Research Article

Is There a Role for Genetic Information in Risk Assessment and Decision Making in Prostate Cancer?

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Abstract

Objectives: Prostate cancer is a neoplasm with a variable natural history and clinical behavior. There is much debate on the use of inherited genetic information in clinical application including risk assessment and treatment decisions. This study was performed to evaluate the relationship between clinical parameters of prostate cancer (PSA, Gleason score, and metastasis) and expression of NKX3.1, AMACR, TMPRSS2-ERG, ERG, and SPINK1 genes.

Methods: Newly diagnosed cases of prostate cancer were selected for this study. Thirty four tissue samples were obtained via open radical prostatectomy and 9 samples were obtained via needle biopsy. Each tissue sample was sectioned into two parts, one used for detection of malignant changes and Gleason score determination, and the other immersed in RNA later solution (Qiagen). The expression of NKX3.1, AMACR, TMPRSS2-ERG, ERG, and SPINK1 genes were assessed by real-time PCR assay. Correlation between expression of each gene and PSA level, Gleason score, and presence of metastasis were examined.

Results: A total number of 43 specimens were studied, from which 9 were obtained from patients with metastatic prostate cancer. The expression of five examined genes had no correlation with PSA level and Gleason score. The expression of AMACR decreased in metastatic prostate cancer ($P = 0.02$). The expression of other genes showed no difference between metastatic and non-metastatic tumors ($P > 0.1$).

Conclusions: Genetic information combined with clinical data can be useful in risk assessment and treatment planning. Based on the results of the current study, the decreased expression of AMACR was a sign of poor prognosis.

Keywords: AMACR, ERG Gene Expression, NKX3.1, Prostate Neoplasm, SPINK1, TMPRSS2-ERG

1. Introduction

Prostate cancer is a neoplasm with a variable natural history that ranges from indolent to aggressive (1). Several clinical features of prostate cancer including tumor stage, Gleason score, serum PSA, and biopsy criteria are used in routine clinical practice to categorize the patients into groups of low, intermediate, high, or very high risk for tumor recurrence following local therapy. Patients with more risk criteria usually suffer substantial disease related mortality (1, 2). However, up to 30% of men undergoing radical prostatectomy will relapse often as a result of micrometastatic disease present at the time of surgery (2).

Currently, there is much debate on the use of inherited genetic information in clinical applications including risk assessment. Therefore, a critical research question is if genotype adds information to risk prediction beyond that of traditional risk factor (3). The challenge is to identify those patients at risk for relapse and to better understand the molecular abnormalities that define tumors at risk of relapse (2). Attempts to explore genetic correlation with tumor behavior have found alterations in a number of candidate genes associated with prostate cancer.

However, no single gene has been shown to have sufficient prognostic utility to warrant clinical implementation. This study attempts to evaluate relationship between clinical parameters of prostate cancer (PSA, Gleason sore, and metastase) and expression of NKX3.1, AMACR, TMPRSS2- ERG, ERG, and SPINK1 genes.

2. Methods

2.1. Tissue Sample Collection

Patients with prostate cancer were selected from individuals who referred to the urology department of uro-

oncology research center (UORC) from June 2011 to March 2013. All the patients were new cases of the disease with no medical history of surgery or therapy regarding prostatic problem. They were first visited by an urologist and underwent imaging and laboratory tests according to the standard diagnostic approaches. Each patient singed a written informed consent before joining the study. The study was approved by the ethics committee of UORC. Thirty four tissue samples were obtained via open radical prostatectomy, and in 9 patients with metastatic disease, tissue sample was obtained via needle biopsy.

Each tissue sample was sectioned into two parts as replicates. One replicate was stained by hematoxylin and Eosin, and examined by a pathologist for detection of malignant changes and evaluation of tumor grade expressed as the Gleason Score. The other replicate was microdissected to obtain tumor and matched normal tissue. The tissue samples were instantly immersed in RNAlater solution (Qiagen) and kept at room temperature for 24 hours. Then, these tissue samples were transferred into liquid nitrogen container for long-term storage.

2.2. Total RNA Extraction and cDNA Synthesis

Total RNA containing small RNAs (e.g.miRNAs) was extracted and purified from tissue sample (50 gr) using miRNeasy® Mini kit (Qiagen, cat. No. 217004) according to the kit instruction. CDNA synthesis was performed using oligo dt or random hexamers (protoscipt kit, new England Biolab, NED # E 6300s) to convert mRNA or non-coding RNAs, respectively.

2.3. Development of Quantitative TaqMan Probe Real Time PCR Assays

Exon-exon junction spanning primers and Taqman probe were designed using primer Express V.3 software (Applied Biosystems) and verified to be specific for their targets by BLAST analysis on NCBI website. NKX3.1 AMACR, SPINK1, ERG, and TMPRSS2-ERG gene fusion were assigned as target genes. GAPDH was considered as normalizing gene in expression analysis experiments. Serially ten-fold diluted plasmids were used as template in triplicate Real-time PCR experiments.

2.4. Data Analysis

Relative mRNA expression was normalized to the geometrical mean of the CT values determined for GAPDH gene using comparative ΔCT method. Correlation between the expression of each gene and PSA level, Gleason score and presence of metastase was evaluated and analyzed using SPSS software.

3. Results

A total number of 43 specimens were studied, from which 9 were obtained from patients with metastatic cancer. The PSA levels were less than 10 ng/mL, 10 - 20 ng/mL and more than 20 ng/mL in 12, 17, 14 patients, respectively.

Gleason score was lower than 7, equal to 7, or above 7 in 10, 20, and 4 patients, respectively. Table 1 shows the relationship between PSA level and expression of these five genes. No relationship was detected between expression of these five genes and PSA level. Table 2 shows the relationship between metastase and expression of these five genes. As can be seen, the expression of AMACR decreased in metastatic prostate cancer (P = 0.02). The expression of other genes showed no difference between metastatic and non-metastatic tumors (P > 0.1). Table 3 shows the relationship between the Gleason score and five examined genes. No relationship was detected between the expression of the five genes and the Gleason score (P > 0.1).

4. Discussion

Up to now, genetic criteria have no role in risk classification, prognosis, and treatment planning of prostate cancer. Establishing a relationship between gene expression and clinical parameters (PSA, Gleason score and clinical stage) can help us in treatment planning. It may be possible to predict clinical behavior of prostate cancer based on gene expression analysis of primary tumor. Such predictive strategies would allow for the rational treatment and application of postsurgical therapeutics to high risk individuals. Here, we appraise five important genes in prostate cancer:

4.1. NKX3.1

NKX3.1 is an androgen-regulated homeodomain gene whose expression is predominantly localized to prostate epithelium and regulates prostate epithelial proliferation. NKX3.1 is located on chromosome 8P 21.2, a region that shows loss of heterozygosity (LOH) in 12 - 89% of high grade prostatic intraepithelial neoplasia (PIN) and 35% to 89% of prostatic adenocarcinomas (4, 5).

Bethel CR et al. reported that the frequency of LOH on chromosome 8P increases with advanced prostate cancer grade and stage (6) although we detected no relationship between the expression of NKX3.1 and PSA level, Gleason score and metastatic disease. Gelmann et al. showed that the expression of NKX3.1 was highly specific of prostate cancer and breast cancer, with little or no staining in a large number of other tumor type (7).

Table 1. Relationship Between PSA Level and Expression of These Five Genes

	Gene													
	NKX3.1			AMACR			TMPRSS2-ERG		ERG			SPINK1		
PSA	Down	No Change	Up	Down	No Change	Up	+	-	Down	No Change	Up	Down	No Change	Up
PSA < 10, ng/mL	12	0	0	5	1	6	10	2	9	1	2	9	0	3
PSA 10 - 20, ng/mL	14	1	2	5	3	9	7	10	6	6	5	10	0	7
PSA > 20, ng/mL	14	0	0	10	0	4	8	6	8	2	4	6	1	7
Total	40	1	2	20	3	18	25	18	23	9	11	25	1	17
	P = 0.5			P = 0.8			P = 0.5		P = 0.3			P = 0.8		

Table 2. The Relationship Between Metastase and Expression of These Five Genes

	Gene													
	NKX3.1			AMACR			TMPRSS2-ERG		ERG			SPINK1		
Metastas	Down	No Change	Up	Down	No change	Up	+	-	Down	No Change	Up	Down	No Change	Up
Metastas	31	1	2	13	2	19	14	20	17	7	10	20	1	13
Metastas	9	0	0	8	0	1	4	25	6	2	1	6	0	3
Total	40	1	2	21	2	20	18	45	23	9	11	26	1	16
	P = 0.6			P = 0.02			P = 0.8		P = 0.5			P = 0.8		

Table 3. The Relationship Between the Gleason Score and Five Examined Genes

	Gene													
	NKX3.1			AMACR			TMPRSS2-ERG		ERG			SPINK1		
Gleason Score	Down	No Change	Up	Down	No Change	Up	+	-	Down	No Change	Up	Down	No Change	Up
GS < 7	9	0	1	5	1	4	4	6	6	3	1	6	0	4
GS = 7	18	1	1	7	1	12	9	11	9	4	7	11	1	8
GS > 7	13	0	0	9	0	4	5	8	8	2	3	9	0	4
Total	40	1	2	21	2	20	18	25	23	9	11	26	1	16
	P = 0.6			P = 0.3			P = 0.9		P = 0.5			P = 0.8		

4.2. AMACR

AMACR is a key enzyme in the Beta-oxidation catabolic pathway of fatty acids and is known to be upregulated in several cancers including prostate cancer (8, 9).

AMACR is highly expressed in the majority of prostate cancer samples, about 6 times higher than the level expressed in the BPH samples (9).

AMACR is abundantly expressed and recognized as a standard tissue biomarker capable of highly sensitive and specific diagnosis of prostate cancer. The value of AMACR and its variants is to develop diagnostic biomarkers that will complement the diagnostic capability of PSA, while addressing the limitation of PSA, specifically its low specificity (8). Sequence variants of AMACR have been previously investigated to find their association with the risk of prostate cancer (10).

Although Jun Luo et al. reported that both untreated metastases and hormone refractory prostate cancer were generally strongly positive for AMACR (9), AMACR expression only decreased in metastatic patients in our study.

Abouassaly et al. also reported that the AMACR expression shows a decrease in metastatic prostate cancer when compared with localized disease that is associated with cancer specific survival (11). Luo et al. found no relationship between AMACR IHC score and Gleason grade, pathological stage, patient age, or preoperative serum PSA (9). The difference may be due to several factors including study design, tumor localization, ethnic background, and detection methods.

4.3. TMPRSS2/ERG and ERG

Several studies have investigated the prevalence and clinical value of TMPRSS2-ERG in prostate cancer. The median prevalence in both clinically localized and castration resistant prostate cancer is around 40 - 50%, with a lower frequency reported in high grade prostatic intraepithelial neoplasia (12). Studies analyzing the association between TMPRSS2-ERG states and clinicopathological parameters such as Gleason score and prognostic value have indicated conflicting results. Moreover, the clinical impact

is still unclear, since while some authors have suggested a worse prognosis for fusion versus non-fusion cancers (13), other either found a favorable prognostic association (14, 15) or did not find any association with clinical outcome (16, 17).

In fact, ERG protein expression is the active form of the gene product and hence could be a better method in documenting any prognostic significance (13). Micheal Taris et al. observed that ERG prevalence significantly increased from high grade PIN (17.5%) to PT2 tumors (27.5%) then to PT3 (43%) and metastases (53%) (13). They showed that the association of ERG fusion with both advanced stage and better outcome in their study could be explained by the fact that fusion positive and fusion negative prostate cancer are likely to progress via different molecular pathways (13).

Stefan Steurer et al. showed that the androgen driven events causing TMPRSS2-ERG fusion and other rearrangements of androgen- dependent genes in prostate epithelial cells of young patients preferentially lead to low grade (and not high grade) prostate cancer. This finding may help explain the slight but significant predominance of low grade cancer in young patients (18).

A study by Fleischmann et al. showed that TMPRSS2-ERG fusion was not overrepresented in high risk population of metastasizing prostate cancer. This finding suggested that this mutation does not characterize particularly aggressive tumors. Investigation of TMPRSS2-ERG fusion prevalence in primary tumor and corresponding metastases might help further elucidate the biological role of this mutation (19).

Studies on the prognostic relevance of TMPRSS2-ERG fusion in cancers treated by radical prostatectomy have shown consistent results. Based on survival curves, some studies have suggested that TMPRSS2-ERG fusion is a poor (20) and conversely a good (14) prognostic factor or some other studies have reported that it is of no prognostic value (21). Moreover, in a large cohort study with more than 2800 prostate cancer patients, the surrogate marker for TMPRSS2-ERG fusion, ERG protein expression, did not predict survival (22). We also found no association with clinical findings. These conflicting results may be due to differences in cohort size and composition, therapy, clinical endpoints, mechanisms of fusion (deletion vs. translocation), and detection methods.

4.4. SPINK1

The serin peptidase inhibitor Kazal type1 (SPINK1), also known as pancreatic secretory trypsin inhibitor (PSTI) or tumor associated trypsin inhibitor (TATI), is an extracellular secreted protein, protecting pancreas from autodigestion by preventing premature activation of pancre-

atic protease (23). Additionally, SPINK1 is described to play an important role in the prevention of apoptosis in benign tissue (23). Several studies suggested SPINK1 expression in malignancies including breast cancer (24), colorectal cancer (25), hepatocellular carcinoma (26), and prostate cancer (27, 28). The expression of SPINK1 has been described to define a subset of aggressive ERG-fusion negative prostate cancer (29). Moreover, SPINK1 was detectable non-invasively in the serum (30) and urine (29) in patients with prostate cancer and pre-clinical models utilize SPINK1 as therapeutic target in SPINK1 positive ERG fusion negative prostate cancer (27).

The analysis of a large and well-defined prostate cancer collection excluded the expression of SPINK1 as a prognostic biomarker in prostate cancer (23). Grupp et al. found no associations between this gene and PSA recurrence, neither in all cancerous cases nor in the relevant subgroup of fusion negative prostate cancers. No association was detected with clinical findings in our study, as well.

4.5. Conclusion

Genetic criteria can include new information to clinical data for better risk assessment and treatment planning, and may identify patients at risk of relapse and metastasis. A decrease in the expression of AMACR may be a sign of poor prognosis and an increase in the risk of metastasis. We found no relationship between NKX3.1, TMPRSS2-ERG, ERG, and SPINK1 expression and clinical findings.

Due to different genetic findings in various studies, it seems necessary to design more studies with matched control expect for genetic information in order to define the exact role of genetic factors.

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