Gene Panel Model Predictive of Outcome in Patients with Prostate Cancer

Nadège Rabiau,^{1–3} Yann Dantal,³ Laurent Guy,^{2,4} Marjolaine Ngollo,^{1,2} Aslihan Dagdemir,^{1,2} Jean-Louis Kemeny,⁵ Benoît Terris,⁶ Annick Vieillefond,⁶ Jean-Paul Boiteux,^{2,4} Yves-Jean Bignon,^{1,2} and Dominique Bernard-Gallon^{1,2}

Abstract

In men at high risk for prostate cancer, established clinical and pathological parameters provide only limited prognostic information. Here we analyzed a French cohort of 103 prostate cancer patients and developed a gene panel model predictive of outcome in this group of patients. The model comprised of a 15-gene TaqMan Low-Density Array (TLDA) card, with gene expressions compared to a standardized reference. The RQ value for each gene was calculated, and a scoring system was developed. Summing all the binary scores (0 or 1) corresponding to the 15 genes, a global score is obtained between 0 and 15. This global score can be compared to Gleason score (0 to 10) by recalculating it into a 0–10 scaled score. A scaled score ≥ 2 suggested that the patient is suffering from a prostate cancer, and a scaled score ≥ 7 flagged aggressive cancer. Statistical analyses demonstrated a strongly significant linear correlation ($p=3.50\text{E-}08$) between scaled score and Gleason score for this prostate cancer cohort ($N=103$). These results support the capacity of this designed 15 target gene TLDA card approach to predict outcome in prostate cancer, opening up a new avenue for personalized medicine through future independent replication and applications for rapid identification of aggressive prostate cancer phenotypes for early intervention.

Introduction

PROSTATE CANCER IS THE MOST FREQUENTLY diagnosed cancer in men in the Western world and the second-leading cause of cancer death in males worldwide (Knudsen and Vasioukhin, 2010). In 2008, prostate cancer represented 66,000 new cases in France and 3.4 millions in Europe (Ferlay et al., 2010). In many Western-world countries, the incidence of prostate cancer is on the rise, although early diagnosis means specific mortality remains stable (Colonna et al., 2008). Prostate cancer is caused by multiple interacting factors (Clapp et al., 2008). Family history of prostate cancer, age, and ethnic origin are well established as risk factors (Brawley, 2012; Kheirandish and Chinegwundoh, 2011; Murthy et al., 2011), and environmental factors also play a role in prostatic carcinogenesis events.

Clinical and pathological parameters such as Gleason score, tumor-node-metastasis (TNM) stage, surgical margin status, and preoperative serum prostate-specific antigen (PSA) after prostatectomy are predictors of outcome in prostate cancer patients (Blute et al., 2001; Humphrey, 2004). However, there is a need to find other markers to improve the prediction of dis-

ease recurrence and the selection of appropriate adjuvant therapy for high-risk patients (Huang et al., 2009).

Recent progress in gene array technology has led to the development of several candidate prognostic cancer models based on multiple gene panels or signatures (Cheville et al., 2008; Kosari et al., 2008; Mesko et al., 2010; Steinbach et al., 2006) where the selected candidate genes are designed to account for the heterogeneity of prostate cancer and provide prognostic information in high-risk patients beyond that provided by established clinical and pathological prognostic features. Candidates have also been tested as predictors of tumor behavior in case studies on patients with radical retropubic prostatectomy and prostate cancer biopsies. These assay-based clinical markers were designed to help clinicians and patients make informed treatment decisions.

Patients and Methods

Biopsy collection

The study included 103 patients; 13 with radical retropubic prostatectomy and samples were preselected by the

¹Department of Oncogenetics, Centre Jean Perrin, Clermont-Ferrand, France.

²Research Team on Individualized Treatments of Cancer, University of Auvergne, Clermont-Ferrand, France.

³Soluscience S.A, Biopôle Clermont-Limagne, Saint-Beauzire, France.

⁴Departments of ⁴Urology and ⁵Anatomo-Cyto-Pathology, Centre Hospitalier Universitaire Gabriel Montpied, Clermont-Ferrand, France.

⁶Department of Anatomo-Cyto-Pathology, Hôpital Cochin, Paris, France.

anatomopathologist in Cochin Hospital (St-Vincent-de-Paul, France) in 2008, and 90 biopsies from patients belonging to the Centre Hospitalier Universitaire of Clermont-Ferrand (France) in 2010. These patients had sextant biopsies and classified by the anatomopathologist (Hodge et al., 1989). All patients gave their informed consent to donate the remaining post-diagnosis samples to research. All prostatectomy samples were conserved in liquid nitrogen in the tumor bank of Anatomico-cyto-pathology department of CHU Cochin-St Vincent de Paul and corresponding RNA samples were prepared in their service. All studied biopsies are housed at the Centre Jean Perrin Tumor Bank (58 Rue Montalembert, BP 392, 63011 Clermont-Ferrand Cedex 1), accredited under No. AC-2008-112, for 5 years (i.e., until December 27, 2013).

RNA extraction

Each prostate biopsy was weighed, disrupted quickly on ice with a tissueRuptor (Qiagen) in RLT+ buffer added with β -mercaptoethanol. Total mRNA isolation was performed using an RNA easy mini kit (Qiagen), following the manufacturer's protocol with an RNeasy spin column and different wash buffers. The quality of total mRNA obtained with the Qiagen kit was verified by electrophoresis on an Agilent® 2100-series BioAnalyzer, and the quality of each sample was determined with the ratio 2 for 18S/28S rRNA. Final RNA concentration (A260:A280 ratio) and purity (A260:A230 ratio) was measured using a NanoDrop ND-8000 spectrophotometer (NanoDrop Technologie, LabTec).

TaqMan mRNA analysis by RT-qPCR

Gene expression data was obtained using TaqMan Low Density Array (TLDA), a 384-well microfluidic card (Cat no: 4342261, Applied Biosystems) capable of performing 384 simultaneous real-time PCR runs, that has been used for gene expression profiling in several studies (Schobesberger et al., 2008; Steinbach et al., 2006). The fluorogenic FAM-labeled probes and sequence specific primers for the list of genes (Table 1) with endogenous control 18S rRNA were obtained as inventoried in the TaqMan array format. This low- to medium-throughput microfluidic card can run 8 triplicate samples against 16 TaqMan Gene Expression Assay targets that are preloaded into each card well. cDNA was generated using the High-Capacity cDNA Reverse Transcription Kit according to the manufacturer's protocol. The RT-PCR runs used 2 μ g of RNA per sample. Each sample used 120 ng cDNA, qsp 60 μ L nuclease-free water, and 60 μ L 2x TaqMan Universal PCR Master Mix (Applied Biosystems) added for the real-time qPCR measurements. We then transferred 100 μ L (100 ng) of the mixture into each sample-loading port of the TLDA connected to one set of the 16 genes of interest. The arrays were centrifuged once (at 1,300 g for 1 min at room temperature) to equally distribute the sample across the wells. The card was then sealed to prevent exchange between wells. RT-qPCR amplification was performed using an Applied Biosystems Prism 7900HT sequence detection system under the following thermal cycler conditions: 2 min at 50°C and 10 min at 94.5°C, followed by 40 cycles of 30 s at 97°C and 1 min at 59.7°C.

Relative gene expression was determined using the comparative C_T (threshold cycle) method, which consists of normalizing the number of target gene copies to an endogenous reference gene (18S rRNA) designated as the calibrator (Fink

TABLE 1. SPECIFIC SEQUENCES OF THE 15 GENE PANEL STUDIED IN THE TLDA

Gene symbols	Gene names	NCBI reference sequences
<i>BRCA1</i>	BRest CAncer1	NM_007294.2
<i>BRCA2</i>	BRest CAncer2	NM_000059.3
<i>AR</i>	Androgen Receptor	NM_001011645.1
<i>IGF1</i>	Insulin like Growth Factor 1	NM_001111283.1
<i>EPHB2</i>	Ephrin B2	NM_017449.3
<i>AMACR</i>	Alpha-Methylacyl-CoA Racemase	NM_203382.1
<i>ESR1</i>	Estrogen Receptor 1	NM_001122742.1
<i>ESR2</i>	Estrogen Receptor 2	NM_001040275.1
<i>BCL2</i>	B-Cell Lymphoma2	NM_000633.2
<i>RNASEL</i>	Ribonuclease L	NM_021133.2
<i>MUC1</i>	Mucin 1	NM_000001.10
<i>IL18</i>	Interleukin 18	NM_001562.2
<i>COL4A3</i>	Collagen, type IV, Alpha 3	NM_031362.2
<i>GSTP1</i>	Glutathion S-Transferase pi 1	NM_000852.2
<i>RASSF1</i>	Ras Association domain Family member 1	NM_170713.2

et al., 1998). The level of each mRNA gene in each sample was then normalized to an RNA reference corresponding to a pool of 32 normal prostate tissues from Caucasian subjects (Clontech, Human Prostate Total RNA, 636550). Values are given as mean $\pm$ SD for three assays.

Results

Patient parameters

Table 2 reports the Gleason score obtained in the 103 studied samples. The Gleason score was classified into three groups: Gleason score ≤ 5 , Gleason score > 7 , and $5 < \text{Gleason score} \leq 7$. We compared gene expression results between healthy samples and the samples from patients in each of the three Gleason groups (Fig. 1). Principal component analysis of these gene sets also distinguished the separate sample groups. The group of patients with Gleason score ≤ 5 showed significant under-expression of the studied panel of 15 genes ($p < 0.01$ for all genes, except *BRCA1* $p < 0.05$). Conversely, the group of patients with Gleason score > 7 showed strong overexpression of the studied panel of 15 genes compared to healthy-sample controls, but only *AMACR* and *BRCA2* were significantly overexpressed ($p < 0.05$), due to the weak number of samples. The group with a Gleason score of between 5 to 7 showed moderate under-expression of the studied panel of 15 genes compared to the group with Gleason score ≤ 5 , but only *COL4A3* ($p < 0.01$) and *BCL2* ($p < 0.05$) were significantly different.

TABLE 2. CLINICAL CHARACTERISTIC OF 103 MALE SUBJECTS (RECRUITED IN 2008 AND 2010) ACCORDING TO GLEASON SCORE

	Gleason score		
	≤ 5	$5 < \text{Gleason} \leq 7$	$7 <$
N=103	56	37	10
Age at diagnosis	65 (51–86)	67 (52–87)	68 (56–86)

Scaled score process and statistical analyses

Table 3 reports RT-qPCR results for a patient presenting a Gleason score of 6 and the corresponding scaled score calculated. The overexpression or underexpression of the 15 studied genes, compared to the RNA reference corresponding to a pool of 32 normal prostate tissues from Caucasian subjects, was calculated using RQ Manager software and charted in Excel. Using LOG (RQ) with reference set to 0, if the calculated value is

positive, the gene is overexpressed, and if the calculated value is negative, the gene is underexpressed. In this method, the global score was calculated with a binary score where an overexpressed gene scores 1 and an underexpressed gene scores 0. However, the scale of the attributed score can be modified by adjusting the score to give greater weight to specific important genes. Thus, a score of 1 is attributed if the difference in expression between the studied sample and control is representative of prostate cancer. For example, for the *IGF1* gene

FIG. 1. Fold changes of 15 studied genes differentiating controls and prostate cancer with different Gleason scores. Gene fold change values showing differential expression between prostate cancer and controls, were generated from RT-qPCR measurements, and represent the difference in means for prostate cancer versus control. The control corresponded to a pool of 32 normal prostate tissues from Caucasian subjects (Clontech). **(A)** shows prostate cancer patients with Gleason score ≤5, **(B)** shows prostate cancer patients with Gleason score ≤7, **(C)** shows prostate cancer patients with Gleason score >7 (***p* < 0.01 and **p* < 0.05).

TABLE 3. EXAMPLE OF 15-GENE PANEL RELATIVE QUANTIFICATION ON A PATIENT PRESENTING A GLEASON SCORE=6 AND THE CORRESPONDING SCALED SCORE CALCULATED

Gene symbols	Normal	Patient with Gleason Score=6		
		RQ	LOG (RQ)	Binary Score
BRCA1	1	1.083	0.035	1
BRCA2	1	2.432	0.386	1
AR	1	0.785	-0.105	1
IGF1	1	1.366	0.135	1
EPHB2	1	1.006	0.003	1
AMACR	1	2.279	0.358	1
ESR1	1	1.023	0.010	1
ESR2	1	8.637	0.936	1
BCL2	1	0.292	-0.535	0
RNASEL	1	0.598	-0.223	0
MUC1	1	0.911	-0.040	0
IL18	1	0.547	-0.262	0
COL4A3	1	1.306	0.116	1
GSTP1	1	0.4	-0.398	0
RASSF1	1	0.961	-0.017	0
Global score total (0-15)				9
Scaled score total (0-10)				6

The relative quantification of each gene is given as median. RQ is calculated as $2^{-\Delta C_T}$, where $\Delta C_T = C_T - C_{T(18s\ rRNA)}$. Fold change indicates the ratio of relative quantification in patients over ratio in normal prostate RNA (i.e., $2^{-\Delta \Delta C_T}$).

known to be overexpressed in cancer, if *IGF1* is overexpressed in the studied sample compared to control, it will score 1, and conversely, if *IGF1* is underexpressed, it will score 0. Similarly, for the *AR* gene known to be underexpressed in adenocarcinoma, if *AR* is underexpressed in the studied sample compared to control, it will score 1, but if *AR* is overexpressed, it will score 0.

Summing all the binary scores (0 or 1) corresponding to the 15 genes gives a global score between 0 and 15. This global score can be compared to Gleason score (0 to 10) by recalculating it into a 0-10 scaled score using the formula:

$$\frac{(\text{global score} - X_{\min})}{(X_{\max} - X_{\min})} \times (X_{\max G} - X_{\min G})$$

where $X_{\min}$ is the minimum global score, $X_{\max}$ is the maximum global score, $X_{\max G}$ is the maximum Gleason score (10), and $X_{\min G}$ is the minimum Gleason score (0). For example, when a global score of 9 is obtained with all 15 genes receiving a binary score (0 or 1), the scaled score, as calculated below, will be 6:

$$\frac{(9 - 0)}{(15 - 0)} \times (10 - 0) = 6$$

A scaled score ≥ 7 suggests that a patient has prostate cancer with an aggressive phenotype, whereas a scaled score ≥ 2 corresponds to a less aggressive cancer phenotype.

Table 3 illustrates the scaled score obtained after relative quantification of the 15-gene panel in a patient presenting a Gleason score=6. Note that the scaled score for this same case also came to 6, correlating with the Gleason score.

Linear correlations between scaled score and Gleason score

Linear correlations were performed between the scaled score and Gleason score, and then between each gene expression level and Gleason score. The formula used was: $p = \text{STUDENT LAW } (r, n-2, 2)$, where r is the correlation coefficient, following a Student's distribution with $n-2$ degrees of freedom, and n is sample size. As shown by Table 4, the linear correlation between scaled score and Gleason score for the whole cohort ($N=103$) was highly significant ($p=3.50E-08$). Moreover, when the 103 prostate cancer samples were studied for the expression levels of specific genes such as *IGF1*, *AMACR*, *BCL2*, *RNASEL*, and *GSTP1*, all the genes were significantly correlated with the Gleason score ($p \leq 0.05$), and *AR* was strongly significant correlated with the Gleason score ($p \leq 0.01$).

Discussion

Candidate prognostic cancer models based on multiple gene panels or signatures have already been proposed (Idikio et al, 2011; Lossos et al., 2004; Ramaswamy et al., 2003; van de Vijver et al., 2002) including several for prostate cancer (Bismar et al., 2006; Cheville et al., 2008; Glinisky et al., 2004; Kosari et al., 2008; Li et al., 2012; Roobol et al., 2011; Ross et al., 2012; Singh et al., 2002).

Molecular profiles can identify characteristics of tumor cells not yet within the scope of pathological and clinical evaluations. When constructing a molecular profile of prostate cancer, it is important to consider the heterogeneity of the disease.

Ideally, it would be possible to find molecular markers of progression by analyzing tissue samples with patient follow-up. However, expression platforms such as TLDA require RNA of a quality that can only be found in frozen tissue samples conserved in tumor banks.

TABLE 4. LINEAR CORRELATION BETWEEN SCALED SCORE AND GLEASON SCORE AND BETWEEN EACH GENE AND GLEASON SCORE

Linear correlations	P	
	N = 103	
Scaled score/Gleason score	3.50E-08	**
BRCA1 expression/Gleason Score	0.400	
BRCA2 expression/Gleason Score	0.210	
AR expression/Gleason Score	0.008	**
IGF1 expression/Gleason Score	0.024	*
EPHB2 expression/Gleason Score	0.075	
ESR1 expression/Gleason Score	0.401	
ESR2 expression/Gleason Score	0.292	
AMACR expression/Gleason Score	0.020	*
BCL2 expression/Gleason Score	0.048	*
RNASEL expression/Gleason Score	0.019	*
MUC1 expression/Gleason Score	0.654	
IL18 expression/Gleason Score	0.054	
COL4A3 expression/Gleason Score	0.094	
GSTP1 expression/Gleason Score	0.031	*
RASSF1 expression/Gleason Score	0.436	

$P = \text{STUDENT LAW } (r, N-2, 2)$, when N is number of samples, * $p \leq 0.05$, ** $p \leq 0.01$.

In order to develop a gene panel model predictive of outcome in patients with prostate cancer, we previously designed a real-time PCR low-density array that included relevant genes for prostate cancer and identified candidate markers by analysis of *BRCA1*, *BRCA2*, *AR*, and *IGF-I* gene expressions in a cohort of 98 prostate biopsies (Rabiau et al., 2011). We used TaqMan RT-qPCR for mRNA detection and immunohistochemistry to find correlations with proteins. Among the 98 studied prostate biopsies, expression levels of the four genes showed strong heterogeneity within the different histological types. However, we found that *BRCA1* and *BRCA2* mRNA were underexpressed, especially in healthy tissues. *AR* expression was tumor stage-dependent. *IGF-I* was specifically expressed in tumors, whereas for *BRCA1*, *BRCA2*, and *AR*, there was only a nonsignificant trend between protein expression and mRNA expression. For *IGF-I*, mRNA expression and staining intensity were significantly correlated ($p < 0.012$). The *AR* biomarker was fairly correlated to diagnosis ($p = 0.013$). *AR* expression was found to be lower in tumors, with 43% sensitivity and 90% specificity. With relative risk = 2.05 [1.13 – 3.69], there was a 2-fold higher chance of cancer occurrence when $AR \leq 0.206$.

MUC1 was also included in the design of the 15 TLDA panel as this gene is expressed at high levels in prostate tumors (Garbar et al., 2008; Rajabi et al., 2012). *MUC1* expression was significantly more frequent in patients with a Gleason score > 7 than in normal prostates, indicating that increased *MUC1* expression is mainly associated with late events in prostate cancer development and *MUC1* is up-regulated during cancer progression (Cozzi et al., 2005). Our previous immunohistochemical analysis of *MUC1* expression in 100 prostate biopsies (Rabiau et al., 2009) found, like others, that tumor prostate tissues showed intensive *MUC1* staining compared to normal tissues. Moreover, Gleason score appeared to correlate to *MUC1* staining.

Other candidate genes were chosen as predictors of tumor behavior. *GSTP1* is a phase-II detoxification enzyme coded by a gene located at 11q3. In 90 of prostate cancers, there is loss of expression of *GSTP1* due to methylation of the CpG islands of its promoter (Lee et al., 1994; Mahapatra et al., 2012; Reibenwein et al., 2007). *RASSF1* is a tumor suppressor gene that shows loss of expression in lung cancer, renal carcinomas with clear cells, and 74% of prostate cancers. This loss was related to CpG island methylation and deacetylation of histones in its promoter located at 3p21 (Kawamoto et al., 2007; Van Neste et al., 2012). *EphB2* is a tumor suppressor gene located at 1p36.1 whose nonsense mutation is found in 10% of sporadic prostate cancers (Adjakly et al., 2011; Kittles et al., 2006; Vardi et al., 2010).

Other genes such as *ER α* and *ER β* were studied because they have been identified in normal and prostate cancer tissues. Loss or downregulation of *ER* expression in prostate cancer has been frequently documented (Latil et al., 2001; Lau et al., 2000; Nakamura et al., 2013; Walton et al., 2009). However, it is known from previous studies that the *ER* promoter is extensively methylated both in prostate cancer cell lines and prostate cancer tissues, leading to *ER* gene inactivation in prostate cancer (Li et al., 2000; Walker, 2011).

Anti-apoptotic *BCL-2* expression resulting in apoptosis resistance in prostate cancer was also investigated in the gene panel (Arbab et al., 2012; Mazzolini et al., 2012).

The panel also included *RNASEL* at chromosome 1q25, which encodes ribonuclease L, part of the interferon-mediated

immune response to viral infection. Meyer et al. (2010) investigated the association between variation in *RNASEL* and prostate cancer risk and progression in a study of 1286 cases and 1264 controls nested within the prospective Physicians' Health Study. The results of this study support a link between *RNASEL* and prostate cancer and suggest that the association may be mediated through inflammation (Jin et al., 2012; Meyer et al., 2010).

Several molecules have been proposed as positive immunohistological markers of prostate carcinoma, the most widely used being α -methylacyl coenzyme A racemase (*AMACR*) which is expressed in 80%–100% of prostate adenocarcinomas (Dabir et al., 2012; Kumaresan et al., 2010; Scarpelli et al., 2012; Trpkov et al., 2009).

Concerning the *IL18* gene, Dwivedi et al. (2011), studying a subpopulation of 149 prostate carcinoma patients with 3-year follow-up, concluded that serum IL-18 has the potential to be a better diagnostic marker with higher specificity and sensitivity and a more valuable prognostic marker than PSA in prostate carcinoma.

Finally, the panel included *COL4A3* gene corresponding to type-IV collagen. This major structural component of basement membranes is a multimeric protein composed of three alpha subunits. The gene encodes alpha 3.

The approach reported here included high-throughput gene expression profiling with quantitative measurements of expression levels of candidate biomarkers. Relative quantification of a 15 target gene TLDA panel in 103 patient tissues was performed, and the corresponding global weighted scores were calculated. Linear correlations were performed between Gleason score and scaled score and then between the Gleason score and the expression level of each gene. We found a highly significant linear correlation ($P = 3.50E-08$) between scaled score and Gleason score for the whole cohort ($N = 103$). Moreover, when the 103 prostate cancer samples were studied for the expression levels of specific genes such as *IGF1*, *AMACR*, *BCL2*, *RNASEL*, and *GSTP*, all the genes were significantly correlated with Gleason score ($p \leq 0.05$), and *AR* was strongly significant correlated with Gleason score ($p \leq 0.01$).

This studied 15 target gene TLDA card approach offers promise as a sample screening method for rapid identification of aggressive prostate phenotypes, opening up potential applications for outbreak investigations and disease surveillance.

Conclusion

The quantification of gene expression is one of the key endpoints for understanding the molecular events initiating prostate cancer and establishing cancer prognosis. This gene panel approach can play a major role in the development of personalized medicine for prostate cancer.

Acknowledgments

N. Rabiau received a grant from "les FondsFEDER Europe." All prostatectomy samples were conserved in liquid nitrogen in the tumor bank held by the Anatomico-cytopathology department at CHU Cochin-St-Vincent-de-Paul and all the biopsies are housed at the Centre Jean Perrin Tumor Bank (58 –Rue Montalembert –BP 392- 63011 Clermont-Ferrand Cedex 1), accredited under No. AC –2008-112, for 5 years (i.e., until 27 December 2013).

Author Disclosure Statement

The authors declare they have no conflicting financial interests.

References

- Adjakly M, Bosviel R, Rabiau N, et al. (2011). DNA methylation and soy phytoestrogens: Quantitative study in DU-145 and PC-3 human prostate cancer cell lines. *Epigenomics* 3, 795–803.
- Arbab IA, Looi CY, Abdul AB, et al. (2012). Dentatin induces apoptosis in prostate cancer cells via Bcl-2, Bcl-xL, survivin downregulation, caspase-9, -3/7 activation, and NF-kappaB inhibition. *Evid Based Complement Alternat Med* 2012, 856029.
- Bismar TA, Demichelis F, Riva A, et al. (2006). Defining aggressive prostate cancer using a 12-gene model. *Neoplasia* 8, 59–68.
- Blute ML, Bergstralh EJ, Iocca A, Scherer B, and Zincke H. (2001). Use of Gleason score, prostate specific antigen, seminal vesicle and margin status to predict biochemical failure after radical prostatectomy. *J Urol* 165, 119–125.
- Brawley OW. (2012). Prostate cancer epidemiology in the United States. *World J Urol* 30, 195–200.
- Cheville JC, Karnes RJ, Therneau TM, et al. (2008). Gene panel model predictive of outcome in men at high-risk of systemic progression and death from prostate cancer after radical retropubic prostatectomy. *J Clin Oncol* 26, 3930–3936.
- Clapp RW, Jacobs MM, and Loechler EL. (2008). Environmental and occupational causes of cancer: New evidence 2005–2007. *Rev Environ Health* 23, 1–37.
- Colonna M, Danzon A, Delafosse P, et al. (2008). Cancer prevalence in France: Time trend, situation in 2002 and extrapolation to 2012. *Eur J Cancer* 44, 115–122.
- Cozzi PJ, Wang J, Delprado W, et al. (2005). MUC1, MUC2, MUC4, MUC5AC and MUC6 expression in the progression of prostate cancer. *Clin Exp Metastasis* 22, 565–573.
- Dabir PD, Ottosen P, Hoyer S, and Hamilton-Dutoit S. (2012). Comparative analysis of three- and two-antibody cocktails to AMACR and basal cell markers for the immunohistochemical diagnosis of prostate carcinoma. *Diagn Pathol* 7, 81.
- Dwivedi S, Goel A, Natu SM, Mandhani A, Khattry S, and Pant KK. (2011). Diagnostic and prognostic significance of prostate specific antigen and serum interleukin 18 and 10 in patients with locally advanced prostate cancer: A prospective study. *Asian Pac J Cancer Prev* 12, 1843–1848.
- Ferlay J, Parkin DM, and Steliarova-Foucher E. (2010). Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer* 46, 765–781.
- Fink L, Seeger W, Ermer L, et al. (1998). Real-time quantitative RT-PCR after laser-assisted cell picking. *Nat Med* 4, 1329–1333.
- Garbar C, Mascaux C, and Wespes E. (2008). Expression of MUC1 and sialyl-Tn in benign prostatic glands, high-grade prostate intraepithelial neoplasia and malignant prostatic glands: A preliminary study. *Anal Quant Cytol Histol* 30, 71–77.
- Glinsky GV, Glinskii AB, Stephenson AJ, Hoffman RM, and Gerald WL. (2004). Gene expression profiling predicts clinical outcome of prostate cancer. *J Clin Invest* 113, 913–923.
- Hodge KK, McNeal JE, Terri, MK, and Stamey TA. (1989). Random systematic versus directed ultrasound guided transrectal core biopsies of the prostate. *J Urol* 142, 71–74; discussion 74–75.
- Huang SP, Huang LC, Ting WC, et al. (2009). Prognostic significance of prostate cancer susceptibility variants on prostate-specific antigen recurrence after radical prostatectomy. *Cancer Epidemiol Biomarkers Prev* 18, 3068–3074.
- Humphrey PA. (2004). Gleason grading and prognostic factors in carcinoma of the prostate. *Mod Pathol* 17, 292–306.
- Idikio HA. (2011). Galectin-3 and Beclin1/Atg6 genes in human cancers: Using cDNA tissue panel, qRT-PCR, and logistic regression model to identify cancer cell biomarkers. *PLoS One* 6, e26150.
- Jin W, Wu DD, Zhang X, Irwin DM, and Zhang YP. (2012). Positive selection on the gene RNASEL: Correlation between patterns of evolution and function. *Mol Biol Evol* 29, 3161–3168.
- Kawamoto K, Okino ST, Place RF, et al. (2007). Epigenetic modifications of RASSF1A gene through chromatin remodeling in prostate cancer. *Clin Cancer Res* 13, 2541–2548.
- Kheirandish P, and Chingwundoh F. (2011). Ethnic differences in prostate cancer. *Br J Cancer* 105, 481–485.
- Kittles RA, Baffoe-Bonnie AB, Moses TY, et al. (2006). A common nonsense mutation in EphB2 is associated with prostate cancer risk in African American men with a positive family history. *J Med Genet* 43, 507–511.
- Knudsen BS, and Vasioukhin V. (2010). Mechanisms of prostate cancer initiation and progression. *Adv Cancer Res* 109, 1–50.
- Kosari F, Munz JM, Savci-Heijink CD, et al. (2008). Identification of prognostic biomarkers for prostate cancer. *Clin Cancer Res* 14, 1734–1743.
- Kumaresan K, Kakkar N, Verma A, Mandal AK, Singh SK, and Joshi K. (2010). Diagnostic utility of alpha-methylacyl CoA racemase (P504S) & HMWCK in morphologically difficult prostate cancer. *Diagn Pathol* 5, 83.
- Latil A, Bieche I, Vidaud D, et al. (2001). Evaluation of androgen, estrogen (ER alpha and ER beta), and progesterone receptor expression in human prostate cancer by real-time quantitative reverse transcription-polymerase chain reaction assays. *Cancer Res* 61, 1919–1926.
- Lau KM, LaSpina M, Long J, and Ho SM. (2000). Expression of estrogen receptor (ER)-alpha and ER-beta in normal and malignant prostatic epithelial cells: Regulation by methylation and involvement in growth regulation. *Cancer Res* 60, 3175–3182.
- Lee WH, Morton RA, Epstein JI, et al. (1994). Cytidine methylation of regulatory sequences near the pi-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. *Proc Natl Acad Sci USA* 91, 11733–11737.
- Li LC, Chui R, Nakajima K, Oh BR, Au HC, and Dahiya R. (2000). Frequent methylation of estrogen receptor in prostate cancer: Correlation with tumor progression. *Cancer Res* 60, 702–706.
- Li J, Zhang ZH, Yin CJ, et al. (2012). Effect of surgical procedures on prostate tumor gene expression profiles. *Asian J Androl* 14, 708–714.
- Lossos IS, Czerwinski DK, Alizadeh AA, et al. (2004). Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. *N Engl J Med* 350, 1828–1837.
- Mahapatra S, Klee EW, Young CY, et al. (2012). Global methylation profiling for risk prediction of prostate cancer. *Clin Cancer Res* 18, 2882–2895.
- Mazzolini R, Dopeso H, Mateo-Lozano S, et al. (2012). Brush border myosin Ia has tumor suppressor activity in the intestine. *Proc Natl Acad Sci USA* 109, 1530–1535.
- Mesko B, Poliska S, Szegedi A, et al. (2010). Peripheral blood gene expression patterns discriminate among chronic inflam-

- matory diseases and healthy controls and identify novel targets. *BMC Med Genomics* 3, 15.
- Meyer MS, Penney KL, Stark JR, et al. (2010). Genetic variation in RNASEL associated with prostate cancer risk and progression. *Carcinogenesis* 31, 1597–1603.
- Murthy VS, Garza MA, Almario DA, et al. (2011). Using a family history intervention to improve cancer risk perception in a black community. *J Genet Couns* 20, 639–649.
- Nakamura Y, McNamara K, and Sasano H. (2013). Estrogen receptor expression and its relevant signaling pathway in prostate cancer: A target of therapy. *Curr Mol Pharmacol Jan. 8* [Epub ahead of print].
- Rabiau N, Dechelotte P, Adjakly M, et al. (2011). BRCA1, BRCA2, AR and IGF-I expression in prostate cancer: Correlation between RT-qPCR and immunohistochemical detection. *Oncol Rep* 26, 695–702.
- Rabiau N, Dechelotte P, Guy L, et al. (2009). Immunohistochemical staining of mucin 1 in prostate tissues. *In Vivo* 23, 203–207.
- Rajabi H, Ahmad R, Jin C, et al. (2012). MUC1-C oncoprotein confers androgen-independent growth of human prostate cancer cells. *Prostate* 72, 1659–1668.
- Ramaswamy S, Ross KN, Lander ES, and Golub TR. (2003). A molecular signature of metastasis in primary solid tumors. *Nat Genet* 33, 49–54.
- Reibenwein J, Pils D, Horak P, et al. (2007). Promoter hypermethylation of GSTP1, AR, and 14-3-3sigma in serum of prostate cancer patients and its clinical relevance. *Prostate* 67, 427–432.
- Roobol MJ, Haese A, and Bjartell A. (2011). Tumour markers in prostate cancer III: Biomarkers in urine. *Acta Oncol* 50, 85–89.
- Ross RW, Galsky MD, Scher HI, et al. (2012). A whole-blood RNA transcript-based prognostic model in men with castration-resistant prostate cancer: A prospective study. *Lancet Oncol* 13, 1105–1113.
- Scarpelli M, Mazzucchelli R, Barbisan F, et al. (2012). Is there a role for prostate tumour overexpressed-1 in the diagnosis of HGPIN and of prostatic adenocarcinoma? A comparison with alpha-methylacyl CoA racemase. *Int J Immunopathol Pharmacol* 25, 67–74.
- Schobesberger M, Baltzer A, Oberli A, et al. (2008). Gene expression variation between distinct areas of breast cancer measured from paraffin-embedded tissue cores. *BMC Cancer* 8, 343.
- Singh D, Febbo PG, Ross K, et al. (2002). Gene expression correlates of clinical prostate cancer behavior. *Cancer Cell* 1, 203–209.
- Steinbach D, Schramm A, Eggert A, et al. (2006). Identification of a set of seven genes for the monitoring of minimal residual disease in pediatric acute myeloid leukemia. *Clin Cancer Res* 12, 2434–2441.
- Trpkov K, Bartczak-McKay J, and Yilmaz A. (2009). Usefulness of cytokeratin 5/6 and AMACR applied as double sequential immunostains for diagnostic assessment of problematic prostate specimens. *Am J Clin Pathol* 132, 211–220.
- van de Vijver MJ, He YD, van't Veer LJ, et al. (2002). A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 347, 1999–2009.
- Van Neste L, Bigley J, Toll A, et al. (2012). A tissue biopsy-based epigenetic multiplex PCR assay for prostate cancer detection. *BMC Urol* 12, 16.
- Vardi A, Bosviel R, Rabiau N, et al. (2010). Soy phytoestrogens modify DNA methylation of GSTP1, RASSF1A, EPH2 and BRCA1 promoter in prostate cancer cells. *In Vivo* 24, 393–400.
- Walker CL. (2011). Epigenomic reprogramming of the developing reproductive tract and disease susceptibility in adulthood. *Birth Defects Res A Clin Mol Teratol* 91, 666–671.
- Walton TJ, Li G, McCulloch TA, et al. (2009). Quantitative RT-PCR analysis of estrogen receptor gene expression in laser microdissected prostate cancer tissue. *Prostate* 69, 810–819.

Address correspondence to:
Dr. Yves-Jean Bignon
Department of Oncogenetics
Centre Jean Perrin
63001 Clermont-Ferrand
France

E-mail: Yves-Jean.BIGNON@cjp.fr