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Citation

Mucci, L. A., Y. Pawitan, F. Demichelis, K. Fall, J. R. Stark, H.-O. Adami, S.-O. Andersson, et al. 2008. "Nine-Gene Molecular Signature Is Not Associated with Prostate Cancer Death in a Watchful Waiting Cohort." *Cancer Epidemiology Biomarkers & Prevention* 17 (1): 249–51. <https://doi.org/10.1158/1055-9965.epi-07-0722>.

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Null Results in Brief

Nine-Gene Molecular Signature Is Not Associated with Prostate Cancer Death in a Watchful Waiting Cohort

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Abstract

Tumor molecular markers hold promise to distinguish potentially lethal from indolent prostate cancer and to guide treatment choices. A previous study identified a nine-gene molecular signature in tumors associated with prostate-specific antigen relapse after prostatectomy. We examined this molecular model in relation to prostate cancer death among 172 men

with initially localized disease. We quantified protein expression of the nine genes in tumors to classify progression risk. Accounting for clinical prognostic factors, the nine-gene model did not provide discrimination to predict lethal and indolent prostate cancer. (Cancer Epidemiol Biomarkers Prev 2008;17(1):249–51)

Introduction

Prostate cancer is the most common male cancer in westernized countries. Most men diagnosed in early stages experience slow-growing or indolent tumors even in the absence of therapy (1, 2). The rising incidence of prostate cancer, chiefly due to increased prostate-specific antigen (PSA) screening and diagnostic intensity, underscores the need to distinguish indolent from lethal prostate cancer to guide treatment decisions.

Clinical nomograms characterize progression risk using pretreatment clinical markers and have predictive power (3, 4), but molecular tumor markers hold promise to improve prognostication (5). A signature of advanced prostate cancer was recently identified by assessing gene expression at the transcriptome and proteome level. The nine-gene model identified markers that were altered in metastatic versus localized prostate cancer and predicted

PSA relapse after surgery in expression array datasets (6). Most men with PSA relapse, however, do not develop metastatic or lethal disease (7). Thus, we tested this molecular signature to predict prostate cancer death among men diagnosed with localized disease and followed prospectively for outcomes over 20 years.

Materials and Methods

Subjects. The Örebro Watchful Waiting Cohort (2, 8) comprises 172 Swedish men with localized (T1a/T1b) prostate cancer diagnosed incidentally by transurethral resection of the prostate for benign prostatic hyperplasia between 1977 and 1991. Following cancer diagnosis, the men were initially managed by watchful waiting and followed with careful monitoring by clinical exams, laboratory tests, and bone scans. Follow-up for development of distant metastases and death is complete through March 2006.

Immunohistochemistry. Archival formalin-fixed, paraffin-embedded tissue specimens were used to construct high-density tissue microarrays (TMA; ref. 8). Two 0.6-mm tumor tissue cores from the dominant prostate cancer nodule of each case were included on TMAs (9). Using immunohistochemistry, we assayed tumor protein expression of the nine genes on TMA sections and assessed staining intensity (0–255) and percent of positive stained area (0–100%) from scanned digital images of

Received 8/6/07; revised 10/23/07; accepted 10/31/07.

Grant support: NIH/National Cancer Institute Prostate Specialized Programs of Research Excellence at the Dana-Farber/Harvard Cancer Center grant NCI P50 CA090381, NIH T32 Training Grant CA009001 (L.A.M.), NIH R01AG21404 (M.A.R. and F.D.), and Deutsche Forschungsgemeinschaft DFG PE1179/1-1 (S.P.).

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doi:10.1158/1055-9965.EPI-07-0722

Table 1. Clinical characteristics comparing risk categories based on the nine-gene model, Örebro Watchful Waiting Cohort, 1977 to 2005

	Risk classification based on nine-gene model*		
	Lowest risk (Q1)	Intermediate risk (Q3)	Highest risk (Q5)
<i>n</i>	17	42	39
Age at diagnosis (y)	75.5 (6.7)	73.6 (7.4)	73.7 (7.9)
Mean (SD) follow-up (y)	8.4 (6.2)	8.2 (6.1)	6.9 (5.2)
Gleason score (%)			
4-5	5.9	16.7	0.0
6	58.8	47.6	48.7
7	29.4	19.1	25.6
8	5.9	16.7	25.6
Tumor extent (%)			
<5%	29.4	45.2	41.0
5-24.9%	64.7	38.1	28.2
25-49.9%	5.9	2.4	7.7
>50%	0.0	14.3	23.1
Nuclear grade (%)			
I	76.5	76.2	56.4
II	17.7	14.3	30.8
III	5.9	9.5	12.8

*Risk classification is based on the molecular risk score divided into quintiles. For presentation, data from Q2 and Q4 are excluded.

TMA cores using the Chromavision image analysis system (10).

Statistical Analysis. Based on the cohort distribution, expression of each marker was divided into quartiles. Expression in the highest quartile for markers up-regulated in metastatic versus localized prostate cancer (AMACR, Itga-5, cIAP, and krip1) received a score of 1 (0 otherwise), whereas expression in the lowest quartile for down-regulated markers (occludin, bm28, p62, lap2, and drbp76) received a score of 1. We summed across each of the genes to create a summary molecular risk score and classified men as having a high, intermediate, or low risk of progression.

We used time-to-event analyses to evaluate the ability of the molecular signature to predict development of lethal prostate cancer. Hazard ratios [HR; 95% confidence intervals (95% CI)] were used as effect measures from proportional hazards model. We compared age-adjusted (continuously) models with models also adjusted for clinical predictors: Gleason grade (categorically,

4-5, 6, 7, and >8), nuclear grade (categorically, grades I, II, and III), and tumor extent (categorically, <5%, 5-24.9%, 25-49.9%, and >50%).

The study was approved by the institutional review boards at the collaborating U.S. and Swedish institutions.

Results

The mean (SD) age of the men at prostate cancer diagnosis was 74.1 (7.1) years. Of the 172 men with localized prostate cancer, 40 developed lethal disease. Mean time to development of metastatic disease was 7.6 years, and mean time from metastasis to time of death was 2.0 years. Men classified as higher risk of lethal prostate cancer based on the nine-gene signature tended to have tumors with higher Gleason grade, higher nuclear grade, and greater tumor extent than men classified at lower risk (Table 1).

First, we individually evaluated each of the gene markers to predict lethal prostate cancer. Dysregulation of cIAP (HR, 1.9; 95% CI, 0.9-3.6) and Itga-5 (HR, 1.7; 95% CI, 0.8-3.3) were the strongest predictors of lethal prostate cancer, although neither was statistically significant.

The nine-gene model was modestly predictive of lethal disease. Of the 17 men classified as lowest risk by the nine-gene model, 11.8% died of cancer, whereas a larger percentage of men (28.2%) classified as highest risk by the nine-gene model died. Comparing highest and lowest risk groups, the HR of lethal prostate cancer was 3.3 (95% CI, 0.7-15.0). However, the model was also correlated with the clinical variables and after adjustment was less of a prognostic predictor (Table 2). The molecular signature was a poorer predictor of lethal prostate cancer (c-index 0.59) compared with clinical markers alone (c-index 0.71).

Discussion

In this cohort of men with initially untreated localized prostate cancer, we found the nine-gene model was not a useful discriminator of lethal versus indolent prostate cancer and did not add information to risk prediction. We tested the signature to predict prostate cancer death, which is the optimal outcome for validation of biomarkers of prostate cancer prognosis.

Table 2. Nine-gene model as a predictor of lethal prostate cancer, Örebro Watchful Waiting Cohort, 1977 to 2005

	Total (<i>n</i>)	Lethal* (%)	Indolent [†] (%)	HR (95% CI)	
				Age adjusted	Multivariable adjusted [‡]
Molecular model only					
Q1 (lowest risk)	17	11.8	35.3	Reference	Reference
Q2	50	22.0	32.0	2.0 (0.4-9.0)	1.7 (0.4-7.8)
Q3 (intermediate risk)	42	28.6	26.2	2.6 (0.6-11.8)	1.7 (0.4-8.2)
Q4	24	16.7	29.2	1.5 (0.3-8.5)	0.6 (0.1-3.2)
Q5 (highest risk)	39	28.2	23.1	3.3 (0.7-15.0)	1.7 (0.3-8.4)
<i>P</i> for trend			0.17	0.78	

*Lethal prostate cancer defined as men who developed distant metastases or died of cancer over follow-up.

[†]Indolent cancer based on long-term survival defined as 10 years or more without development of distant metastases or death from prostate cancer.

[‡]Hazard ratios and confidence intervals adjusted for age at diagnosis, Gleason score, nuclear grade, and tumor extent.

Although biochemical failure is associated with an increased likelihood of cancer death, most men who experience a relapse would not die of their disease (11, 12).

Our cohort included prospective long-term and complete clinical follow-up, which is important because prostate cancer deaths can occur many years after diagnosis (2, 13). Our study population was derived from a well-defined catchment area, with similar clinical care for all patients, thus reducing potential selection biases. Although the Swedish Watchful Waiting Cohort was small, we had a relatively large number of events (40 lethals). Our study had 80% power to exclude for the nine-gene signature HR of 1.85 comparing extreme risk groups, a clinically meaningful risk estimate for biomarker studies. The tissue samples underwent standardized histopathologic review for Gleason grading to address potential grade migration over time (13). Although the Örebro cohort was assembled in the pre-PSA era, the cancers were incidentally detected and likely resemble PSA detected cases given the distribution of Gleason grade and stage. These TURP tended to be transitional zone as opposed to peripheral tumors, but there is little evidence to suggest meaningful differences in the biology of tumors in these zones. We had no baseline PSA levels, a clinical predictor of outcome (14-16). However, it is unlikely that such information would have altered our results.

Although we could not validate this nine-gene model, tumor biomarkers still hold promise to characterize a patient's prostate cancer prognosis to target appropriate therapy. Molecular tools can potentially distinguish men for whom aggressive treatment would be indicated and thereby reduce the number needed to treat to avoid one prostate cancer death. The challenge for the future is to use cohorts with appropriate clinical outcomes and follow-up for development and validation of molecular signatures.

Acknowledgments

We thank Kelly Lamb and Lela Schumacher for technical support critical to this study. The TMA arrays were constructed at the Dana-Farber/Harvard Cancer Center Tissue Microarray Core Facility.

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Cancer Epidemiol Biomarkers Prev 2008;17:249-251.

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