RETRACTED ARTICLE: Serum levels of PTEN mRNA in colorectal cancer: a case-control study

Yang Yan\textsuperscript{a}, Xiaohui Du\textsuperscript{a}, Shaoyou Xia\textsuperscript{a}, Songyan Li\textsuperscript{a}, Da Teng\textsuperscript{a}, Shidong Hu\textsuperscript{a}, Yufeng Wang\textsuperscript{b} and Rong Li\textsuperscript{a}

\textsuperscript{a}Department of General Surgery, First Medical Center, Chinese PLA General Hospital, Beijing, 100853 China; \textsuperscript{b}Department of Patient Admission Management, Chinese PLA General Hospital, Beijing, 100853 China

\textbf{ABSTRACT}

\textbf{Background:} The purpose of this study was to identify the expression of phosphatase and tensin homolog (PTEN) and its diagnostic value in colorectal cancer (CRC).

\textbf{Methods:} The expression level of serum PTEN at mRNA and protein level were determined using quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot analyses, respectively. Chi-square test was employed to explore the relationship between PTEN expression and clinical features of CRC patients. The receiver operating characteristics (ROC) curve was established to evaluate the diagnostic performance of PTEN in CRC.

\textbf{Results:} The expression of serum PTEN was significantly lower in patients with CRC than that in healthy controls both at mRNA and protein level ($p < 0.05$). Also, the low PTEN expression was significantly related to serosal invasion, lymph node metastasis and Ki-67, but had no relation with age, sex, tumor depth and tumor site. The area under ROC curve of 0.810 corresponding with a sensitivity of 97.79\% and a specificity of 70.31\% were obtained, which suggested PTEN could act as a diagnostic marker for CRC.

\textbf{Conclusion:} Altogether, serum PTEN expression was down-regulated and it participated in the development of CRC. Besides, it could act as an efficient and independent diagnostic marker for CRC patients.

\section*{Introduction}

Colorectal cancer (CRC) is one of the most frequent tumors with high malignancy all over the world, especially in developed countries [1–3]. Although the incidence of CRC in China is lower than that in Western countries, it is still a substantial burden in China for its increasing morbidity in recent years [4]. Metastasis is a major cause for CRC-related deaths, including liver metastasis and lymph node metastasis [5,6]. Currently, treatments for CRC mainly consist of surgical resection, radiotherapy and chemotherapy [7]. What is more, a series of clinical examinations have been used for CRC screening, such as colonoscopy, fecal occult blood test and sigmoidoscopy [8,9]. However, CRC patients still suffer a poor prognosis and high mortality because of the diagnosis in advanced stage [10]. Therefore, it is urgently needed to identify novel diagnostic molecules for CRC patients.

Phosphatase and tensin homolog (PTEN), also known as MMAC1 or TEPI, are located on chromosome 10p23.31 [11,12]. It encodes a protein with a weight of 47 kDa, which contains 403 amino acids [13,14]. PTEN gene consists of a C2 domain binding to the membrane phospholipids and an N-terminal phosphatase domain whose active site is responsible for the enzymatic function of this protein [15]. PTEN is initially identified to be disrupted in plenty of sporadic tumors and targeted by germline mutations [16]. It is the first tumor that inhibits gene with the activity of phosphatase and it plays a suppressor role in the process of tumor profile, differentiation, infiltration and apoptosis in many malignant cells [17]. It was also considered to be down-regulated in CRC according to previous studies [18,19]. However, little is reported about the relationship between the expression of PENT with the diagnosis of CRC.

In this study, we detected the expression of PTEN and investigated its relationship with clinical factors of patients with CRC. Besides, we attempted to verify the diagnostic value of PTEN in the early detection of CRC via establishing ROC curve.

\section*{Materials and methods}

\textbf{Patients and specimens}

A total of 136 patients (52 males and 84 females), who were pathologically diagnosed as CRC were enrolled in the study from May 2008 to April 2013 at Chinese PLA General Hospital. In addition, 64 healthy blood donors were selected as healthy controls. All the patients had received no radio- or chemotherapy before blood collection. This study was approved by the Ethics Committee of Chinese PLA General Hospital. The informed consents were provided by all participants.

\section*{CONTACT} Xiaohui Du fegyanb@163.com Department of General Surgery, First Medical Center, Chinese PLA General Hospital, Beijing, 100853 China

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
Serum specimens were taken from all the participants for routine biochemical investigations and put into the blood collection tubes of EDTA, respectively. Then, all the samples were stored at −80 °C for use. Also, the clinicopathological features of the CRC patients were collected in a database.

**RNA extraction and qRT-PCR analysis**

Total RNA was isolated from all serum samples using mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA), respectively. Then reverse-transcription was conducted to synthesize the first chain of cDNA with TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). RT-PCR reaction was conducted in Applied Biosystems 7900 Fast Real-Time PCR system (Foster City, CA, USA). The relative mRNA expression of PTEN was calculated via the $2^{-\Delta\Delta Ct}$ method and normalized to GAPDH. Each sample was in triplicate.

**Western blot**

Total protein was obtained from serum of the patients with CRC and healthy controls and separated on SDS-PAGE gels. Then, the brands on gels were transferred onto nitrocellulose membrane. The membranes were blocked with 5% non-fat milk (Bio-Rad, Hercules, CA, USA) and incubated with primary anti-PTEN antibody at 4 °C overnight. The secondary antibody was added to horseradish peroxidase-conjugate after the membrane was washed. Reaction with the enhanced chemiluminescence kit (Forevergen Biosciences, Guangzhou, Guangdong, China) was performed for detecting the quantification of PTEN protein.

**Statistical analysis**

All data were presented as means ± SD. Statistical analyses were conducted using SPSS 18.0 software (IBM Corporation, Armonk, NY, USA). The differences between the two groups were analyzed by t-test. The relationship between PTEN expression and clinical characteristics was elucidated by the Chi-square test. The receiver operating characteristic (ROC) curve was designed to assess the diagnostic performance of PTEN for distinguishing patients with CRC from healthy controls. It was considered to be statistically significant when $p$ values were less than .05.

**Results**

**The mRNA expression of PTEN was reduced in patients with CRC**

The relative mRNA expression level of PTEN in CRC patients and healthy controls were detected by qRT-PCR analysis, respectively. The result showed that serum PTEN mRNA expression was significantly lower in patients with CRC than that in healthy controls (1.408 ± 0.282 vs. 2.182 ± 0.616) ($p < .05$) (Figure 1).

**The protein expression of PTEN was decreased in patients with CRC**

The protein expression of PTEN in CRC patients and healthy controls were measured with Western blot. As shown in Figure 2, the protein expression of serum PTEN mRNA expression was significantly decreased in patients with CRC compared to that in healthy controls (0.172 ± 0.035 vs. 0.264 ± 0.075) ($p < .05$).

**Relationship between PTEN expression and clinical factors of CRC patients**

The clinical information of CRC patients, including sex, age, tumor depth, serosal invasion, tumor site, lymph node metastasis and Ki-67 were recorded at the diagnosis time. Also, the low PTEN expression was proved to be significantly associated with lymph node metastasis ($p = .017$), serosal invasion ($p = .045$) and Ki-67 ($p = .039$) in CRC (Table 1). However, no significant relationship was found between PTEN expression and sex, age, tumor depth as well as tumor site ($p > .05$).
Diagnostic value of PTEN for CRC

The diagnostic performance was analyzed using ROC curves and the optimal cut-off point was determined according to the maximum sum of specificity and sensitivity. As shown in Figure 3, the optimal cut-off point was 2.000, providing the specificity and sensitivity of 70.31 and 97.79%, respectively. Besides, the area under ROC curve (AUC) was 0.810, indicating PTEN had a great diagnostic value for CRC.

Discussion

CRC is a type of malignancy with high mortality and morbidity. Most of the sporadic CRCs could develop from normal epithelium to adenoma with considerable genetic and epigenetic molecular alterations [20]. It is a heterogeneous disease caused by progressive accumulation of genetic and epigenetic aberrations and defects in immune surveillance [21–23]. The implementation of population-based CRC screening programmes has reduced the mortality of CRC, but most CRCs are still detected symptomatically and prompt symptomatic diagnosis remains a priority [24,25]. Therefore, the early detection of CRC with an effective biomarker is necessary.

Recently, a large number of efforts have been paid on finding molecules to improve the diagnosis and prognosis of CRC as well as provide the treatment strategies. For instance, Xiao et al. explained that methylated NDRG4 gene expression had a high sensitivity and specificity for the diagnosis of CRC [26]. Interleukin-8 was reported to be of great diagnostic value for CRC by Jin et al. [27]. Ghanbari et al. found that the down-regulated plasma microRNA-142-3p and microRNA-26a-5p could be a potential diagnostic marker in CRC [28]. As yet, they were all not specific or most effective. Hence, we were engaged in finding more novel and efficient biomarkers for the diagnosis of CRC.

PTEN is one of the most frequently muted genes among those who act as tumor suppressors in human cancers [29]. It plays the suppressor roles through participating in the transmission of the chemical pathway and transferring the signal to cells to induce programmed cell death. Down-regulation or dysfunction of PTEN is often determined in numerous human cancers and has been confirmed to be tightly related to the development and progression of these diseases. Liu et al. declared that PTEN was reduced in human solid salivary adenoid cystic carcinoma and might be effective in the targeted therapy by targeting PI3K/mTOR pathway [30]. Bai et al. showed the decreased expression of PTEN at mRNA level could influence the cell proliferation via regulating the expression of miR-205 in lung cancer [31]. Carriet et al. suggested that the expression of a wild type PTEN tumor suppressor protein predicted sensitivity to 2ME2 and justify exploration of 2ME2 combined with pan-PI3K inhibitors for the treatment of preclinical glioblastoma [32]. It was also found that in multiple myeloma subgroup with an aberrant PTEN status had longer overall survival with respect to the unmethylated subgroup by Piras et al. [33]. In the present study, we detected the expression of PTEN and found it was down-regulated, which revealed it was a tumor suppressor in CRC. This result was consistent with previous studies.

Based on the PTEN expression, we investigated whether it was related to the development of CRC through assessing its relationship with clinical factors of CRC patients. Chi-square test manifested that the low PTEN expression was significantly associated with such characteristics such as lymph node metastasis, serosal invasion and Ki-67, indicating that PTEN might be involved in the development and progression of CRC.

With regard to our hypothesis, we further established the ROC curve to evaluate the diagnostic value of PTEN for CRC. According to the results, the AUC was 0.810 with specificity and a sensitivity of 70.31 and 97.79%, respectively,
suggesting PTEN was a potential diagnostic molecule for CRC. To our knowledge, this was the first time to evaluate the diagnostic value of PTEN in CRC. However, the diagnostic specificity of serum PTEN for CRC was only 70.31%, which might cause high false-positive rate. The combination of serum PTEN with other biomarkers might be an effective approach to improve the diagnostic value of serum PTEN for CRC.

In conclusion, serum PTEN is down-regulated in CRC and it serves as a tumor suppressor. Besides, low PTEN expression is significantly correlated with Ki-67, lymph node metastasis and serosal invasion. What is more, PTEN has the capacity to be employed as an indicator for the diagnosis of CRC. However, the precise mechanism of PTEN on CRC remains poorly understood, which needs further studies. Due to the relatively small sample size, our results require further verification with larger sample size.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**References**

[1] Mariani M, He S, McHugh M, et al. Integrated multidimensional analysis is required for accurate prognostic biomarkers in colorectal cancer [Randomized Controlled Trial Research Support, Non-U.S. Gov't]. PloS One. 2014;9:e101065.

[2] Perez-Carbonell L, Balabuque F, Toiyama Y, et al. IGFBP3 methylation is a novel diagnostic and predictive biomarker in colorectal cancer [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. PloS One. 2014;9:e104285.

[3] Lee S, Lee K, Yoon S, et al. Anomalies in network bridges involved in bile acid metabolism predict outcomes of colorectal cancer patients [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. PloS One. 2014;9:e107925.

[4] Liu Z, Zhang Y, Niu Y, et al. A systematic review and meta-analysis of diagnostic and prognostic serum biomarkers of colorectal cancer [Meta-Analysis Research Support, Non-U.S. Gov't]. PloS One. 2014;9:e103910.

[5] Zhou Y, Wu J, Fu X, et al. OTUB1 promotes metastasis and serves as a marker of poor prognosis in colorectal cancer [Research Support, Non-U.S. Gov't]. Mol Cancer. 2014;13:258.

[6] Han J, Rong LF, Shi CB, et al. Screening of lymph node metastasis associated IncRNAs in colorectal cancer patients [Research Support, Non-U.S. Gov't]. WJG. 2014;20:8139.

[7] Ayyildiz T, Dolar E, Adim S8, et al. Lack of prognostic significance of SOCS-1 expression in colorectal adenocarcinomas [Comparative Study]. Asian Pac J Cancer Prev. 2014;15:8469–8474.

[8] Parente F, Beom C, Ardizzone A, et al. Outcomes and cost evaluation of the first two rounds of a colorectal cancer screening program based on immunochemical fecal occult blood test in northern Italy [Research Support, Non-U.S. Gov't]. Endoscopy. 2013;45:27–34.

[9] Khalid-de Bakker C, Jonkers D, Smits K, et al. Participation in colorectal cancer screening trials after first-time invitation: a systematic review [Meta-Analysis Review]. Endoscopy. 2011;43:1059–1086.

[10] Zhang Q, Wang XQ, Wang J, et al. Upregulation of spondin-2 predicts poor survival of colorectal carcinoma patients [Research Support, Non-U.S. Gov't]. Oncotarget. 2015;6:15095–15110.

[11] Seol JE, Park IH, Lee W, et al. Cowden syndrome with a novel germline PTEN mutation and an unusual clinical course. Ann Dermatol. 2015;27:306–309.

[12] Kafshdooz L, Kafshdooz T, Tabrizi AD, et al. Role of exon 7 PTEN gene in endometrial carcinoma [Research Support, Non-U.S. Gov't]. Asian Pac J Cancer Prev. 2015;16:4521–4524.

[13] Collaud S, Tischler V, Atanasosf A, et al. Lung neuroendocrine tumors: correlation of ubiquitinylation and sumoylation with nucleo-cytosolic partitioning of PTEN [Research Support, Non-U.S. Gov't]. BMC Cancer. 2015;15:74.

[14] Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. Nat Genet. 1997;15:356–362.

[15] Gbelcova H, Bakes P, Priscaková P, et al. PTEN sequence analysis in endometrial hyperplasia and endometrial carcinoma in Slovak women [Research Support, Non-U.S. Gov't]. Anal Cell Pathol (Amst). 2015;2015:746856.

[16] Nakakido M, Deng Z, Suzuki T, et al. Dysregulation of AKT pathway by SMYD2-mediated lysine methylation on PTEN. Neoplasia. 2015;17:387–373.

[17] Moss ET, Hollingworth J, Reynolds TM. The role of CA125 in clinical practice. J Clin Pathol. 2005;58:308–312.

[18] Chen Y, Shi Y, Lin J, et al. Combined analysis of EGFR and PTEN status in patients with KRAS wild-type metastatic colorectal cancer. Medicine. 2015;94:e1698.

[19] Sun Y, Tian H, Wang L. Effects of PTEN on the proliferation and apoptosis of colorectal cancer cells via the phosphoinositol-3-kinase/Akt pathway [Research Support, Non-U.S. Gov't]. Oncol Rep. 2013;33:1828–1836.

[20] Bertuzzi M, Morelli C, Bagnati R, et al. Plasma clusterin as a candidate pre-diagnosis marker of colorectal cancer risk in the Florence cohort of the European Prospective Investigation into cancer and nutrition: a pilot study [Research Support, Non-U.S. Gov't]. BMC Cancer. 2015;15:56.

[21] Grady WM, Pritchard CC. Molecular alterations and biomarkers in colorectal cancer [Research Support, N.I.H., Extramural mResearch Support, Non-U.S. Gov't Review]. Toxicol Pathol. 2014;42:124–139.

[22] Lao VV, Grady WM. Epigenetics and colorectal cancer [Research Support, Non-U.S. Gov't]. Endoscopy. 2010;42:1630–16342.

[23] Sun Y, Tian H, Wang L. Effects of PTEN on the proliferation and apoptosis of colorectal cancer cells via the phosphoinositol-3-kinase/Akt pathway [Research Support, Non-U.S. Gov't]. WJG. 2013;19:607–615.

[24] Atkin WS, Edwards R, Kralj-Hans I, et al. Once-only flexible sigmoidoscopy screening in prevention of colorectal cancer: a multicentre randomised controlled trial [Multicenter Study Randomized Controlled Trial Research Support, Non-U.S. Gov't]. Lancet. 2010;375:1624–1633.

[25] Astin M, Griffin T, Neal RD, et al. The diagnostic value of syndecan-1 in colorectal cancer [Editorial Review]. Br J Gen Pract. 2011;61:e231–e243.

[26] Xiao W, Zhao H, Dong W, et al. Quantitative detection of methylated NDRG4 gene as a candidate biomarker for diagnosis of colorectal cancer. Oncol Lett. 2015;9:1383–1387.

[27] Jin WJ, Xu JM, Xu WL, et al. Diagnostic value of interleukin-8 in colorectal cancer: a case-control study and meta-analysis [Meta-Analysis Review]. WJG. 2014;20:16334–16342.

[28] Ghanbari R, Mosakhani N, Asadi J, et al. Downregulation of plasma MiR-142-3p and MiR-26a-5p in patients with colorectal carcinoma. Iran J Cancer Prev. 2015;8:16332.

[29] Gil A, Rodriguez-Escudero I, Stumpf M, et al. A functional dissection of PTEN N-terminus: implications in PTEN subcellular targeting and tumor suppressor activity [Research Support, Non-U.S. Gov't]. PloS One. 2015;10:e0119287.
[30] Liu H, Du L, Wang R, et al. High frequency of loss of PTEN expression in human solid salivary adenoid cystic carcinoma and its implication for targeted therapy [Research Support, Non-U.S. Gov't]. Oncotarget. 2015; 6:11477–11491.

[31] Bai J, Zhu X, Ma J. et al. miR-205 regulates A549 cells proliferation by targeting PTEN [Research Support, Non-U.S. Gov't]. Int J Clin Exp Pathol. 2015;8:1175–1183.

[32] Muh CR, Joshi S, Singh AR, et al. PTEN status mediates 2ME2 antitumor efficacy in preclinical glioblastoma models: role of HIF1alpha suppression [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. J Neurooncol. 2014;116:89–97.

[33] Piras G, Monne M, Palmas AD, et al. Methylation analysis of the phosphates and tensin homologue on chromosome 10 gene (PTEN) in multiple myeloma. Clin Epigenetics. 2014;6:16.