

PTEN – clinical significance in colorectal cancer

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ABSTRACT

Phosphatase and tensin homolog deleted on chromosome ten (*PTEN*) is a human suppressor gene. Its protein product is a bispecific phosphatase playing the complex role in the cell cycle regulating processes and apoptosis by the mechanism of signal transduction into the cell via tyrosine kinase B signaling pathway (PI3K/Akt/mTOR). Reduction or loss of *PTEN* function is implicated in the pathogenesis of many malignancies, including colorectal cancer. A gradual decrease in the function of *PTEN* in the sequence of transformations: normal tissue–polyp–adenocarcinoma – disseminated cancer was indicated. The relation between the *PTEN* loss and the higher clinical severity of colorectal cancer was observed, i.e. higher TNM status and higher tendency to form metastases, leading in some of the studies to shortened patients survival during the observation period. The potential predictive value of the *PTEN* function loss for the EGFR-targeted therapy in patients with advanced colorectal cancer is the subject of controversy. The potential application of *PTEN* assessment in clinical practice as a prognostic and/or predictive factor requires further well-designed prospective studies on larger patient population, using the unified methodology.

The aim of the study is to summarize the current knowledge on the role of *PTEN* gene and PTEN protein in the pathogenesis of colorectal cancer and the role of *PTEN* in clinical practice.

KEY WORDS: PTEN, colorectal cancer, EGFR-targeted therapy

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INTRODUCTION

In Poland, colorectal cancer (CRC) is the 2nd common cause of cancer-related deaths in males and the 3rd one in females in 2013. In 2013, CRC was detected in 15,899 people [1]. The forecasts for 2015 indicate an increase in the incidence to 18,286 per year [2].

The aetiology of CRC is multifactorial and the oncogenesis is a multistep process. Studies conducted in experimental models allowed Fearon and Vogelstein to formulate the concept of neoplastic transformation in 1990, whereby the CRC development involves an alternating sequence from normal epithelium to adenoma, and thereafter a gradual increase of lesion size and invasiveness, finally effecting in cancer development [3]. An important role in adenoma – carcinoma transformation sequence is played by molecular processes such as DNA repair defects, genomic instability, aberrations and mutations resulting in suppressor factors inactivation (e.g. *APC*, *p53*, *TGF-α*) or activation of proto-oncogenes including *RAS*, *BRAF* and *PTEN*. The epigenetic processes are also attributed to have an impact on tumorigenesis.

PTEN (phosphatase and tensin homolog deleted on chromosome ten), previously described as *MMAC1* (mutated in multiple advanced cancers or *TEP-1* – *TGFβ*-regulated and epithelial cell-enriched phosphatase) is a suppressor gene located on the long arm of chromosome 10 (10q23.31). The *PTEN* gene protein product has got the enzymatic functions exhibiting the lipid phosphatase and serine, threonine and tyrosine phosphatase activity and participates in the regulation of many cellular metabolic processes.

PTEN is the second most common human gene undergoing somatic mutations (after the *TP53*) [4]. Loss of *PTEN* expression may occur as a result of various genomic changes, including somatic mutations, chromosomal deletion or epigenetic changes such as promoter region hypermethylation [5, 6].

Decrease or loss of *PTEN* function are described in the course of many medical condition as well as malignant tumours. Germline mutations of *PTEN* gene underlie some rare autosomal dominant diseases: the Cowden syndrome, Bannayan–Riley–Ruvalcaba syndrome and Proteus syndrome, which are known as *PTEN* hamartoma tumor syndromes (PHTS) [7]. In turn, the somatic mutations, deletions or epigenetic changes of the *PTEN* gene are involved in the pathogenesis of many malignant tumours: CRC [5–7], stomach cancer [8], pancreatic cancer [9], hepatocellular carcinoma [10], thyroid cancer [11], prostate [12],

breast [13] and ovarian cancer [14], endometrial cancer [15], lung cancer [16, 17], renal cell carcinoma [18], melanoma [19] and glioblastoma [20]. Data obtained from studies on human tumor cell lines and animal models indicate that the effects of *PTEN* inactivation are specific for the tissue in which the lesion occurs [21].

PTEN – THE MECHANISMS OF ACTION

The lipid phosphatase activity is the best known *PTEN* protein property and determines its tumour suppressor function. *PTEN* is currently the only known lipid phosphatase acting as the phosphatidylinositol 3-kinase (PI3K) agonist [6]. *PTEN* protein catalyses the disconnection of the 3'-phosphate residue from the phosphatidylinositol (3,4,5)-trisphosphate (PIP₃), leading to the inactivation of the PI3K-dependent signalling pathway.

PI3K occurs in physiological conditions in resting cells in S phase as an inactive form. PIP₃ serves as a second messenger in the into-the-cell signalling transduction process via tyrosine kinase B (PI3K/Akt/mTOR) pathway. This way, it determines the response of the cells to stimulation by growth factors (i.a. by epithelial/epidermal growth factor receptor [EGFR], cytokines and hormones [including insulin]) and activates the Akt-1 kinase. In actively dividing cells, the PIP₃ level is high and the Akt kinase complex is activated by phosphorylation. The initiation of apoptosis is associated with the decrease of PIP₃ levels and Akt kinase dephosphorylation under high *PTEN* concentration [13, 22]. The functional *PTEN* deficit leads to the PI3K activation and continuous Akt kinase pathway stimulation, resulting in the promotion of carcinogenesis processes, such as apoptosis inhibition, cellular growth independent from growth factors, lack of response to growth inhibitors, unlimited division potential of cells, genomic instability, angiogenesis stimulation, cell migration, surrounding tissue invasion and metastasis [6, 23].

On the other hand, *PTEN* protein also exhibits poor phosphatase properties to some proteins, including the *PTEN* protein and growth factors receptors [23]. *PTEN* affects the cell functions also in mechanisms that are partially independent of its phosphatase activity.

PTEN function enables the maintenance of normal tissues architecture by affecting the intercellular connections stabilisation, cell polarity and migration [24]. The inactivation of *PTEN* in combination with agents in tumour micro-environment, such as TNF-α, may increase the CRC invasiveness [25].

THE PTEN EXPRESSION AND THE STAGE OF CARCINOGENESIS

There is considerable evidence indicating a gradual reduction of PTEN expression in the sequence of transformations: normal tissue–polyp–adenocarcinoma–distant metastases [5, 26–28].

Different results were obtained by Colakoglu et al., indicating more frequent lack of PTEN expression (referred to as “0” by immunohistochemistry, IHC) in colonic adenomatous polyps vs. colorectal cancer specimens (40% vs. 5.3%; $p < 0.0001$). The authors hypothesized that a decrease in PTEN function may play a particular role in the early stages of neoplastic transformation. There was no difference in the PTEN expression intensity between polyps appearing synchronously with CRC and polyps occurring as the single colonic lesion [29].

On the other hand, Hsu et al. described a significantly higher reduction of PTEN expression in the cell nuclei of colonic adenomas and adenocarcinomas compared to the normal mucosa [preserved PTEN activity, defined as present in $> 10\%$ of cells assessed by IHC, was observed in 55.6% (5/9) of adenomas, 53.4% (71/133) of adenocarcinomas and 89.2% (33/37) normal mucosa specimens; $p < 0.05$]. No differences in the PTEN activity in the cytoplasm were observed [30].

PTEN AND CANCER ADVANCEMENT

There are abundant data demonstrating that reduced PTEN expression may be associated with increased severity of the disease, manifested by higher TNM and Dukes' stage [22, 28, 31], greater invasion depth [22], larger tumour size [22, 30], lower histological grade [32, 33], more frequent lymph node involvement [22, 34], blood vessels infiltration [30], distant metastases [33] and, as a consequence, shorter patients survival during follow-up [22]. The relation between the loss of PTEN expression and greater clinical advancement and high CEA antigen concentration was indicated [5]. There are also a few reports suggesting that a PTEN expression reduction may be an independent predictor of the local recurrence when the regression model was used [29].

Studies on experimental models show that PTEN participates in the CRC cell polarity regulation, affecting their migration, invasiveness and metastatic potential. However, it is not the factor that autonomously initiates processes which lead to the formation of metastases [24].

Sawai et al. observed more frequent low PTEN expression in CRC specimens collected from the patients who have developed liver metastases (reduced expression in 75.4% of CRC specimens, loss of expression in metastatic tumour samples), compared to the control group without distant metastases (high PTEN expression preserved in 62.9%, the evaluation was performed by IHC). Low PTEN expression was associated with more advanced TNM stage, higher incidence of lymph node metastasis and local recurrence. A significant difference was noted in 5-year survival of CRC patients with liver metastases depending on whether the PTEN expression occurred or not [34].

PTEN AND EGFR-TARGETED THERAPY OUTCOMES

The results of studies on the predictive value of PTEN function loss in EGFR-targeted therapy of patients with advanced CRC remain ambiguous.

Loupakis et al. noted the relationship between the loss of PTEN expression and poor treatment response in patients with metastatic CRC unresponsive to irinotecan and cetuximab-based therapy. This relationship was observed in the subgroup of patients with metastatic disease, but it was not significant in patients with a primary tumour. The survival analysis showed longer progression-free survival (PFS) in patients with preserved PTEN function, the improved overall survival (OS) was observed only in the subgroup of PTEN-positive patients with wild-type *KRAS* tumours [35, 36].

The meta-analysis including 9 studies involving 634 patients with CRC treated with EGFR-targeted therapy (including 4 studies of patients with primary tumours and patients with metastatic disease and 5 trials involving exclusively patients with primary tumours) showed that loss of PTEN function was associated with poorer treatment response in terms of decreased objective response rate (ORR), shorter PFS and OS. Authors noted lower ORR and shorter PFS in the subgroup of patients with primary tumours compared to patients with metastatic CRC [37].

The authors of another meta-analysis, involving 698 patients who underwent second-line CRC treatment based on cetuximab, observed the relationship between PTEN function loss and decreased ORR and PFS, with no significant effect on OS. In the group of patients with wild type *KRAS*, the preserved PTEN function was not associated with prolonged PFS and OS [38].

Yang et al. conducted a meta-analysis on the predictive role of PTEN function loss, *BRAF* mutation and *PIK3CA* exon 9 and 20 mutations in patients with wild-type *KRAS* metastatic CRC receiving EGFR-targeted therapy. 20 studies were analysed. The presence of *BRAF* mutation was assessed in 13 trials, *PIK3CA* mutations – in 6, PTEN status – in 9. The results of the meta-analysis may suggest higher individual predictive value of *BRAF* and *PIK3CA* exon 20 mutations, compared with the PTEN function loss. However, the *BRAF* and *PIK3CA* exon 20 mutations frequency was relatively low in the analysed population (respectively in 10.9% and 2.3% of patients; wherein the loss of PTEN function was reported 32.1%). Some patients who only had one of these mutations (especially *BRAF* mutation or loss of PTEN) were still noted to benefit from the targeted therapy. According to the authors, significantly better predictive significance could be achieved by the simultaneous assessment of the three biomarkers [36, 39].

The problem of using the PTEN status assessment in making therapeutic decisions has been raised in the current guidelines for the biomarkers testing in CRC by the Spanish Society of Pathology and the Spanish Society of Medical Oncology published in 2015. According to the recommendations, at the present state of knowledge, the PTEN status should not be routinely assessed in patients with CRC (level of evidence IIIc) [40].

PTEN IN CLINICAL PRACTICE – LIMITATIONS

A significant problem in the interpretation of the results of available studies is the differences in the used methodology – i.e. various methods of PTEN gene or PTEN protein detection, lack of standardised techniques and unified protocol of collecting

tissue samples (specimens from primary tumours or metastases). Furthermore, the loss of PTEN function may be a consequence of different genetic mechanisms. In most of published studies, PTEN assessment was performed using IHC as a technique that reflects the loss of function of PTEN in the different mechanisms, but the available IHC methods of PTEN protein expression testing have not been validated yet. In addition, the terms “loss” or “preserved PTEN function” have not been clearly defined and the PTEN assessment by IHC staining bears the hallmarks of subjectivity.

In some of the studies, PTEN testing was assessed by evaluation of the PTEN gene alleles using fluorescent in situ hybridization (FISH), evaluation of gene mutation type or presence of promoter region methylation. The consistency of the results obtained using these methods with the results of IHC testing remains uncertain.

Another limitation for the use of available knowledge on PTEN in clinical practice in the European population is the fact that a significant part of the studies has been conducted in Asian populations.

CONCLUSIONS

Despite the evidence of the important role of PTEN in the CRC pathogenesis and numerous reports on the association between the PTEN status and clinical parameters and the effectiveness of targeted therapy for CRC patients, the use of the PTEN status assessment in clinical practice remains questionable. A potential use of PTEN as a prognostic or predictive factor in clinical practice requires further well-designed prospective studies on larger patient population, with the use of unified methodology.

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