Angiogenic systemic response to the hypoxic microenvironment in prostate tumorigenesis: A pilot study

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Abstract. The present paper aimed to investigate the altered angiogenetic mechanisms in hypoxic conditions in patients with prostate tumours, in correlation with common clinico-pathologic variables. A case-control study was developed and included 87 patients with prostate tumours [40 diagnosed with benign prostatic hyperplasia (BPH) and 47 diagnosed with prostate cancer (PCa), using prostate transrectal biopsy] and 40 healthy subjects. The following parameters were evaluated in the serum of volunteers: Hypoxia-inducible factor (HIF)-1α, fibroblast growth factor (FGF)-2, vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-2 and -9, thrombospondin (TSP)-1 and soluble VEGF-1 receptor. Experimental data analysis demonstrated increasing amounts of inflammation in patients with PCa (IL-6, 18.1±4.7 ng/ml) and BPH (IL-6, 16.3±5.1 ng/ml) vs. control (IL-6, 4.1±1.2 ng/ml); overregulation of HIF1α in patients with PCa (129.3±21.8 ng/ml) compared with patients with BPH (65.6±18.2 ng/ml) and control (61.3±12.7 ng/ml); angiogenesis abnormalities in patients with PCa (upregulation of FGF-2, VEGF, MMP-2 and -9, suppression of TSP-1 and soluble VEGR-1) and BPH (upregulation FGF-2 and VEGF) compared with the control group. In conclusion, a greater understanding of the biological mechanism, the pathological roles and the clinical significance of various proangiogenic parameters and angiogenic-suppressor proteins seem useful in clinical practice for establishing an early diagnosis of prostate pathology and finding an individualized therapeutic approach.

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Abbreviations: BPH, benign prostate hyperplasia; PCa, prostate cancer; HIF, hypoxia-inducible factor; FGF-2, fibroblast growth factor; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; TSP-1, thrombospondin; sVEGFR-1, soluble VEGF-1 receptor; PHD, Prolyl hydroxylase domain; FGF, fibroblast growth factor; PDGF, platelet derived growth factor; TIMP, tissue inhibitors of metalloproteinase; CTGF, connective tissue growth factor; EGF, epidermal growth factor; AR, androgen receptor; FIH, hypoxia-inducible factor

Key words: prostate tumours, microenvironment, hypoxia, angiogenesis

Introduction

Over the past decade, the immune microenvironment has been considered a possible element of pathogenicity, especially in solid tumors (1,2). Previous studies have demonstrated that the immune microenvironment in solid malignancies plays an important role in uncontrolled cell proliferation, resistance to apoptosis, stimulation of angiogenesis, suppression of biological host defence mechanisms, metastasis and exacerbation of invasion due to inflammation (1-3), tumour immune surveillance, immunological evasion, cancer therapeutic efficacy and immune checkpoint targeting drug aiming at individualized therapy (4,5). The clinical and experimental results obtained so far suggest that tumour biology is influenced by the bidirectional relationship between genetic/epigenetic changes of tumour cells and the permanent reorganization of the immune microenvironment in solid malignancies (5-7). In solid types of cancer (such as lung cancer and melanoma), knowing the composition of the tumour microenvironment may help in an early diagnosis (2).

The tumor environment is defined as the space around a tumour, consisting of an extracellular matrix, non-tumour cells that are present in the matrix and numerous bioactive molecules. The cellular matrix is described as a three-dimensional, non-cellular network consisting of collagen, fibrillin, proteoglycans, elastin, fibronectin, laminin, hyaluronic acid and a multitude of other specialized macromolecules used for adhesion, recognition and signalling (6-8). The extracellular tumour matrix is permanently being remodelled by the action of matrix metalloproteinases, hypoxia, oxidative stress, expression/activation of inflammasomes and other bioactive molecules present in the tumour microenvironment (such as cytokines, growth factors, chemokines and signal

proteins) (8-10). Bioactive molecules, exogenous biogenesis of exosomes and microRNA are secreted through the cooperation between tumour cells and the constituent cells of the tumour microenvironment (stromal, immune, vascular cells, tumour associated fibroblasts, endothelial or epithelial cells, tumour-associated macrophages, mesenchymal stem cells) (11-13). These events are associated with metabolic reprogramming in cancer cells (14).

The interaction between prostatic epithelial cells and the tumour environment plays an important role in prostatic oncogenesis. Hypoxia is a common feature in the microenvironment of solid tumours. HIF1α modulates the following biological pathways of cancer cells that protect or save cell functions and facilitate cellular adaptation to hypoxia-ischemia: DNA damage response, neo-angiogenesis, tumour growth and angiogenesis, immune evasion, metabolism reprogramming, epithelial mesenchymal transition, tumour microenvironment, the response of therapeutic agent, mitochondrial function, apoptosis and resistance to oxidative stress (6,15,16). Hypoxia-inducible factors (HIFs) are activated by intra-tumour hypoxia. HIF-1α is a protein produced as a cellular response to hypoxia. HIF-1α is frequently upregulated in human prostate cancer (PCa) cells and plays an important role in angiogenesis. HIF1α is stabilized by reactive oxygen species (ROS), which blocks prolyl hydroxylase domain (PHD) and also activates MAPKs. PHD1, PHD2, PHD3 and factor-inhibiting HIF (FIH) are negative regulators of HIF signalling (16,17).

The activation of the tumour hypoxic response and altered levels of nitric oxide, cytokines, metzincins and growth factors can regulate malignant phenotype. Angiogenesis plays an important role in the development, growth and progression of prostate tumours. Various molecules, including vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), matrix metalloproteinases (MMPs), tissue inhibitor of metalloproteinases (TIMPs), thrombospondins (TSPs), soluble VEGF-1 receptors (sVEGFRs), cytokines and chemokines, can induce/suppress angiogenesis and have been extensively studied *in vivo* and *in vitro* (17-19). HIF-1α controls VEGF-A gene transcription and VEGFs/VEGFRs signalling (20,21). The pathological and clinical significance of various soluble proangiogenic parameters and tumour-suppressor proteins remain incompletely characterized in prostate tumorigenesis (22). The MMP signature has an essential role in tumorigenesis and biochemical recurrence in patients with PCa (22). The peptide growth factors, including VEGFs, sVEGFRs, human growth factor, FGF2, connective tissue growth factor (CTGF), PDGF, TNF, transforming growth factor β1, epidermal growth factor, MMPs, TIMPs, TSPs, insulin-like growth factors, IL-1b, angiotensin-2 and thrombin significantly differ among non-tumor cases (healthy), benign and malignant tumours (11,19,23-25).

The present study aimed to investigate molecular responses to hypoxia in prostate tumorigenesis using the dynamic changes in circulating HIF1α and angiogenic/antiangiogenic factors. The analysis of the correlations between the clinicopathological particularities of the studied groups and the molecular imbalances reported in hypoxic conditions may improve the personalized treatment strategies in patients with BPH and PCa.

Materials and methods

Characteristics of included groups. The present study is a case-control study developed for a period of 3 years (January 2018 to December 2020) that includes 87 male patients [40 diagnosed with benign prostatic hyperplasia (BPH) and 47 diagnosed with PCa using prostate transrectal biopsy] and 40 healthy male subjects (Control group). All the patients signed the informed consent, and all the procedures were performed according to the Declaration of Helsinki from 1975. Patients were selected from those who attended the Clinical Hospital of Nephrology 'Carol Davila' (Bucharest, Romania) and Clinical Hospital of Emergency 'St. John' (Bucharest, Romania), and the study protocol was approved by the Ethics Committee of the Clinical Hospital of Emergency 'St. John' (4/4.12.2017; Bucharest, Romania). The study group was homogenous and all the patients were included after signing an informed consent approved by the ethical committee. The data were collected by the study team, who were in contact with every patient for data acquisition and blood sample collection. The subjects with prostate pathology were diagnosed by histological exam and using prostate transrectal biopsy. The patients with prostatic cancer did not present metastasis on thoracic-abdominal-pelvic CT scan nor locally advanced disease. The maximal Gleason score for the included patients was 7 (3+4). All subjects with negative microbiologic examinations were included. The inclusion criteria in the study were also, subjects over 18 years old, with adequate nutritional status. The exclusion criteria were: Patients with signs or symptoms of acute infection; patients who did not receive any treatment for prostatic pathology before the inclusion in the study; patients with any cardiovascular, hepatic, thyroid, gastrointestinal, recent history of viral or bacterial infections, tobacco use, drug abuse, alcoholism or use of vitamin or other antioxidant supplements. The biological samples were collected before the prostate biopsy and the CT scan confirmed the eligibility of patients.

Biological samples and ethics statement. The blood samples were collected from all the study participants after 12 h of fasting using a holder-vacutainer system. Centrifugation of the blood samples was performed at 3,000 x g, for 10 min, after 1 h of keeping the blood at room temperature. The sera were separated and frozen at -80˚C before analysis. The haemolysed, icteric, lactescent or microbiologically contaminated samples were excluded.

Quantitative determinations. Serum levels of HIF1α, VEGF, FGF2, MMP-2, MMP-9, TSP-1 and sVEGFR-1, were identified using enzyme-linked immunosorbent assay (ELISA). Reactive kits were exclusively designated for research studies and do not provide reference values for the selected parameters. Manufacturers specifications for HIF1α were: Quantitative solid phase sandwich immunoassay ELISA kit (E367Hu), analytical sensitivity 0.01 ng/ml, detection range 0.05-15 ng/ml (Shanghai Korain Biotech Co., Ltd.). The concentration of HIF1α in the human serum was determined by comparing the optical density of the samples at 450 nm to the standard curve using a colorimetric microplate reader (Tecan Trading AG). The antibodies were coated onto the microwells and incubated with the serum of the patients. HIF protein was captured by

the coated antibody. After extensive washing, an HIF detection antibody was added to detect the captured HIF protein, afterwards a HRP substrate, 3,3',5,5'-tetramethylbenzidine, to develop the colour.

FGF2 concentration (pg/ml) was determined using the Quantikine solid phase sandwich immunoassay ELISA kit (cat. no. DFB50; R&D Systems, Inc.) with an analytical sensitivity 3 pg/ml and assay range 10-640 pg/ml. The colour intensity which proportional to the quantity of FGF2 was measurable at 450 nm using TECAN analyser.

VEGF concentration (pg/ml) was determined using the human VEGF Quantikine ELISA kit (cat. no. DVE00; R&D Systems, Inc.) that has an analytical sensitivity 9 pg/ml and assay range 15.6-2,000 pg/ml, at 450 nm using a TECAN colorimetric microplate reader analyser.

MMP-2 concentration (ng/ml) was quantified using the RayBio® human MMP-2 ELISA kit (cat. no. ELH-MMP-2; RayBiotech Life, Inc.) that has an analytical sensitivity 3.5 pg/ml and an assay range 3.5-800 ng/ml, using a TECAN colorimetric reader at 450 nm.

MMP-9 concentration (ng/ml) was determined using the human MMP-9 ELISA kit (cat. no. ELH-MMP-9; RayBiotech Life, Inc.) that has an analytical sensitivity 10 pg/ml and assay range 10-6,000 pg/ml using a TECAN detection colorimetric reader at 450 nm.

TSP-1 concentration (ng/ml) was determined using the human TSP-1 Quantikine ELISA kit (cat. no. DTSP10; R&D Systems, Inc.; analytical sensitivity, 0.944 ng/ml; detection range, 7.8-500 ng/ml). The intensity of signal directly proportional to the concentration of TSP-1 was determined by colorimetric detection at 450 nm using a TECAN analyser.

sVEGFR1/FLTI concentration (ng/ml) was quantitatively measured using solid-phase quantitative sandwich ELISA kit (cat. no. CSB-P17498; Cusabio Technology, LLC); analytical sensitivity, 0.039 ng/ml; assay range, 0.156-10 ng/ml) using a TECAN analyser.

Statistical analysis. The data are presented using the mean and standard deviation. SPSS version 27 (IBM Corp.) was used for the statistical analysis. The data between groups were compared using either ANOVA with Tukey post-hoc test or Kruskal-Wallis test with Dunn's post-hoc test for normally and non-normally distributed data, respectively. The relation between the studied markers was assessed by Pearson's correlation coefficient, but not before the assessment of data normality by the Kolmogorov-Smirnov test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Disease activity parameters in the patient groups. The subject's characteristics are presented in Table I. The study groups are homogenous concerning age. As activity disease factors, PSA and free PSA were used as markers of inflammation (especially for IL-6). Statistically significant differences were detected between the BPH, PCa and control groups in prostate volume, PSA, free PSA, IPSS and IL-6 (Table I).

Abnormalities of HIF1α in tumour-genesis. In the PCa group, HIF1α expression was significantly increased by 1.97-fold compared with the BPH group ($P<0.05$) and 2.10-fold compared with the control group ($P<0.001$). HIF1α did not vary significantly when comparing between the BPH and control group ($P>0.05$; Table II).

Molecular regulators of angiogenesis. The study groups presented different variations between pro-angiogenic factors (FGF-2, VEGF, MMP-2 and MMP-9) and anti-angiogenic factors (TSP-1 and sVEGFR-1). In the PCa group, FGF-2 was significantly increased by 1.16-fold compared with BPH ($P<0.05$) and by 4.77-fold compared with the control group ($P<0.05$). FFP-2 increased also significantly, 4.10 folds when compared to BPH and the PCa groups ($P<0.05$). VEGF increased statistically significantly in the PCa group by 2.12-fold compared to BPH ($P<0.05$) and 4.12-fold compared with the control group ($P<0.05$). VEGF increased also significantly, 1.94-fold in the BPH group when compared to the control group ($P<0.05$). MMP-2 was also significantly increased in the PCa group by 1.99-fold compared with BPH ($P<0.05$) and by 2.15-fold compared with the control group ($P<0.05$). MMP-9 was significantly increased in the PCa group by 2.50-fold compared with BPH ($P<0.05$) and by 2.15-fold compared with the control group ($P<0.05$). MMP-2 and MMP-9 did not present statistical differences between BPH and the control group ($P<0.05$).

Regarding the anti-angiogenic factors, significant variations were detected as follows. TSP-1 was significantly decreased in the PCa group by 1.85-fold compared with BPH ($P<0.05$) and by 1.83-fold compared with the control group ($P<0.05$). sVEGFR-1 was significantly decreased in the PCa group by 1.50-fold compared with BPH ($P<0.05$) and by 1.46-fold compared with the control group ($P<0.05$). TSP-1 and sVEGFR-1 did not present statistical differences between BPH and control groups ($P>0.05$; Table III).

Interplays between microenvironment soluble factors and clinicopathological variables. The relationship between prostate hypertrophy/neoplasia microenvironment soluble factors, clinicopathologic variables, angiogenesis and related molecules were investigated. In the PCa group it was observed that there were strong significant correlations between pro-angiogenic factors (HIF1α, FGF-2, VEGF, MMP-2, MMP-9) and IL-6 and medium significant correlations between HIF1α, MMP-2, MMP-9 and PSA. Concerning anti-angiogenic factors in PCa groups it was observed that there were inverse correlations between TSP-1 and sVEGFR-1 compared with IL-6. In the BPH groups, only FGF-2 and VEGF were correlated with IL-6, while FGF-2 was correlated with PV (Table IV).

As observed in Table V, in PCa, HIF1α induces the overexpression of FGF-2, VEGF, MMP-2, MMP-9 and exerts and an inhibitor effect over TSP-1. Whereas in BPH, HIF1α exerts a low positive effect over FGF-2 and VEGF. It could be observed that both in PCa and BPH, FGF-2 had a significant correlation with VEGF, and the strongest correlation was in PCa (Table V).

Discussion

The hypoxic microenvironment constitutes a developing area of investigation in solid tumours. Several studies have

Table I. Clinical and pathological characteristics of the patients.

Parameter	BPH group (A, n=40)	PCa group (B, n=47)	Control group (C, n=40)	p1	p2
Age, years	68.1±11.3	65.1±9.3	65.6±10.2	0.237	
Prostate volume, cm^3	50.3±15.1	39.0±11.6	18.5±2.1	0.032	A vs. B=0.014
					A vs. C: =0.009
					B vs. C: =0.006
PSA, ng/ml	2.5±1.4	11.3±4.9	1.6±0.6	0.024	A vs. B=0.004
					A vs. C: =0.039
					B vs. C: =0.011
Free PSA, ng/ml	0.25±0.05	0.38±0.04	0.13±0.03	0.031	A vs. B=0.026
					A vs. C: =0.021
					B vs. C: =0.004
IPSS	18.3±4.2	17.8±3.8	4.2±1.2	0.042	A vs. B=0.074
					A vs. C: =0.018
					B vs. C: =0.014
IL-6, ng/ml	16.3±5.1	18.1±4.7	4.1±1.2	0.011	A vs. B=0.052
					A vs. C: =0.011
					B vs. C: =0.007

BPH, benign prostate hyperplasia; PCa, prostate cancer; PSA, prostate-specific antigen; IPSS, international prostate symptom score; p, significance level; p1, triple comparison of the groups; p2, pairwise comparison of the groups.

Table II. HIF1α levels in studied groups.

Parameter	BPH group (A; n=40)	PCa group (B; n=47)	Control group (C; n=40)	p1	p2
HIF1α, ng/ml	65.6±18.2	129.3±21.8	61.3±12.7	0.039	A vs. B=0.002
					A vs. C=0.196
					B vs. C: =0.001

BPH, benign prostate hyperplasia; PCa, prostate cancer; HIF, hypoxia-inducible factor; p, significance level; p1, triple comparison of the groups; p2, pairwise comparison of the groups.

identified various soluble factors (such as different interleukins) in the neoplastic microenvironment involved in cancer development and progression (4-6,13,16,17,26-29). The current study explored the contribution of the angiogenic response to hypoxia and inflammation in PCa and BPH biology. Under hypoxic conditions, patients with PCa had an imbalance between soluble antiangiogenic and proangiogenic circulating factors.

In the current study, experimental data analysis showed an exacerbation of inflammation in patients with PCa and BPH vs. the control group. Serum IL-6 levels were elevated in prostate tumours and correlated with HIF1α and proangiogenic/antiangiogenic factors. These results demonstrated that IL-6 was produced by the tumour cells or the tumour microenvironment. IL-6, derived from cancer-associated fibroblasts, plays an important role in regulating tumour growth and progression of PCa cells, modulation of p53 turn-over, induction of epithelial to mesenchymal transition, cell survival, modulation of chemoresistance, neuroendocrine differentiation in the prostate, androgen receptor activation and androgen synthesis (30). In prostate tumours, IL-6 modulates multiple signalling pathways, including JAK/STAT, ERK1/2/MAPK and PI3K (25,30,31).

In the present study, HIF-1α was upregulated in the human PCa compared with BPH and the control group. Notably, there was a moderate correlation between circulating levels of HIF1α vs. PSA and free PSA concentrations in patients with PCa. Based on these results, overexpression of HIF1α was hypothesised to be an early event in prostate carcinogenesis. In patients with BPH, periodic measurement of HIF1α may help to identify patients with BPH early according to the risk of developing complications. The increase in HIF1α is consistent with overexpression of angiogenesis in prostate pathology. HIF1α is involved in the adaptation to insufficient oxygen or hypoxia, expression of androgen receptor (AR), vascularization, tumour angiogenesis, the pathophysiology of ischemic conditions and resistance to androgen/AR-targeted therapy. HIF1α interacts with the AR on the PSA gene promoter (6,8,9,32,33). In inflammatory conditions, hypoxia/HIF1α signalling may play a possible role in the neoplastic transformation of benign

Table III. Angiogenesis-related factors in studied groups.

Parameter	BPH group (A; n=40)	PCa group (B; n=47)	Control group (C; n=40)	p1	p2
FGF-2, pg/ml	27.9±6.1	32.5±5.4	6.8±3.7	0.017	A vs. B=0.028 A vs. C: =0.005 B vs. C: =0.001
VEGF, pg/ml	237.35±39.1	503.9±129.2	122.3±21.2	0.029	A vs. B=0.005 A vs. C: =0.018 B vs. C: =0.001
MMP-2, ng/ml	502.2±91.7	1002.5±207.4	465.3±78.4	0.040	A vs. B: =0.004 A vs. C=0.054 B vs. C: =0.001
MMP-9, ng/ml	178.3±33.1	409.1±103.5	163.3±29.5	0.026	A vs. B=0.002 A vs. C: =0.181 B vs. C: =0.007
TSP-1, ng/ml	1112.8±122.3	600.4±137.4	1100.3±100.6	0.027	A vs. B=0.0029 A vs. C=0.453 B vs. C=0.001
sVEGFR-1, ng/ml	15.2±3.3	10.1±4.1	14.8±2.1	0.006	A vs. B=0.001 A vs. C=0.202 B vs. C=0.002

BPH, benign prostate hyperplasia; PCa, prostate cancer; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; TSP, thrombospondin; sVEGFR, soluble vascular endothelial growth factor receptor; p, significance level; p1, triple comparison of the groups; p2, pairwise comparison of the groups.

prostate cells. This remark could be supported by the immunohistochemical observations, according to which HIF is activated by intratumoral hypoxia, and is modulated by ROS, PHD and FIH (16). HIF1α, PHD1, PHD2, PHD3 and FIH expression levels in PCa are not significantly correlated with PSA level, tumour stage, Gleason score, presence of positive lymph nodes or recurrence (16). The HIF1α gene is upregulated in localized PCa, but not in metastatic, hyperplastic and non-tumoral adjacent tissue (16). The increased concentration of HIF1α is associated with angiogenic factors and aggressive cell phenotype progression of patients suffering from PCa. In hypoxic conditions, HIF1α can promote the secretion of IL-6, VEGFs/sVEGFRs and CTGF in cancer-associated fibroblasts (20,21,33).

The present data showed that, in hypoxic and inflammatory conditions, patients with PCa had an imbalance between circulant proangiogenic and antiangiogenic soluble factors. BPH was characterized by non-variation values of HIF1α, but moderately increased values only for VEGF and FGF2 compared with normal subjects. In hypoxic conditions, PCa was characterized by an overregulation of stimulators of angiogenesis (VEGF, FGF2, MMP-2 and MMP-9) and downregulation of suppressors of angiogenesis (TSP-1) compared with patients with BPH and healthy subjects. The evolution of these factors was correlated with the prostate volume, PSA and free PSA in patients with PCa. A clinicopathological significance of immunohistochemical relations between cellular response to hypoxia quantified by HIF-1α immunoreactions, and micro-vessel density calculated by CD34 immunostaining was observed in a previous study (17). The angiogenetic profile evaluated by VEGF cytoplasmic immunoreactivity was examined in hyperplastic and malignant prostate tissue. The interrelationship between CD34 and HIF-1α, VEGF and HIF-1α and VEGF and CD34 were stronger in PCa compared with in BPH and were also significantly associated with high-grade carcinomas (17).

On the other hand, several studies report that alteration of VEGF/VEGFR and FGF/FGFR are associated with PCa development and progression via Ras, Src, MAPK, PKC, PI3K-AKT, and STAT (34,35). FGF2 is a potent mediator of angiogenesis and is upregulated in response to inflammatory stimuli and prostate tumours; is modulated by heparin, integrin α5β3, soluble FGFR1, FGF-BP, free gangliosides, TSP-1, pentraxin 3/TSG-14, fibrinogen, α2 macroglobulin, PDGF and CXCL4/PF4 (34,35). The interaction of these molecules and co-receptor or adhesion partners is required for the binding and activation of FGF receptors (34,35). VEGF is a growth factor of both angiogenesis and vasculogenesis and its synthesis is induced by hypoxia and cytokines such as IL-1, IL-6, IL-8, oncostatin, M and TNF-α (34). The role of IL-6 in the inflammatory microenvironment and prostatic tumorigenesis has also been underlined in a previous study by Ene *et al* (36). The interaction of VEGF with VEGFR1 and VEGFR2 promotes tumour angiogenic activity and circulating VEGF levels are correlated with disease activity in PCa (34,35).

In the present study, evaluating the prospective associations from the profiles between the angiogenic/antiangiogenic factors and clinical-biological status of the patients with prostatic diseases in initial development phases may offer another perspective over tumour mechanisms and become an efficient

Table IV. Correlations analysis between angiogenesis-related molecules and disease characteristics.

A, PCa (47 cases)

Parameters	IL-6	PV	PSA	Free PSA	IPSS
HIF1α	r=0.59[a]	NS	r=0.49[b]	r=0.16[c]	NS
FGF-2	r=0.57[a]	r=0.28[b]	NS	r=0.10[c]	NS
VEGF	r=0.63[a]	NS	NS	NS	r=0.19[c]
MMP-2	r=0.31[a]	NS	r=0.21[b]	NS	NS
MMP-9	r=0.68[a]	NS	r=0.15[b]	NS	NS
TSP-1	r=-0.22[b]	NS	r=-0.19[c]	NS	NS
sVEGFR-1	r=-0.18[a]	NS	NS	NS	NS

B, BPH (40 cases)

Parameters	IL-6	PV	PSA	Free PSA	IPSS
HIF1α	NS	NS	NS	NS	NS
FGF-2	r=0.41[b]	r=0.10[b]	NS	NS	NS
VEGF	r=0.32[b]	NS	NS	NS	NS
MMP-2	NS	NS	NS	NS	NS
MMP-9	r=0.12[c]	NS	NS	NS	NS
TSP-1	NS	NS	NS	NS	NS
VEGFR-1	NS	NS	NS	NS	NS

[a]P<0.01; [b]P<0.05; [c]P=0.05. BPH, benign prostate hyperplasia; PCa, prostate cancer; IL, interleukin; PV, prostate volume; PSA, prostate-specific antigen; IPSS, international prostate symptom score; HIF, hypoxia inducible factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; TSP, thrombospondin; sVEGFR, soluble vascular endothelial growth factor receptor; NS, insignificant; r, correlation coefficient; P, significance level.

instrument for patient monitoring and management. Another study showed that angiogenesis plays an important role in PCa pathogenesis, but the outcomes of the antiangiogenic therapy are not very promising. The possible explanations of the low efficiency of antiangiogenic therapy in PCa might be due to the redundance of the alternative angiogenic ways; molecular heterogeneity of the prostatic tumoral environment; inactivation of the suppressive genes of tumour; or genetic and epigenetic variability of pro and anti-angiogenic factors (36-38).

The present study indicated that MMPs-2 and -9 were produced in inflammation or hypoxic conditions in patients with PCa. MMPs behaved as an angio-modulator in PCa, playing a decisive role in regulating pro- and anti-angiogenic factors. Angiogenesis controlled by the MMPs/TIMPs axis is associated with the malignant progression of PCa (39). MMP-2 and -9 are negatively regulated by the androgen pathway (40,41). MMP-2 is involved in vessel remodelling, angiogenesis, tissue repair, tumour invasion, inflammation and extracellular matrix degradation. The C-terminal non-catalytic fragment of MMP-2, PEX, has anti-angiogenic and anti-tumour properties and inhibits cell migration and cell adhesion to FGF-2 and vitronectin. PEX is secreted by fibroblasts and it is produced by prostate tumours. Autocatalytic cleavage of PEX is facilitated by binding integrin 5/β3 (39-41). MMP-9 is secreted in the extracellular space and extracellular matrix; it exerts a physiological and pathological angiogenic and remodelling effect on the vasculature. Its circulating levels are increased in inflammatory disorders (39,40). Biomedical evidence indicates serum MMPs as potential biomarkers for the diagnosis or prognosis of prostatic malignancies (42). MMP-2 in combination with PSA can increase the sensitivity for the diagnosis and monitoring of PCa. Serum levels of MMP-9 can indicate the presence of malignancy and metastases (42).

Additionally, negative regulatory molecules TSP-1 and sVEGFR-1 strongly influence angiogenesis. In patients with PCa, a reciprocal inhibitory effect between TSP-1/HIF1α, TSP-1/FGF-2 and sVEGFR-1/VEGF was observed. These results suggest that decreased circulatory TSP-1 and sVEGFR-1 levels due to hypoxia could contribute to angiogenesis. TSP-1 and sVEGFR-1 could inhibit angiogenesis in prostate neoplasia. Notably, there was a lack of correlation between HIF1α and sVEGFR-1, which meant that serum variations of sVEGFR-1 were independent of HIF1α signalling in patients with PCa. Experimental studies have not demonstrated whether the interaction between hypoxia and the molecular mechanisms that generate soluble forms of VEGFR1 (alternative splicing of mRNA, proteolytic cleavage) are coordinated by HIF1α. Some reports suggest that sVEGFR-1 is released from macrophage/monocytes after exposure to GM-CSF, via HIF-2α. Other data indicate the origin of sFtl1-14 in activation of the growth arrest and DNA damage-inducible Gadd45a factor

Table V. Correlation analysis between angiogenesis-related molecules.

A, PCa (47 cases)

Parameters	FGF-2	VEGF	MMP-2	MMP-9	sVEGFR-1	TSP-1
HIF1α	r=0.59[a]	r=0.63[a]	r=0.48[a]	r=0.51[a]	NS	r=-0.64[a]
FGF-2	-	r=0.57[a]	NS	NS	NS	r=-0.39[a]
VEGF	-	-	NS	r=0.17[b]	r=-0.29[b]	NS
MMP-2	-	-	-	NS	NS	NS
MMP-9	-	-	-	-	NS	r=-0.23[b]
sVEGFR-1	-	-	-	-	-	NS

B, BPH (40 cases)

Parameters	FGF-2	VEGF	MMP-2	MMP-9	sVEGFR-1	TSP-1
HIF1α	r=0.19[a]	r=0.21[a]	NS	NS	NS	NS
FGF-2	-	r=0.12[c]	NS	NS	NS	NS
VEGF	-	-	NS	NS	r=0.10[c]	NS
MMP-2	-	-	-	NS	NS	NS
MMP-9	-	-	-	-	NS	NS
sVEGFR-1	-	-	-	-	-	NS

[a]P<0.01; [b]P<0.05; [c]P=0.05. BPH, benign prostate hyperplasia; PCa, prostate cancer; HIF, hypoxia inducible factor; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; TSP, thrombospondin; sVEGFR, soluble vascular endothelial growth factor receptor; NS, insignifiant; r, correlation coefficient; P, significance level.

and p38 phosphorylation (19,43,44). Also, the influence of a few cytokines secreted in the microenvironment (IL-4, IL-6, GM-CSF) and the effect of cyclooxygenase-1, hydroxylases and demethylases, glycosyltransferases/sialidases, heparinases, protein kinases C, focal adhesion kinase or of HSP27, GM3 gangliosides, the presence of some integrins type α5β3 and α5β1 over the secretion and of stability of HIF1α have been presented in the research literature (19,21,43). Other data concludes that sVEGFR-1 can function as an inhibitor in the autocrine regulation of angiogenesis by competing with VEGF and PLGF. Hypoxia regulates sVEGFR-1/sFlt-1mRNA levels by cis-elements involved in mRNA alternative processes (44). sVEGFR-1 contributes to cancer pathogenesis (cell survival, angiogenesis, proliferation, extracellular matrix invasion) by regulating inflammatory responses and recruitment of tumour-infiltrating macrophages by the VEGF/Flt-1 and/or VEGF/KDR signalling via MAPK1/ERK2, MAPK3/ERK1 and MAPK/AKT1 pathways (15,19). VEGFR-1 supports cancer immune escape and stimulates the release of immunosuppressive cytokines (20). sVEGFR-1, which derives from alternative splicing of the VEGFR-1, interacts with VEGF-A, VEGF-B, placenta growth factor and VEGFR-2 to block the activity of these ligands (20). Extracellular granzyme K induces *de novo* synthesis and release of sVEGFR-1 protein and mRNA levels from endothelial cells independently of PAR-1, sequestering VEGF and inhibiting VEGFR signalling and angiogenesis (19,45).

The biological effects of TSP-1 in the microenvironment have been previously studied. TSP-1 is a potent mediator that modulates cell-to-cell and cell-matrix adhesion, proliferation, migration and angiogenesis (46). TSP-1 mediates interactions between cells and the extracellular matrix. Recently, it has been reported that two serum proteins, TSP-1 and cathepsin D, can improve the diagnosis of high-grade PCa (46). In the tumour microenvironment TSP-1 regulates metabolic response to ischemic and genotoxic stress in several types of cancer (melanoma or breast carcinoma) (47).

According to the present results, modulatory mechanisms of HIF1α, cytokines, growth factors, MMPs and matricellular proteins might be involved in the molecular angiogenic response of the microenvironment in prostate tumorigenesis. Inflammation, immunity and complex interactions between tumour cells and the microenvironment remain to be elucidated.

To the best of our knowledge, the present study was the first to evaluate angiogenetic mechanisms in hypoxic conditions in patients with prostate tumours, correlated with common clinicopathologic variables (48). However, some limitations should be noted. In the evaluation of the prospective associations between the profiles of angiogenic/antiangiogenic factors and clinical-biological status of the patients with prostatic affections in initial phase, the immune-enzymatic techniques for detection of serum metabolites were used for the following reasons: i) The availability and the compliance of the patients in collection of biological samples; and ii) the use of fast procedures of collecting and processing the samples, with anterior optimization of the analysis methods and with the fast implementation of the outcomes in the clinical context. The

present study only followed patients with early PCa for a short period. Also, the number of patients was relatively reduced and randomization was not performed. For an improved understanding of physiopathological angiogenic/antiangiogenic processes in the tumour microenvironment in PCa, follow-up of the patients should be longer and more data according to cancer progression should be collected. Moreover, all the studied factors should be evaluated in advanced and metastatic neoplastic diseases.

In conclusion, the complex interactions between tumour cells and the hypoxic microenvironment could alter the angiogenesis in prostate tumours. The BPH was characterized in the present study by non-variation values of HIF1α, and moderately high values for VEGF and FGF2; while PCa was characterized by overregulation of endogenous positive regulatory molecules of angiogenesis (VEGF, FGF2, MMP-2, MMP-9) and downregulation of suppressors of angiogenesis (TSP-1, sVEGFR-1). The assessment of circulating HIF1α and angiogenic stimulators/inhibitors could contribute to the differential diagnosis between patients with BPH and PCa. The present data suggested that the overproduction of HIF1α was an early event in prostate carcinogenesis. Modulatory mechanisms of HIF1α, cytokines, growth factors, matrix metalloproteinases and matricellular antiangiogenic proteins could be involved in the development of prostate malignancies.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

All the authors had equal contributions. IN was responsible for the acquisition of data, CE was responsible for the analysis and interpretation of data, and CDE was responsible for study conception and design. CE, IN and CDE confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All the patients signed the informed consent, and all the procedures were performed according to the Declaration of Helsinki from 1975. Patients were selected from those who attended the Clinical Hospital of Nephrology 'Carol Davila' and Clinical Hospital of Emergency 'St. John', and the study protocol was approved by the Ethics Committee of the Clinical Hospital of Emergency 'St. John' (approval no. 4/4.12.2017).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Hanahan D and Coussens ML: Accessories to the crime: Functions of cells recruited to the tumor microenvironment. Cancer Cell 21: 309-322, 2012.
2. Nisticò P and Ciliberto G: Biological mechanisms linked to inflammation in cancer: Discovery of tumor microenvironment-related biomarkers and their clinical application in solid tumors. Int J Biol Markers 35 (Suppl 1): S8-S11, 2020.
3. Sfanos KS, Yegnasubramanian S, Nelson WG and De Marzo AM: The inflammatory microenvironment and microbiome in prostate cancer development. Nat Rev Urol 15: 11-24, 2018.
4. Tang T, Huang X, Gang Z, Zhengtao H, Xueli B and Tingbo L: Advantages of targeting the tumor immune microenvironment over blocking immune checkpoint in cancer immunotherapy. Signal Transduct Target Ther 6: 72, 2021.
5. Baghban R, Roshangar L, Jahanban-Esfahlan R, Seidi K, Ebrahimi-Kalan A, Jaymand M, Kolahian S, Javaheri T and Zare P: Tumor microenvironment complexity and therapeutic implications at a glance. Cell Commun Signal 18: 59, 2020.
6. Bahmad HF, Jalloul M, Azar J, Moubarak MM, Samad TA, Mukherji D, Al-Sayegh M and Abou-Kheir W: Tumor microenvironment in prostate cancer: Toward identification of novel molecular biomarkers for diagnosis, prognosis, and therapy development. Front Genet 12: 652747, 2021.
7. Archer M, Dogra N and Kyprianou N: Inflammation as a driver of prostate cancer metastasis and therapeutic resistance. Cancers (Basel) 12: 2984, 2020.
8. Barthes J, Özçelik H, Hindié M, Ndreu-Halili A, Hasan A and Vrana NE: Cell microenvironment engineering and monitoring for tissue engineering and regenerative medicine: The recent advances. Biomed Res Int 2014: 921905, 2014.
9. Emami Nejad A, Najafgholian S, Rostami A, Sistani A, Shojaeifar S, Esparvarinha M, Nedaeinia R, Haghjooy Javanmard S, Taherian M, Ahmadlou M, *et al*: The role of hypoxia in the tumor microenvironment and development of cancer stem cell: A novel approach to developing treatment. Cancer Cell Int 21: 62, 2021.
10. Jang JH, Kim DH and Surh YJ: Dynamic roles of inflammasomes in inflammatory tumor microenvironment. NPJ Precis Oncol 5: 18, 2021.
11. Yang E, Wang X, Gong Z, Yu M, Wu H and Zhang D: Exosome-mediated metabolic reprogramming: The emerging role in tumor microenvironment remodeling and its influence on cancer progression. Signal Transduct Target Ther 5: 242, 2020.
12. Riemann A, Reime S and Thews O: Acidic extracellular environment affects miRNA expression in tumors in vitro and in vivo. Int J Cancer 144: 1609-1618, 2019.
13. Ene CD, Penescu MN, Georgescu SR, Tampa M and Nicolae I: Posttranslational modifications pattern in clear cell renal cell carcinoma. Metabolites 11: 10, 2020.
14. Nicolae I, Nicolae CD, Schipor S and Caragheorgheopol A: Influence of iron deficiency on angiogenesis in melanoma patients. Endocr Abstr 32: P547, 2013.
15. Schipor S, Caragheorgheopol A, Nicolae CD, Nicolae I and Paun D: Serum levels of VEGF in cutaneous melanoma. Clin Chem Lab Med 49 (Suppl): S1-S874, 2011.
16. Pavlakis D, Kampantais S, Gkagkalidis K, Gourvas V, Memmos D, Tsionga A, Dimitriadis G and Vakalopoulos I: Hypoxia-inducible factor 2a expression is positively correlated with gleason score in prostate cancer. Technol Cancer Res Treat 20: 1533033821990010, 2021.
17. Lekas A, Lazaris AC, Deliveliotis C, Chrisofos M, Zoubouli C, Lapas D, Papathomas T, Fokitis I and Nakopoulou L: The expression of hypoxia-inducible factor-1alpha (HIF-1alpha) and angiogenesis markers in hyperplastic and malignant prostate tissue. Anticancer Res 26: 2989-2993, 2006.
18. Binder MJ and Ward AC: The role of the metzincin superfamily in prostate cancer progression: A systematic-like review. Int J Mol Sci 22: 3608, 2021.

19. Li S, van Dijk CGM, Meeldijk J, Kok HM, Blommestein I, Verbakel ALF, Kotte M, Broekhuizen R, Laclé MM, Goldschmeding R, *et al*: Extracellular granzyme K modulates angiogenesis by regulating soluble VEGFR1 release from endothelial cells. Front Oncol 11: 681967, 2021.
20. Ceci C, Atzori MG, Lacal PM and Graziani G: Role of VEGFs/VEGFR-1 signaling and its inhibition in modulating tumor invasion: Experimental evidence in different metastatic cancer models. Int J Mol Sci 21: 1388, 2020.
21. Failla CM, Carbo M and Morea V: Positive and negative regulation of angiogenesis by soluble vascular endothelial growth factor receptor-1. Int J Mol Sci 19: 1306, 2018.
22. Geng JH, Lin VC, Yu CC, Huang CY, Yin HL, Chang TY, Lu TL, Huang SP and Bao BY: Inherited variants in Wnt pathway genes influence outcomes of prostate cancer patients receiving androgen deprivation therapy. Int J Mol Sci 17: 1970, 2016.
23. Soulitzis N, Karyotis I, Delakas D and Spandidos DA: Expression analysis of peptide growth factors VEGF, FGF2, TGFB1, EGF and IGF1 in prostate cancer and benign prostatic hyperplasia. Int J Oncol 29: 305-314, 2006.
24. Ene CD, Nicolae I, Ene C, Tampa M, Matei C and Georgescu SR: Effect of tobacco alkaloids on the endocrine system. Rev Chim 66: 628-633, 2015.
25. Matsuoka Y, Nakayama H, Yoshida R, Hirosue A, Nagata M, Tanaka T, Kawahara K, Sakata J, Arita H, Nakashima H, *et al*: IL-6 controls resistance to radiation by suppressing oxidative stress via the Nrf2-antioxidant pathway in oral squamous cell carcinoma. Br J Cancer 115: 1234-1244, 2016.
26. Ene CD, Tampa M, Nicolae I, Mitran CI, Mitran MI, Matei C, Caruntu A, Caruntu C and Georgescu SR: Antiganglioside antibodies and inflammatory response in cutaneous melanoma. J Immunol Res 2020: 2491265, 2020.
27. Anghel AE, Ene CD, Nicolae I, Budu VA, Constantin C and Neagu M: Interleukin 8-major player in cutaneous melanoma metastasic process. Rom Biotechnol Lett 20: 10911-10920, 2015.
28. Ene CD and Nicolae I: Gangliosides and antigangliosides in malignant melanoma. Melanoma-chapter 14, 361-401. Current Clinical Management and Future Therapeutics; Murph M (ed). InTech: London, UK, 2015.
29. Ene CD, Anghel AE, Neagu M and Nicolae I: 25-OH vitamin D and interleukin-8: Emerging biomarkers in cutaneous melanoma development and progression. Mediators Inflamm 2015: 904876, 2015.
30. Cheteh EH, Sarne V, Ceder S, Bianchi J, Augsten M, Rundqvist H, Egevad L, Östman A and Wiman KG: Interleukin-6 derived from cancer-associated fibroblasts attenuates the p53 response to doxorubicin in prostate cancer cells. Cell Death Discov 6: 42, 2020.
31. Culig Z: Response to androgens and androgen receptor antagonists in the presence of cytokines in prostate cancer. Cancers (Basel) 13: 2944, 2021.
32. Tran MGB, Bibby BAS, Yang L, Lo F, Warren AY, Shukla D, Osborne M, Hadfield J, Carroll T, Stark R, *et al*: Independence of HIF1a and androgen signaling pathways in prostate cancer. BMC Cancer 20: 469, 2020.
33. Huang M, Du H, Zhang L, Che H and Liang C: The association of HIF-1α expression with clinicopathological significance in prostate cancer: A meta-analysis. Cancer Manag Res 10: 2809-2816, 2018.
34. Teishima J, Hayashi T, Nagamatsu H, Shoji K, Shikuma H, Yamanaka R, Sekino Y, Goto K, Inoue S and Matsubara A: Fibroblast growth factor family in the progression of prostate cancer. J Clin Med 8: 183, 2019.
35. Rivera-Pérez J, Monter-Vera MDR, Barrientos-Alvarado C, Toscano-Garibay JD, Cuesta-Mejías T and Flores-Estrada J: Evaluation of VEGF and PEDF in prostate cancer: A preliminary study in serum and biopsies. Oncol Lett 15: 1072-1078, 2018.
36. Ene CV, Nicolae I, Geavlete B, Geavlete P and Ene CD: IL-6 signaling link between inflammatory tumor microenvironment and prostatic tumorigenesis. Anal Cell Pathol (Amst) 2022: 5980387, 2022.
37. Melegh Z and Oltean S: Targeting angiogenesis in prostate cancer. Int J Mol Sci 20: 2676, 2019.
38. Ioannidou E, Moschetta M, Shah S, Parker JS, Ozturk MA, Pappas-Gogos G, Sheriff M, Rassy E and Boussios S: Angiogenesis and anti-angiogenic treatment in prostate cancer: mechanisms of action and molecular targets. Int J Mol Sci 22: 9926, 2021.
39. Franko A, Berti L, Hennenlotter J, Rausch S, Scharpf MO, de Angelis MH, Stenzl A, Peter A, Birkenfeld AL, Lutz SZ, *et al*: Increased expressions of matrix metalloproteinases (MMPs) in prostate cancer tissues of men with type 2 diabetes. Biomedicines 8: 507, 2020.
40. Gong Y, Chippada-Venkata UD and Oh WK: Roles of matrix metalloproteinases and their natural inhibitors in prostate cancer progression. Cancers (Basel) 6: 1298-1327, 2014.
41. Dinu L, Ene CD, Nicolae I, Tampa M, Matei C and Georgescu SR: Serum levels of 8-hydroxy-deoxyguanosine under the chemicals influence. Rev Chim 65: 1319-1326, 2014.
42. Quintero-Fabián S, Arreola R, Becerril-Villanueva E, Torres-Romero JC, Arana-Argáez V, Lara-Riegos J, Ramírez-Camacho MA and Alvarez-Sánchez ME: Role of matrix metalloproteinases in angiogenesis and cancer. Front Oncol 9: 1370, 2019.
43. Nicolae CD, Coman OA, Ene C, Nicolae I and Fulga I: Hepcidin in neoplastic disease. J Med Life 69: 365-360, 2013.
44. Ikeda T, Sun L, Tsuruoka N, Ishigaki Y, Yoshitomi Y, Yoshitake Y and Yonekura H: Hypoxia down-regulates sFlt-1 (sVEGFR-1) expression in human microvascular endothelial cells by a mechanism involving mRNA alternative processing. Biochem J 436: 399-407, 2011.
45. Persu C, Braschi E and Lavelle J: A review of prospective clinical trials for neurogenic bladder: Pharmaceuticals. Cent European J Urol 67: 264-269, 2014.
46. Macagno A, Athanasiou A, Wittig A, Huber R, Weber S, Keller T, Rhiel M, Golding B and Schiess R: Analytical performance of thrombospondin-1 and cathepsin D immunoassays part of a novel CE-IVD marked test as an aid in the diagnosis of prostate cancer. PLoS One 15: e0233442, 2020.
47. Kaur S, Bronson SM, Pal-Nath D, Miller TW, Soto-Pantoja DR and Roberts DD: Functions of thrombospondin-1 in the tumor microenvironment. Int J Mol Sci 22: 4570, 2021.
48. Vacaroiu IA, Cuiban E, Geavlete BG, Gheorghita V, David C, Ene CE, Bulai C, Lupusoru GE, Lupusoru M, Balcangiu-Stroescu AE, *et al*: Chronic kidney disease-an underestimated risk factor for antimicrobial resistance in patients with urinary tract infections. Biomedicines 10: 2368, 2022.