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Telomerase Activity and Hepsin Gene Expression in Romanian Patients with Prostate Cancer: Preliminary Data

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Abstract

Purpose: Since PSA is not well associated with prostate cancer, there is a need in finding new more specific biomarkers for the diagnosis and/or follow-up prostate cancer patients. We have investigated the expression of telomerase activity and hepsin gene expressions in different prostate cancer tissues from Romanian patients. Prostate cancer diagnosis was revealed by total prostate-specific antigen levels and pathological assessments.

Patients and methods: We have selected 38 patients with proven biopsy prostate cancer (8 patients with metastasis and 30 patients without metastasis) and 20 patients with benign prostatic hyperplasia. The average age was 66.5 year in patients with PCa and 55.8 years in patients with BPH. Telomerase activity was analyzed with TRAPEzE ELISA Telomerase Detection (Millipore). Hepsin gene expression was revealed by Real-Time Polymerase Chain Reaction methodology.

Results: Our preliminary data showed that in all patients with proven prostate cancer metastasis both high telomerase activity and hepsin genes overexpression were found. 30% of patients with BPH had different grades of telomerase activity while the rest of them had no telomerase activity. In 3 patients with normal PSA levels, we have noticed an increased telomerase activity.

Conclusion: Telomerase activity and hepsin genes expressions could be linked with local invasion and metastasis of prostate cancer. We think that the level of telomerase activity and hepsin gene expression could be considered a valuable prognostic biomarker in the outcome of Romanian patients with prostate cancer.

Keywords: Telomerase; Hepsin; Prostate cancer; Prognostic; Biomarkers; Gene expression; PSA

Abbreviations: BPH: Benign Prostatic Hyperplasia; PCa: Prostate Cancer; PIN: Prostatic Intraepithelial Neoplasia; PSA: Prostate Specific Antigen; PCR: Polymerase Chain Reaction; RT-PCR: Real-Time Polymerase Chain Reaction; DNA: Deoxyribonucleic Acid; RNA: Ribonucleic Acid; TRAP: Telomerase Repeat Amplification Protocol; hTR: human Telomeric RNA; HRP: Horseradish Peroxidase.

Introduction

Prostate cancer is the second most common cancer, after lung cancer, in men worldwide, with very large geographical and racial variations [1]. In Romania, the incidence varies depending on the age from 6 to 151 per 100,000 inhabitants [2,3]. In solid organ donors we have noticed a prevalence of high serum values of total PSA of about 12% (Constantinescu et al., Unpublished Data). Thus, strategies to detect prostate cancer in early stages are very important for the treatment monitoring and patient's life prognosis [4].

PSA for early diagnosis of prostate cancer remains controversial due to the poor correlation between PSA serum values and different stages of prostate cancer [5]. PSA is organ specific but is not cancer specific [6]. PSA high levels could lead to overdiagnosis and overtreatment of prostate cancer. Therefore, efforts are being made to find alternative prostate cancer biomarkers that can predict the aggressiveness of the disease. Hence, there is a critical need for the development of non-invasive prostate cancer biomarkers with a better diagnostic and prognostic potential.

In the diagnosis and monitoring of prostate cancer treatment, telomerase is used as tumor marker. Telomerase is a ribonucleoprotein with reverse transcriptase activity (hTERT). Along with human telomeric RNA (hTR), telomerase maintains the length of telomere. Telomeres are conserved nucleoprotein structures localized at the end of linear chromosomes. They contain 15 kilobases (kb) of tandem hexamer repeat TTAGGG. Telomeres go through the degradation process with aging at the somatic cell site. Telomerase maintain chromosome stability by adding d(TTAGGG)n repeats to the ends of the linear chromosomes. The introduction of telomerase into normal human cells is enough to immortalize cells [7-11].

Telomerase activity is typically absent from most normal human cells but is expressed in nearly all human cancer. In addition, telomerase activity has also been correlated with prostate tumor aggressiveness. Particularly, compared with low-grade tumors, high-grade tumors have maximally activated telomerase and a significant correlation between the telomerase activity and the Gleason score has been found [12,13]. The development of the TRAP (telomerase repeat amplification protocol) assays has allowed screening of telomerase activity in different tumor types [14].

Several proteins, such as hepsin, are associated with the occurrence of prostate cancer [15]. Hepsin protein is a type II transmembrane serine proteinase. Hepsin is encoded by the HPN human gene located on chromosome 19 at q11-13.2 and contains 14 exons.

Hepsin is linked with intracellular adhesion, transmembrane signal transduction and degradation of extracellular matrix. Nandana et al. found that hepsin is involved in occurrence and progression in different malignant tumors. Hepsin acts as a proteolytic enzyme. By degrading the extracellular matrix, hepsin allows cancer cells to disseminate [16,17].

Therefore, our aim was to investigate the telomerase acitvity and expression of hepsin in prostate cancer tissues and its implication in proliferation of tumor cells.

Patients and Methods

A total of 38 patients with PCa (age: 51~82 years, average age: 66.5 years) admitted in Fundeni Clinical Institute from January 2018 to December 2018 were recruited for the present study. Another 20 patients (age: 45~65 years, average age: 53.1 years) who passed through a surgical resection of benign prostatic hyperplasia (BPH) during the same period of time were recruited as the control group. Informed written consent was previously obtained from all patients, and research protocol was approved by the Fundeni Clinical Institute Ethical Committee.

Fragments containing neoplastic prostatic tissue and benign prostatic tissue have been collected. In order to be included in telomerase activity and hepsin gene expression assessment, all samples were evaluated by the routine clinical histopathological methods. All the tumor samples were characterized according to international tumor-node-metastasis classification and Gleason system.

The quantitative determination of total PSA in human serum was done by Chemiluminescent Microparticle Immunoassay Method (Architect i2000, Abbott Laboratories, USA).

Detection of telomerase activity was performed with TRAPEzE ELISA Telomerase Detection (Millipore) kit which is highly sensitive for detecting telomerase activity in tumor tissues.

Hepsin gene expressions were revealed by Real-Time PCR. For the study of hepsin expression, we have extracted total RNA using RNeasy kit (Qiagen, Inc., Valencia, California), followed by the measurement of RNA concentration by spectrophotometry. Extracted total RNA was reverse transcribed into first strand cDNA with Superscript reverse transcriptase (Invitrogen). Further, cDNA was used for quantitative RT-PCR. For the amplification of cDNA were used LC-FastStart DNA Master SYBR Green kit (Roche Diagnostics) and the following primers: forward 5'-GGGACCCTGCTACTTCTGA-3', reverse 5'-ACGTCCCTTCCGTCTTGTC-3'. The PCR reactions were performed in the LightCycler from Roche.

Statistics

All results have been analyzed using Minitab software, version 18.1.

Results

We have combined telomerase activity and hepsin genes expression with PSA levels in order to reveal the following things: diagnostical aggression and invasion of prostate cancer. Most of the patients included in the study didn't have any information related to the prostate cancer. The most frequent symptom at admittance was frequent mictions. We have been studied the correlations between telomerase activity and hepsin gene expressions in patients with prostate cancer and benign prostatic hyperplasia.

All 38 patients with PCa had an average age of 66.5 years and average of preoperative PSA levels of 19.3 ng/ml. Patients with BPH had an average age of 55.8 years and average of preoperative PSA levels of 9.4 ng/ml. Normal PSA levels were met only in 5 patients from the control group.

In all patients with PCa, high telomerase activity and hepsin genes overexpression were found.

30% of patients with BPH had different grades of telomerase activity while the rest of them had no telomerase activity. In 3 patients with normal PSA levels, we have noticed an increased telomerase activity (Figure 1). A small percentage of patients (8.8) without PCa had hepsin overexpression (Figure 2).

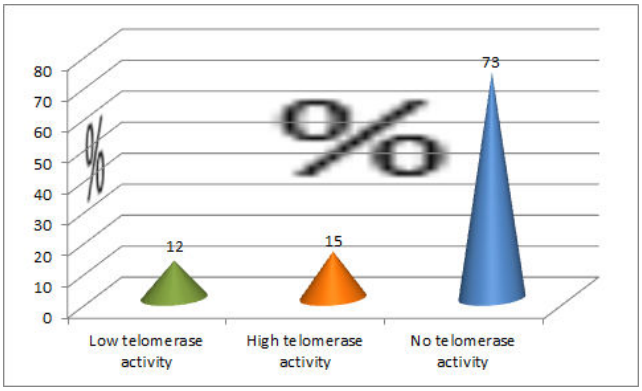

Figure 1: Telomerase activity in 20 patients with benign prostatic hyperplasia.

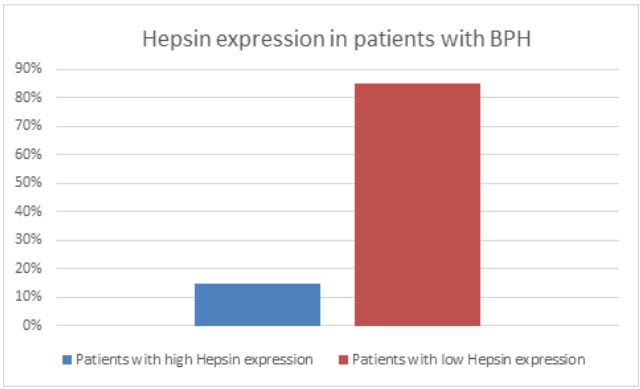

Figure 2: Hepsin expression in patients with BPH.

In case of tumours stages, we have noticed that the frequency of telomerase activity and hepsin gene expressions was bigger in patients with stages PT4 compared to other tumours stages (PT1, PT2, PT3).

This preliminary data show that telomerase and hepsin are two reliable markers for PCa diagnosis and follow-up of the patients.

Discussion

Over the past two decades, prostate cancer treatment decisions have been based almost exclusively on histological criteria (Gleason score), PSA levels and local disease state (TNM, WHO 2009), without attention to biomarkers characteristics [18].

Gleason score is a tool used to classify prostate cancer based on its ability to develop and expand. Pathologists examine the two most common types of cancer cells collected during biopsies and designate each type a grade from 1 to 5. The combination of two grades-for example 3+4-increases Gleason score from 2 to 10. The lowest Gleason score indicates slow-growing cancer that is unlikely to extend beyond the prostate (metastasis). In general, Gleason score, PSA scores and/or biopsy results (tumours at stage T2a or lower) could indicate early cancer onset.

PSA is the most widely used marker in PCa, for screening, diagnostic purposes, and response to treatment monitoring. PSA is usually present in serum in low concentrations. Serum PSA forms stable complexes with α1-antichymotrypsin (ACT) and α2-macroglobulin and the rest is free-PSA. In cases of prostate diseases and prostatic biopsy, PSA levels are usually increased. A systematic analysis of Cochrane (2013) revealed that PCa screening was associated with an increase in the number of cases of PCa diagnosed and PCa screening has disadvantages due to the risk of overdiagnosis and overtreatment [19-21].

The recommendations in the international guidelines for the early detection of prostate cancer have changed over time. Thus, in 2012, the U.S. Preventive Services Task Force (USPSTF) recommended that screening of PCa based on PSA should not be performed. In May 2018, USPSTF decided that PCa screening will be offered to men aged 55-69 who want an early diagnosis and take the risks of excessive invasive investigations [22,23].

None of Gleason score or PSA serum levels alone could precisely give information about the prostate cancer and predict the aggressiveness of the disease.

So, we have evaluated the association of telomerase activity and hepsin gene expressions in Romanian patients with prostate cancer in order to see if they can identify men with prostate cancer and benign prostatic hyperplasia.

In clinical studies, aggressive metastatic disease and poor prognosis have been linked with high telomerase activity in prostate cancer patients [24]. Sensitivity of telomerase in the early stages of cancer is significantly higher than in cytological methods: 75% vs. 80% [25-27].

Hepsin overexpression causes disorganization and destruction of the basal membrane of the prostate leading to metastasis [28]. Stephan C et al. reported in their research that hepsin overexpression in prostate cancer tissues correlates with the Gleason score, the prognosis and recurrence rate of the tumor [29]. Some researchers showed that hepsin expressions could indicated PCa relapsed following prostatectomy [30,31].

Conclusion

In our selected patients, PSA levels were not correlated in many cases with the aggressiveness of the disease and its prognosis.

Telomerase activity and hepsin gene expression gave us additional information related to support personalized prostate cancer diagnostics.

We think that telomerase activity and hepsin genes expression could be used as a non-invasive biomarker in Romanian patients for early and complete assessment of prostate cancer. Also, they could be better biomarkers for therapy monitoring.

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