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Original Article

Establishment of a combined detection system for the serum tumor markers in mouse prostate cancer

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Abstract: Prostate specific antigen (PSA) is a major indicator of the prostate cancer, PSA similarity is not found in serum of mouse, but the serum biomarker in prostate cancer of mouse is deficient. Our experiment is to investigate the expression of PSP94 in serum of mice to determine serum biomarker for prostate cancer and improve the inspection system. Tag SV40 gene was inserted into PSP94, and the KIMAP model was established, according to growth cycle which were divided into three groups, including 20 weeks, 40 weeks and 60 weeks old. Serum PSP94 was detected in the model of prostate cancer which was knocked in the detected gene in mice. Compared with wild type mice, the levels of PSP94 in KIMAP mice serum were significantly increased in 20 weeks, 40 weeks and 60 weeks old, with significant differences ($P<0.01$). Compared with 20 weeks, the serum levels of PSP94 in KIMAP mice were significantly increased at 40 weeks and 60 weeks old, with significant difference ($P<0.01$). There was no difference of PSP94 expression in serum of KIMAP mice between 40 weeks and 60 weeks old. PSP94 increased with the age of the KIMAP mouse model with prostate cancer, and was positively correlated with the change of tumor. The result showed that the grading of prostate cancer was closely related to PSP94 in serum, which can be used as a standard for the detection serum markers of mouse prostate cancer.

Keyword: Prostate cancer, serum tumor markers, PSA, combined detection

Introduction

In recent years, prostate cancer is a common malignant tumor in old men, which incidence rate is the first place in male malignant tumor in Europe and the United States. In recent years, because of the changes in the structure of human diet, the increase of the aging population, the incidence of prostate cancer in China has been increasing year by year [1]. However, the early prostate cancer attacks insidiously, which early clinical symptoms are similar to the symptoms of benign prostatic hyperplasia, which incubation period is long. When the hematuria, pelvic pain and even weight loss and other symptoms appear, the distant metastases have had, so the early diagnosis and early detection of prostate cancer have important clinical significance for the treatment. The PAS is an important test basis of prostate cancer in clinical diagnosis in addition to rectal examination syndrome, which is widely used in clinical detection [2]. Under normal physiological conditions, PSA of prostate specific expression was secreted into semen by prostatic duct, which concentration in seminal plasma is 100 times higher than that in serum, while there is a tight tissue barrier between the prostate gland and the blood circulation system, which hinder the exchange of material between the two obstacles. The tissue barrier is damaged when prostatic cancer appears, which causes PSA to seep into the blood and the contend of blood increase, so the detection of PSA can increase the diagnosis rate of prostate cancer and have contribute to the treatment and detection of prostate cancer [3]. In addition, prostate gland can secrete proteins, by which the most three proteins that prostate gland PSP94 (prostate specific protein 94), PSA and PAP (prostatic acid phosphatase) were secreted [4, 5]. Our experiment will take the method of gene KIMAP (knock-in mouse adenocarcinoma prostate model) and analyze the competition of serum

PSP94 by ELISA. According to the curve of PSP4, the serum tumor markers in mouse models were established.

Materials and methods

Animal's selection

Eighty Wistar male mice were selected, which were in the clean grade and whose weights were at the range of 45-65 g, all the animals were purchase from Weifang Medical University with permission number SCXK 2014-0011. Each rat was kept in cages, the temperature of the animal laboratory was at the range of (20~25)°C, and the relative humidity was of (50~60)%.

Mice were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Affiliated Hospital of Weifang Medical University.

Reagents and equipments

Alendronate (ALN) was purchased from Shijiazhuang Haisen Chemical Co. td.; polyclonal antibody of Col II, MMP-13 and β-catenin were purchased from Beijing Zhongshan Biological Technology Co. Ltd.; Recombinant human IL-1 beta was purchased from Shanghai Kelaite Biotechnology Company; Real-time PCR MasterMix was purchased from America Medical Engineering Department of Northwestern University; Motic 6.0true color medical image acquisition and analysis system was purchased from the United States Gloud company.

Experimental model preparation and grouping

KIMAP model of mouse [6]: Tag SV40 gene was produced in the prostate tissue of mice, which were specially induced by promoter/enhancer region of mouse PSP94 consisting 3842 bp. Tag SV40 gene was inserted into the PSP94 site, and the KIMAP model was established, prostate tumor grew slowly, which diameter and quality reached 4 mm and 0.4 g when 50 weeks old respectively, KIMAP mouse was found multiple organ metastasis in advanced prostate cancer. The experimental models were divided into three groups according to the growth cycle, with 20 mice in each group. The pathological types of prostate cancer in mice of 20 weeks old group were normal/low differentiation and high differentiated prostatic intraepithelial neoplasm, while the pathological type of prostate cancer in mice of the 40 weeks old group were early prostate cancer and advanced prostate cancer respectively.

Specimen collection of blood

In our experiment we adopted the mixed narcotic drugs that Ketamine combined with toluene (Ketamine:toluene = 5:1) to anesthetized the mice in accordance with the proportion of 0.03 mL/10 g. The venous blood collection of each mouse did not exceed 300 μL, and the collection period of blood samplings were 1 time/2 weeks. The establishment of ELISA method for the determination of PSP94

Competition in serum

Firstly, PSP94 level in serum was measured. The recombinant PSP94 (TrcHism PSP94) of mouse was set to the antigen (100 ng/hole), the carbonate coating buffer were placed into the ELISA plate (96 holes), which had been placed for 24 h in 4°C. After PBS-T flushing 3 times, the plate had been closing in 1.5% bovine serum albumin for 1 h at 37°C. After the PSP94 antiserum was diluted with BSA/PBS-T with the ratio of 1:40000, the mixture were placed into the standard hole or sample hole of enzyme label plate, after 30 min culture, PBS-T was used to wash 3 times. Once again, swine anti rabbit IgG serum labeling with HRP was added to dilute, culturing 1 h. After PBS-T washing 3 times, 0.4 mg/mL o-diaminobenzene; o-phenylenediamine were added, which had been placed in developing buffer named 0.05% H_2O_2 for 20 min for developer. The absorbance of the standard/sample hole OD595 was measured by using the porous enzyme standard meter (Bio-Rad), and the final curves were generated. According to the relative absorbance (B/B0) and the standard of competition protein concentration (ng/mL) the standard curve was drew. The formula of B/B0 is: B = OD595-NSB; when maximum antibody binding, B = OD-595-NSB.

Serum PSP94 detection

According to the previous standard, in this experiment, the serum samples of each group (50 μL/hole) were detected continuously for 3 times, and the mice were grouped based on the

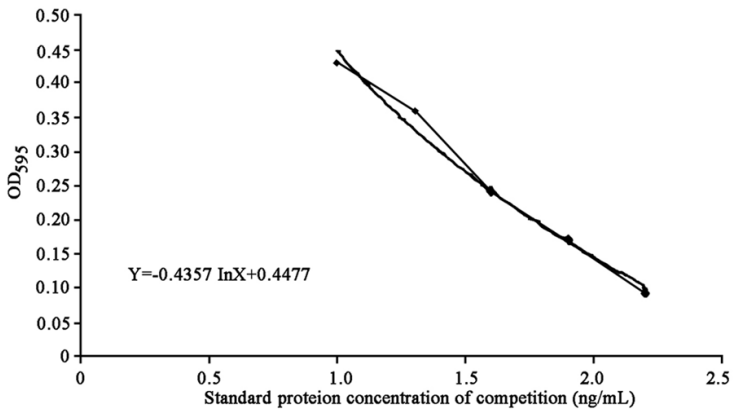

Figure 1. The establishment of standard curve by adopting recombinant TrcHis mPSP94 as Envelope antigen and competition antigen.

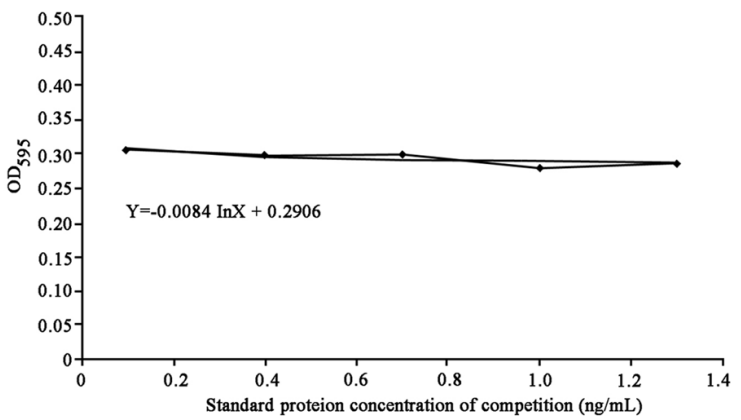

Figure 2. The establishment of standard curve by adopting recombinant TrcHis mPSP94 as envelope antigen and wild mouse PSP94 as competition antigen.

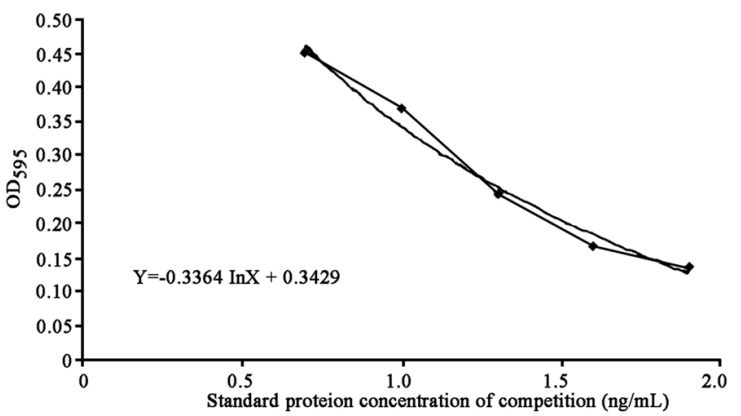

Figure 3. Enclosing polyclonal antibody with Escherichia coli lysate, the establishment of standard curve by adopting recombinant TrcHis mPSP94 as envelope antigen and competition antigen.

age according to tumor progress of KIMAP model.

Statistical analysis

Data were analyzed by SPSS17.0 software, all measurement data are used x ± S (mean ± standard deviation) said, the analysis of variance were used, $P<0.05$ said there was statistically significant difference.

Results

Establish standard curve of competitive ELISA

In order to illustrate whether serum PSP94 has detected, two kinds of immune detection method were used, according to the results of the immunoassay, a standard curve quantitation of serum PSP94 in mice was obtained, and the experimental results show that the method can also be applied to the quantitative analysis of PSP94 in wild type mice. **Figure 1**, according to the recombinant TrcHis mPSP94, the linear log curve of negative slope was established, the negative slope of the curve showed that the method operates normally, and the sensitivity of wild-type mice PSP94 was determined by the standard curve of **Figure 1**. **Figure 2**, the standard curve without inclination was established by the recombinant TrcHis envelope antigen of mPSP94, because of the specificity of carrier protein isomerase, the PSP94 of different levels of mice couldn't be distinguished from the curve. Moreover, due to its major components as immunoassay, isomerase was regarded as antigen to competitive binding the immune detection materials. And we adopted the lysate of Escherichia coli containing isomerase with the characteristics of close polyclonal antibody, which can prevent the signal of isomerase competition. So in the application of recombinant TrcHis mPSP94 as a competitive anti-

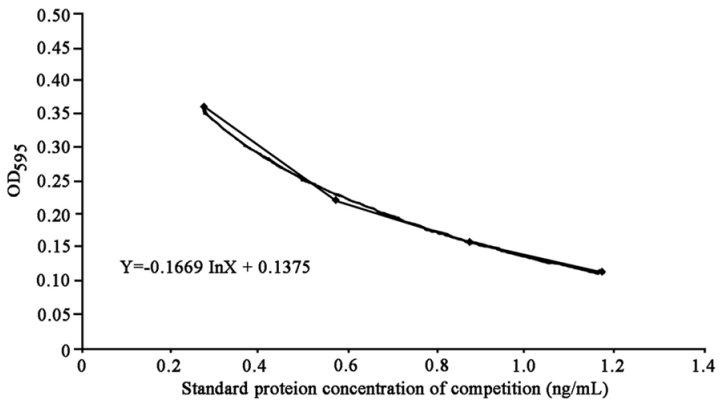

Figure 4. Enclosing polyclonal antibody with Escherichia coli lysate, the establishment of standard curve by adopting recombinant TrcHis mPSP94 as envelope antigen and wild mouse PSP94 as competition antigen.

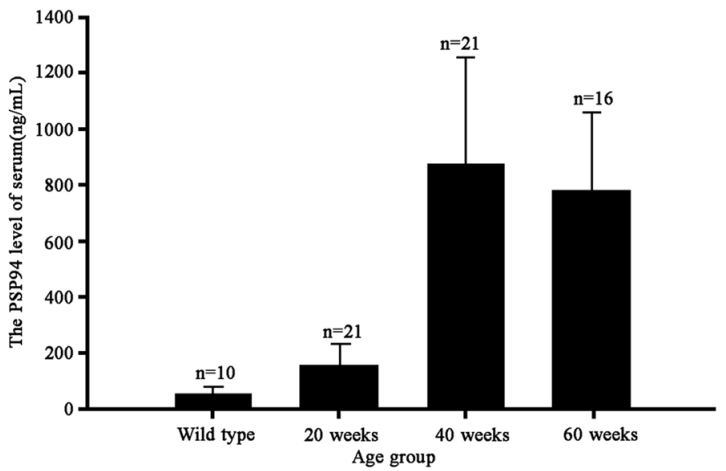

Figure 5. Comparison of serum PSP94 levels in three groups of KIMAP mice.

gen, the standard curve cannot be established. According to the above principle, we set up the new standard curves of **Figures 3** and **4**. **Figure 3** was based on TrcHis mPSP94 method, established the competitive antigen, a line formed by the negative slope. And **Figure 4** was a similar curve of the wild-type mouse PSP94 in which the method of taking a competitive antigen was also formed, which can detect PSP94 of mouse.

Detection of serum PSP94 concentration in mice by competitive ELISA method

We adopted the Escherichia coli containing isomerase, closes polyclonal by cleavage of 18-solution in order to eliminate the signal generated by the isomerase competition, the polyclone antibody was closed by the lysate. The standard curve of **Figure 3** was formed, according to which the concentration ofPSP94 was detected. In our experiment, each hole was dropped into 50 μL serum from tail vein of mice, ELISA method was used. The average serum concentration of wild type mice were 49.8 ng/mL. The average serum concentration of mice in the 20 weeks and 40 weeks old group were 149.5 ng/mL and 798.5 ng/mL, respectively, and the average serum concentration of mice in the 60 weeks was 725.4 ng/mL. Compared with wild-type mice serum, the levels of serum PSP94 were significantly increased in KIMAP mice of 20 weeks, 40 weeks and 60 weeks, there were significant differences ($P<0.01$). Compared with 20 weeks group of mice, the serum levels of PSP94 in KIMAP mice were significantly increased at 40 weeks of age and 60 weeks of age, with significant difference ($P<0.01$). There was no difference in serum PSP94 between 40 weeks and 60 weeks old KIMAP mice, **Figure 5**.

Discussion

PSA as a tumor marker, is one of the main indicators of diagnosis, in recent years, PSA has been used as a gold standard for early detection and follow-up of prostate cancer progression. When the patients with prostate cancer undergo surgery or radiotherapy and chemotherapy, PSA was monitored increasing continuously, indicating prostate cancer recurrence. Current clinical guidelines have been used PSA as a serum tumor marker in the diagnosis of survival rate of prostate cancer patients [7, 8].

The study found that the tumor makers were less in mouse model of prostate cancer, there was no fixed markers corresponding to PSA [9]. Prostate specific serumtumor markers were as effective or more efficient as PSA, and can be used as an evaluation indicator of prostate cancer in animal experimental studies of prostate cancer [10]. Serum marker has the advantages

of low cost and practical features, which can detect prostate cancer therapeutic effects and progress of recurrence [11]. In recent years, prostate cancer treatment of pre-clinical trials need mouse animal model experiment, however, there were lack of the serum tumor markers for rodent prostate cancer, furthermore, limitation of statistical variability, large technical expenses as well as high standard requirements for testing the operator's technology, which can't carry out the data analysis of the large sample data of mice [12]. So the study on the results of clinical anatomy may be regarded as reference of treatment Evaluation [13, 14]. So we established the model of prostate cancer to determine whether PSP94 may be regarded as a tumor marker in mouse. PSP94 is a conservative and rapidly evolving protein. Study find the expression of PSP94 in mice is specifically expressed in the prostate tissue, and is similar to that of serum PSA by binding to PSP94 in serum. In addition, PSP94 is able to express the result of a similar PSA transcription in the serum or the isoform. Which demonstrate that PSP94 and PSA have similar regulation mechanism of secretion [15-17]. Because of the similarity with PSP94, PSA can be used as a serum marker for prostate cancer. In addition, the PSP94 has the characteristics of small molecular weight and weak immunogenicity [18-20]. Our experiment uses Escherichia coli containing isomerase, closes polyclonal by cleavage of 18-solution in order to eliminate the signal generated by the competition isomerase [18-20]. According to the above method, two new standard curves are formed (**Figures 3**, **4**), in addition, the TrcHis mPSP94 is used to form a negative slope of the line through the competition antigen (**Figure 3**), while the PSP94 of wild type mouse also develops a similar curve in the method of competing antigen (**Figure 4**), so the experimental results show the TrcHis mPSP94 can be used to detect mouse PSP94 by method of competition antigen.

In summary, the ELISA results show that PSP94 increased with age growth in KIMAP mice model with prostate cancer, and which is positively correlated with the change of tumor. The results show classification of prostate cancer is closely related to serum PSP94, and which can be used as a standard for the detection of serum markers of mouse prostate cancer.

Disclosure of conflict of interest

None.

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