Differential Expression of Genes Related to Androgen and Estrogen Metabolism in Hereditary versus Sporadic Prostate Cancer

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Abstract

The hereditary predisposition to prostate cancer is rare and accounts for <5% of cases. Except for younger age at diagnosis, no phenotypic features have been clearly associated with hereditary prostate cancer. The aim of the study was to analyze the expression of genes related to androgen and estrogen metabolism in both hereditary and sporadic prostate cancers in patients matched for clinicopathologic features. Tissues were obtained from patients included in a national familial prostate cancer registry. From the 120 cases of hereditary forms suggesting autosomal dominant Mendelian inheritance, 21 patients were treated by radical prostatectomy for whom formalin-fixed tissue was available. Twenty-one sporadic cases were then matched according to age, Gleason score, and pathologic stage. Immunohistochemistry was done on tissue microarray using antibodies directed against androgen receptor (AR), estrogen receptor α (ERA), estrogen receptor β, 5α-reductase I and II, aromatase, and the proliferation marker Ki67. The percentage of AR-positive cancer cells was higher in hereditary cancer compared with sporadic cases ($P < 0.004$). In contrast, the mean number of ERA-positive stromal cells was lower in hereditary versus sporadic cancer ($P < 0.03$). This differential expression of AR and ERA suggests that a specific pattern of hormone receptors is associated with hereditary predisposition to prostate cancer. (Cancer Epidemiol Biomarkers Prev 2008;17(6):1505–9)

Introduction

Familial forms of prostate cancer account for ~20% of cases and are defined by at least two first-degree relatives affected. Hereditary transmission compatible with Mendelian inheritance accounts for only 5% of prostate cancer cases. The recognition of this hereditary pattern needs strict criteria, either three prostate cancer cases in first- or second-degree relatives or two cases diagnosed before the age of 55 years (1). Except for early age at diagnosis, no clinical, pathologic, or evolutionary patterns have been clearly associated with hereditary prostate cancer predisposition when compared with sporadic cases. The role of the androgen pathway in the development and progression of prostate cancer has been well established, and overexpression of the *androgen receptor* (*AR*) gene has been associated with poor prognosis (2). Circulating testosterone is metabolized in the prostate to the more active androgen dihydrotestosterone by the enzymes 5α-reductase type I and II (5AR1 and 5AR2), which are likely to play a role in the initiation and maintenance of prostate cancer. In addition to androgens, estrogens also play a role in prostate carcinogenesis. Aromatase, which converts testosterone into estradiol, is expressed in the human prostate (3). The effects of estrogens on target tissues are mediated by estrogen receptor α and β (ERA and ERB), both receptors being present in the prostate (4). Some variants of genes related to estrogen metabolism, including aromatase and the *ERA* gene, have been associated with increased risk of prostate cancer development (5-7). The aim of the present study was to compare the expression of genes involved in hormonal prostatic homeostasis between hereditary and sporadic prostate cancer in patients matched for clinicopathologic features.

Materials and Methods

Patients and Tissues. The Centre de Recherche sur les Pathologies Prostatiques national collection of prostate cancer families has been previously described (8): the families are all Caucasian, and informed consent was obtained according to the institutional review board. Within this national cohort of familial prostate cancers, 120 hereditary cases were obtained from families with at least three first-degree relatives affected. Sixty-one of these patients were treated by radical prostatectomy. In 40 cases, tissues were fixed in Bouin and inappropriate for immunostaining. The study therefore included 21 patients with hereditary prostate cancer treated by radical prostatectomy between 1995 and 2005, with available tissues for immunohistochemistry. Patient characteristics are summarized in Table 1. Sporadic

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Table 1. Patient characteristics and gene expression in hereditary and sporadic prostate cancer

	Hereditary	Sporadic	
Age (y), median (range)	63 (55-70)	61 (55-70)	
PSA (ng/mL), median (range)	9.8 (5.3-19)	9.4 (4.7-17)	
Gleason score	G5-6 (n = 5), G7 (n = 14), G8 (n = 2)	G5-6 (n = 5), G7 (n = 14), G8 (n = 2)	
Gleason grade (majority)	G3 (n = 8), G4 (n = 13)	G3 (n = 8), G4 (n = 13)	
Pathologic stage	pT2 (n = 14), pT3 (n = 7)	pT2 (n = 14), pT3 (n = 7)	
AR (%), median (range)	90 (10-100)	40 (0-90)	$P < 0.004$
ERA (cells/core), median (range)	10+ (0-100)	30+ (10-200)	$P < 0.03$
Ki67 (%), median (range)	2 (0-13)	1 (0-10)	$P = 0.2$
5AR1	− (n = 8), + (n = 9), ++ (n = 4)	− (n = 10), + (n = 7), ++ (n = 4)	$P = 0.7$
5AR2	− (n = 3), + (n = 3), ++ (n = 10), +++ (n = 5)	− (n = 3), + (n = 8), ++ (n = 5), +++ (n = 4)	$P = 0.3$
Aromatase	− (n = 12), + (n = 9)	− (n = 15), + (n = 6)	$P = 0.5$
ERB	− (n = 4), + (n = 17)	− (n = 7), + (n = 14)	$P = 0.5$

Abbreviation: PSA, prostate-specific antigen.

prostate cancer tissues were obtained from patients undergoing radical prostatectomy at the ''Institut Mutualiste Montsouris'' between 1995 and 2005. Patients were selected as sporadic cases if they had at least two nonaffected brothers >50 y old. Among these sporadic cases, we selected 21 patients strictly matched with the 21 hereditary prostate cancers according to age (three groups: 55 to <60, 60 to <65, and 65-70), prostate-specific antigen (three groups: 4 to <10, 10 to <15, and 15 to <20), Gleason score, and pathologic stage (Table 1).

Tissue Array. The tissue microarray block was prepared from the 42 formalin-fixed, paraffin-embedded tumors, including the 21 hereditary prostate cancers matched with 21 sporadic prostate cancers. For each case, three cores of tumor and three cores of normal prostate tissue were transferred from the selected areas to the recipient block.

Immunohistochemistry. Immunostaining was done on tissue sections from the tissue microarray block using the following antibodies: AR and Ki67 (1:40 and 1:50 dilution, respectively; DakoCytomation), ERA (1:60 dilution; Novocastra), ERB (1:1,000 dilution; Chemicon), 5AR1 and 5AR2 (1:100 dilution; Santa Cruz Biotechnology), and aromatase (1:200 dilution; Affinity BioReagents). Negative controls were obtained after either omission of the primary antibody or incubation with an irrelevant antibody (mouse monoclonal immunoglobulin). Colonic samples included in the tissue microarray as orientation cores were also used as negative controls for most markers except Ki67. Samples from other tissues known to express each marker were used as positive controls, including breast carcinoma for ERA and ERB and hormone-refractory prostate cancer for AR. The prostate cancer cell line DU145 was used as a positive control for 5AR1, 5AR2, and aromatase (8).

Scoring of Antibody Staining. Slides were analyzed by two pathologists (G.F. and M.Y.) in a blind fashion. For AR and Ki67, positive cells were expressed as a percentage of total epithelial cells. ERA was scored as a mean number of positive stromal cells per core. We observed that staining for ERB and aromatase on tumor epithelial cells was either diffusely positive or with negative or weak signal, and results were therefore classified as positive or negative. 5AR1 staining was scored as follows on stromal cells: −, no stained cells; +, focal staining; and ++, diffuse staining. The staining pattern of 5AR2 expression on tumoral cells was classified as follows: −, no stained cells; +, rare stained cells; ++, <50% positive cells; and +++, >50% positive cells. In case of interobserver variability (different categories in case of categorical data, or variability >10% in case of continuous data), which occurred in <5% of cases, slides were rescored with both pathologists until a consensus was reached.

Statistical Analysis. All statistical analyses were done using the StatView 5.0 software (Abacus Concepts, Inc.). Comparison between groups was analyzed using the χ^2 test or the Fisher's exact test for categorical data and nonparametric Mann-Whitney or Kruskal-Wallis test for continuous data. The relation between two continuous variables was measured using the nonparametric Spearman rank correlation test.

Table 2. Relationship between hormone metabolism–related gene expression and prognostic factors

Markers	PSA	Gleason grade (G3 vs G4)	Pathologic stage (pT2 vs pT3)	Proliferation
AR	$P = 0.1$ (S) ($r = 0.33$)	$P = 0.7$ (MW)	$P = 0.3$ (MW)	$P < 0.004$ (S) ($r = 0.56$)
ERA	$P = 0.4$ (S) ($r = 0.16$)	$P < 0.05$ (MW)	$P = 0.6$ (MW)	$P = 0.1$ (S) ($r = 0.28$)
ERB	$P = 0.7$ (MW)	$P = 0.7$ (F)	$P = 0.3$ (F)	$P = 0.1$ (MW)
5AR1	$P = 0.8$ (KW)	$P = 0.9$ (χ^2)	$P = 0.2$ (χ^2)	$P = 0.8$ (KW)
5AR2	$P = 0.6$ (KW)	$P = 0.08$ (χ^2)	$P = 0.2$ (χ^2)	$P = 0.5$ (KW)
Aromatase	$P = 0.2$ (MW)	$P = 0.2$ (F)	$P = 0.06$ (F)	$P = 0.1$ (MW)

Abbreviations: MW, Mann-Whitney test; KW, Kruskal-Wallis test; S, Spearman correlation test; F, Fisher's exact test.

Results

Immunostaining was located in the nucleus for AR, ERA, and ERB and in the cytoplasm for aromatase, 5AR1, and 5AR2. In normal prostate tissue, AR was expressed mainly in luminal epithelial cells, ERA and 5AR1 staining was located on stromal cells, and ERB- and 5AR2-positive cells were found in the basal epithelial compartment. Only weak aromatase staining was found on luminal cells. No difference of staining was observed between normal prostate tissues from sporadic and hereditary cases, except for ERA. In fact, the median number of ERA-positive stromal cells in normal prostate tissue was significantly lower in hereditary cases (median, 10 cells per core) when compared with sporadic cases (median, 40 cells per core; $P < 0.003$, Mann-Whitney test).

In prostate cancer, AR was expressed on 0% to 100% (median, 70%) of the tumor cells. ERA-positive cells were identified only in the stromal compartment from 0 to 200 (median, 20) positive cells per core. ERB and aromatase staining on cancer cells was scored positive in 27 and 12 cases, respectively. 5AR1 stained only stromal cells, with a focal or diffuse staining in 18 cases. 5AR2 expression on cancer cells was scored ++ or +++ in 23 cases. Ki67-positive cells ranged from 0% to 13% (median, 1%) of cancer cells.

The relationships between the expression in cancer tissues of genes related to hormone metabolism and prognostic factors are summarized in Table 2. AR expression shows a significantly positive correlation with cancer cell proliferation determined by Ki67 staining. The number of ERA-positive stromal cells in tumoral tissue significantly increased with the Gleason grade, with a mean number of 27 cells per core in Gleason grade 3 versus 44 in Gleason grade 4. Cancers with intense aromatase staining were predominantly pT3 (8 of 12), but the difference was not significant ($P = 0.06$).

The expression of hormone metabolism–related genes in hereditary and sporadic cancers is summarized in Table 1. The percentage of AR-positive cells was higher in hereditary cancers when compared with sporadic cases (Fig. 1). Moreover, the number of ERA-positive cells per core was significantly lower in hereditary cancers than in sporadic tumors (Fig. 1). No significant difference of expression was found between the hereditary and sporadic groups for ERB, 5AR1, 5AR2, and aromatase.

Discussion

Hereditary prostate cancer accounts for <5% of prostate cancer cases and needs to be better defined in terms of pathologic characteristics. Most reports found no phenotypic differences between familial or hereditary and sporadic cancers, except for a younger age at diagnosis (9-11). However, others have described different pathologic features in familial cases (12, 13). We therefore choose to match hereditary and sporadic cancer cases for clinicopathologic features to allow comparison of gene expression between two populations distinct only on the familial history of prostate cancer. That study pertains to an analysis of the largest series of hereditary cases to date.

We observed that AR expression on cancer cells correlated with increased Ki67 staining, in accordance with the direct regulation of AR signaling on cell proliferation (14). The enzymes 5AR1 and 5AR2, responsible for the local production of dihydrotestosterone, have been previously shown to be expressed in prostate cancer (15-17). In the present study, we observed that 5AR1-positive cells were located in the stroma, and 5AR2 was expressed in epithelial cancer cells. The estrogen signaling pathway is regulated by ligand-specific transcription factors ERA and ERB (18), which have been suggested to mediate different downstream effects (19). We observed, like others (4, 20), that ERA is mainly located in the stromal cells, and ERB in the basal epithelial cells of the normal prostate. We found that ERA expression positively correlated with prostate cancer differentiation, as previously reported (21).

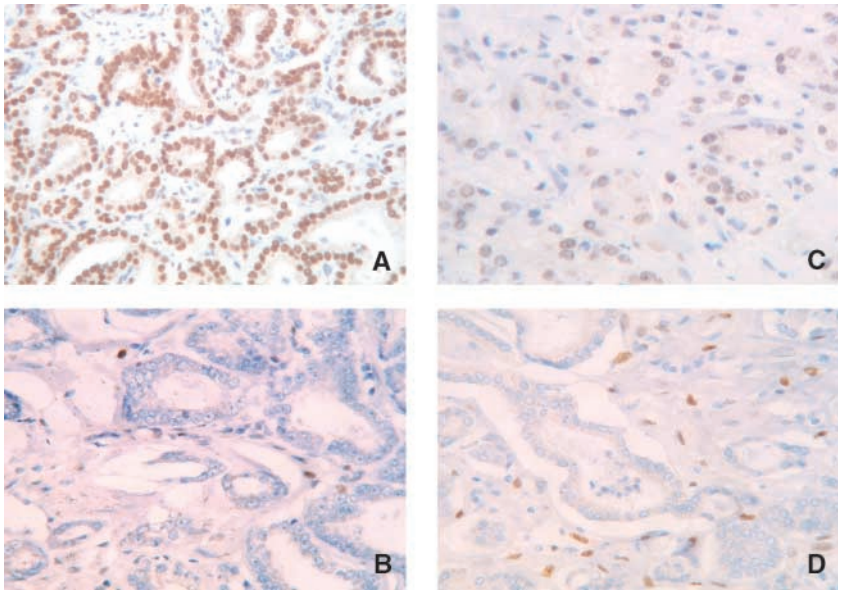

Figure 1. Immunostaining in hereditary and sporadic prostate cancers matched for clinicopathologic features: AR expression is increased in the hereditary cancer (**A**) when compared with the sporadic case (**C**). In contrast, ERA-positive expressing stromal cells are less numerous in the hereditary cancer (**B**) than in the sporadic case (**D**).

However, in contrast with other studies (4), we did not observe any significant relationship between ERB expression and cancer differentiation. This discrepancy could be explained by the use of either different antibodies or different methods of scoring. The aromatase enzyme, responsible for the local production of estrogens, is also present in the human prostate (3). We observed that aromatase expression was increased in prostate cancer when compared with normal tissue, mainly in pT3 tumors. Such a dysregulation in aromatase expression has previously been described (3) and suggests that this enzyme could play a role in prostate carcinogenesis and progression.

We showed a higher ratio of AR-positive cancer cells in hereditary prostate cancer when compared with sporadic cases. AR expression in prostate cancer has been previously reported to be increased in black Americans in comparison with Caucasian patients, supporting the hypothesis that differential expression of AR protein may be driven at least in part by genetic events (22). Moreover, short variants of the *AR* gene have been shown to induce increased transcriptional activity and high AR protein levels (23). The present study also showed that the number of ERA-positive stromal cells is decreased in hereditary cases when compared with sporadic cases not only in cancer tissue but also in normal prostate. Some polymorphisms of the *ERA* gene have been associated with prostate cancer risk (6, 7), but the effects of these genetic variations on the transcriptional activity of the *ERA* gene and on ERA protein expression remain unknown.

Although it could be interesting to confirm the differential expression of AR and ERA in hereditary and sporadic cancer at level of mRNA by quantitative PCR, it is rare to obtain frozen prostate cancer specimens from patients with hereditary prostate cancer. Moreover, we previously observed a very good correlation between AR, ERA, and ERB gene expression obtained with reverse transcription-PCR and immunohistochemistry (24). In Transgenic adenocarcinoma mouse prostate (TRAMP) mice, characterization of androgen receptor expression has been well studied: somatic mutations in the *AR* gene occur spontaneously in TRAMP tumors, and AR and ERA, but not ERB, were significantly increased in prostates of TRAMP compared with C57BL/6 mice (25). It is, however, difficult to extrapolate results from TRAMP model carcinogenesis to human prostate carcinogenesis associated to hereditary predisposition.

Differences in hormone receptor expression have also been shown in hereditary breast tumors when compared with age- and grade-matched sporadic counterparts. BRCA1 breast cancers exhibit a down-regulation of both ERA and ERA target genes, and BRCA1 regulates ERA gene transactivation (26). These data support the concept that some susceptibility genes may regulate hormone receptor expression in familial breast cancer. Our findings strongly suggest that similar interactions are likely to occur in hereditary prostate cancer. Despite the fact that hereditary prostate cancers are genotypically heterogeneous, they seem to share common phenotypic expression of both androgen and estrogen receptors. High AR to ERA expression ratio may therefore be related to increased risk of tumor development in men with a family history of prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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