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ORIGINAL ARTICLE

Gene expression profiles in prostate cancer: Identification of candidate non-invasive diagnostic markers☆

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KEYWORDS
Prostate cancer;
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Abstract
Objective: To analyze gene expression profiles of prostate cancer (PCa) with the aim of determining the relevant differentially expressed genes and subsequently ascertaining whether this differential expression is maintained in post-prostatic massage (PPM) urine samples.
Materials and methods: Forty-six tissue specimens (36 from PCa patients and 10 controls) and 158 urine PPM-urines (113 from PCa patients and 45 controls) were collected between December 2003 and May 2007. DNA microarrays were used to identify genes differentially expressed between tumor and control samples. Ten genes were technically validated in the same tissue samples by quantitative RT-PCR (RT-qPCR). Forty-two selected differentially expressed genes were validated in an independent set of PPM-urines by qRT-PCR.
Results: Multidimensional scaling plot according to the expression of all the microarray genes showed a clear distinction between control and tumor samples. A total of 1047 differentially expressed genes (FDR ≤ 0.1) were identified between both groups of samples. We found a high correlation in the comparison of microarray and RT-qPCR gene expression levels ($r = 0.928$, $p < 0.001$). Thirteen genes maintained the same fold change direction when analyzed in PPM-urine samples and in four of them (*HOXC6*, *PCA3*, *PDK4* and *TMPRSS2-ERG*), these differences were statistically significant ($p < 0.05$).
Conclusion: The analysis of PCa by DNA microarrays provides new putative mRNA markers for PCa diagnosis that, with caution, can be extrapolated to PPM-urines.

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PALABRAS CLAVE
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Perfil de expresión génica en el cáncer de próstata: identificación de marcadores candidatos para el diagnóstico no invasivo

Resumen

Objetivo: Analizar los perfiles de expresión génica del cáncer de próstata (CaP) e identificar los genes diferencialmente expresados. Determinar si la expresión diferencial en tejido se mantiene en muestras de orina-posmasaje prostático (PMP).

Material y métodos: Un total de 46 muestras de tejido prostático (36 de pacientes con CaP y 10 controles) y 158 orinas-PMP (113 de pacientes con CaP y 45 controles) se recogieron entre diciembre de 2003 y mayo de 2007. Se utilizaron microarrays de ADN para identificar los genes diferencialmente expresados entre las muestras de tejido tumorales y las controles. Diez genes fueron seleccionados para la validación técnica de los microarrays en las mismas muestras tisulares mediante PCR cuantitativa (RT-qPCR). Se seleccionaron 42 genes para ser validados en muestras de orina-PMP mediante RT-qPCR.

Resultados: El gráfico de escalado multidimensional mostró una clara separación entre las muestras de tejido tumorales y las controles. Se han identificado 1.047 genes diferencialmente expresados (FDR $\leq$ 0,1) entre los 2 grupos. La correlación entre los datos de microarrays y RT-qPCR fue alta ($r = 0{,}928$, $p < 0{,}001$). Trece genes mantuvieron el mismo sentido de expresión diferencial al ser analizados en orinas-PMP y 4 de ellos (*HOXC6*, *PCA3*, *PDK4* y *TMPRSS2-ERG*) mostraron diferencias de expresión estadísticamente significativas entre orinas-PMP tumorales y controles ($p < 0{,}05$).

Conclusión: Existe un perfil de expresión génica diferencial en el CaP. Aunque la extrapolación de la expresión génica obtenida en tejido prostático a orina-PMP se debe realizar con precaución, el análisis del tejido prostático permite la identificación de nuevos biomarcadores para diagnóstico no invasivo del CaP.

Introduction

Prostate cancer (PCa) is one of the most common male malignancies in our population.[1] During the last 2 decades, the prostate specific antigen (PSA) has been widely used for screening, diagnosis, and follow-up of PCa. Routine use of PSA has been the object of continuous controversy due to its limited specificity, which is derived from the fact that elevated serum PSA levels are produced in a variety of non-neoplastic conditions, such as prostatitis and benign prostatic hyperplasia (BPH). On the contrary, a substantial number of men with PSA in the normal range (<4 ng/ml) have PCa.[2] Currently, the diagnosis of PCa is made by means of prostate biopsy guided by transrectal ultrasound, which does not detect approximately 20–30% of the cases.[3] Therefore, there is a need for additional specific markers for more accurate and early detection of PCa, which allows for a reduction in the number of unnecessary prostate biopsies. Furthermore, it would be of great interest that these markers could be determined non-invasively, like in urine or serum samples.

The development of new technologies of medium–high yield for the analysis of gene expression, such as DNA microarrays or new platforms based on quantitative real-time PCR (RT-qPCR, reverse transcription quantitative PCR), has had a significant effect on the discovery of new molecular markers for diagnosis and prediction of disease progression in several types of cancer, including PCa.[4–8] In addition, the detection of biomarkers for PCa in non-invasive samples has already been described in the literature,[9–16] but so far no biomarker has replaced the routine use of PSA as a screening and follow-up method of PCa.

In this study we used the DNA microarray technology to identify the differentially expressed genes in PCa. Furthermore, we analyzed a selection of these genes in samples of post-prostatic massage urine (PPM urines) of an independent cohort of patients in order to assess the correlation between gene expression in the tissue sample and the PPM urine and identify potential new molecular markers for the non-invasive diagnosis of PCa.

Materials and methods

Patients and samples

The samples used in this study were collected between December 2003 and May 2007 in the Fundació Puigvert and the Clínic Hospital of Barcelona. This study was approved by the ethics committee and all patients and controls included in it were duly informed of its objectives before being included and they signed the informed consent sheet.

In order to identify the differentially expressed genes in PCa, 36 PCa tissue samples of different tumor stages and Gleason scores[17,18] were collected and 10 samples of prostate tissue from patients with BPH without evidence of malignancy in the prostate (supplementary material). The tumor samples were grouped into 3 categories: (1) pT2G7 samples (15 pT2G7); (2) pT3-4 samples, independent of Gleason score (5 T3G7, 1 T3G8, 7 T3G9 and 1 T4G7); and (3) Gleason samples >8, independent of tumor stage (7 T3G9).