1.0000).

The samples examined immunohistochemically (43 samples) were categorized as follows: samples without mutation or harboring silent mutation (25 samples), samples harboring missense mutation (7 samples), and samples harboring frameshift mutation or mutation that results in premature stop codon (11 samples). All the mutations present in the immunohistochemically evaluated samples, were heterozygous.

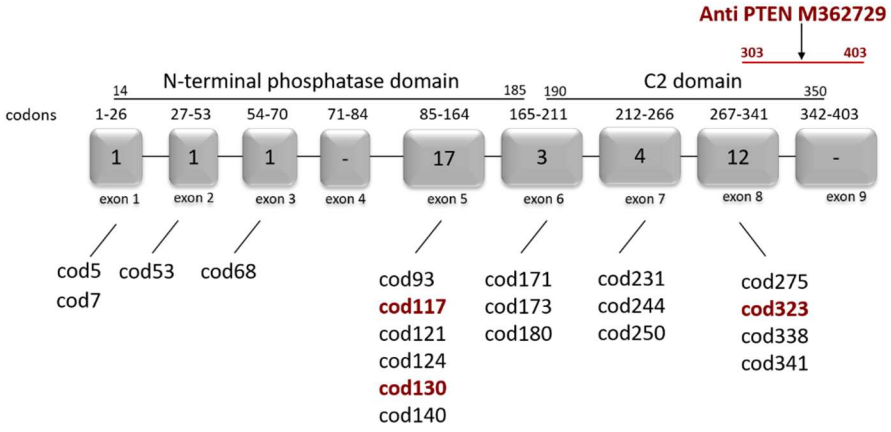

Fig. 2. *PTEN* protein domains arrangement and most frequent mutations´ localization. Red codons – codons containing the most common mutations in population of Slovak women [22], codons 303 – 403 – Anti PTEN M362729 binding domain

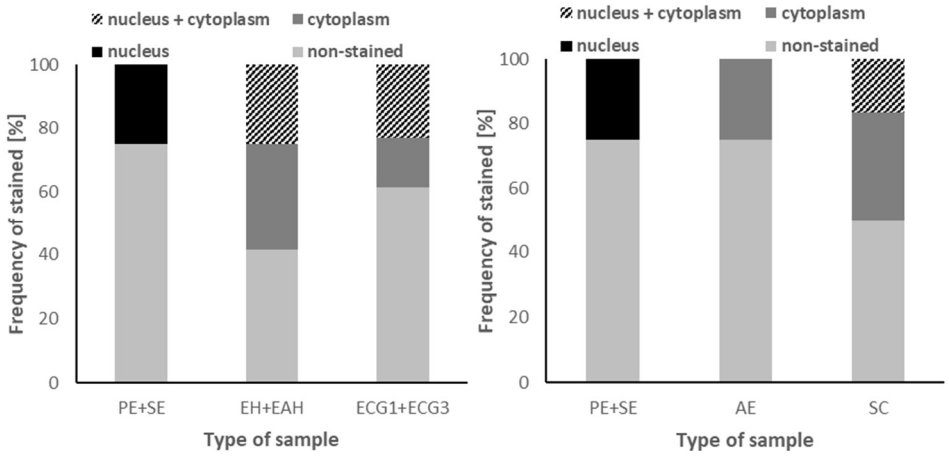

Fig. 3. Localization of the accumulated PTEN in the cell in regard to severity of diagnosis. PE – proliferative endometrium, SE – secretory endometrium, AE – atrophic endometrium, EH – endometrial hyperplasia without atypia, EAH – endometrial atypical hyperplasia, ECG1/3 – endometrioid carcinoma, grade 1 or 3, SC – serous carcinoma

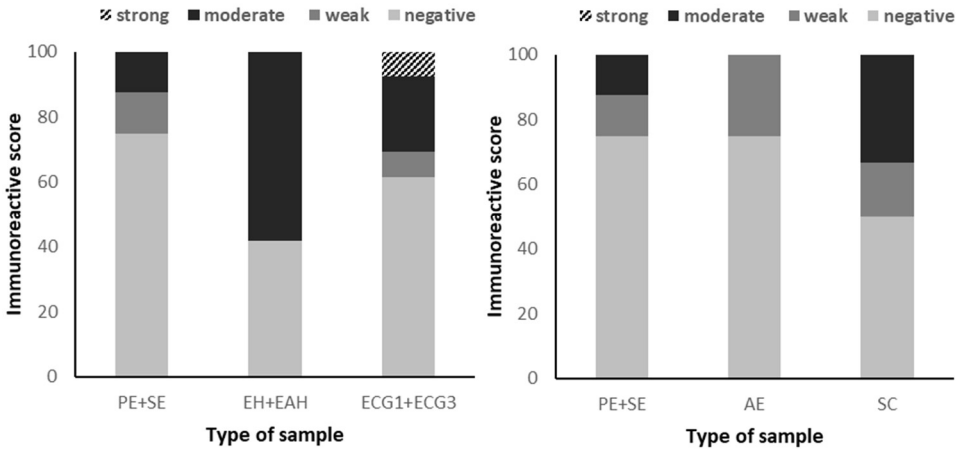

Fig. 4. Immunoreactive score in regard to severity of diagnosis. PE – proliferative endometrium, SE – secretory endometrium, AE – atrophic endometrium, EH – endometrial hyperplasia without atypia, EAH – endometrial atypical hyperplasia, ECG1/3 – endometrioid carcinoma, grade 1 or 3, SC – serous carcinoma

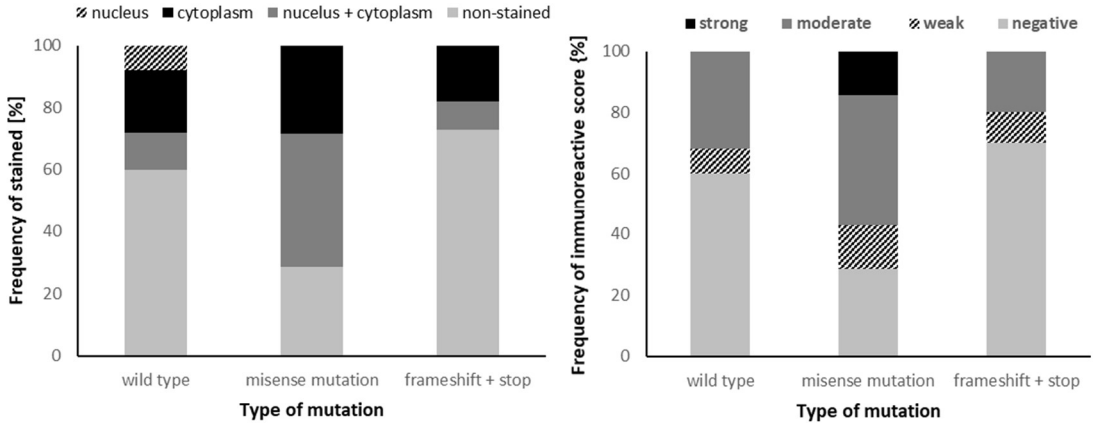

Fig. 5. Localization of the accumulated PTEN in the cell (**a**) and immunoreactive score (**b**) in regard to type of mutation

Most samples (58.1 %) subjected to immunohistochemical analysis for the presence and localization of PTEN protein did not yield either the nucleus staining or the cytoplasm staining. Exclusive nucleus staining occurred only in two samples, both represented proliferative endometrium without any mutation. The remaining samples of proliferative and secretory endometrium did not yield neither nucleus nor cytoplasm staining. While in samples of endometrial hyperplasia and carcinoma tissue, nucleus staining occurred concurrently with cytoplasmic staining at a frequency approximately the same as nucleus staining in healthy tissue, nucleus staining was completely absent in atrophic endometrium tissue (Fig. 3). We observed lower IRS in normal and atrophic endometrial samples (Fig. 4). However, we did not observe a correlation between either the localization of PTEN in the cell and the severity of the diagnosis (Fig. 3), nor the increasing IRS and the severity of the diagnosis (Fig. 4).

Samples harboring missense mutations were more frequently stained (Fig. 5a). IRS of samples harboring missense mutations are higher as well (Fig. 5b). Only one sample shown strong IRS, concretely sample harboring a missense mutation.

Discussion

Cancer Genome Atlas Research Network identified four distinct molecular categories of ECa – ultramutated, hypermutated, copy number low and copy number high (mainly serous EC). *PTEN* mutations were found in 94 %, 88 %, 77 % and 15 % respectively of specimens [23]. We identified *PTEN* mutations in 72 % of endometrioid cancer samples. Surprisingly we detected

higher frequency, 40 %, of non-silent *PTEN* mutations in serous carcinomas that we expected, that suggests populational diversity in the frequency of somatic *PTEN* mutations in serous endometrial carcinomas.

According to COSMIC [11], the most frequent mutations are missense substitutions (43.8 %), followed by frameshift deletions (28.5 %) and nonsense substitutions (23 %) [24]. In our dataset, frameshift mutations creating the stop codon were most common (54.2 %), followed by missense substitutions (39.6 %) and silent substitutions (6.2 %).

The frequency of non-silent *PTEN* mutations was rising according to severity, from normal endometrium of proliferative and secretory phase through precancerous lesions (hyperplasia) to endometroid ECa. Only silent mutations were found in normal endometrium of proliferative and secretory phase. Non-silent mutations were found in all types of hyperplasia what suggests that the damaging of PTEN function represents an early event in the endometrial tumorigenesis what is in line with previous studies [25]. Interestingly, we observed that mutations harming the C2 domain (codons 190 - 350) of PTEN protein are typical in the early phases of the neoplastic process while there is rising frequency of mutations that harm the N-terminal phosphatase domain (codons 14 – 185) as neoplastic processes progress. Only mutations affecting the C2 domain of PTEN were found in endometrial hyperplasias without atypia while in endometrial atypical hyperplasia and endometroid carcinomas, mutations harming the N-terminal phosphatase domain are present with high frequency (most frequent are mutations in exon 5). The possible explanation of this phenomenon is that mutations harming the N-terminal phosphatase domain, which contains the catalytic site, are leading to the complete loss of PTEN phosphatase function leading more rapidly to the development of a more malignant phenotype while mutations harming the C2 domain (most frequent are mutations in exon 8) lead to the partial loss of PTEN function due to disturbing its localization but participation of other genes is indispensable to accelerate the progression of the neoplastic process [16,26]. The frequency of *PTEN* mutations is the highest in endometroid endometrial carcinomas.

We also found one homozygous non-silent mutation in atrophic endometrium. Mutation (COSV64297582) is classified as pathogenic in COSMIC and is localized in C2 domain. This suggest that sometimes pathogenic non-silent *PTEN* mutations are present in physiological tissue but mutation is insufficient to cause endometrial cancer. Hence, it is important to prove the pathogenic character of mutations before their application in the diagnostic process or for therapeutic decisions.

In summary, we identified several novel mutations that are not annotated in COSMIC or they were not detected in ECa in Slovak population.

In physiological tissue, PTEN is localized in cytoplasm and in nucleus where it fills tumor-suppressive functions [27]. The absence of nuclear PTEN is associated with more aggressive cancers [28]. PTEN protein contains several signaling motif for transportation to nucleus and mutations causing the truncation of protein can lead to abnormal localization or the deletion of the C-terminal tail of PTEN decreased the cellular half-life of the protein due to rapid protein degradation [29].

We did not observe significant change of PTEN localization in nucleus in different histological samples or according to the type of mutation. We detected higher percentage of non-stained cells in samples with frameshift mutation but the difference was not significant.

Guidelines recommend PTEN immune-histochemistry for detection of PTEN loss for differentiating between endometrial hyperplasia without atypia and endometrial atypical hyperplasia / endometrioid intraepithelial neoplasia, which is a precancerous lesion [30]. We did not observe significant difference in IRS between endometrial hyperplasia without atypia and endometrial atypical hyperplasia, thus we consider them as one group.

Loss of PTEN is observed in 19 % of benign endometrial tissue, even 55 % in endometrial cancer [31]. Most of our samples (58.1 %), subjected to immunohistochemical analysis for the presence and localization of PTEN protein, did not yield either the nucleus staining or the cytoplasm staining, over 70 % in proliferative endometrium and secretory endometrium which were used as control healthy tissue. However, we did not observe a correlation between either the localization of PTEN in the cell and the severity of the diagnosis, nor the increasing IRS and the severity of the diagnosis even though the frequency of non-silent *PTEN* mutations was rising according to severity of the sample.

The antibody, that was used, binds to last 100 amino acids of C-domain. Not all mutations of *PTEN* and their effect on PTEN protein can be detected as altered protein´s level or localizations. If mutations are causing the truncation of protein or they are changing the amino acids sequence, null staining of PTEN could be observed. The

effect of nonsense mutations truncating the protein will depend of localization and in some cases some of the functions of PTEN can be maintained.

In daily routine, immunostaining of PTEN has been proven to be technically challenging, and its pan-cellular distribution is often difficult and confusing to interpret [32]. Several criteria are used to define PTEN loss. PTEN loss appears barely a moderately accurate marker of precancerous hyperplasia [30]. All of these, including our findings, show that combination of traditional immunohistochemistry and sequencing of *PTEN* would be more beneficial for patients and for evaluation of disorder prognosis.

Findings about impact of PTEN loss are contradictory. The absence or mutation of *PTEN* leads to subsequent loss of the p53 protein and an inability of cells to respond to DNA-damaging agents with an apoptotic response which could contribute to uncontrolled proliferation and progression of tumor [33] or the absence of nuclear PTEN is associated with more aggressive cancers [28]. On other hand tumors with loss of *PTEN* expression may have less aggressive biological behavior compared with tumors without PTEN loss. *PTEN* mutations leading to loss of protein are associated with favorable prognosis and has impact on survival [34,35]. These statements underline the prognostic significance of PTEN aberration.

Inhibitors of mTOR, such as everolimus, temsirolimus have been developed. These inhibitors are targeting PI3-kinase/PTEN/Akt pathway. The tumor sensitivity to treatment can be affected by genetic variability of targeted genes. Patients whose tumor contains particular PTEN protein truncating mutations with no detectable levels of PTEN may benefit more from the therapeutics targeting PI3-kinase/PTEN/Akt pathway [35]. On other hand, the Y68 frameshift mutation of *PTEN*, that leads to the truncation of PTEN protein and to the loss of phosphatase activity, is responsible for resistance to docetaxel treatment [36]. The effect of mutations identified by us on treatment sensitivity is still unknown. Experimental data and first clinical trials suggest that PTEN loss can sensitizes cancer cells to inhibitors of PI3K/Akt/mTOR pathway while agents targeting signaling nodes upstream of PI3K will be less useful [20].

Poly (ADP-ribose) polymerase (PARP) inhibitors have emerged as promising cancer therapeutics especially for tumors with deficient homologous recombination (HR) repair. PTEN plays role in DNA double-strand break repair. Sensitivity of PTEN-deficient endometrioid endometrial cancers to PARP inhibition remain controversial but several studies reported combined effect of PARP and PI3K inhibitors as promising therapeutic approach [37,38].

Based on our findings it is not possible to say that loss of PTEN, changed PTEN quantity or specific mutation are predictive marker of endometrial cancer or progression of endometroid hyperplasia into endometrial cancer. Low level of reproducibility is typical for diagnosis of certain histotypes of endometrial tumors what can lead to inappropriate clinical management. Molecular categorization would improve accuracy of diagnosis and prognostication. Not all molecular methods and extensive complex genetic testing are available for every clinical institution and that's why is beneficial to identified concrete genes and concrete variants that can affect therapy and management of disorder.

Importance of *PTEN* sequencing also emerge in therapies and resistance to it. Based on sequencing would be possible to pinpoint patients with higher or lower benefits from treatment but this would not be doable without thorough identification of *PTEN* mutations in specific histological stages of endometrium.

Conclusions

In our study, we observed considerable genetic and immunological heterogeneity of the endometrial samples in individual histopathological categories without any trend in regard to severity of diagnosis. In our work we detected twelve novel mutations in *PTEN* in Slovak population. This suggests the importance of the genetic variability characterization of ECa, as genetic background affects the course and progression of endometrial cancer, as well as the sensitivity of tumors to therapeutics. Therefore, patients should be included based on the molecular profile of the tumor rather than on the basis of the histopathological character in clinical trials focusing on the effectiveness of treatment.

Conflict of Interest

There is no conflict of interest.

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