Immunohistochemical Expression of BRCA1 and Lethal Prostate Cancer

Michelangelo Fiorentino1,9, Gregory Judson2,3, Kathryn Penney2,3, Richard Flavin1, Jennifer Stark2,3, Christopher Fiore1, Katja Fall2,3,10, Neil Martin3,6, Jing Ma2, Jennifer Sinnott2,5, Edward Giovannucci2,3,4, Meir Stampfer2,3,4, Howard D. Sesso3,7, Philip W. Kantoff8,11, Stephen Finn1, Massimo Loda1,11, and Lorelei Mucci2,3

Abstract

BRCA1 functions as a tumor suppressor; recent work suggests that BRCA1 may also induce cell cycle arrest to allow for DNA repair. We hypothesized that BRCA1 expression in prostate tumor tissue may be associated with prostate cancer progression through regulation of the cell cycle. We used immunohistochemistry to evaluate BRCA1 protein expression in archival tumor samples from 393 prostate cancer cases in the Physicians’ Health Study. The men were followed prospectively from diagnosis to development of metastases and mortality. Fifteen percent of tumors stained positive for BRCA1. BRCA1-positive tumors had substantially increased tumor proliferation index compared with negative tumors (47.0 Ki67-positive nuclei versus 10.3, P = 0.0016) and were more likely to develop lethal cancer compared with BRCA1-negative tumors (hazard ratio, 4.6; 95% confidence interval, 2.4–8.7). These findings strengthen the hypothesis that BRCA1 plays a role in cell cycle control and show that BRCA1 is a marker of clinical prostate cancer prognosis.

Introduction

BRCA1 is a multifunctional tumor suppressor protein implicated in regulating the maintenance of genome integrity through the activation of DNA repair genes, heterochromatin formation, double strand–break repair, homologous recombination events, and ubiquitination (1–3). Recently, a more complex role for BRCA1 was proposed, whereby BRCA1 can induce arrest at different cell cycle check points to allow for DNA repair (4–6).

Mutations in BRCA1 have been associated with increased risk of breast, ovarian, and, more recently, prostate cancer—particularly high-grade disease (7–12). However, although mutations in BRCA1 may influence familial prostate cancer risk and progression, few studies have examined BRCA1 protein expression in prostate cancer tumor tissue, and, to our knowledge, none have correlated expression with cancer progression and mortality. Recently, Schayek and colleagues showed that BRCA1 protein expression in prostate differentially regulates IGF-IR gene expression in an androgen-dependent manner and found significantly elevated BRCA1 levels in prostate cancer in comparison with normal prostate tissue (13). We hypothesized that BRCA1 expression could have prognostic relevance in prostate cancer through its regulation of the cell cycle regardless of germ-line mutations.

Materials and Methods

We undertook a prospective study among 392 men in the Physicians’ Health Study (refs. 14, 15; http://clinicaltrials.gov identifier: NCT00000500) who were diagnosed with prostate cancer from 1983 to 2004. We constructed tumor tissue microarrays from archival prostatectomy and trans urethral resection of the prostate tumor tissue specimens using three 0.6-mm cores of tumor per case. Immunohistochemical staining was performed on 5-μm sections of the tissue microarrays (TMA) to assess BRCA1 expression [monoclonal MS110 antibody specific for the NH2 terminus of the 220 kDa BRCA1 protein (Calbiochem), diluted 1:50 after EDTA-based antigen retrieval] and cell proliferation [polyclonal anti Ki67 antibody (Vector Labs), diluted 1:2,000 after citrate-based antigen retrieval]. MCF7 and HCC1937 cell lines were used as positive and negative controls for BRCA1 immunostaining, respectively. Because of the small proportion of stained nuclei and the homogeneous intensity of the immunostaining, the study pathologists (M.F. and R.F.) scored tumor expression of BRCA1 manually as positive or negative; Ki67 proliferation index was scored by quantitative image analysis (Ariol SL-50,
Applied Imaging; Fig. 1). The possible heterogeneity of the immunohistochemical staining for BRCA1 was also controlled using whole sections of 14 prostate cancer cases included in the TMAs. RNA expression levels of BRCA1 were available from a subset of participants (n = 116) using a gene expression profiling study that applied the DASL Illumina 6K array (16). The study was approved by Partners Health Care Institutional Review Board.

We abstracted data on age, stage, and prostate specific antigen (PSA) levels at diagnosis from medical records, and conducted a standardized histopathologic review for Gleason score (17, 18). The men were followed prospectively since diagnosis for the development of bony metastases and mortality through March 2009, without loss to follow-up.

We evaluated whether BRCA1-positive and BRCA1-negative tumor status based on immunohistochemistry differed according to Gleason score, tumor stage, PSA level, and age at diagnosis using generalized linear regression for continuous data and $\chi^2$ tests for categorical data. In addition, we assessed BRCA1-positive and BRCA1-negative prostate tumors for the number of Ki67-positive nuclei as well as BRCA1 RNA expression levels using ANOVA. Mean Ki67-positive

Table 1. Clinical characteristics of 392 men in the Physicians’ Health Study according to BRCA1 status, 1983 to 2008

<table>
<thead>
<tr>
<th></th>
<th>BRCA1 negative</th>
<th>BRCA1 positive</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>332</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis (95% CI)</td>
<td>66.5 (65.7–67.2)</td>
<td>67.3 (65.6–69.0)</td>
<td>0.37</td>
</tr>
<tr>
<td>PSA at diagnosis (95% CI)</td>
<td>10.2 (6.0–14.4)</td>
<td>27.0 (15.9–38.1)</td>
<td>0.0056</td>
</tr>
<tr>
<td>Mean follow-up time</td>
<td>11.0 (10.5–11.4)</td>
<td>8.8 (7.7–9.9)</td>
<td>0.0006</td>
</tr>
<tr>
<td>n dead/metastases (% of total)</td>
<td>24 (7.2)</td>
<td>16 (26.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Gleason score, n (%)</td>
<td></td>
<td></td>
<td>0.004*</td>
</tr>
<tr>
<td>4–6</td>
<td>97 (29.2)</td>
<td>10 (16.7)</td>
<td></td>
</tr>
<tr>
<td>3+4</td>
<td>116 (34.9)</td>
<td>19 (31.7)</td>
<td></td>
</tr>
<tr>
<td>4+3</td>
<td>65 (19.6)</td>
<td>10 (16.7)</td>
<td></td>
</tr>
<tr>
<td>8–10</td>
<td>52 (15.7)</td>
<td>21 (35.0)</td>
<td></td>
</tr>
<tr>
<td>Stage, n (%)</td>
<td></td>
<td></td>
<td>0.0005*</td>
</tr>
<tr>
<td>pT2</td>
<td>207 (62.3)</td>
<td>26 (43.3)</td>
<td></td>
</tr>
<tr>
<td>pT3</td>
<td>72 (21.7)</td>
<td>4 (6.7)</td>
<td></td>
</tr>
<tr>
<td>pT4/N1</td>
<td>4 (1.2)</td>
<td>4 (6.7)</td>
<td></td>
</tr>
</tbody>
</table>

*P for trend.
nuclei scores were log_{10} transformed before analysis to account for the uneven distribution of scores in the raw data. To assess the extent to which BRCA1 status was associated with poor progression, we used Cox proportional hazards models and examined the association between BRCA1 status and lethal prostate cancer, defined as development of distant metastases or prostate cancer–specific mortality. All statistical tests were two-sided.

This project was approved by the Partners Health Care Institutional Review Board.

Results

Normal prostate tissue did not stain for BRCA1; however, 15.3% (n = 60) of prostate tumor samples showed patchy nuclear clear positive immunostaining with a punctuate pattern (Fig. 1). There was a total correspondence between BRCA1 staining in the TMA cores and in the whole sections obtained from the selected 14 corresponding donor blocks in terms of signal intensity and percentage of positive nuclei. Cases that stained positively for BRCA1 had substantially and significantly higher Gleason score, higher PSA levels at diagnosis, and more advanced stage compared to those with tumors that did not stain for BRCA1 (Table 1). Moreover, BRCA1-positive tumors were marked by substantially increased tumor proliferation index compared with BRCA1-negative tumors (47.0 Ki67-positive nuclei versus 10.3, P = 0.0016). Tumors staining positive for BRCA1 also showed increased BRCA1 mRNA relative expression [mean, 10.5; 95% confidence interval (95% CI), 10.2–10.8] compared with tumors negative for BRCA1 (mean, 9.9; 95% CI, 9.7–10.1, P for difference = 0.008).

During a mean follow-up of 10.6 years, 40 men died of cancer or developed bony metastases. Sixteen of the 60 men (26.7%) with BRCA1-positive tumors died of prostate cancer, compared with 24 of 332 (7.2%) men who were BRCA1 negative [hazard ratio (HR), 4.6; 95% CI, 2.4–8.7]. This association remained statistically significant after adjusting for age at diagnosis and Gleason score (HR, 2.5; 95% CI, 1.3–4.8). Interestingly, although BRCA1-positive tumors had substantially increased tumor proliferative index, the association of BRCA1 and lethal prostate cancer remained significant after controlling for log_{10}-transformed Ki67 expression (HR, 3.6; 95% CI, 1.6–8.0).

Discussion

This study represents the first demonstration of a direct correlation between the expression of BRCA1 and the Ki67 proliferative index in prostate cancer and further strengthens the hypothesis that BRCA1 may play a role in cell cycle control and is a potent independent marker of clinical prognosis. Ki67 is a well-known predictor of adverse prognosis and resistance to therapy in prostate cancer (19, 20). In addition, association of increased proliferation and BRCA1 protein immunohistochemical expression was recently described in breast cancer epithelial cells from BRCA1 mutation carriers possibly as a result of epidermal growth factor receptor pathway activation (21). In agreement with the recent observation by Schayek and colleagues (13), we found that BRCA1 was not expressed in normal prostate tissue. We hypothesize that this localization of BRCA1 only to the most aggressive tumors may reflect an inefficient attempt to upregulate DNA repair mechanisms in prostate epithelial cells with high proliferative rate and extensive genetic instability.

Cases whose prostate tumors stained positive for BRCA1 had significantly higher Gleason score, PSA at diagnosis, and tumor proliferation as well as significantly worse prognosis than those with negative BRCA1 staining. In addition, mRNA levels were also increased in the BRCA1 protein–positive tumors, indicating a transcriptional-level control in these cases. Taken together, these observations support the hypothesis that the BRCA1 gene may hold another biological function apart from its tumor suppressor activity.

Although the mechanism of cell cycle regulation by BRCA1 still requires further exploration, we can conclude that the immunohistochemical investigation of BRCA1 protein expression represents a new tool for understanding the cell cycle regulation in the development of prostate cancer to lethal disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank the participants in the Physicians’ Health Study for their long-standing participation, and Julia Fleet, Joanne Smith, Vadim Bubes, and Haiyan Zhang for their assistance with data collection and programming.

Grant Support

Department of Defense (W81XWH-05-1-0562) and the Dana-Farber/Harvard Cancer Center Prostate Specialized Programs of Research Excellence. The Physicians’ Health Study is supported by grants CA34944, CA40360, and CA097193 from the National Cancer Institute, and grants HL-26490 and HL-34595 from the National Heart, Lung, and Blood Institute. L. Mucci is a Michael Milken Scholar of the Prostate Cancer Foundation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 11/09/2009; revised 12/15/2009; accepted 01/06/2010; published OnlineFirst 04/06/2010.

References


5. MacLachlan TK, Somasundaram K, Sgagias M, et al. BRCA1 effects...


Immunohistochemical Expression of BRCA1 and Lethal Prostate Cancer

Michelangelo Fiorentino, Gregory Judson, Kathryn Penney, et al.

Cancer Res 2010;70:3136-3139. Published OnlineFirst April 13, 2010.

Updated version  Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-09-4100

Cited articles  This article cites 21 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/70/8/3136.full#ref-list-1

Citing articles  This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/70/8/3136.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.