Expression and significance of PTEN and Claudin-3 in prostate cancer

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Abstract. Expression and significance of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and Claudin-3 in the blood of patients with prostate cancer (PCa) were investigated. Retrospective analysis of 84 cases of PCa patients confirmed by pathological diagnosis were studied, as the experiment group. Moreover, the physical examination data of 84 healthy volunteers examined in the Affiliated Hospital of Beihua University were the control group. The expression levels of blood in the PTEN and Claudin-3 of both the experiment group and the control group were determined by enzyme-linked immunosorbent assay. According to the blood expression in PTEN and Claudin-3 between both the experiment group and the control group, the test value of the ROC curve in PTEN and Claudin-3 were detected by both single detection and joint detection. The expression levels of PTEN in the experiment group were significantly lower than the control group (P<0.05). The expression levels of Claudin-3 were higher in the experiment group than the control group (P<0.01). The expression levels of PTEN and Claudin-3 in the experiment group were significantly associated with the distant metastasis of cancer cells, preoperative prostate-specific antigen levels, tumor diameter and pathological stages (P<0.01). The expression levels of PTEN in the pathological stage of T1-T2 group was lower than that of the T3-T4 group (P<0.01). The expression levels of PTEN and Claudin-3 are closely related to the distant metastasis of cancer cells, preoperative prostate-specific antigen level, tumor diameter and pathological stage. Combined detection of both PTEN and Claudin-3 can improve the specificity levels of PCa for diagnosis and has an important diagnostic value for PCa. It can be used as a biological indicator for PCa diagnosis, disease severity analysis and efficacy evaluation.

Introduction

Prostate cancer (PCa) is a very common tumor in the male urinary system, and is a clinically complex multifactorial disease. Approximately 22,000 American men are diagnosed with PCa each year (1). In recent years, with the growth of aging population and improvement of living conditions, both the incidence and mortality rates due to PCa have increased (2). PCa is a malignant tumor of prostate epithelium, with slow development of tumor cells, insidious pathogenesis and no obvious symptoms in early stage (3). The cancer is often in the late stage when there are obvious symptoms such as dysuria, hematuria, impotence and urodynia. The cancer cells have undergone distant metastasis and local infiltration, and invaded organs and tissues other than prostate capsule, therefore missing the best time for treatment (4). Studies have shown that timely and effective detection of early cancer with well-timed treatment can reduce PCa mortalities, which can be conducive to the prognosis of patients with PCa and reduce the recurrent rate of PCa patients (5). Therefore, finding effective and convenient diagnostic methods and indicators can improve the early diagnosis rate of PCa patients, also it is the key to improve the cure and survival rates of PCa patients.

Claudins is a transmembrane with connexin protein and is currently found in approximately 30 family members with molecular weights between 20 and 27 kDa (6). Changes in the expression levels of mRNA and protein are often associated with a variety of disease pathogenesis in the body. Medical scientists have confirmed that elevated levels of Claudin-4 can lead to the development and spread of cancer cells in patients with pancreatic cancer and breast cancer (7,8). Claudin-1 is overexpressed in colon cancer patients (9). Claudin-3 is a member of the Claudins connexin protein and is closely associated with the transmembrane protein. It has an important effect in the transmission and transportation of cells. Some studies have suggested that the abnormal expression of Claudin-3 is closely related to the occurrence and development of tumors (10).

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is composed of 9 exogenous factors and 8 inclusive factors, 1212 base pairs located at zone 2 and band 3 of the long arm in human chromosome 10, with a total length of 200 kb. The functional protein encoded is a bispecific phosphate synthase, a polypeptide chain consisting of 403 amino
acids residues (11). PTEN is the first tumor suppressor gene with phosphatase activity, which can inhibit tumors by promoting cell apoptosis (12). This gene has a high frequency of deletions and mutations in prostate cancer (13) and has received extensive attention in recent years. PTEN can mainly inhibit the growth, invasion and metastasis of cells and the pathologic adhesion of tumor cells (14). It is involved in cell differentiation, cell attachment, cell migration and apoptosis, it can maintain the body's immune system stability and has an important effect in a variety of physiological activities (15). The abnormal expression of PTEN can induce the growth of cells, which benefits the occurrence of tumors. Also induces cell invasiveness and adhesion ability, which benefits the metastasis of tumor cells (16).

Clinically, PCa examination methods mainly include prostate biopsy guided by rectal ultrasound, digital rectal examination and nuclear magnetic resonance detection (17). With the advancement of medical science, the diagnostic rate of PCa in the discovery of PCa tumor markers such as prostate specific antigen and prostate specific membrane antigen has also improved. However, some studies have found that in the autopsy of males who died normally over the age of 70, >80% of the patients with PCa were not fully detected when they were alive (18). This suggests that further investigations into the pathogenesis of PCa are required in order to find more accurate examination methods for tumor markers.

This study explored the blood expression levels and significance of PTEN and Claudin-3 in patients, investigated the expression and significance of PTEN and Claudin-3 in the blood of patients with PCa to provide a basis for clinical practice.

Patients and methods

General information. Retrospective analysis of 84 cases of PCa patients confirmed by pathological diagnosis, and the medical records were the experiment group. Moreover, the physical examination data of 84 healthy volunteers examined in the Affiliated Hospital of Beihua University (Jilin, China) were the control group. The average age in the experiment group was 67.65 years of age, according to the gleason scoring methods, the experiment group was divided into different subgroups. There were 3 cases in the high differentiation group (Gleason 2-4 unit), 16 cases in the medium differentiation group (Gleason 5-6 unit) and 65 cases in the low differentiation group (7-10 unit). According to the TNM staging of prostate, the experiment group was divided into 26 cases of T1-T2 stage, treated as T1-T1 subgroup, and 58 cases of T3-T4 stage, treated as T3-T4 subgroup.

The study was approved by the ethics committee of Affiliated Hospital of Beihua University. All the subjects were informed and agreed to participate in the clinical study, and informed consents were obtained.

Inclusion and exclusion criteria

Inclusion criteria. Patients with PCa diagnosed by Clinical Pathology in the Affiliated Hospital of Beihua University; age ≥18 years; tumor grading and clear stage. No radiotherapy, chemotherapy and other anticancer treatments were used before serum was taken. No congenital genetic disease; perfect clinical medical records for patients were available.

Exclusion criteria. Patients who had taken antibiotics within three months before sampling; patients with liver dysfunction; autoimmune system defects, and suffering from other tumors; PCa for recurrence at admission and patients with urinary system diseases.

Main reagents and instruments. Automatic washing machine (model: RT-3100; Shanghai Tiancheng Technology Co., Ltd., Shanghai, China); automatic quantitative enzyme-labeling instrument (model: Bole 680; Shanghai Dingqian Biotechnology Co., Ltd., Shanghai, China); Claudin-3 ELISA kit (item no. PRE8808; Beijing Huaxia Ocean Technology Co., Ltd., Beijing, China); PTEN ELISA kit (item no. YD2717; Shanghai Yudu Biotechnology Co., Ltd., Shanghai, China); UV-visible spectrophotometer (model: UV1700; Shanghai Jeanqi Instrument Technology Co., Ltd., Shanghai, China); and a low speed normal temperature centrifuge (model: 3-5N; Hunan Hengnuo Instrument Equipment Co., Ltd., Hunan, China) were used in the present study.

Collection of specimens. All the participants in the experiment were fasted for >8 h the night before blood collection. The next morning, 5 ml of elbow venous blood was taken on an empty stomach. After standing at 30°C for 25 min, the serum was separated in a centrifuge of 2,300 x g at 20˚C and centrifugation time was approximately 15 min. After the end of the centrifugation, it was let to stand for 10 min. After the specimens were layered, the supernatant was carefully collected and stored in a refrigerator at -20° C until re-use.

Detection of PTEN and Claudin-3 expression levels. In this experiment, the expression levels of PTEN and Claudin-3 in the blood between the experiment group and the control group were determined by enzyme-linked immunosorbent assay (ELISA).

The specimen and kit were taken out of the refrigerator and melted at 30°C. Then 20-fold concentration of 30 ml of washing liquid and extra 570 ml of distilled water were added prior to dilution in the original washing solution. Subsequently, the ELISA plate was removed. Standard solution (50 µl) was poured into the wells, then 10 µl of sample and 60 µl of sample diluent were added to the sample wells. Chromogenic reagent (100 µl) was added to each well except the blank one; the ELISA plate was removed after 60 min in a 37°C water tank and 50 µl of washing solution was added to each well. After leaving it for 1 min, the liquid in the well was removed and the plate was washed 5 times. Enzyme standard solution (50 µl) was added to each well (except for the blank control well). The chromogenic reagent was added after 15 min in a 37°C reciprocal shaking bath and avoid colored-light for 15 min. Then the ELISA plate was taken out and termination solution was added to stop the reaction. After the reaction was terminated for 10 min, the absorption value of each well was measured at a wavelength of 450 nm. The R-value was calculated according to the absorption value corresponding to the concentration of the standard product, and the product is a good when the R-value has an accuracy of 99% or more. The linear regression equation was calculated using a fully automatic enzyme-labeling instrument, and the sample concentration was calculated based on the measurement of absorption values.
Statistical analysis. Data analysis was performed using SPSS 17.0 (Shanghai Yuchuang Network Technology Co., Ltd., Shanghai, China) statistical software. The measurement data were expressed as mean ± standard deviation (Means Network). The measurement data between groups were compared by t-test. ANOVA was used for the comparison of multiple groups with lSD post hoc test. The receiver operating characteristic (ROC) curve was used to evaluate the diagnostic efficacy of serum PTEN and Claudin‑3 expression on PCa. When P<0.05, the difference was statistically significant.

Results

Expression levels of PTEN and Claudin‑3 in the experiment group and the control group. The experimental results showed that the expression level of PTEN in the experiment group was 7.32, which was lower than the control group (14.58), (t=15.560, P<0.01). The expression level of Claudin‑3 in the experiment group was 10.36, which was higher than the control group (expression level of 2.43), (t=26.790, P<0.01) (Fig. 1).

The expression levels of PTEN and Claudin‑3 of different baseline data in the experiment group. The results showed that the expression levels of PTEN and Claudin‑3 in the experiment group were not significantly associated with age, smoking, alcoholism, body mass index, preoperative blood glucose, preoperative Hb, preoperative Alb and preoperative CRP (P>0.05) (Table I).
Relationship between the expression levels of PTEN, Claudin-3 and the clinicopathological features. The results showed that the expression levels of PTEN and Claudin-3 in the experiment group were significantly associated with distant metastasis of cancer cells, preoperative prostate specific antigen level, tumor diameter and pathological stage (P<0.01) (Table II).

Expression levels of PTEN and Claudin-3 in different clinical graded experiment groups. The results showed that the lowest expression level of PTEN in the poor differentiation group was 6.46±0.78, which was lower than the medium and high differentiation groups (7.59±1.32 and 8.27±0.69, respectively) (P<0.01). The expression level of PTEN in the medium differentiation group was lower than that in high differentiation group (P<0.01). Claudin-3 had the highest expression level in the low differentiation group (11.75±1.19), and was higher than both the medium and high differentiation groups (9.87±2.09 and 8.12±0.34, respectively) (P<0.01). The expression level of Claudin-3 in the medium differentiation group was higher than that in high differentiation group (P<0.01) (Fig. 2).
and Claudin-3 were evaluated. The AUC of PTEN expression and the ROC curve diagram of the expression level of PTEN in the blood of PCa and the normal blood of healthy volunteers showed that the diagnostic specificity was 88%, sensitivity was 64% and the optimal cut-off point for diagnosing PCa was 8.895. The AUC of Claudin-3 expression level for diagnosis of PCa was 0.7375 (95% CI, 0.6617-0.8133), the diagnostic specificity was 85%, sensitivity was 57% and the optimal cut-off point for diagnosing PCa was 3.310. Further combined with PTEN and Claudin-3 to map the ROC curve diagram of PCa, the AUC of both PTEN and Claudin-3 was 0.8576 (95% CI, 0.8018-0.9134), and the optimal cut-off point for diagnosing PCa was 0.5697, the specificity was 69% and sensitivity was 88% (Fig. 4).

### Discussion

PCa is a unique and common tumor that occurs in males, and has the second highest incidence rate worldwide, exceeded only by lung cancer (19). The causes of PCa include inheritance, pathogenic microorganism, drugs and diet. It occurs very often in middle-aged and elderly males, and also is one of the main causes of death among them (20). The pathogenesis of PCa is relatively insidious, which is prone to early metastasis, and there is often no obvious specificity in the early stage. It is often at the late stage of cancer when patients have symptoms such as impotence, premature ejaculation, blood essence, dysuria and ejaculation pain. Therefore, it usually fails to achieve the desired therapeutic effect (21). PCa has a poor chemosensitivity and complicated treatment process, most of the patients are elderly, and their physical functions and organ functions have decreased to some extent. In addition, the treatment is more difficult; the side effects of late-staged cancer radiotherapy and chemotherapy are greater; older patients have poor-tolerance and poorer living conditions (22). The biological behavior of PCa is quite complicated, with the current development of PCa; the mechanism is still unclear, and finding more accurate PCa tumor markers is a focus for international research.

Claudin-3 is a member of the Claudins protein and is one of the most important structural molecules that make up the tight junction of epithelial cells. It mainly has an effect in maintaining intercellular barrier function and cell polarity in the body (23). Abnormal expression of Claudin-3 often leads to loss of cell polarity, and the adhesion of intercellular force is reduced to varying degrees, destroyed the epithelial permeability barrier, thereby promoting the occurrence and development of tumors. Studies have shown that in a variety of tumor patients, the expression of Claudin-3 is significantly higher than the adjacent normal tissues (10), indicating that the Claudin-3 is closely related to the occurrence and development of tumors. It is often at the late stage of cancer when patients have symptoms such as impotence, premature ejaculation, blood essence, dysuria and ejaculation pain. Therefore, it usually fails to achieve the desired therapeutic effect (21). PCa has a poor chemosensitivity and complicated treatment process, most of the patients are elderly, and their physical functions and organ functions have decreased to some extent. In addition, the treatment is more difficult; the side effects of late-staged cancer radiotherapy and chemotherapy are greater; older patients have poor-tolerance and poorer living conditions (22). The biological behavior of PCa is quite complicated, with the current development of PCa; the mechanism is still unclear, and finding more accurate PCa tumor markers is a focus for international research.

Expression levels of PTEN and Claudin-3 in different TNM stages of the experiment groups. The results showed that the expression level of PTEN in T1-T2 group was 7.94±1.02, which was higher than the T3-T4 group (6.47±0.79), there was a significant difference between the groups (P<0.01). The expression level of Claudin-3 in the T1-T2 group was 8.94±1.16, which was lower than the T3-T4 group at 11.52±1.42. There was a significant difference between the groups (P<0.01) (Fig. 3).

Diagnostic value of PCa in the expression levels of PTEN and Claudin-3. The expression levels of PTEN and Claudin-3 in the blood of PCa and the normal blood of healthy volunteers and the ROC curve diagram of the expression level of PTEN and Claudin-3 were evaluated. The AUC of PTEN expression level for diagnosis of PCa was 0.7943 (95% CI, 0.7243-0.9643), the diagnostic specificity was 88%, sensitivity was 64% and the optimal cut-off point for diagnosing PCa was 8.895. The AUC of Claudin-3 expression level for diagnosis of PCa was 0.7375 (95% CI, 0.6617-0.8133), the diagnostic specificity was 85%, sensitivity was 57% and the optimal cut-off point for diagnosing PCa was 3.310. Further combined with PTEN and Claudin-3 to map the ROC curve diagram of PCa, the AUC of both PTEN and Claudin-3 was 0.8576 (95% CI, 0.8018-0.9134), and the optimal cut-off point for diagnosing PCa was 0.5697, the specificity was 69% and sensitivity was 88% (Fig. 4).

We have found that the expression levels of PTEN in the blood of patients with PCa is significantly lower than in normal people, and the difference was statistically significant.
The results of this study are similar to the research of Yue et al (26), and they found that irregular expression of PTEN in PCa patients of human can cause abnormal accumulation of esterified cholesterol, cholesterol esterification promotes cancer cell invasion. The expression levels of Claudin-3 in the blood of patients with PCa was higher than in normal people, and the difference was statistically significant (P<0.01). The results of this study are similar to those of Chinni et al (27), who found that Claudin-3 and Claudin-4 were overexpressed in PCa patients. The expression levels of PTEN and Claudin-3 in the blood of PCa patients were significantly correlated with distant metastasis in cancer cells, preoperative prostate specific antigen levels, tumor diameter and pathological stage (P<0.01). Phattarataratip and Sappayatosok (28) found that family members of the Claudin proteins have an important effect in oral squamous cell carcinoma. The irregular expression of Claudin-7 was associated with pathological grade, late-staged TNM grade, tumor size, fibropathopathy, vascular invasion and involvement of regional lymph nodes. Moreover, Koperek et al (29) studied the expression of PTEN protein in papillary thyroid cancer and found that the expression of PTEN is related to sex, metastasis of lymph nodes and pathological stages. This can be used as a prognostic factor for the evaluation of papillary thyroid tumors. There are some similarities between the research stated above and our studies, therefore it is confirmed that both PTEN and Claudin-3 have important effects in the growth, development and metastasis of tumors.

In our further studies, we found that the lowest expression levels of PTEN in the blood of PCa patients in the low differentiation group were lower than both the medium and high differentiation groups (P<0.01). The highest expression levels of Claudin-3 in the low differentiation group were higher than both the medium and high differentiation groups (P<0.01). The expression levels of PTEN in T1-T2 group were higher than the T3-T4 group, and the difference between both groups were significant (P<0.01). The expression levels of Claudin-3 in T1-T2 group were lower than the T3-T4 group, and the difference between both groups were significant (P<0.01). By examining the ROC curve diagram of PCa between both PTEN and Claudin, we have found that the AUC of PCa was 0.8576 (95% CI, 0.8018-0.9134); the optimal cut-off point was 0.5697; specificity was 69% and the sensitivity was 88%; The ROC curve of PCa was diagnosed by plotting the expression levels of PTEN and Claudin-3. The combined diagnosis can improve the sensitivity levels of PCa.

This study strictly selected the research objects according to the inclusion and exclusion criteria, which ensured the reliability of the study results. However, this study still has certain defects. We only included a few patients in this study, and more PCa patients with different pathological types should be collected for research. The present study did not conduct an in-depth study of the association between PTEN, Claudin-3 and other clinical symptoms of PCa patients. Therefore, there are certain limitations.

PTEN is lowly expressed in the blood of PCa patients and Claudin-3 is highly expressed in the blood of PCa patients. The expression levels of PTEN and Claudin-3 are closely related to the distant metastasis of cancer cells, preoperative prostate specific antigen level, tumor diameter and pathological stage. Combined detection of PTEN and Claudin-3 can improve the specificity of PCa for diagnosis, which has an important diagnostic value for PCa. It can be used as a biological indicator for PCa diagnosis, disease severity analysis and efficacy evaluation.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors‘ contributions

XY was involved in writing the manuscript. XY and LZ performed ELISA. XY and JK collected the patient general data and specimens. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Affiliated Hospital of Beihua University (Jilin, China). Patients who participated in this research had complete clinical data. Signed informed consents were obtained from the patients or the guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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