BRCA1, *BRCA2*, *AR* and *IGF-I* expression in prostate cancer: Correlation between RT-qPCR and immunohistochemical detection

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Abstract. Identification and characterization of biomarkers in prostate cancer are important for improving the diagnosis. The aim of this study was to determine differences in the expression of 4 genes according to the stage of malignancy in prostate cancer. We analyzed *BRCA1*, *BRCA2*, androgen receptor (*AR*) and *IGF-I* gene expression in a cohort of 98 prostate biopsies. We used TaqMan RT-qPCR for mRNA detection, and correlation with proteins was performed using immunohistochemistry. Among the 98 studied prostate biopsies, high heterogeneity in the expression of the 4 genes was detected among the different histological types. However, down-regulation of *BRCA1* and *BRCA2* mRNA was detected, particularly in the normal tissues. The expression of *AR* was dependent on the stage of the tumor. The *IGF-I* gene was specifically expressed in the tumor tissues. Upon comparison between protein and mRNA expression for BRCA1, BRCA2 and AR, we obtained a trend; however, this did not achieve statistical significance. Regarding IGF-I, a correlation between mRNA expression and staining intensity of the protein was found to be significant ($p<0.012$). The AR biomarker was found to be slightly correlated with the prostate cancer diagnosis ($p=0.013$). AR was found to be decreased in the tumors with a 43% sensitivity and 90% specificity. The relative risk of 2.05 (1.13-3.69) indicated a 2-fold higher chance of cancer occurence when AR was ≤0.206.

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Introduction

Prostate cancer is the most commonly diagnosed malignancy among males in Western countries. There were 679,000 new cases of prostate cancer worldwide in 2002 (1) with 40,000 new cases being reported in France in 2000 (2,3). The incidence of prostate cancer has increased 4-fold during the last two decades, and in the US, one in eight men will develop prostate cancer during his lifetime (4). The most significant risk factor is age with race being another risk factor; African-American men appear to have a worse prognosis than Caucasian men (5). Since the discovery of the prostate-specific antigen (PSA) in 1970 (6), this assay remains the reference for prostate cancer detection (7). However, at present, most men are diagnosed with localized disease in the absence of symptoms (8), and the value of PSA screening, 4.0 ng/ml as the upper limit of normal, actually fails to detect a significant percentage of cancers (9). The use of PSA for detecting cancer has been heavily criticized by Stamey *et al* (10) who believe that increases in PSA from 4.0 to 10.0 ng/ml result more frequently in a diagnosis of benign prostate hyperplasia than in cancer. These findings underscore the need for more accurate biomarkers, that not only detect prostate cancer, but also distinguish indolent from aggressive disease. Improved markers are needed to determine which patients may benefit from a more aggressive treatment, and which patients may be spared unnecessary and potentially harmful interventions (11).

When prostate cancer is suspected due to prostate volume or/and the PSA level, a prostate biopsy is routinely requested by the urologist. In recent years, many hospitals have used a sextant biopsy protocol (12). Tissues examined by the anatomopathologist are then classified.

Epidemiologic studies have shown the possible association of prostate cancer with other types of cancers particularly with breast cancer (13). This implies the existence of similar predisposition genes such as *BRCA1* and *BRCA2*, well-known tumor-suppressor genes in breast and ovarian cancers. BRCA1 and BRCA2 proteins act in DNA damage repair and cell cycle control (14). The loss of *BRCA1* and *BRCA2* gene functions increases cell proliferation, genetic instability and tumorigenesis (15). In addition to breast and ovarian cancer in women, recent evidence suggests that germ-line mutations of *BRCA1* also confer an increased lifetime risk for prostate cancer in males (16). Consistent with a potential tumor-suppressor role, human prostate cancer DU-145 cells transfected with wild-type BRCA1 were found to exhibit a slightly decreased proliferation rate, an increase in sensitivity to chemotherapy drugs, increased susceptibility to drug-induced apoptosis, reduced repair of single-strand DNA breaks and alterations in expression of key cellular regulatory proteins (including BRCA2) (17). Furthermore, *BRCA1* splice variant *BRCA1* a/p110 was recently shown to display antitumoral activity in triple-negative (TN) prostate cancer cells (18).

Previous studies have suggested that BRCA1 functions as an AR coregulator and plays a positive role in androgen-induced cell death (19,20). Hormonal factors are suspected to influence the development of prostate cancer due to the interaction between androgens in normal prostate or cancer. A meta-analysis revealed that a high testosterone level increased the risk of development of prostate cancer (21). AR is a member of the nuclear hormone receptor family of transcription factors, necessary for the normal growth, terminal differentiation and function of male urogenital structures, including the prostate gland. One function of AR involves the activation of gene expression which controls proliferation and differentiation of prostate cells. It is a transcription factor coded by the *AR* gene (Xq11-12) (22). The transcription activity of the *AR* gene is determined by the N-terminal region and the associated protein. This domain is encoded by exon 1 of the *AR* gene containing CAG and GGC repeats. A shorter CAG repeat sequence in the *AR* gene was found to be associated with an increased incidence of prostate cancer. A possible association may exist between *AR* polymorphisms and the development of prostate cancer (23). Another study also supported the hypothesis of increased *AR* mRNA levels being associated with the occurrence of prostate cancer (24-26).

The insulin-like growth factors, IGF-I and IGF-II, are a family of mitogenic polypeptides with important roles in normal growth and differentiation as well as in tumor development and progression (27,28). In prostate cancer, it has been demonstrated that the IGF plays an important role in the transformation of the prostate epithelium (29,30). Epidemiologic studies also showed a significant increase in serum IGF-I levels in patients who later developed prostate cancer (31). Another study demonstrated that the change in IGF-IR expression exhibited in a metastatic model resulted in significant alterations in AR signaling (32). Recently, Schayek *et al* demonstrated that BRCA1 enhanced IGF-IR levels in LNCaP C4-2 cells expressing an endogenous AR (33).

Due to the heterogeneity and multifoci of prostate cancer, the search for potential novel markers is required for a more precise diagnosis. In order to identify new predictive factors based on the degree of malignancy of prostate cancers, the present study investigated the expression of the *BRCA1*, *BRCA2*, *AR* and *IGF-I* genes. We quantified the expression of these genes by quantitative RT-PCR in mRNAs of 98 prostate biopsies and compared their protein expression levels using immunohistochemistry.

Patients and methods

Patients. This study examined 98 men who underwent a prostate biopsy to establish a diagnosis of prostate cancer. They were hospitalized at the CHU of Clermont-Ferrand and were enrolled in the study after providing informed consent. This approach was suggested by the PPC (People Protection Committee) within the framework of the constitution for collection of biological samples. All of the patients in the series underwent sextant biopsies (34). Upon agreement of the patient, a sample was used for research. The procedures were carried out using a local anaesthesia. The identity of each patient was confidential. The stage of cancer was diagnosed by anatomopathologic examination. Each patient was *BRCA1* and *BRCA2* non-carrier. Each biopsy was stored in a cryotube conserved in liquid nitrogen at -196˚C.

mRNA extraction. Each prostate biopsy was immediately weighed and ground thoroughly using a French press. The powder was disrupted in a potter with the appropriate quantity of RLT buffer [lysis buffer provided in the Direct Cell Lysis kit (Qiagen)] added to β-mercaptoethanol, according to the weight of the sample. The solution was then homogenized by passing the lysate at least 10 times through a 30-G needle fitted to an RNase-free syringe. Afterwards, total mRNA isolation was performed using the RNeasy mini kit (Qiagen) following the manufacter's protocol and an RNeasy spin column and various wash buffers. The quality of total mRNA obtained with the Qiagen kit was verified by electrophoresis on a BioAnalyser 2100 Agilent®, and the quality of each sample was determined by the ratio of 18s/28s rRNA equal to 2.

Real-time quantitative PCR. The total mRNA obtained with the Qiagen kit was amplified and reverse transcribed using a kit from Pharmacia according to the manufacturer's conditions. The reference used for this study was a commercial RNA reference of a pool of 32 prostate tissues obtained from the Ozyme society. These tissues were non-malignant and obtained from 32 Caucasian men. This reference was amplified and retro-transcribed identically to the other samples used in our study.

In the present study, the expression of *BRCA1*, *BRCA2*, *IGF-I* and *AR* mRNAs was measured with quantitative real-time PCR (TaqMan® System) (31,35).

The probes and the primers used for the quantification were chosen with the help of a primer express computer program, the results of which are summarized in Table I. All doubly labeled probes, 18s rRNA probe and primers, and

Table I. Oligonucleotide primer pairs and TaqMan probes.

Gene	Forward primer	TaqMan probe	Reverse primer
BRCA1	CAGAGGACAATGGCTTCCATG	AATTGGGCAGATGTGTGAGGCACCTG	CTACACTGTCCAACACCCACTCTC
BRCA2	CCAAGTGGTCCACCCCAAC	ACTGTACTTCAGGGCCGTACACTGCTCAAA	CACAATTAGGAGAAGACATCAGAAGC
rRNA 18S	CGGCTACCACATCCAAGGAA	TGCTGGCACCAGACTTGCCCTC	GCTGGAATTACCGCGGCT
	Assays on Demand		
AR	CTTGCCTGGCTTCCGCAACTTACAC		
IGF-I	CGTCTGCCGAGGTTGTCCATCTGGG		

AR and *IGF-I* were from Assays on Demand synthesized by Applied Biosystems.

TaqMan universal PCR Master mix were obtained from Applied Biosystems. Only 78 samples were evaluated for *IGF-I* and *AR* due to a loss of a quantity of several extracted RNAs.

PCR was carried out on cDNA in 96-well plates. Single-stage PCR was carried out in a final volume of 25 μl containing 5 μl of the RT reaction mix (diluted to 25 ng) and 12.5 μl of TaqMan Universal PCR Master mix, containing 200 nM of each primers and probes (*BRCA1*, *BRCA2*, *IGF-I* or *AR*), 200 μM dNTP, 400 μM dUTP, 5 mM $MgCl_2$, 1.25 AmpliTaq Gold, 0.5 unit of AmpErase uracil N-glycosylase (UNG) and 50 nM of both 18s rRNA primers and probe.

Thermal cycling conditions were set at 2 min at 50˚C, 10 min at 95˚C followed by 50 cycles at 95˚C for 15 sec and 60˚C for 1 min for *BRCA1*, *BRCA2* and *AR* and by 40 cycles for *IGF-I*. Data were collected using an ABI PRISM 7700 SDS analytical thermal cycler (Applied Biosystems).

Relative gene expression was determined using the $\Delta\Delta C_T$ method ($\Delta C_{T(sample)}$-$\Delta C_{T(reference)}$, threshold cycle). The comparative C_T method is a mathematical model that calculates changes in gene expression as a relative fold difference between an experimental and calibrator sample (36). It consists of normalizing the number of target gene copies to an endogenous reference gene (18s rRNA), designated as the calibrator (37). To guarantee reproducibility, two independent reverse transcriptions were carried out for one RNA extraction. Each reverse transcription was analyzed in triplicate and expressed as a mean ± SD (38). Each value represents the mean ± SD for three assays. Statistical analysis of the results was carried out using the Student's t-test with differences considered significant at $p<0.05$.

Immunohistochemistry. Alcohol-formalin-acetic acid-fixed and paraffin-embedded sections (4-μm) were cut using a microtome. They were mounted on silanized glass slides (Starfrost), and dried overnight at 37˚C. After deparaffinization and rehydration through a series of graded alcohols (100 and 70%) and distilled water, a heat-induced antigen retrieval method was used. This included a 3-min incubation in citrate buffer, pH 5.9, in a pressure cooker, followed by a 15-min cool-down period in a water bath. Further processing was performed with a NexES automated immunostainer using an AEC kit (Ventana Medical Systems Inc., Tucson, AZ). Slides were then incubated at 37˚C for 32 min with anti-BRCA1 [mouse, (17F8), GeneTex®], anti-BRCA2 [mouse, (5F6), GeneTex®], anti-AR (mouse, 53226, Tebu-Bio®), and finally with anti-IGF-I (rabbit, 905-429, Assay Designs®) primary antibodies. BRCA1 and BRCA2 antibodies were used at a dilution of 1:20, the IGF-I antibody was used at 1:100 and the AR antibody was used at a dilution of 1:10. Subsequent incubations with a biotinylated secondary antibody and avidin-conjugated peroxidase complex were carried out in a Ventana NexES immunostainer in accordance with the manufacturer's protocol.

Slides were then counterstained with hematoxylin for 3 min, rinsed in distilled water, and placed on coverslips with an aqueous Faramount mounting media (Dako, Glostrup, Denmarck). The primary polyclonal antibody was omitted and replaced with PBS as a negative control (39).

Statistical correlation between mRNA and protein expression. Correlation between gene and protein expression was carried out using the non-parametric Kruskal-Wallis H test since distributions were not Gaussian and/or variances were different. Relations between diagnosis and other clinical

Table II. Clinical characteristics of the studied patients.

Case (n)	ADC	PIN	PTT	NM
Total cases (n=98)	31	18	23	24
Age at diagnosis (years) n (%)				
<49	0	0	0	1 (4.1)
50-59	3 (9.7)	4 (22.3)	8 (34.8)	3 (12.5)
60-69	17 (54.8)	5 (27.7)	8 (34.8)	20 (8.4)
>70	11 (35.5)	9 (50)	7 (30.4)	0
PSA baseline (ng/ml) n (%)				
<4	1 (3.3)	8 (44.5)	3 (13.1)	2 (8.3)
4-10	13 (41.9)	4 (22.2)	14 (60.8)	14 (58.4)
10-20	6 (19.3)	3(16.65)	4 (17.4)	7 (29.2)
>20	11 (35.5)	3(16.65)	2 (8.7)	1 (4.1)

ADC, adenocarcinoma; PIN, prostatic intraepithelial neoplasia; PTT, peritumoral tissue; NM, no malignancy; PSA, prostate-specific antigen.

Table III. Gleason sum score and TNM score in the studied patients with adenocarcinoma.

	n (%)
Gleason score	
≤7 (5-7)	29 (92.8)
>7 (8-9)	3 (7.2)
TNM stage	
T1c	13 (40.7)
T2	1 (3.1)
T2a	3 (9.4)
T2b	5 (15.6)
T2c	2 (6.2)
T3	8 (25.0)

characteristics used the χ^2 test. The standard cutoff of p=0.05 was used for significance. Sem software was used to perform the tests (40).

Results

Patient characteristics. Ninety-eight patients who underwent a prostate biopsy for prostate cancer diagnosis were included in the study. Among the study population, one case of no malignancy (NM) was found in a patient <49 years of age. In contrast, diagnoses of the patients 50-59 years of age consisted of symptomatic diseases. In regards to the diagnoses of the patients 60-69 years of age and greater than 70 years, the majority of cases were matched adenocarcinoma (ADC).

According to the PSA levels (Table II), patients were classified into subgroups: <4, 4-10, 10-20, and >20 ng/ml. An increase in serum PSA level was found to be associated with the onset of the disease.

The prostatic ADC tissue samples were next assigned a Gleason score (Table III) which describes the differentiation of the prostate tissue and is indicative of the degree of malignancy. The Gleason score is expressed as the sum of the two most prominent Gleason patterns. A Gleason score >7 is correlated with a highly malignant tumor whereas a low Gleason score ≤7 is an indicator of less agressive disease (41,42). Our study population included 3 patients with a Gleason score >7. Prostate cancer was also classified as localized disease with tumor-node-metastasis (TNM) stage ≤T2; and advanced disease (regional-distant) with TNM stage ≥T3 (Table III).

Relative levels of BRCA1, BRCA2, AR and IGF-I transcripts in prostate biopsies. Variations of *BRCA1*, *BRCA2*, *AR* and *IGF-I* mRNA expression were determined by RT-qPCR in the 98 biopsies at different stages of malignancy (Table IV). They were classified into four groups according to their degrees of malignancy: NM, no malignancy; PIN, prostatic intraepithelial

Table IV. Level of *BRCA1*, *BRCA2*, *AR* and *IGF-I* mRNA expression according to the different degrees of malignancy of prostate cancer.

	Cases	No expression (<0.2) n (%)	Underexpression (0.2-1) n (%)	Overexpression (1-5) n (%)
BRCA1				
ADC	31	7 (22.6)	12 (38.7)	12 (38.7)
PIN	10	2 (20.0)	4 (40.0)	4 (40.0)
PTT	23	2 (8.7)	7 (30.4)	14 (60.9)
NM	24	10 (41.2)	6 (26.5)	8 (32.3)
BRCA2				
ADC	31	12 (38.7)	10 (32.3)	9 (29.0)
PIN	10	4 (40.0)	3 (30.0)	3 (30.0)
PTT	23	3 (13.0)	11 (47.8)	9 (39.2)
NM	24	12 (47.0)	6 (26.5)	6 (26.5)
AR				
ADC	23	11 (43.9)	7 (30.4)	5 (21.7)
PIN	10	3 (30.0)	7 (70.0)	0 (0.0)
PTT	20	2 (10.0)	9 (45.0)	4 (20.0)
NM	24	4 (12.6)	8 (33.4)	12 (45.9)
IGF-I				
ADC	23	14 (60.9)	6 (26.1)	3 (13.0)
PIN	10	5 (50.0)	4 (40.0)	1 (10.0)
PTT	20	11 (55.0)	6 (30.0)	3 (5.0)
NM	24	13 (54.2)	11 (45.8)	0 (0.0)

ADC, adenocarcinoma; PIN, prostatic intraepithelial neoplasia; PTT, peritumoral tissue; NM, no malignancy. The relative expression was expressed as a function of the reference corresponding to 1 with RT-qPCR.

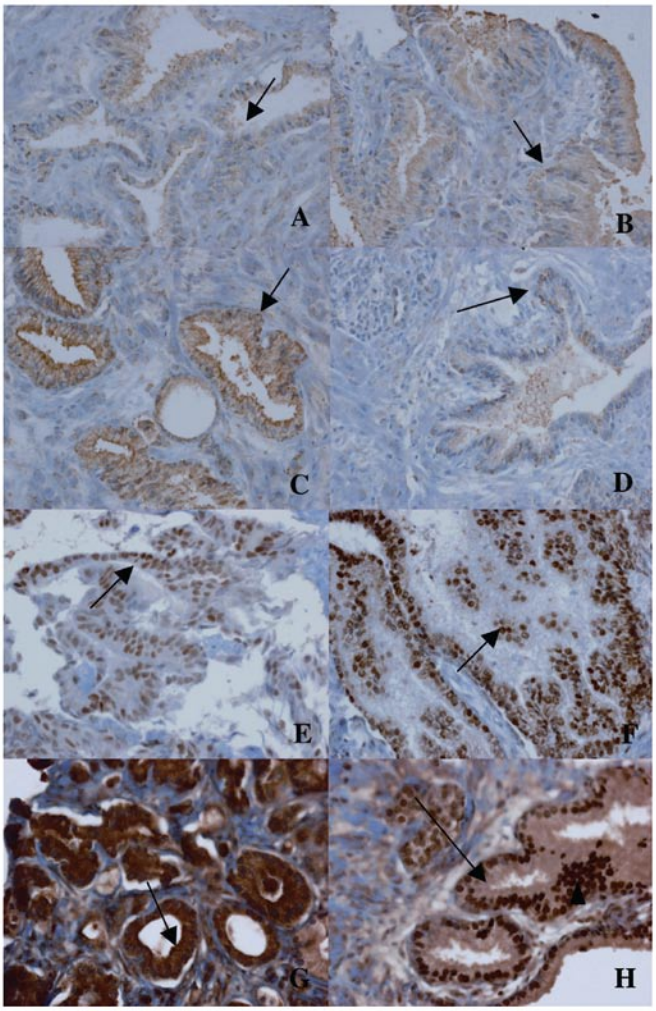

Figure 1. Immunoperoxidase staining of human prostate biopsies on paraffin-embedded sections (x40). (A) Cytoplasmic staining with a BRCA1 monoclonal antibody in adenocarcinoma (ADC) (arrow). (B) Cytoplasmic staining with a BRCA1 monoclonal antibody in a non-malignant biopsy (NM) (arrow). (C) Cytoplasmic staining with a BRCA2 monoclonal antibody in ADC (arrow). (D) Cytoplasmic staining with a BRCA2 monoclonal antibody in NM (arrow). (E) Nuclear staining with an AR monoclonal antibody in ADC (arrow). (F) Nuclear staining with an AR monoclonal antibody in NM (arrow). (G) Cytoplasmic staining with an IGF-1 polyclonal antibody in ADC (arrow). (H) Negative control without the primary antibody, replaced with PBS.

neoplasia; PTT, peritumoral tissue and ADC, adenocarcinoma. Thirty-four NM cases, 10 PIN, 23 PTT and 31 ADC cases were studied. The results as shown in Table IV demonstrated a high heterogeneity of expression of the *BRCA1*, *BRCA2*, *AR* and *IGF-I* genes in the different degrees of prostate malignancy. *BRCA1* and *BRCA2* mRNA expression was found to be heterogeneous in the prostate biopsies. Overexpression of *BRCA1* was found in 14 of the 23 (60.9%) PTT cases. No expression was found for *BRCA1* and *BRCA2* mRNA in 10 of 24 (41.2%) and in 12 of 24 (47%) NM tissues, respectively.

Overexpression of *AR* mRNA was noted in 12 of 24 (45.9%) normal tissues. An intermediate expression was also exhibited in 7 of 10 (70.0%) PIN cases. *IGF-I* mRNA was not expressed in 13 of 24 (54.2) NM cases. In contrast, in ADC, *IGF-I* was up-regulated.

Table V. Location and staining intensity of BRCA1, BRCA2, AR, IGF-I proteins according to the different grades of the prostate biopsies.

Protein localization	Diagnosis				Total
	ADC	PIN	PTT	NM	
BRCA1					
Cytoplasmic					
+/-	7	5	10	7	29
+	9	10	12	12	43
++	4	0	3	4	11
+++	0	0	0	0	
Nuclear					
+/-	0	1	1	1	3
+	0	1	1	0	2
Not detected	2	2	0	1	5
Cases (n)	22	17	25	24	88
BRCA2					
Cytoplasmic					
+/-	7	2	3	2	14
+	16	13	18	14	61
++	1	2	4	5	12
+++	1	1	1	2	5
Nuclear					
+/-	1	1	1	1	4
+	2	0	1	0	3
Not detected	0	0	0	1	1
Cases (n)	26	19	28	25	98
AR					
Nuclear					
+/-	8	4	2	5	19
+	10	12	15	13	50
++	3	1	7	5	16
+++	3	0	0	0	3
Cytoplasmic					
-	24	16	25	23	88
Not detected	0	1	1	1	3
Cases (n)	24	16	25	23	88
IGF-I					
Cytoplasmic					
+/-	3	5	10	9	27
+	12	7	12	11	42
++	3	4	2	0	9
+++	4	0	1	0	5
Nuclear					
+	3	3	5	6	17
Not detected	2	0	0	3	5
Cases (n)	24	18	25	24	91

ADC, adenocarcinoma; PIN, prostatic intraepithelial neoplasia; PTT, peritumoral tissue; NM, no malignancy. -, negative; +/-, weakly intense; +, intense; ++, very intense; +++, strongly intense.

Immunohistochemistry. BRCA1 and BRCA2 proteins were preferentially detected from a less intense (+/-) to intense (++)

Table VI. Correlation between mRNA and protein expression.

	Protein expression				
	-, -/+, +		++, +++		
	Average	Standard deviation	Average	Standard deviation	P-value
mRNA expression					
BRCA1	2.529	5.618	4.410	8.076	0.22
BRCA2	1.893	4.296	2.103	4.196	0.60
AR	4.028	11.312	2.546	6.389	0.72
IGF-I	0.287	0.317	1.522	2.550	0.12 (0.012)[a]

A non-parametric Kruskal-Wallis H test was used. The staining intensities corresponding to -, negative; +/-, weakly intense; +, intense; ++, very intense; +++, strongly intense, were classified into two classes -, +/-,+ and ++, +++ for BRCA1, BRCA2, AR and IGF-I. [a]For IGF-I, when five classes (-, -/+, +, ++, +++) were considered separately, p=0.012.

staining located more frequently in the cytoplasm in NM and tumors than in the nucleus. AR protein was located with intense (+) nuclear staining in 5 of 23 NM cases and was slightly decreased in the tumor biopsies. IGF-I was overexpressed in 4 of 24 ADC cases with strongly intense (+++) staining to intense (+) staining in 12 of 24 cases (Table V, Fig. 1).

Correlation between mRNA and protein expression. Statistical analysis was performed using the non-parametric Kruskal-Wallis H test (Table VI). The staining intensities corresponding to -, negative; +/-, weakly intense; +, intense; ++, very intense; +++, strongly intense were classified into two classes for BRCA1, BRCA2, AR and IGF-I. In the first class, the average corresponded to the patients exhibiting a weak protein staining and in the second class the average corresponded to patients with more intense protein staining. Upon comparison, we obtained a non-significant trend for the two classes between the protein and mRNA expression for *BRCA1*, *BRCA2*, *AR* and *IGF-I*. For *IGF-I*, we also considered the five classes of staining intensity, and the correlation with the mRNA expression was significant ($p<0.012$). Concerning *AR*, no correlation was found. Thus, ROC analysis was carried out for the prediction of the pathology by comparison of the normal and tumor tissues. The AR biomarker was correlated with diagnosis, but the relation was weak ($p=0.013$). AR was found to be decreased in the tumor tissues. AR values under the optimum threshold of 0.206 appeared to discriminate between tumor and normal tissues with a 43% sensitivity and 90% specificity. The positive predictive value (PPV) was 60% and the negative predictive value (NPV) was 82%, while the estimation of the area under the ROC curve (AUC) 95% confidence interval (0.44-0.79) included 0.5, and the statistical analysis reached significance only at the optimum threshold ($p=0.018$). The relative risk of 2.05 (1.13-3.69) indicated a 2-fold higher chance for cancer occurence, when AR was ≤0.206.

Discussion

The purpose of this study was to determine differences in *BRCA1*, *BRCA2*, *AR* and *IGF-I* expression in prostate cancer of various malignancies and to establish a correlation between mRNA assayed by RT-qPCR and proteins detected using immunohistochemical analysis. The present study focused on these genes in order to ascertain whether they could be used as predictive factors. It has been reported in previous studies that *BRCA2* is not expressed in normal tissue (43). In our series of 24 normal tissues, this result was confirmed as 47.06% of cases did not express the *BRCA2* gene. Overexpression of *BRCA1* mRNA has been shown to accelerate cell proliferation in breast cancer (44), and the present study revealed that the majority of tumor cases overexpressed the *BRCA1* gene. At the protein level, BRCA1 and BRCA2 expression was preferentially cytoplasmic.

Several investigators reported a stronger response to chemotherapy in prostate cancer cells stably expressing *AR* (13). In our study, *AR* was preferentially expressed in normal tissues. Other researchers demonstrated *in vivo* the antitumor effect of the *AR* on prostate cancer growth. *AR*-expressing cells formed tumors in male mice at a much lower rate than the AR-negative controls and, moreover, the *AR*-expressing tumors showed decreased vascularity and massive apoptosis (45). By immunohistochemistry, we found a greater frequency of nuclear overexpression of AR in normal prostate tissues than in the adenocarcinomas. This was in agreement with Agoulnik and Weigel (46) who also found down-regulated AR expression in prostate tumors.

Despite evidence implicating the IGF system in the pathogenesis of prostate cancer, its precise role remains unclear. Other studies have used immunochemistry and *in situ* hybridization techniques. They compared prostate cancer with PIN and its normal adjacent prostate counterpart and found that levels of *IGF* increased significantly from normal tissue to PIN to prostate cancer (47). Moreover, the circulating level of *IGF-I* was correlated with the degree of malignancy and the risk of prostate cancer (31). This is in agreement with the present study which demonstrated an increase in *IGF-I* mRNA expression in ADC. IGF-I accelerates the progression of precancerous changes to invasive lesions. In our immunohistochemical results, we confirmed the correlation of the IGF-I level and the degree of malignancy. Hence, *IGF* may serve to predict the outcome of prostate cancer.

This study demonstrated a potential correlation between the expression of mRNA and protein staining for the four genes. We found that at the mRNA level, in normal tissues,

BRCA1 and *BRCA2* were underexpressed. Regarding *AR*, the expression was decreased in tumor tissues and for *IGF-I*, an overexpression was found. Upon comparison of the protein and mRNA expression for BRCA1 and BRCA2, an unsignificant trend was noted. For IGF-I, the correlation with mRNA expression and staining intensity of the protein was significant ($p<0.012$).

In conclusion, our results demonstrated that *BRCA1*, *BRCA2*, *AR* and *IGF-I* genes may be used as potential biomarkers to determine the malignant potential in prostate cancer in order to facilitate diagnosis.

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