ELSEVIER

Cancer Detection and Prevention 30 (2006) 449–454

Cancer Detection and Prevention

www.elsevier.com/locate/cdp

Kinetic fluorescence reverse transcriptase-polymerase chain reaction for alpha-methylacyl CoA racemase distinguishes prostate cancer from benign lesions☆

Martin Schostak MD[a,*], Kurt Miller MD[a], Hans Krause PhD[a], Mark Schrader MD[a], Carsten Kempkensteffen MD[a], Jens Kollermann MD[b]

[a] *Department of Urology, Charité–Campus Benjamin Franklin, Universitätsmedizin Berlin, Germany*
[b] *Institute of Pathology, University hospital of Hamburg-Eppendorf, Germany*

Accepted 31 July 2006

Abstract

Background: High-throughput gene expression profiling has recently shown that the mRNA for alpha-methylacyl CoA racemase (AMACR or P504S) is overexpressed in prostate carcinomas (PCa). Several immunohistochemical studies have reported the usefulness of anti-AMACR/P504S for detecting prostate cancer over the full range of prostate specimens encountered in surgical pathology. We tested real-time reverse transcriptase-polymerase chain reaction (RT-PCR) for specific and sensitive detection of AMACR transcripts as a supplementary measure for discriminating between malignant and benign lesions in prostatic tissues. *Methods*: Total RNA was isolated from snap-frozen chips in 55 cases of benign prostatic hyperplasia (BPH) and from frozen sections in 57 prostatectomy cases. The latter were analyzed by an uropathologist (J.K.) and found to contain at least 50% malignant epithelia. Relative quantification of AMACR transcripts was performed by RT-PCR using hybridization probes for detection and PBGD for normalization. *Results*: Normalized AMACR transcript levels showed an average 3.75-fold increase in 57 prostate carcinomas cases when compared to 55 cases of BPH ($p < 0.0001$). A 85.6% specificity and 64.9% sensitivity can be achieved if the cutoff is set at 12.95. AMACR expression levels among PCa cases were not statistically associated with the tumor and lymph node stage, the grading, the surgical margins, the Gleason score or progression. *Discussion*: The present study demonstrates the usefulness of quantitative AMACR-mRNA transcript detection in prostatic tissues as an alternative to immunological staining techniques. Since the latter clearly predominate in the laboratory routine, PCR-based detection of AMACR has the potential to gain widespread acceptance as a suitable future tool for monitoring prostate cancer patients.

Keywords: Prostate cancer; AMACR; P504S; RT-PCR; mRNA; Quantitative detection; Prostate-specific antigen (PSA); Immunohistochemical antibodies; Benign prostatic hyperplasia (BPH); Nuclear atypia; Cytokeratin; mRNA analysis; Tumor stage; Pathological classification; Gleason score; Clinical progression; Predictive values

1. Introduction

Since 2003, prostate cancer has been the tumor with the highest incidence among men in the Federal Republic of Germany [1]. A precise pathological classification is of essential importance for treating both localized and advanced tumors. Prostate-specific antigen (PSA) currently plays a predominant role in the detection, immunohistological testing, and follow-up. However, PSA is not an explicit

☆ Part of the results was presented at the 96th annual meeting of the American Association for Cancer research (April 16–20, 2005, Anaheim, California, USA).

* Corresponding author at: Urologische Klinik & Hochschulambulanz, Charité–Campus Benjamin Franklin; Universitätsmedizin Berlin, Hindenburgdamm 30, 12200 Berlin, Germany. Tel.: +49 30 8445 2577; fax: +49 30 8445 4620.

E-mail address: martin.schostak@charite.de (M. Schostak).

0361-090X/$30.00
doi:10.1016/j.cdp.2006.07.010

tumor marker for cancer. Both benign and malignant tissue changes can lead to elevated values. With an appropriate number of biopsies, the relatively high sensitivity enables detection of a prostate carcinomas (PCa) in virtually any man. However, the specificity of PSA is low. This in turn leads to a high percentage of essentially superfluous prostate biopsies [2]. The subsequent histological ''confirmation'' likewise shows considerable shortcomings. Thus great importance is attached to detection of the so-called basal cells. However, they are difficult or impossible to demonstrate by routine staining methods. PSA- and PAP-negative cells may even escape immunohistological detection, though it is quite possible to have benign findings *without* and malignant ones *with* basal cells.

Alpha-methylacyl CoA racemase (AMACR) is involved in the conversion of *R* stereoisomers of branched-chain fatty acids to *S* stereoisomers (racemization) [3]. Known dietary prostate cancer risk factors connected with elevations of the enzyme in prostate carcinoma cells have recently led to the development of AMACR-specific immunohistochemical antibodies that is otherwise basal-cell-independent and shows markedly higher specificity than the hitherto existing cytokeratin-based markers. First reports showed overexpression of the corresponding gene product compared to benign cells in a quantitative mRNA analysis [4].

This study examines possibilities for detecting prostate cancer by quantitative fluorescence-based real-time RT-PCR (Light-Cycler). Our aim was to find an objective molecular biological marker that can complement conventional histology and immunohistochemistry in doubtful cases.

2. Materials and methods

2.1. Patients and tissues

Probe retrieval was done in agreement with the Helsinki Declaration. Institutional review board approval was obtained for this study. Each patient signed a consent form approved by the Committee on Human Rights in Research at our institution.

The study examined tissues obtained by transurethral resection (TUR-P) in 55 patients with benign prostatic hyperplasia (BPH) and 57 patients with prostatic adenocarcinoma as well as specimens from a radical prostatectomy in 50 cases, and tissue chips from a palliative TUR-P in 7 cases.

In all cases, tissues were removed and immediately snap frozen in liquid nitrogen and stored at −80 °C for further processing. Ten 30 μm frozen sections were prepared from each tissue sample with the aid of a Cryostat 2800 (Leica Instruments GmbH, Nussloch, Germany) and stained with hematoxylin–eosin. Samples from cancer patients were microscopically examined by an uropathologist (J.K.). Only specimens with a carcinoma component of at least 50% were selected for further mRNA analysis.

2.2. Follow-up

At the time of sample processing, information on the current PSA and any clinical progression was obtained by telephone. Here a PSA of over 0.02 ng/ml was assessed as progression in the 50 radical prostatectomy cases, whereas progression had to be clinical, e.g. new metastases, to be evaluated as such in the 7 palliative TUR-P cases.

2.3. RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR)

RNA was prepared from tissue samples using EZ1 BioRobot™ (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The RNA concentration and quality (28S/18S) was determined after capillary electrophoresis using RNA Nano LabChip® technology in an AGILENT Bioanalyzer 2100 instrument (Agilent GmbH, Waldbronn, Germany).

2.4. Quantitative RT-PCR

Real-time one-step RT-PCR for detecting AMACR mRNA was performed using a RT-PCR thermal cycler system according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany).

Primers for AMACR were sense (5′-TGGCTTTGTCA-GGTGTTC-3′) and antisense (5′-TATGCTGTTCCTTC-CACCATAT-3′). Hybridization probes specific for the AMACR gene were the FL probe (CCCAGTGCACACA-TAAGGCC-FL) and the LC Red640-probe (CCACCAG-CAAAGTCAGCCAC-PH).

For one-step RT-PCR, 250 ng of RNA were first reverse-transcribed into cDNA for 10 min at 55°C and later amplified during 45 cycles (1 s at 95 °C, 15 s at 60 °C and 20 s at 72 °C).

For amplification of the housekeeping gene the porphobilinogen deaminase (PBGD) Housekeeping gene Kit (Roche Diagnostics GmbH) was used. For amplification of the target gene AMACR the RT-PCR RNA Amplification Kit in combination with AMACR-specific primers (Roche Diagnostics GmbH) was used.

2.5. Evaluation

The Light-Cycler Software Version 3.3 (Roche Diagnostics GmbH) was used to analyze PCR kinetics and calculate quantitative data. A standard curve generated in a separate run was loaded into runs of patient samples (without standard curves). Each run included one sample of known concentration and in the range covered by the standard curve, thus allowing estimation of exact copy numbers by the second derivative maximum method.