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ORIGINAL ARTICLE

How reliable and useful are angiogenesis blood mediators for prostate cancer diagnosis?

Ovidiu Balacescu[1]*, Nicolae Crisan[2,8]*, Loredana Balacescu[1], Simona Visan[1], Oana Tudoran[1], Bogdan Fetica[3], Catalina Bungardean[4], Catalin Marian[5,6], Bogdan Petrut[7,8]

[1]Department of Functional Genomics, Proteomics and Experimental Pathology, The Oncology Institute "Prof Dr. Ion Chiricuta", Cluj-Napoca, Romania; [2]Department of Urology, Municipal Clinical Hospital, Cluj-Napoca, Romania; [3]Department of Pathology, The Oncology Institute "Prof Dr. Ion Chiricuta", Cluj-Napoca, Romania; [4]Department of Pathology, Municipal Clinical Hospital, Cluj-Napoca, Romania; [5]Department of Biochemistry & Pharmacology, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania; [6]The Ohio State University Comprehensive Cancer Center, Division of Cancer Prevention and Control, Columbus, Ohio, United States of America; [7]Department of Surgery, The Oncology Institute "Prof Dr. Ion Chiricuta", Cluj-Napoca, Romania; [8]Department of Surgical Oncology, University of Medicine and Pharmacy, "I. Hatieganu", Cluj-Napoca, Romania.

* These authors contributed equally to this work

Summary

Purpose: *To evaluate if a blood panel of genes involved in the modulation of the immune system, angiogenesis and tumor development could be used for prostate cancer detection.*

Methods: *Gene expression profiling of blood samples was assessed with the human angiogenesis RT² Profiler™ pcr array. The study group was divided into training and a testing/validation set. In total, 36 blood samples from 6 heatlhy men, 19 patients with prostate cancer (PCa) and 11 patients with benign prostate pathology (BP) were included in this study.*

Results: *Transcriptional analysis revealed a supervised signature of 28 genes which discriminated the PCa samples from control on the training set (fold regulation [FR] cut off 1.5, p<0.05). This signature was further validated on the testing set. All 28 genes used for this classification were differentially expressed in the new set of 12 PCa samples compared to control but also compared to benign samples (FR cut off 1.5, p<0.05).*

Conclusions: *Our data could provide new insight into PCa, as a non-invasive predictive tool which along with other factors could improve PCa diagnosis. However, our findings have to be confirmed in a larger cohort of patients before having a clear picture of how this molecular profile will help to increase the accuracy of diagnosis.*

Key words: *prostate cancer, diagnosis, blood, angiogenesis, gene profiling*

Introduction

With an annual incidence of more than 1.1 million cases and about 300.000 deaths, PCa represents the second most commonly diagnosed cancer in the male population worldwide [1].

Prostate-specific antigen (PSA) was the first FDA-approved test that has significantly contributed to the early diagnosis of PCa [2]. Although it is still regarded as the best conventional serum marker supporting early diagnostic and management of PCa, PSA is limited by a significant lack of specificity leading to overdiagnosis and overtreatment [3]. Serum level of PSA may increase in benign prostatic hyperplasia (BPH) and inflammation such as chronic prostatitis as well as due to different

Corresponding author: Ovidiu Balacescu, PhD. The Oncology Institute "Prof. Dr. Ion Chiricuta" Department of Functional Genomics, Proteomics and Experimental Pathology Cluj-Napoca, 34-36 Republicii Street, Zip code 400015, Cluj-Napoca, Romania.
Tel/Fax: +40 264 590 638, Email: obalacescu@yahoo.com
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lifestyle factors [4,5]. Considering the benefits and harms of PSA screening, the recommendations were updated to balance these aspects [6].

Recently, trying to improve the PCa diagnosis in the 4-10 ng/ml PSA range, FDA approved a new PSA-based test named Prostate Health Index (PHI) which has been shown to perform well also in the 2-10 ng/ml PSA range [7]. Although FDA-approved biomarkers and several additional tests [8] have been commercially developed lately, the lack of specificity and sensitivity of these tests in detecting PCa remains a major problem [9,10]. Even including the artificial neural networks (ANNS) for PCa diagnosis did not bring significant results [11].

Blood is considered a surrogate tissue that can monitor the body's physiological status, whereas modifications of gene expression in its cells are depending on pathological conditions. New concepts stemming from genomics technology in the discovery of biomarkers have shown that patterns of transcription can lead to better diagnosis. Moreover, peripheral blood profiling can provide data that will lead to the early diagnosis, prognosis and treatment response of solid tumors [12-15]. However, to identify the molecules involved in tumor-blood communication as well as to demonstrate their possible role as biomarkers still represent a great challenge.

Investigations into the molecular basis of tumorigenesis have previously demonstrated that angiogenesis is one of the earliest events in tumor development [16]. Tumors release autocrine and paracrine factors that activate or facilitate this process, thus tumor angiogenesis is the result of changes in the balance between positive and negative angiogenic factors [17]. Alteration of the expression of some mediators of angiogenesis, such fibroblast growth factors (FGFs), vascular endothelial growth factors (VEGFs), transforming growth factors (TGF-α and TGF-β), platelet-derived growth factor (PDGF), angiogenin (ANG), interleukins (IL-8) or tumor necrosis factor-alpha (TNFα), has been previously associated to PCa [18-21].

The aim of this study was to determine if a blood panel of genes involved in the modulation of immune system, angiogenesis and tumor development could provide supplementary information for PCa diagnosis, in addition to the currently used evaluations.

Methods

Patient selection

Thirty-six patients from the Municipal Clinical Hospital and The Oncology Institute "Prof. Dr. Ion Chiricuta", Cluj-Napoca, Romania, participated in this study. The ethical approval was given by the Institutional Ethics Committee while all patients signed an informed consent according to the Declaration of Helsinki. Thirty subjects were enrolled in the study either based on PCa suspicions (PSA>4 ng/ml and/or abnormal digital rectal examination (DRE) or already having PCa diagnosis (biopsy-confirmed). After histopatological diagnosis, the study group consisted of 19 patients with PCa, 7 patients with benign prostate hyperplasia (BPH) and 4 patients with chronic prostatitis (CP). Additionally, 6 patients with serum PSA<2.1 ng/ml and normal DRE were considered as control group (Ctr).

Sample collection and preparation

About 2 ml of peripheral blood were collected from each patient in EDTA anticoagulant tubes, at the time of study enrollement, in the same condition for all pa-

Table 1. Clinical and pathological features of the patients in the training group

Sample number	*Blood sample code*	*Study group*	*Age, years*	*PSA (ng/ml)*	*Gleason score*	*HP exam*
1	Ctr 1	Control	51	0.6	-	-
2	Ctr 2	Control	53	0.5	-	-
3	Ctr 3	Control	62	1.4	-	-
4	Ctr 4	Control	56	1.2	-	-
5	Ctr 5	Control	55	1.8	-	-
6	Ctr 6	Control	36	0.6	-	-
7	PCa 1	Cancer	79	168	5+4=9	Acinar prostate adenocarcinoma
8	PCa 2	Cancer	73	79.3	3+5=8	Acinar prostate adenocarcinoma
9	PCa 3	Cancer	61	862	3+4=7	Acinar prostate adenocarcinoma
10	PCa 4	Cancer	71	13.5	4+3=7	Acinar prostate adenocarcinoma
11	PCa 5	Cancer	55	92	3+5=8	Acinar prostate adenocarcinoma
12	PCa 6	Cancer	57	351.8	5+3=8	Acinar prostate adenocarcinoma
13	PCa 7	Cancer	71	5.24	1+3=4	Acinar prostate adenocarcinoma

HP: Histopathology, Ctr: Control, PCa: Prostate Cancer

tients (before noon). None of the patients had fever or any acute diseases or received hormonal therapy, radiotherapy or chemotherapy before harvesting the blood samples. The samples were stored on ice and processed as quickly as possible following a standardized protocol, including plasma removing, erythrocyte lysis and total RNA extraction from nucleated blood cells using TriReagent (Ambion/Thermo Fisher Scientific, Waltham, MA, USA). RNA concentrations were measured with Nanodrop spectrophotometer (NanoDrop Technologies/Thermo Fisher Scientific) while the quality of RNA was assessed with Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) based on the RNA integrity number (RIN) values. All extracted RNA was stored at -80°C until their further processing for PCR array analysis.

Assessment of the study

The study group was divided into a training set and a testing/validation set. The PCa samples were included into the analysis consecutively as they were collected.

The training set consisting of 13 samples (7 PCa and 6 Ctr) was used to identify a gene expression signature that discriminated between PCa patients and the control group. The mean age of the patients in the PCa group was 66.7 years (range 55-79) and 52.2 years (range 36-62) in the control group. PCa patients had a Gleason score between 4 and 9 (Table 1).

The testing set (12 PCa and 11 benign prostate pathologies) was used to validate the supervised signature obtained in the training set. The mean age of the

Table 2. Clinical and pathological features of the patients in the testing/validation set.

Sample number	*Blood sample code*	*Study group*	*Age, years*	*PSA (ng/ml)*	*Gleason score*	*Prostate volume (mm)*	*Tumor presence (%)*	*HP exam*
14	PCa 8	Cancer	67	13.9	4+5=9	45/40/30	-	Acinar prostate adenocarcinoma; pT3bN0MxL0V0
15	PCa 9	Cancer	58	3.4	3+4=7	66/40/34	30	Acinar prostate adenocarcinoma; pT2cNxMxL0V0
16	PCa 10	Cancer	56	17	4+3=7	45/50/37	80	Acinar prostate adenocarcinoma; multifocal HGPIN pT3bN1MxL0V0R1
17	PCa 11	Cancer	55	10.8	3+4=7	-	-	Acinar prostate adenocarcinoma pT3bN0MxL0V1R1
18	PCa12	Cancer	65	6.7	3+3=6	-	5	Acinar prostate adenocarcinoma; HGPIN; pT2N0MxL0V0R0
19	PCa 13	Cancer	61	7	3+3=6	45/50/40	5	Acinar prostate adenocarcinoma; multifocal HGPIN pT2cNxMxL0V0R0
20	PCa 14	Cancer	65	11.3	2+3=5	50/65/40	15	Prostate adenocarcinoma pT2cN0MxL0V0
21	PCa 15	Cancer	61	8.9	3+3=6	45/50/35	5	Acinar prostate adenocarcinoma; multifocal HGPIN pT2cNxMxL0V0R0
22	PCa 16	Cancer	59	7	3+3=6	45/50/40	10	Acinar prostate adenocarcinoma; extensive HGPIN pT2cNxMxL0V0R0
23	PCa 17	Cancer	66	9.5	4+3=7	45/45/20	25	Acinar Prostate adenocarcinoma; HGPIN; pT3bNoMx
24	PCa 18	Cancer	67	8	3+3=6	-		Acinar prostate adenocarcinoma; extensive HGPIN
25	PCa 19	Cancer	70	6.8	3+2=5	65/65/45	1	Acinar prostate adenocarcinoma; extensive HGPIN pT2aN0MxL0V0
26	BP1	Benign	60	4.5	-	-	-	Chronic Prostatitis
27	BP2	Benign	67	15.9	-	-	-	Chronic Prostatitis
28	BP3	Benign	65	5.5	-	-	-	Chronic Prostatitis
29	BP4	Benign	68	4.7	-	-	-	Chronic Prostatitis
30	BP5	Benign	53	20	-	-	-	Benign Prostatic Hyperplasia
31	BP6	Benign	72	6	-	-	-	Benign Prostatic Hyperplasia
32	BP7	Benign	60	5.2	-	-	-	Benign Prostatic Hyperplasia
33	BP8	Benign	69	7	-	-	-	Benign Prostatic Hyperplasia
34	BP9	Benign	70	5	-	-	-	Benign Prostatic Hyperplasia
35	BP10	Benign	67	4.6	-	-	-	Benign Prostatic Hyperplasia
36	BP11	Benign	62	4.1	-	-	-	Benign Prostatic Hyperplasia

BP: benign prostate, PCa: prostate cancer, HGPIN: high-grade prostatic intraepithelial neoplasia, HP: histopathology

Table 3. The panel of 84 genes evaluated by PCR array (PAH-024 plates)

Angiogenic factors	
Growth factors and receptors	ANGPT1, ANGPT2, ANPEP, ECGF1, EREG, FGF1, FGF2, FIGF, FLT1, JAG1, KDR, LAMA5, NRP1, NRP2, PGF, PLXDC1, STAB1, VEGFA, VEGFC
Adhesion molecules	ANGPTL3, BAI1, COL4A3, IL8, LAMA5, NRP1, NRP2, STAB1
Proteases, inhibitors and other matrix proteins	ANGPTL4, PECAM1, PF4, PROK2, SERPINF1, TNFAIP2
Transcription factors and others	HAND2, SPHK1
Other factors involved in angiogenesis, immune response and tumor development	
Cytokines and chemokines	CCL11, CCL2, CXCL1, CXCL10, CXCL3, CXCL5, CXCL6, CXCL9, IFNA1, IFNB1, IFNG, IL1B, IL6, MDK, TNF
Other growth factors and receptors	EDG1, EFNA1, EFNA3, EFNB2, EGF, EPHB4, FGFR3, HGF, IGF1, ITGB3, PDGFA, TEK, TGFA, TGFB1, TGFB2, TGFBR1
Adhesion molecules	CCL11, CCL2, CDH5, COL18A1, EDG1, ENG, ITGAV, ITGB3, THBS1, THBS2
Proteases, inhibitors and other matrix proteins	LECT1, LEP, MMP2, MMP9, PLAU, PLG, TIMP1, TIMP2, TIMP3
Transcription factors and others	AKT1, HIF1A, HPSE, ID1, ID3, NOTCH4, PTGS1

Table 4. Differentially expressed genes between PCa and control group (1.5<FR<-1.5, p <0.05)

Symbol	*Description*	*PCa vs. Control*	
		FR	*p value*
CXCL1	Chemokine (C-X-C motif) ligand 1	2.38	0.0221
IL8	Interleukin 8	3.87	0.0143
ITGAV	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	2.85	0.0007
ANGPT2	Angiopoietin 2	-5.57	0.0001
ANGPTL3	Angiopoietin-like 3	-7.39	0.0003
COL18A1	Anti-angiogenic agent; collagen alpha-1(XVIII) chain; endostatin; multi-functional protein MFP	-1.73	0.0317
COL4A3	Collagen, type IV, alpha 3 (Goodpasture antigen)	-5.10	0.0333
CXCL3	Chemokine (C-X-C motif) ligand 3	-6.39	0.0001
FGF1	Fibroblast growth factor 1 (acidic)	-5.03	0.0140
FIGF	C-fos induced growth factor (vascular endothelial growth factor D)	-9.45	0.0001
FLT1	Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	-6.47	0.0257
HGF	Hepatocyte growth factor (hepapoietin A; scatter factor)	-2.13	0.0016
HPSE	endo-glucuronidase; heparanase	-1.55	0.0197
ID1	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	-5.47	0.0002
ID3	Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	-1.90	0.0112
IFNB1	Interferon, beta 1, fibroblast	-4.10	0.0228
IL6	Interleukin 6 (interferon, beta 2)	-4.73	0.0412
LECT1	Leukocyte cell derived chemotaxin 1	-5.91	0.0001
LEP	Leptin	-4.20	0.0040
MDK	Midkine (neurite growth-promoting factor 2)	-5.51	0.0001
NOTCH4	Notch homolog 4 (Drosophila)	-2.39	0.0011
PGF	Placental growth factor	-6.83	0.0000
SERPINF1	Serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	-2.46	0.0010
TGFBR1	Transforming growth factor, beta receptor 1	-1.77	0.0114
THBS2	Thrombospondin 2	-3.46	0.0005
TIMP1	TIMP metallopeptidase inhibitor 1	-2.74	0.0000
VEGFA	Vascular endothelial growth factor A	-2.08	0.0033
VEGFC	Vascular endothelial growth factor C	-3.00	0.0034

FR: fold regulation

patients was 62.5 (range 55-70) for the PCa group and 64.81 (range 53-72) for the benign group (BP). The total PSA ranged from 3.4 to 13.9 ng/ml for PCa patients and from 4.1 to 15.9 ng/ml for BP patients. Nine of 12 tumors had extensive or multifocal high-grade prostatic intraepithelial neoplasia (HGPIN). For most samples, the tumor area in the prostatectomy pieces was relatively small (1-15%). Regarding tumor stage, 50% of the tumors (n=6) were T2c and 33.3% (n=4) were T3b (Table 2).

PCR array evaluation

Eighty-four genes were simultaneously evaluated using the Human Angiogenesis RT² Profiler™ PCR Array PAH-024F plates (SABiosciences, a Qiagen Company, Hilden, Germany). The array includes growth factors and their receptors, chemokines and cytokines, matrix and adhesion molecules, proteases and their inhibitors, as well as transcription factors that are involved in the modulation of angiogenesis and tumor development (Table 3).

A total of 300 ng RNA from each sample was used for cDNA synthesis by reverse transcription with RT² First Strand Kit (SABiosciences). The cDNA was subsequently amplified with the ready-to-use RT² SYBR Green qPCR Master Mix (SABiosciences) in a 98-well plate using a LightCycler 480 device (Roche Applied Science, Penzberg, Germany). The reaction condition was as follows: 10 min at 95°C for enzyme activation followed by 45 cycles of 15 s at 95°C and 1 min at 60°C for the amplification step.

PCR array data analysis

Changes in the expression of target genes were measured in relation to the mean cycle threshold (CT) values of five different housekeeping genes (*B2M, HPRT1, RPL13A, GAPDH and ACTB*) by the $\Delta\Delta C_t$ method [22] and *t*-test was used to compare gene expression changes between groups of interest. The genes with fold regulation (FR) threshold of ±1.5 and p value<0.05 were considered differentially expressed between groups.

Results

Eighty-four genes involved in immune system, angiogenesis, and tumor development were simultaneously profiled in the whole blood of the patients with PCa, BPH, CP and without prostate disease. A gene was regarded as being constitutively expressed if it was detected at a CT value of less than 35. The dataset reflected a pattern of constitutive expression for the majority of the genes. The percentage of genes expressed in the training set and the testing set had similar values.

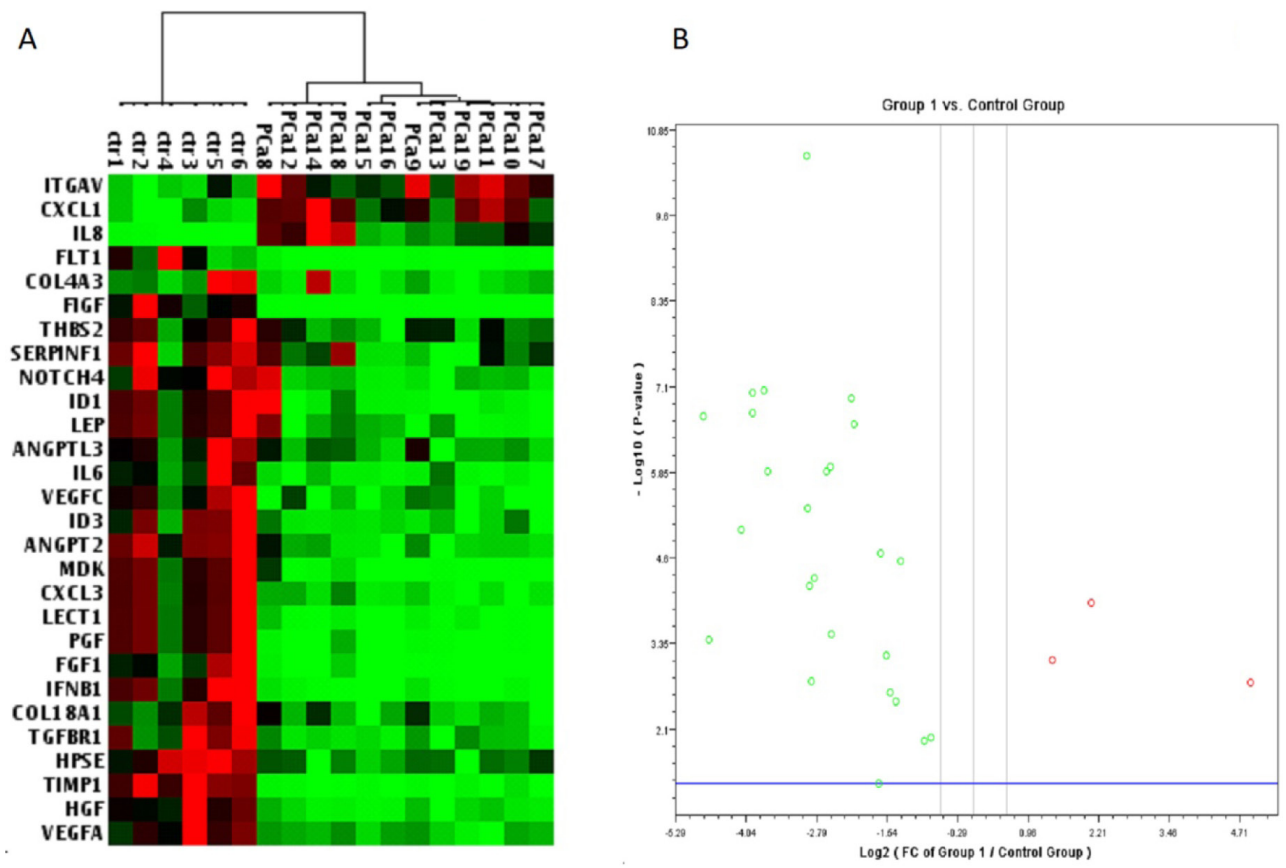

Figure 1. A: Supervised hierarchical clustering of the samples in the first validation step, based on the panel of genes identified on training set. The distances between clusters were calculated using average linkage method. **B:** Volcano Plot for differentially expressed genes in the 12 PCa group versus control.

Out of the 84 genes investigated, we identified a panel of 28 genes differentially expressed between PCa and control group (1.5<FR<-1.5, p-value<0.5). Three genes were significantly up-regulated more than 2.3 times, while the other 25 genes were down-regulated with the range extending from -9.45 to -1.55 (Table 4).

The panel of genes identified using the training set was validated on a new group of 12 PCa samples (Table 2). The supervised hierarchical clustering using average linkage method revealed two broad clusters corresponding to the PCa samples and control group (Figure 1A). All 28 genes used for this classification were also differentially expressed between the new PCa samples and control (1.5<FR<-1.5 and p-value<0.05) (Figure 1B). The FR and p-values are presented in Table 5.

To check if this supervised signature could also discriminate between PCa and benign prostatic forms, we extended the testing/validation group by adding an additional set of 11 benign prostate samples (Table 2). The supervised hierarchical clustering showed a clear separation of the samples resulting in two main clusters, one of them including 12 PCa samples (left cluster) and the other including 17 non-cancer samples (6 controls and 11

Table 5. The FR and p value of the genes in the PCa supervised signature, for training and testing/validation set. All the genes identified in the training set have maintained their statistical significance when were used as discriminator in testing set

Gene symbol	*Training set*		*Testing set*			
	PCa vs Ctr		*PCa vs Ctr*		*PCa vs. Ctr+Benign*	
	FR	*pvalue*	*FR*	*p value*	*FR*	*p value*
Up-regulated						
CXCL1	2.38	0.022	4.24	<0.001	2.72	<0.001
IL8	3.87	0.014	30.04	0.002	16.24	<0.001
ITGAV	2.85	0.001	2.63	0.001	2.14	0.028
Down-regulated						
ANGPT2	-5.57	<0.001	-4.34	<0.001	-4.11	<0.001
ANGPTL3	-7.39	<0.001	-2.60	0.003	-2.46	0.042
COL18A1	-1.73	0.032	-2.79	0.002	-2.59	0.001
COL4A3	-5.10	0.033	-3.23	0.050	-2.97	0.039
CXCL3	-6.39	<0.001	-5.83	<0.001	-5.07	<0.001
FGF1	-5.03	0.014	-17.47	<0.001	-16.59	<0.001
FIGF	-9.45	<0.001	-27.86	<0.001	-22.72	<0.001
FLT1	-6.47	0.026	-26.01	<0.001	-22.61	0.001
HGF	-2.13	0.002	-4.51	<0.001	-2.95	0.000
HPSE	-1.55	0.020	-7.69	<0.001	-5.69	<0.001
ID1	-5.47	<0.001	-7.33	0.002	-6.72	0.002
ID3	-1.90	0.011	-3.14	<0.001	-2.84	<0.001
IFNB1	-4.10	0.023	-15.18	<0.001	-13.66	<0.001
IL6	-4.73	0.041	-7.13	<0.001	-6.84	0.001
LECT1	-5.91	<0.001	-13.22	<0.001	-11.98	<0.001
LEP	-4.20	0.004	-5.76	<0.001	-5.93	0.005
MDK	-5.51	<0.001	-12.63	<0.001	-9.22	<0.001
NOTCH4	-2.39	0.001	-2.93	0.001	-2.23	0.001
PGF	-6.83	<0.001	-15.19	<0.001	-11.29	<0.001
SERPINF1	-2.46	0.001	-1.85	0.012	-1.51	0.029
TGFBR1	-1.77	0.011	-2.46	<0.001	-2.19	0.001
THBS2	-3.46	<0.001	-1.70	0.011	-1.99	0.041
TIMP1	-2.74	<0.001	-7.79	<0.001	-4.40	<0.001
VEGFA	-2.08	0.003	-6.12	<0.001	-5.46	<0.001
VEGFC	-3.00	0.003	-7.51	<0.001	-7.23	0.006

FR: fold regulation

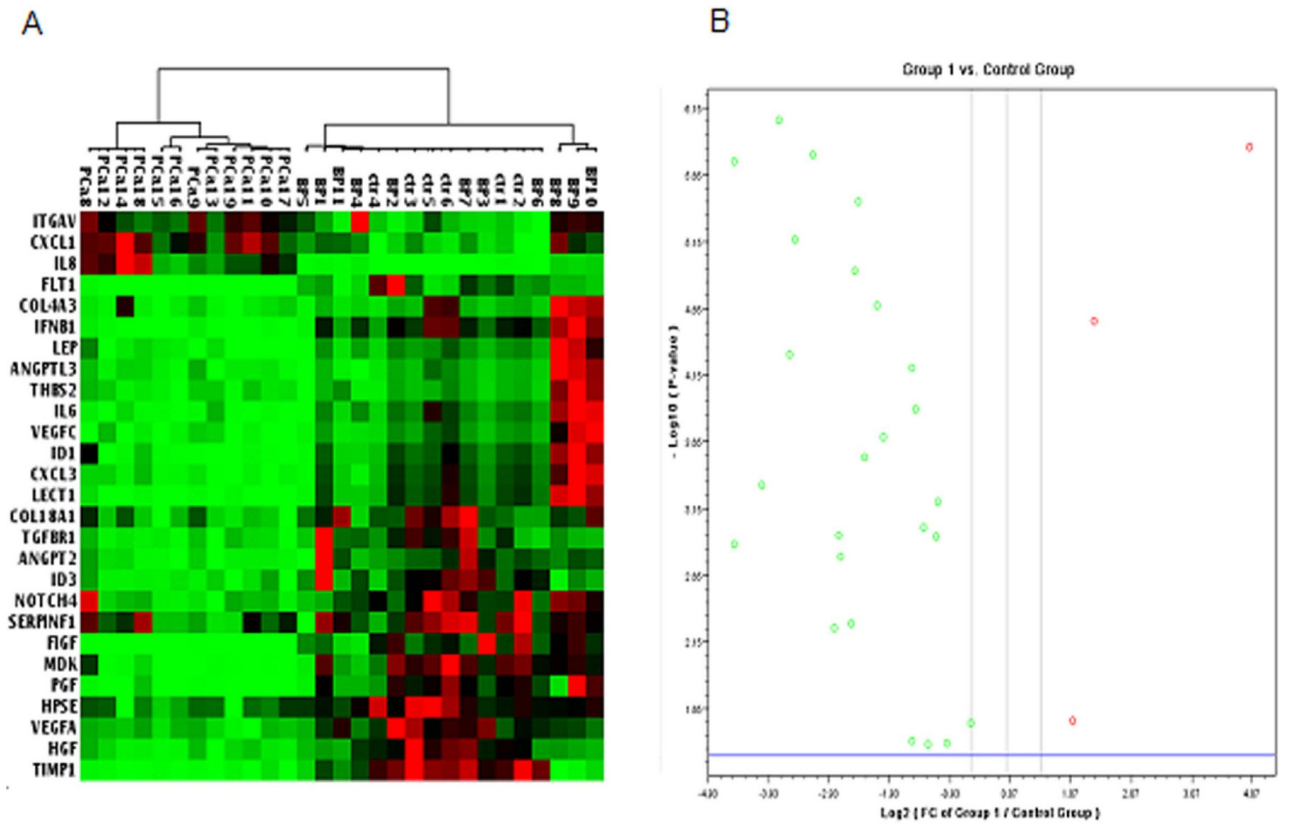

Figure 2. A: Supervised hierarchical clustering of the samples in the extended validation group, based on the panel of genes identified on training set. The distances between clusters were calculated using average linkage method. **B:** Volcano Plot for differentially expressed genes in PCa versus benign and control samples.

benign prostate) (right cluster) (Figure 2A). All 28 genes have maintained differential expression in PCa versus benign and control group (1.5<FR<-1.5 and p-value<0.05) (Figure 2B) (Table 5).

Discussion

Peripheral blood represents an important source for the detection of biomarkers in any pathology, including PCa. Since PSA was associated with the diagnosis of PCa, different approaches to identify new biomarkers have been made. Although new molecules such as mRNA, miRNA or lncRNA were associated with PCa diagnosis and some of them presented higher sensitivity, their singular use did not succeed to increase the specificity of current clinical methods used for diagnosis [23,24]. Lately, gene expression profiling has proved its utility in predicting clinical outcome and treatment response. MammaPrint [25] and OncotypeDX [26] are two examples of such useful tests that highlight the importance of assessing multigene panels for prognosis and prediction of metastasis in breast [27], colon [28] and PCa [29]. Therefore, we took the advantage of PCR array technology to investigate in blood the expression of a panel of genes involved in angiogenesis and tumor progression, which could provide supplementary data for PCa diagnosis, in addition to the currently used evaluations. We found a panel of 28 genes that discriminate the patients with PCa from those with benign prostate pathologies. This molecular signature is independent of parameters such as Gleason score, PSA value, age or extent of tumor (%) within the prostate gland. This signature detected PCa with a fairly low tumor burden of 5% tumor cells, such as PCa 18, PCa 19 and PCa 21 or even cancers having as low as 1% tumor cells, like the case of PCa 25 (Table 2). As we presented above, the genes included in the molecular signature are involved in angiogenesis both directly, by stimulating the endothelial cells proliferation, and indirectly, by modulating the tumor microenvironment, supporting communication between tumor and host via immune response.

In a previous study, we found distinct molecular signatures in the blood of patients with two breast cancer subtypes, showing an enrichment of the canonical pathways and molecules related to innate and adaptive immune response and tumor-related inflammation [30]. Growing tumors communicate with the tissue in which they

develop and also affect the cells of the immune system of the host [31]. The high rate of spontaneously occurring tumors in immunocompromised animals [32] and humans [33] reflects the inhibitory role of the immune system on tumor growth. The tumor-blood communication involves a broad spectrum of signaling molecules, including modulators of angiogenesis and immune system and such an active cellular crosstalk could be reflected in the molecular blood signature of PCa patients.

The results of this study suggest an alteration of immune signaling molecules in the blood of PCa patients compared to healthy subjects and benign prostate pathologies. We observed overexpression of IL8 and CXCL1 chemokines involved in immune response, and cell adhesion molecule ITGAV, while the rest of 23 down-regulated genes are related to many processes, such as modulation of immune and inflammatory response, invasion, angiogenesis, and endothelial cell sprouting and development. Tumors and activated stromal cells secrete pro-inflammatory chemokines and cytokines that stimulate inflammation, angiogenesis and recruitment of leukocytes [34]. Moreover, tumor infiltrating lymphocytes (TILs) and particularly tumor-associated macrophages (TAMs), two important classes of immune cells are involved in tumor immunoediting [35,36]. Previous studies have shown that some chemokines and cytokines, particularly CXCL12, CXCL1, IL6 and IL8, stimulate the growth, survival or invasive behavior of tumor cells, including the prostate epithelium [37,38]. Furthermore, the interaction between proinflammatory and cancer-promoting chemokines such as CXCL1, CXCL2, CXCL3 and IL8, secreted by prostate epithelial cells, might lead to initiation and progression at early stages of PCa [39]. The CXCL1 protein was identified in both benign disease and normal prostate epithelium glands, but significantly overexpressed in prostate cancers [40]. Interleukin-8 is a potent member of the supergene family of CXC chemokines that has angiogenic and angiostatic potency. These angiogenesis-related activities are correlated with tumorigenesis in many tumor types and are distinct by their ability to recruit neutrophils [41]. IL-8 expression was elevated in blood samples of PCa patients, suggesting that IL-8 may play a role in tumor progression by stimulating angiogenesis, which would be in accord with previous results [42].

Macrophages may be the source of many angiogenic factors, such as CCL-2, IL-1β, FGF-2, TGFβ and MMP-9, which are increased in tumors *in vivo* compared to tumor cells *in vitro* [43,44]. Matrix metalloproteinases, particularly MMP-9, are involved in tissue remodeling, facilitating tumor growth, migration, invasion and angiogenesis. Conversely, the majority of MMPs are regulated by a network of proteases and inhibitors of metalloproteinase (TIMPs). Consequently, the proteolytic activity of MMPs is dependent of the TIMPs levels, low levels of TIMPs being associated with increased proteolytic activity of MMPs. The down-regulation of TIMP1 observed in our study has been confirmed by another study, where the loss of TIMP1 immune-expression was correlated with tumor recurrence [45].

Our results show that among the investigated cell adhesion molecules, only ITGAV was up-regulated, whereas COL4A3, ANGPTL3, NRP2, CDH5, and THBS2 were down-regulated. ITGAV encodes the integrin alpha chain V and can interact with extracellular matrix ligands. In the initial stage of prostate tumorigenesis, ITGAV was observed to be up-regulated in prostatic intraepithelial neoplasia (PIN) compared to normal prostate [46]. In the tested samples, almost all of the tumors had extensive or multifocal HGPIN.

VEGF exists as multiple isoforms of two families, the pro-angiogenic family and the anti-angiogenic family which are generated by an alternative splicing event at the terminal exon 8 of the VEGF mRNA. Some of the isoforms of VEGFA and VEGFC can become inhibitors of pro-angiogenic factors, but not of angiogenesis [47]. In our study, we found decreased levels of the mRNAs for both VEGFA and VEGFC isoforms.

The angiopoietins are principal regulators of vascular growth and regression, but in normal prostate and prostate tumors the role of the angiopoietins is unknown. Levels of ANGPT2, an important regulator of angiogenesis, have been correlated with histological grade, vascular density, metastases, and outcome in PCa [48]. However, we found decreased gene expression of ANGPT2 and ANGPTL3 in the blood pf PCa patients. In a previous study, we also confirmed lower levels of serum ANGPT2 and TIMP1 proteins in PCa vs. normal prostate [49].

It has to keep in mind that molecular data from blood is different from those from tissue and an association between blood gene profiling and tumor have to be interpreted in the context of tumor-blood communication, involving a large spectrum of different cells, not just cancer cells.

Conclusions

Our results highlighted a blood gene expression signature which discriminates PCa from normal subjects and benign pathologies of the pros-

tate. This panel of genes could provide new insight in PCa as a non-invasive predictive tool, which in combination with other factors could improve diagnosis of PCa and consequently avoid PCa overdiagnosis and unnecessary biopsy. However, our data have to be confirmed in larger cohorts of patients before having a clear picture about how this molecular profile will help to increase the accuracy of diagnosis.

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Conflict of interests

The authors declare no conflict of interests.

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