Rom J Morphol Embryol 2020, 61(3):803–811 ISSN (print) 1220–0522, ISSN (online) 2066–8279 doi: 10.47162/RJME.61.3.18

ORIGINAL PAPER

RJME Romanian Journal of Morphology & Embryology http://www.rjme.ro/

The role of androgen receptors in vascular and cell proliferation of the prostate adenocarcinomas

ANDREI PĂNUŞ[1)], CLAUDIU MĂRGĂRITESCU[2)], PETRU OCTAVIAN DRĂGOESCU[3)], PAUL IOAN TOMESCU[3)], MIHAI LUCIAN ŞTEFĂNESCU[3)], ALEX EMILIAN STEPAN[2)]

[1)]*PhD Student, Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania*

[2)]*Department of Pathology, University of Medicine and Pharmacy of Craiova, Romania*

[3)]*Department of Urology, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

Prostate adenocarcinoma (PA) is by incidence and prognosis a unique model for investigating the biomolecular mechanisms involved in tumor progression. In this study, we analyzed the immunoexpression of androgen receptor (AR), cluster of differentiation 105 (CD105) and Ki67 for 61 cases of PA, in relation to the main clinicopathological parameters of the lesions. The AR scores, CD105 microvessel density (MVD) and Ki67 proliferation index (PI) were significantly higher in patients with serum prostate-specific antigen (PSA) above 20 ng/mL, in ductal, colloid and sarcomatoid types of PA, in growth patterns 4–5 or mixed, respectively in the case of high-grade advanced stage tumors, with perineural and vascular invasion, as well as in groups with a reserved prognosis. The results obtained, reflected in the positive linear correlation of AR, CD105 and Ki67 expression, indicate synchronous endocrine, angiogenic and proliferative mechanisms involved in tumor progression, which can be used to optimize the targeted tumor therapy.

Keywords: androgen receptor, CD105, Ki67, prostate adenocarcinoma.

Introduction

Prostate adenocarcinoma (PA) ranks second among malignant neoplasms worldwide, most cases being diagnosed after 70 years age [1–3]. The mortality rate of PA is relatively low compared to other malignancies, and the access to screening programs increases the ability to diagnose the lesions in early stages and thus the life expectancy of patients [2–4].

However, the rate of PA-associated morbidity greatly influences patients' quality of life and the biological behavior of tumors is relatively difficult to be assessed, given that aggressive, metastatic, and hormone-independent PAs place these lesions on the fifth place as the cause of death in men [2, 3, 5, 6]. In this context, the study of the biomolecular mechanisms involved in the initiation and progression of PA is a permanent concern.

The hormone dependence of PA, the incidence and prognosis of lesions designate these tumors as a unique model for carcinogenesis investigation. In addition to the central role of androgens in the development of PA, the involvement of androgen receptors (ARs) in the growth, invasion and metastasis of the tumors was the subject of many studies, with heterogeneous results mainly due to the different investigation methods [6].

At the same time, there are relatively few recent studies that have addressed to AR expression in endothelial cells and the involvement of these receptors in prostate tumor angiogenesis, with results that suggest the dependence of mechanisms and that support and enhances the tumor cell proliferation [7, 8]. Although angiogenesis is a mechanism that is shown to be involved in tumor development and survival, the results obtained in the prostate are controversial in relation to the clinicopathological parameters of the lesions, especially due to a complex architecture of neoformation vessels and different designs of investigation used to quantify the microvessel density (MVD) and expression of proangiogenic factors [9–12]. The identification of relation between hormonal status and prostate tumor angiogenesis can open new perspectives on targeted antiproliferative therapy and can contribute to improving the prognosis of patients, especially in lesions with aggressive biological behavior.

Aim

In this study, we analyzed the immunoexpression of AR, cluster of differentiation 105 (CD105) and Ki67 in relation to the clinicopathological aggressivity parameters for the PAs in order to establish the role of AR in the progression of lesions.

Materials and Methods

The study included 61 PAs from patients investigated, operated and diagnosed in the Clinic of Urology and Department of Pathology, Emergency County Hospital of Craiova, Romania, during 2016–2020. The biological material was represented by radical total prostatectomy specimens, fixed in 10% neutral buffered formalin, processed by classical paraffin embedding and Hematoxylin–Eosin (HE) staining.

The histopathological (HP) assessment of the PA was

done according to the criteria developed by the *Working Group for Tumors of the Urinary System and Male Genital Organs* within *World Health Organization* (WHO) [13].

The study analyzed the clinicopathological aggressivity parameters of PA represented by age, serum prostate-specific antigen (PSA) values, histological type, growth pattern, Gleason score, simplified grading groups, vascular and perineural invasion, tumor stage, prognostic groups in relation with the immunoexpression of AR and specific markers for vascular (CD105) and cellular (Ki67) tumor proliferation, which are shown in Table 1.

Table 1 – ***Monoclonal antibodies used and immunostaining protocol***

Antibody	Clone/Manufacturer	Dilution	Pretreatment	External positive control
AR	AR441/Dako (mouse antihuman)	1:50	Microwaving in Tris-EDTA buffer, pH 9	Normal prostate
CD105 (endoglin)	EP274/Abcam (rabbit antihuman)	1:100	Microwaving in Tris-EDTA buffer, pH 9	Kidney
Ki67	MIB-1/Dako (mouse antihuman)	1:70	Microwaving in citrate buffer, pH 6	Tonsil

AR: Androgen receptor; CD105: Cluster of differentiation 105; EDTA: Ethylenediaminetetraacetic acid.

The immunohistochemical (IHC) technique included dewaxing in xylene, rehydrating in alcohols, endogenous enzyme blocking with hydrogen peroxide, and unspecific blocking with bovine serum albumin (BSA) and incubation with primary antibodies at 4°C, overnight. The working system was represented by EnVision™ FLEX System (code K8002, Dako). To visualize the reactions, we used 3,3'-Diaminobenzidine (DAB) tetrahydrochloride (as chromogen from the same IHC working kit). To validate the IHC reactions were used external positive controls, external negative controls, and internal positive controls.

The semiquantitative assessment of the immuno-reactions was performed according to literature data based on the investigated antibody. For the AR, the Allred score was used, which resulted by adding the score resulting from the determination of the percentage of positive cells (0: no staining, 1: <1%, 2: 1–10%, 3: 11–33%, 4: 34–66%, 5: >66%) and the score resulting from determining the intensity of the reaction (0: no staining, 1: weak, 2: moderate, 3: strong); Allred final scores between 0–2 were considered negative, and those between 3–8 positive [14]. The CD105-positive neoformation vessels were quantified by establishing the MVD, which consisted of setting at 40× microscopic objective the five most intensely vascularized tumor areas ("hot spots"), counting the vessels marked at 20× objective and establishing for each case of the mean value for the used microscopic fields [15]. The counting of the vessels was performed individually by two authors, being considered for counting only the vessels with completely visible clear contour, including unicellular reactions. The Ki67 proliferation index (PI) was expressed as a percentage by the ratio of the number of positive cells to the total number of cells 20× microscope objective, for each case being counted five fields with maximum stainings and then averaging for each case.

The inclusion criterion of cases in the study was represented by the diagnosis of PA, confirmed on biopsy, on tumor transurethral resection of the prostate (TURP) fragments or directly on prostatectomy specimens. Only primitive PAs were included in this study, without history of oncological treatments and no history of cancer with other locations.

For the statistical analysis, we used the comparison tests represented by Student's *t*-test and one-way Analysis of Variance (ANOVA), χ^2 (*chi*-squared) and Pearson's comparison tests within Statistical Package for the Social Sciences (SPSS) 10 software, the results being considered significant for values of $p<0.05$.

For the images acquisition, the Nikon Eclipse E600 microscope equipped with Lucia 5 software was used.

The local Ethics Committee approved the study, which was done with the informed consent of the patients.

Results

In this study, the analysis of clinicopathological data indicated the predominance of patients over 70 years old (62.3%), who presented more frequently serum PSA values over 20 ng/mL (80.3%). The most common histological types of PA were conventional (78.7%), followed by ductal (6.5%) and foamy cells (4.9%). Regardless of the histological type, areas of the conventional adenocarcinoma were present, which were used to grade the lesions. Pure tumor growth patterns were present in 33 (54.1%) cases and mixed patterns in 28 (45.9%) cases. The most common Gleason score observed was score 8 (37.7%), followed by score 6 (19.7%), score 9 (18%), and scores 7 and 10 (14.8% and 9.8%, respectively), while depending on the simplified grading groups were group 4 (37.7%), group 5 (27.9%), group 1 (19.7) and groups 2 and 3 (9.8% and 4.9%, respectively). The perineural invasion was present in most patients (50.8%), being more common compared to vascular invasion (41%). In this study, the category of tumor extension (pT) coincided with the tumor stage, the most common being the lesions in pT2/stage II (44.2%) (Table 2). At the same time, most patients were classified in prognostic group 2b (49.2%) and group 3 (40.1%).

AR immunoexpression was identified in all cases analyzed in the nucleus of luminal cells of tumor glands, the signal being present in non-tumor glands but also in stromal elements represented mainly by fibroblasts, but also in rare lymphocytes and macrophages. For the whole analyzed group, the average number of marked tumor cells was 64.4±14.5, the intensity of the reactions being mainly moderate/strong and an average value of the Allred score of 6.7.

In this study, we found significant differences in AR immunoexpression in relation to the analyzed clinico-pathological parameters. Thus, age over 70 years was associated with Allred maximum scores ($p=0.02$, *chi*-squared test), PSA values >20 ng/mL were associated with Allred scores of 7 and 8 ($p<0.001$, *chi*-squared test) and ductal, colloid, and sarcomatoid tumor types were associated with Allred scores 6–8 ($p<0.001$, *chi*-squared test). At the same time, the pure growth patterns 5 and 4 and mixed patterns revealed average Allred scores of 8,

7 and 6.9, compared to pattern 3, which presented an average value of 5.2 (p<0.001, *chi*-squared test). This aspect was also reflected on the classical tumor grading, in which Gleason scores 8–10 were associated only with Allred scores 6–8 (p<0.001, *chi*-squared test) (Table 3).

In relation to the simplified tumor grading, for group 1, the number of marked tumor cells was 49.1±19.4, the intensity of the reaction was low/moderate, and the average score was 5.2, while for groups 2 and 3, the number of marked cells was 56.6±10.3 and 60, respectively, moderate predominant reaction intensity and mean staining score of 6.3 in both cases (Figure 1, A and B).

In comparison, for groups 4 and 5, the number of labeled cells was 66.5±10.3 and 75.5±6.4, respectively, the intensity of reactions predominantly strong, and the average final scores with values of 6.9 and 7.7, respectively (Figure 1, C and D), differences that were statistically significant (p<0.001, *chi*-squared test) (Figure 2A). In relation to the tumor stage, for the tumors in stage I, the average value of the number of AR-positive tumor cells was 47.7±20, the reactions intensity was weak/moderate and the average score 5, while for those in stage II and III, the mean values for positive cells were 61.8±11.4 and 73.2±7.8, respectively, with moderate/strong reaction intensity and mean scores of 6.7 and 7.4, respectively, differences that were statistically significant (p<0.001, *chi*-squared test) (Figure 2B).

Table 2 – ***Distribution of cases depending on clinico-pathological parameters***

Clinicopathological parameters	No. of cases
Age [years]	<70: 23, >70: 38
Serum PSA [ng/mL]	≤10: 7, 11–19: 5, 20-50: 36, >50: 13
Histological type	conventional: 48, ductal: 4, foamy cells: 3, colloid: 2, atrophic: 2, pseudo-hyperplastic: 1, sarcomatoid: 1
Growth pattern (pure and mixed)	pure – pattern 3: 12, pattern 4: 15, pattern 5: 6 mixed – pattern 3: 17, pattern 4: 20, pattern 5: 19
Gleason score	score 6: 12, score 7: 9, score 8: 23, score 9: 11, score 10: 6
Grade simplified groups	group 1: 12, group 2: 6, group 3: 3, group 4: 23, group 5: 17
Perineural invasion	present: 31, absent: 30
Vascular invasion	present: 12, absent: 49
Tumoral extension (pT)/Tumor stage	pT1/stage I: 9, pT22/stage II: 27, pT3/stage III: 25
Prognostic groups	group 1: 3, group 2a: 3, group 2b: 30, group 3: 25

PSA: Prostate-specific antigen.

Table 3 – ***Average values of the semiquantitative assessment for the analyzed markers depending on clinicopathological parameters***

Clinicopathological parameters		AR (Allred score)	CD105 (endoglin) (MVD)	Ki67 (PI)
Age [years]	*<70*	6.6	33.2±14.8	25.3±18.1
	>70	6.7	35.5±11.3	20.8±12
Serum PSA [ng/mL]	*≤10*	6.4	29.2±12.3	14.5±5
	11–19	5	19±13.4	10.6±9.3
	20–50	6.7	35±12	20.4±11.3
	>50	7.4	42.6±7.5	37.3±17.7
Histological type	*conventional*	6.8	35.2±12.1	22.5±14.2
	ductal	7.5	42.5±8.6	30±9.1
	foamy cells	5.6	33.3±7.6	14.3±4
	colloid	7.5	37.5	39
	atrophic	3.5	10	4
	pseudohyperplastic	4	10	5
	sarcomatoid	8	50	40
Growth pattern (pure and mixed)	*pattern 3*	5.2	17±6.8	8.2±2.8
	pattern 4	7	36.3±7.8	18.4±3.3
	pattern 5	8	50.8±3.7	50±12.6
	mixed	6.9	37.8±9.8	24.9±12.8
Gleason score	*score 6*	5.2	17±6.8	8.2±2.8
	score 7	6.3	27.7±7.9	12.8±2.1
	score 8	6.9	36.9±6.6	18.6±3.2
	score 9	7.6	45.9±6.2	39±7.6
	score 10	8	50.8±3.7	50±12.6
Grade simplified groups	*group 1*	5.2	17±6.8	8.2±2.8
	group 2	6.3	27.5±7.5	12.3±2.2
	group 3	6.3	28.3±10.4	14±1.7
	group 4	6.9	36.9±6.6	18.6±3.2
	group 5	7.7	47.6±5.8	42.9±10.7
Perineural invasion	*present*	7.2	39.3±11.9	30.5±16
	absent	6.2	29.8±11.9	14.2±6.3
Vascular invasion	*present*	7.9	47.5±6.5	42±12.5
	absent	6.4	31.5±11.8	17.7±10.6

Clinicopathological parameters		AR (Allred score)	CD105 (endoglin) (MVD)	Ki67 (PI)
Tumoral extension (pT)/Tumor stage	*pT1/stage I*	5	15.5±6.3	7.6±3
	pT2/stage II	6.7	34.2±10.8	19.1±10.1
	pT3/stage III	7.4	42±8.2	31.5±15.5
Prognostic groups	*group 1*	5.6	16.6±2.3	9.6±2.5
	group 2	3.6	10	4.3±1.1
	group 3	6.6	32.8±11.4	18.1±10.1
	group 4	7.4	42±8.2	32±15.7

AR: Androgen receptor; CD105: Cluster of differentiation 105; MVD: Microvessel density; PI: Proliferation index; PSA: Prostate-specific antigen.

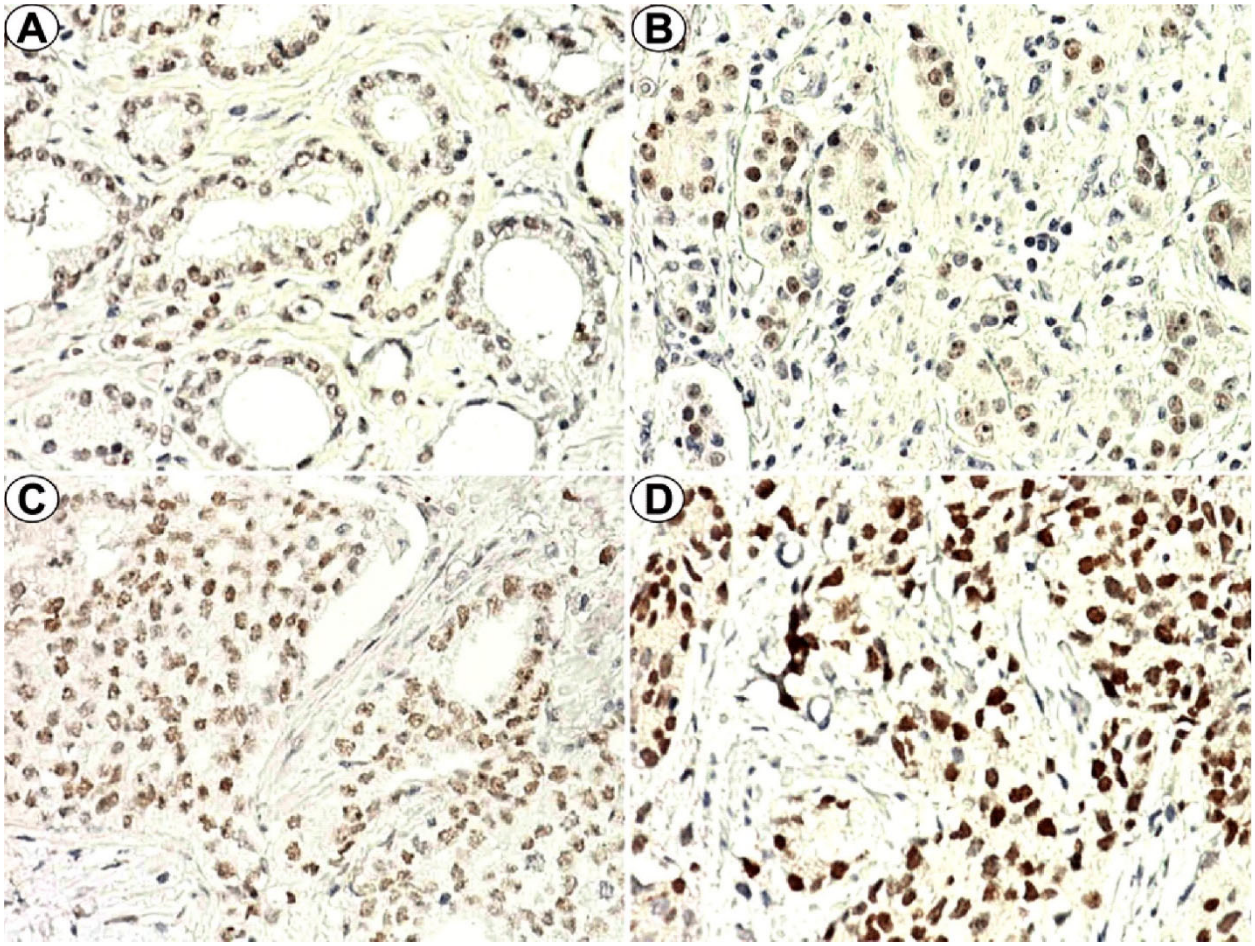

Figure 1 – ***Prostate adenocarcinoma: (A) Gleason grade 6 (group 1); (B) Gleason grade 7 (group 3); (C) Gleason grade 8 (group 4); (D) Gleason grade 9 (group 5). Anti-AR antibody immunostaining: (A–D) ×200. AR: Androgen receptor.***

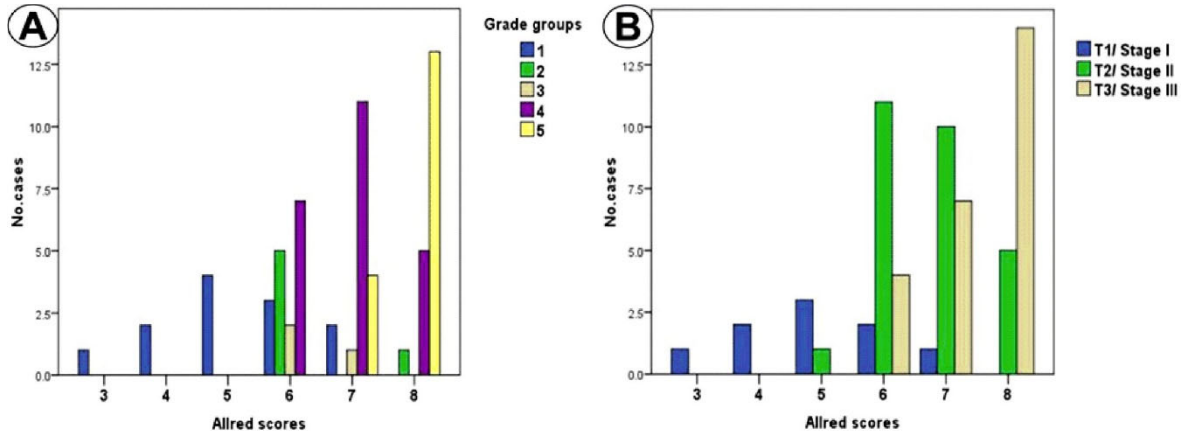

Figure 2 – ***(A) Distribution of cases depending on AR Allred scores and simplified tumor grades; (B) Distribution of cases depending on AR Allred scores and tumor stages. AR: Androgen receptor.***

The Allred mean values were significantly higher for tumors with perineural invasion (p=0.006, *chi*-squared test) and vascular invasion (p<0.001, *chi*-squared test), while prognostic groups 2b/3 were more frequently associated with Allred scores 6–8 (p<0.001, *chi*-squared test).

The analysis of CD105 (endoglin) immunoexpression was identified in all cases in the cytoplasm of endothelial cells of tumor neovessels. The vascular network of PA has been complex and relatively difficult to assess with numerous branches and vascular anastomoses around tumor islands. The tumor neoformation vessels presented variable sizes and shapes, with aberrant morphology and predominance of small, irregular, tortuous, and sometimes single cellular vessels.

Depending on the clinicopathological parameters analyzed, the MVD of CD105-positive vessels was superior in patients over 70 years of age (p=0.504, Student's t-test), with PSA >20 ng/mL (p=0.002, ANOVA test) and in the case of ductal, colloid and sarcomatoid tumor types (p=0.017, ANOVA test). In this study, the maximum values of CD105 MVD were present for pure growth patterns 4 and 5 and mixed patterns (p<0.001, ANOVA test), the highest MVD values being associated with Gleason scores 8–10 (p<0.001, ANOVA test). Regarding simplified tumor grading, there were differences in MVD (Table 3). Thus, for group 1, the mean value of CD105 MVD was significantly lower (17±6.8) (Figure 3A) compared to the mean value of groups 2–3 (27.7±7.3) (Figure 3B) and groups 4–5 (41.5±8.2) (p<0.001, ANOVA test) (Figure 3, C and D; Figure 4A).

At the same time in relation to the tumor stage, the mean value of CD105 MVD for stage I lesions (15.5±6.3) was significantly lower than those for stage II–III (37.9 ±10.3) (p<0.001, ANOVA test) (Figure 4B).

CD105 MVD values were significantly higher in the case of PA with perineural invasion (p<0.001, ANOVA test), vascular invasion (p<0.001, ANOVA test) and for lesions in prognostic groups 2b/3 (p<0.001, ANOVA test).

Ki67 immunoexpression was identified in all cases analyzed in the nucleus of tumor cells and in rare stromal lymphocytes. The Ki67 PI was higher in patients less than 70 years old (p=0.256, Student's t-test), with PSA values >20 ng/mL (p<0.001, ANOVA test) and was higher in case of ductal, colloid and sarcomatoid histological types (p=0.095, ANOVA test). Ki67 PI presented maximum values in the case of growth pattern 5 (p<0.001, ANOVA test) and Gleason scores 9–10 (p<0.001, ANOVA test) (Table 3). Depending on the simplified tumor grading, the mean Ki67 PI values increased from groups 1–4 (8.2±2.8, 12.3±2.2, 14±1.7 and 18.6±3.2, respectively) to group 5 (42.9±10.7) (Figure 5, A–D), the aspect being statistically significant (p<0.001, ANOVA test) (Figure 6A).

Depending on the tumor stage, the mean Ki67 PI values were significantly lower in stage I (7.6±3) compared to stage II (19.1±10.1) and stage III (31.5±15.5) (p<0.001, ANOVA test) (Figure 6B).

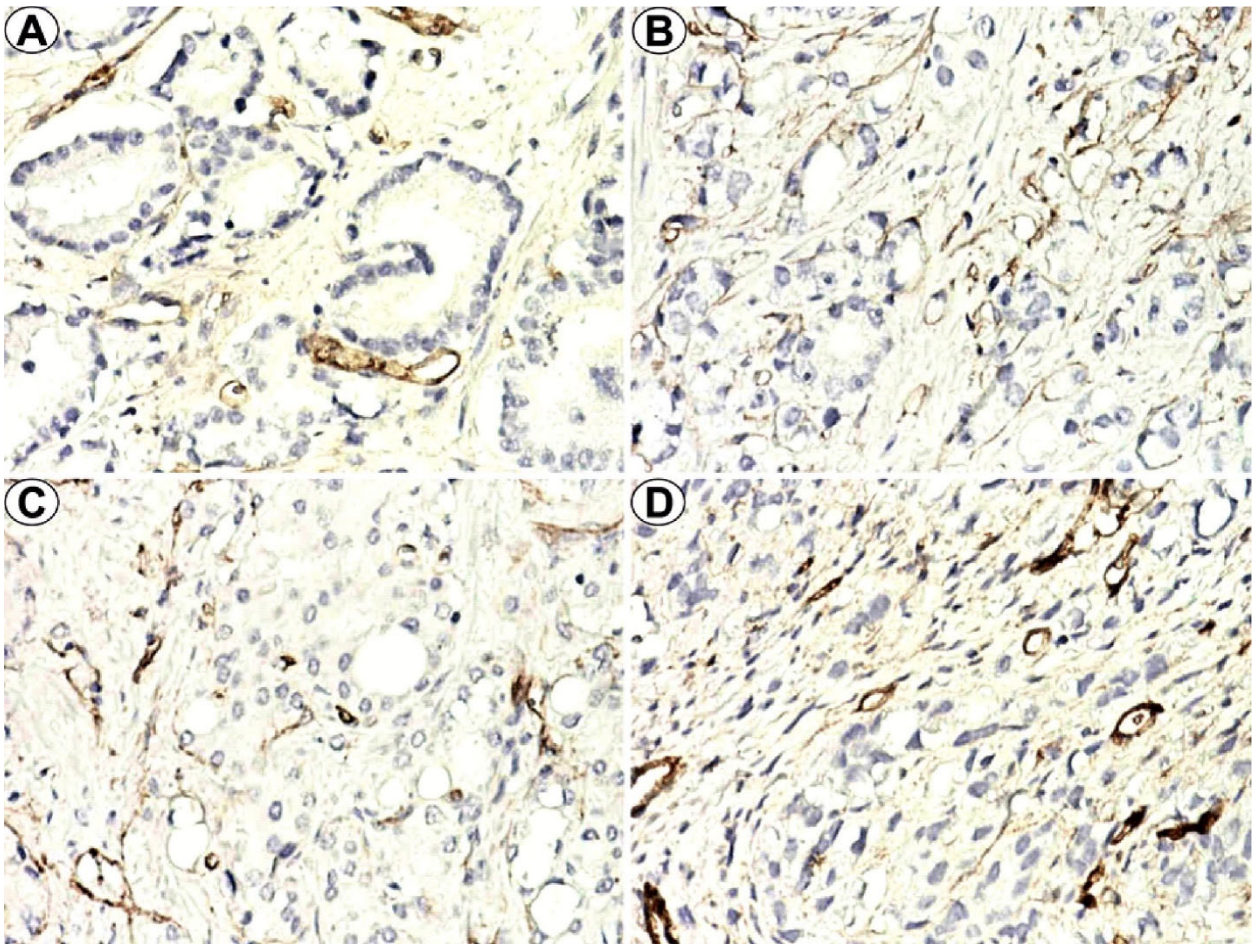

Figure 3 – ***Prostate adenocarcinoma: (A) Gleason grade 6 (group 1); (B) Gleason grade 7 (group 3); (C) Gleason grade 8 (group 4); (D) Gleason grade 9 (group 5). Anti-CD105 antibody immunostaining: (A–D) ×200. CD105: Cluster of differentiation 105.***

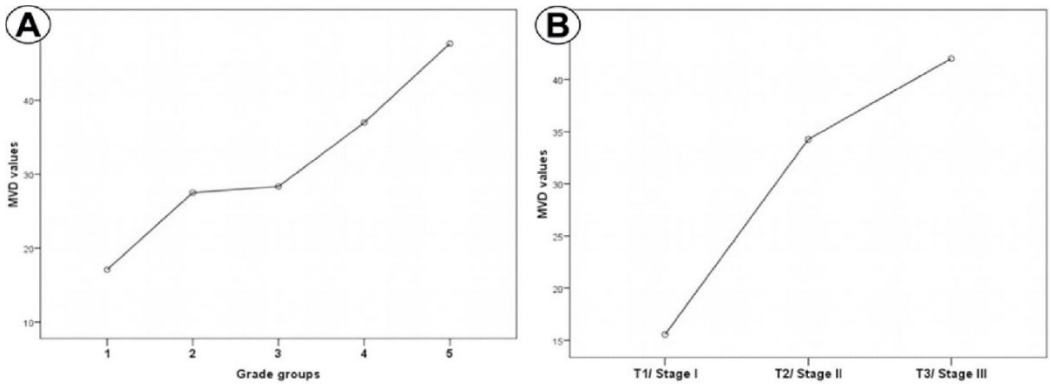

Figure 4 – ***(A) Distribution of cases depending on CD105 MVD average values and simplified tumor grades; (B) Distribution of cases depending on CD105 MVD average values and tumor stages. CD105: Cluster of differentiation 105; MVD: Microvessel density.***

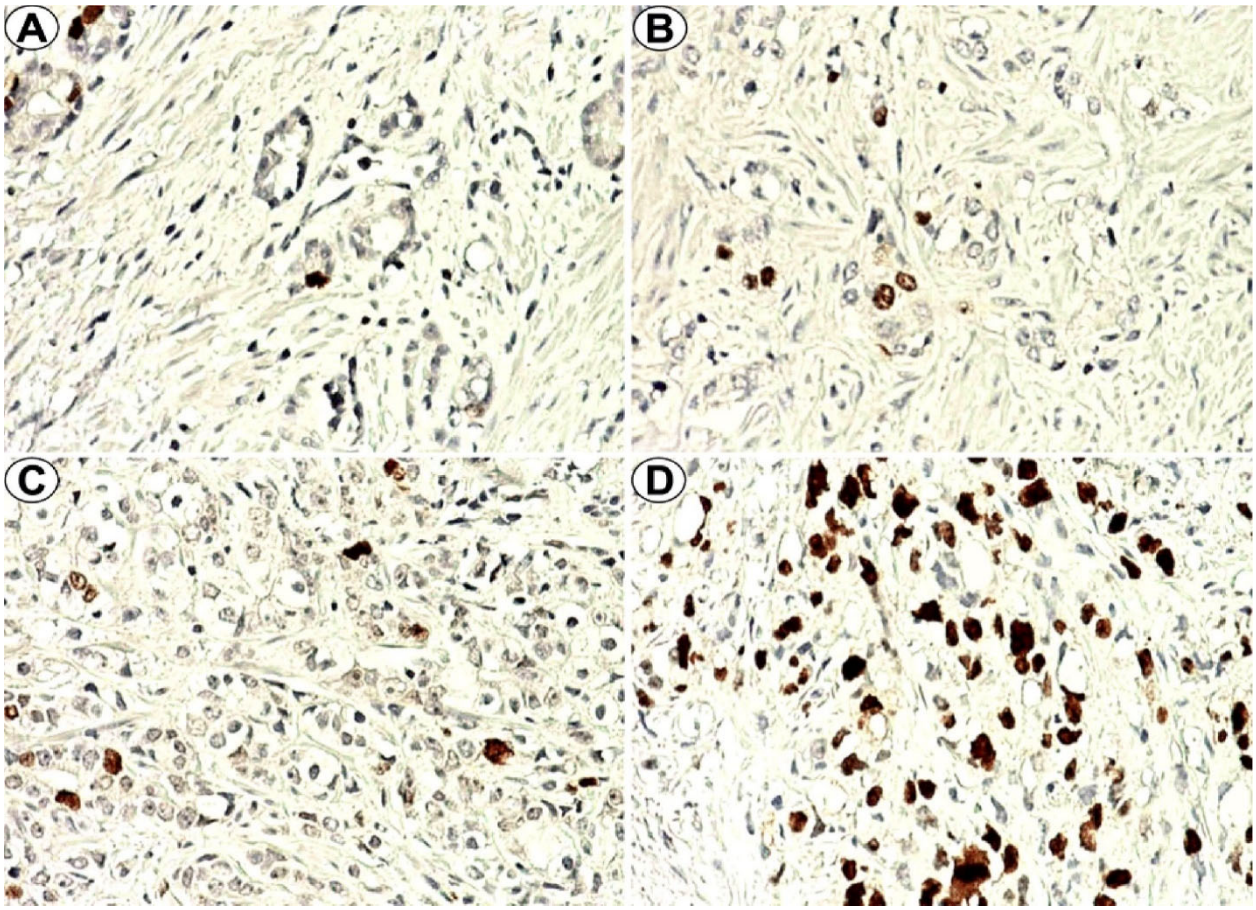

Figure 5 – ***Prostate adenocarcinoma: (A) Gleason grade 6 (group 1); (B) Gleason grade 7 (group 3); (C) Gleason grade 8 (group 4); (D) Gleason grade 9 (group 5). Anti-Ki67 antibody immunostaining: (A–D) ×200.***

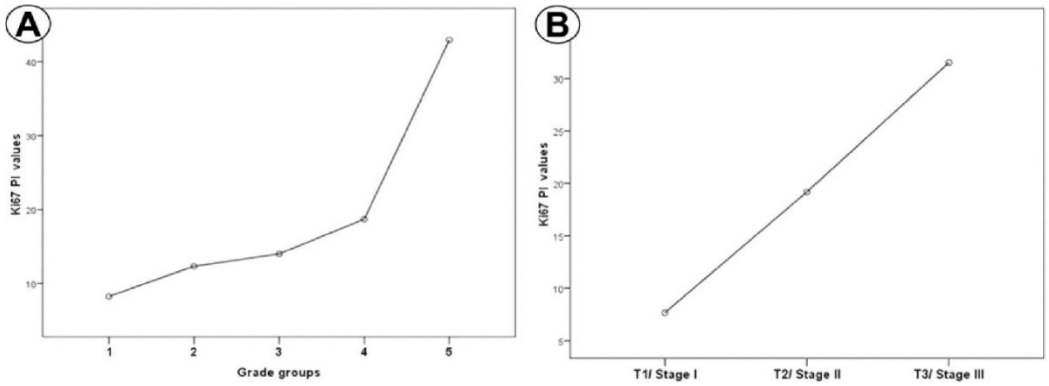

Figure 6 – ***(A) Distribution of cases depending on Ki67 PI average values and simplified tumor grades; (B) Distribution of cases depending on Ki67 PI average values and tumor stages. PI: Proliferation index.***

Ki67 PI values were significantly higher in PA with perineural invasion (p<0.001, ANOVA test), vascular invasion (p<0.001, ANOVA test) and for lesions in prognostic groups 2b/3 (p<0.001, ANOVA test).

The analysis of the mean percentage values of the AR scores, the mean values of CD105 MVD and Ki67 PI values indicated a positive linear correlation of the three analyzed markers (p<0.001, Pearson's test) (Figure 7).

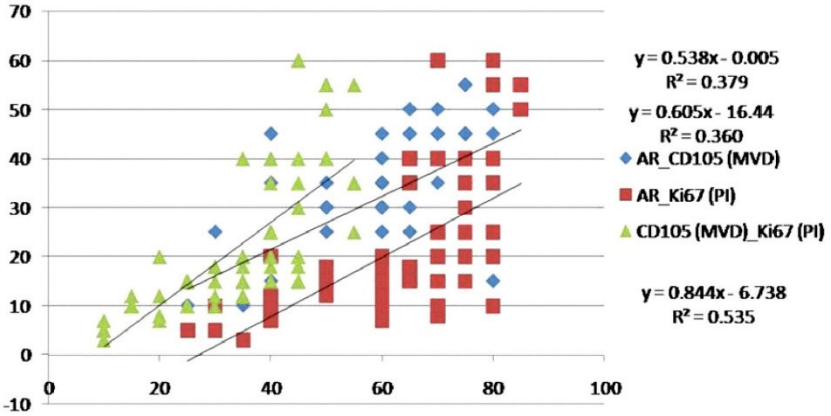

Figure 7 – ***Distribution of the average values for AR (%), CD105 (MVD) and Ki67 (PI). AR: Androgen receptor; CD105: Cluster of differentiation 105; MVD: Microvessel density; PI: Proliferation index.***

Discussions

Androgens are essential for normal prostate morpho-functional development and play a central role in the development of PA [6, 7, 16]. Androgen signaling requires the AR, which activates gene transcription after phosphorylation and translocation at the nuclear level [8]. In this way, AR is considered a ligand-dependent transcriptional activator that regulates the activity of genes involved in proliferation, migration, differentiation, cell cycle and apoptosis [2, 9]. Thus, castration of men with high blood pressure or administration of luteinizing hormone antagonists (androgen deprivation therapy) was one of the first treatment options, which had the effects of temporarily stopping for tumor progression [2, 6]. However, the evolution towards hormone-resistant PA seems inevitable through mechanisms, such as AR over-expression, AR mutations, independent AR activation, and additional or second line antihormonal therapies are required [6, 17, 18]. Thus, androgen deprivation therapy finally seems to increase AR activity, especially in conditions where the existence of independent AR stem or progenitor cells are suspected in the prostate [17]. In this context, there are some studies that have indicated the association of increased AR expression with imaging or histological aggression markers, such as Gleason score or tumor stage [19].

In our study, AR immunoexpression was identified in all cases and high Allred scores were statistically associated with age over 70 years, PSA over 20 ng/mL, high grade, advanced stage, perineural and vascular invasion, and reserved prognostic groups. The data from the literature on AR expression are variable due to the size and homogeneity of the study groups, the types of surgical specimens analyzed, the quantification methods, the histological parameters considered and the compartment in which the evaluation is performed [6].

There are studies that have analyzed AR in the stroma and that have come to suggest that decreased expression in this compartment is associated with histological parameters of aggression and a poor prognosis for PA [2, 6]. In our study, most of the lesions were of intermediate/high grade and in stages II/III, and the stromal staining was poorly represented, located especially in fibroblasts, lymphocytes and macrophages, aspects that are consistent with the data in the literature.

Angiogenesis is a complex multistage process involved in the progression and survival of many solid malignancies, among the inducers of the prostate process being in addition to vascular endothelial growth factor (VEGF), the fibroblast growth factor-2 (FGF2) receptor expressed especially in independent androgen PA, transforming growth factor-beta (TGFβ), as well as the TGFβRI receptor and matrix metalloproteinases (MMPs) [9]. The usefulness of quantifying neoformation vessels in PA is controversial due to the variable results obtained depending especially on the quantification methods, the antibody used, the homogeneity of the group, the cut-off reference values that can guide the tumor progression [11]. At the same time, MVD is also dependent on the type of surgical specimen and the primary HP processing, as there are no standard methods that offer a high reproducibility, with all the attempts to introduce automatic quantification methods [12, 20]. Besides all these aspects, the prostatic vascular geometry is a complex one, sometimes difficult to appreciate [9, 21].

In this study, MVD values were associated with high PSA levels, high grade, advanced stage, perineural and vascular invasion, and reserved prognostic groups. Some studies have indicated that MVD is higher in prostate cancer compared to normal tissue or benign lesions and correlates with histological prognostic parameters of PA [9, 10]. Thus, high MVD values were observed in metastatic primary tumors compared to localized disease, in the case of high Gleason scores, in advanced stages [9, 15, 20, 22–25]. On the contrary, there are studies that have not identified such statistical relations [9, 26, 27].

Endoglin (CD105) is a TGFβ receptor and is considered a specific and sensitive marker for the quantification of neoformed vessels [10]. CD105 is involved in normal vascular development and is expressed in proliferative endothelial cells and during tumor angiogenesis, with superior results on MVD quantification compared to other panendothelial markers, such as CD31 or CD34 [10, 15,

20, 28, 29]. However, there are also studies indicating the absence of CD105 expression correlations with PA parameters [30].

The involvement of AR in tumor angiogenesis is unclear, but there are data indicating that it promotes endothelial proliferation through an AR/VEGF-A/cyclin A-mediated mechanism [2, 31]. In our study, the relation of AR to CD105 was linearly positive, suggesting the synergistic or sequential involvement of hormonal and angiogenic mechanisms in the progression of PA.

Prostate functional endothelial cells appear to have an AR, being susceptible to androgenic action, with the participation of VEGF in a paracrine mechanism of endothelial stimulation by tumor cells, the induction of proangiogenic factor being mediated by AR transcription factor binding [7, 32]. On the other hand, one of the genes regulated by the AR transcription factor is *VEGF*, a proangiogenic mitogen secreted by tumor cells [32]. In a recent study, Jia *et al.* indicated a positive correlation between AR expression and MVD in human tissues with neurofibroma and suggested that AR enhances VEGF-A transcription by direct interaction with the VEGF-A promoter in these tumors [33]. At the same time, the AR relation with VEGF is supported by the autocrine sequence hypoxia-inducible factor-1 (HIF-1)/ cyclooxygenase-2 (COX2) [9].

For the analysis of tumor proliferation, in this study was used Ki67, cell cycle regulatory protein and one of the most used markers for establishing the biological behavior of PA and patient prognosis, used for all types of prostate specimens, and which has independent associations with the clinicobiological tumoral parameters [34].

The results indicated a significant association of high Ki67 PI with PSA values above 20 ng/mL, with high grade, advanced stages, perineural and vascular invasion and reserved prognostic groups of PA. In most studies in the literature, Ki67 immunoexpression has been associated with tumor grade and/or tumor stage, some of these studies being performed on large groups of over 500 prostatectomies [3, 5, 34–36]. Also, some authors have indicated the association of Ki67 with positive resection margins [35], with non-recurrent survival and overall survival [34] or with tumor size [5]. Due to the simplicity of quantification and low intra- and interobserver variations regardless of the histological specimen used, it is proposed to use Ki67 as a routine prognostic marker for PA in current clinical practice [5, 34, 37].

In our study there were differences in the expression of the markers used in relation to some histological forms of PA. Although there are rare studies, some authors indicate the association of high AR levels with increased Ki67 PI [19], aspect observed also in our study. At the same time, Ki67 revealed a positive linear correlation with CD105 MVD, suggesting that high values of these markers may be associated for predicting PA behavior.

Conclusions

High values of AR, Ki67 and CD105 immunoexpression were associated with clinicopathological parameters that define aggressive, high-grade PA classified in advanced stages. The positive linear relations of AR with markers associated with vascular and cell proliferation suggests the synergistic or sequential involvement of endocrine and angiogenic mechanisms in prostate tumor progression. The results obtained can be used to improve the stratification criteria of patients for prostate antineoplastic therapy.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin, 2015, 65(2): 87–108. https://doi.org/10.3322/caac.21262 PMID: 25651787

[2] Cioni B, Zwart W, Bergman AM. Androgen receptor moon-lighting in the prostate cancer microenvironment. Endocr Relat Cancer, 2018, 25(6):R331–R349. https://doi.org/10.1530/ERC-18-0042 PMID: 29618577

[3] Verma R, Gupta V, Singh J, Verma M, Gupta G, Gupta S, Sen R, Ralli M. Significance of p53 and Ki-67 expression in prostate cancer. Urol Ann, 2015, 7(4):488–493. https://doi.org/10.4103/0974-7796.158507 PMID: 26692671 PMCID: PMC4660702

[4] Cooperberg MR, Carroll PR. Trends in management for patients with localized prostate cancer, 1990–2013. JAMA, 2015, 314(1):80–82. https://doi.org/10.1001/jama.2015.6036 PMID: 26151271

[5] Richardsen E, Andersen S, Al-Saad S, Rakaee M, Nordby Y, Pedersen MI, Ness N, Grindstad T, Movik I, Dønnem T, Bremnes R, Busund LT. Evaluation of the proliferation marker Ki-67 in a large prostatectomy cohort. PLoS One, 2017, 12(11):e0186852. https://doi.org/10.1371/journal.pone.0186852 PMID: 29141018 PMCID: PMC5687762

[6] Poelaert F, Van Praet C, Beerens AS, De Meerleer G, Fonteyne V, Ost P, Lumen N. The role of androgen receptor expression in the curative treatment of prostate cancer with radiotherapy: a pilot study. Biomed Res Int, 2015, 2015: 812815. https://doi.org/10.1155/2015/812815 PMID: 25793207 PMCID: PMC4352440

[7] Torres-Estay V, Carreño DV, San Francisco IF, Sotomayor P, Godoy AS, Smith GJ. Androgen receptor in human endothelial cells. J Endocrinol, 2015, 224(3):R131–R137. https://doi.org/10.1530/JOE-14-0611 PMID: 25563353 PMCID: PMC4700832

[8] Eisermann K, Fraizer G. The androgen receptor and VEGF: mechanisms of androgen-regulated angiogenesis in prostate cancer. Cancers (Basel), 2017, 9(4):32. https://doi.org/10.3390/cancers9040032 PMID: 28394264 PMCID: PMC5406707

[9] Russo G, Mischi M, Scheepens W, De la Rosette JJ, Wijkstra H. Angiogenesis in prostate cancer: onset, progression and imaging. BJU Int, 2012, 110(11 Pt C):E794–E808. https://doi.org/10.1111/j.1464-410X.2012.11444.x PMID: 22958524

[10] Grivas N, Goussia A, Stefanou D, Giannakis D. Microvascular density and immunohistochemical expression of VEGF, VEGFR-1 and VEGFR-2 in benign prostatic hyperplasia, high-grade prostate intraepithelial neoplasia and prostate cancer. Cent European J Urol, 2016, 69(1):63–71. https://doi.org/10.5173/ceju.2016.726 PMID: 27123329 PMCID: PMC4846728

[11] Bono AV, Celato N, Cova V, Salvadore M, Chinetti S, Novario R. Microvessel density in prostate carcinoma. Prostate Cancer Prostatic Dis, 2002, 5(2):123–127. https://doi.org/10.1038/sj.pcan.4500572 PMID: 12497001

[12] Miyata Y, Sakai H. Reconsideration of the clinical and histopathological significance of angiogenesis in prostate cancer: usefulness and limitations of microvessel density measurement. Int J Urol, 2015, 22(9):806–815. https://doi.org/10.1111/iju.12840 PMID: 26153072

[13] Moch H, Humphrey PA, Ulbright TM, Reuter VE (eds). World Health Organization (WHO) Classification of tumours of the urinary system and male genital organs. 4th edition, vol. 8, International Agency for Research on Cancer (IARC) Press, Lyon, France, 2016, 135–184.

[14] Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol, 1998, 11(2):155–168. PMID: 9504686

[15] Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman J. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol, 1993, 143(2):401–409. PMID: 7688183 PMCID: PMC1887042
[16] Toivanen R, Shen MM. Prostate organogenesis: tissue induction, hormonal regulation and cell type specification. Development, 2017, 144(8):1382–1398. https://doi.org/10.1242/dev.148270 PMID: 28400434 PMCID: PMC5399670
[17] Chua CW, Epsi NJ, Leung EY, Xuan S, Lei M, Li BI, Bergren SK, Hibshoosh H, Mitrofanova A, Shen MM. Differential requirements of androgen receptor in luminal progenitors during prostate regeneration and tumor initiation. Elife, 2018, 7: e28768. https://doi.org/10.7554/eLife.28768 PMID: 29334357 PMCID: PMC5807048
[18] Huggins C, Hodges CV. Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. CA Cancer J Clin, 1972, 22(4):232–240. https://doi.org/10.3322/canjclin.22.4.232. PMID: 4625049
[19] Li R, Wheeler T, Dai H, Frolov A, Thompson T, Ayala G. High level of androgen receptor is associated with aggressive clinicopathologic features and decreased biochemical recurrence-free survival in prostate: cancer patients treated with radical prostatectomy. Am J Surg Pathol, 2004, 28(7):928–934. https://doi.org/10.1097/00000478-200407000-00013 PMID: 15223964
[20] Miyata Y, Mitsunari K, Asai A, Takehara K, Mochizuki Y, Sakai H. Pathological significance and prognostic role of microvessel density, evaluated using CD31, CD34, and CD105 in prostate cancer patients after radical prostatectomy with neoadjuvant therapy. Prostate, 2015, 75(1):84–91. https://doi.org/10.1002/pros.22894 PMID: 25307287 PMCID: PMC4282783
[21] Chung LWK, Baseman A, Assikis V, Zhau HE. Molecular insights into prostate cancer progression: the missing link of tumor microenvironment. J Urol, 2005, 173(1):10–20. https://doi.org/10.1097/01.ju.0000141582.15218.10 PMID: 15592017
[22] Brawer MK, Deering RE, Brown M, Preston SD, Bigler SA. Predictors of pathologic stage in prostatic carcinoma. The role of neovascularity. Cancer, 1994, 73(3):678–687. https://doi.org/10.1002/1097-0142(19940201)73:3<678::aid-cncr2820730329>3.0.co;2-6 PMID: 7507798
[23] Silberman MA, Partin AW, Veltri RW, Epstein JI. Tumor angiogenesis correlates with progression after radical prostatectomy but not with pathologic stage in Gleason sum 5 to 7 adenocarcinoma of the prostate. Cancer, 1997, 79(4):772–779. https://doi.org/10.1002/(sici)1097-0142(19970215)79:4<772::aid-cncr14>3.0.co;2-x PMID: 9024715
[24] Dugonjić AS, Usaj SK, Eri Z, Latinović LT. Significance of microvessel density in prostate cancer core biopsy. Vojnosanit Pregl, 2015, 72(4):317–327. https://doi.org/10.2298/VSP131111004S PMID: 26040177
[25] Bostwick DG, Wheeler TM, Blute M, Barrett DM, MacLennan GT, Sebo TJ, Scardino PT, Humphrey PA, Hudson MA, Fradet Y, Miller GJ, Crawford ED, Blumenstein BA, Mahran HE, Miles BJ. Optimized microvessel density analysis improves prediction of cancer stage from prostate needle biopsies. Urology, 1996, 48(1):47–57. https://doi.org/10.1016/s0090-4295(96)00149-5 PMID: 8693651
[26] Rubin MA, Buyyounouski M, Bagiella E, Sharir S, Neugut A, Benson M, de la Taille A, Katz AE, Olsson CA, Ennis RD. Microvessel density in prostate cancer: lack of correlation with tumor grade, pathologic stage, and clinical outcome. Urology, 1999, 53(3):542–547. https://doi.org/10.1016/s0090-4295(98)00561-5 PMID: 10096381
[27] Erbersdobler A, Isbarn H, Dix K, Steiner I, Schlomm T, Mirlacher M, Sauter G, Haese A. Prognostic value of microvessel density in prostate cancer: a tissue microarray study. World J Urol, 2010, 28(6):687–692. https://doi.org/10.1007/s00345-009-0471-4 PMID: 19714336
[28] Dallas NA, Samuel S, Xia L, Fan F, Gray MJ, Lim SJ, Ellis LM. Endoglin (CD105): a marker of tumor vasculature and potential target for therapy. Clin Cancer Res, 2008, 14(7):1931–1937. https://doi.org/10.1158/1078-0432.CCR-07-4478 PMID: 18381930
[29] Stepan D, Simionescu C, Stepan A, Muntean M, Voinea B. VEGF and CD105 immunoexpression in squamous cervical carcinomas and associated precancerous lesions. Rom J Morphol Embryol, 2012, 53(3):585–589. PMID: 22990551
[30] Steiner I, Jung K, Miller K, Stephan C, Erbersdobler A. Expression of endothelial factors in prostate cancer: a possible role of caveolin-1 for tumour progression. Oncol Rep, 2012, 27(2):389–395. https://doi.org/10.3892/or.2011.1539 PMID: 22075971
[31] Cai J, Hong Y, Weng C, Tan C, Imperato-McGinley J, Zhu YS. Androgen stimulates endothelial cell proliferation *via* an androgen receptor/VEGF/cyclin A-mediated mechanism. Am J Physiol Heart Circ Physiol, 2011, 300(4):H1210–H1221. https://doi.org/10.1152/ajpheart.01210.2010 PMID: 21257919 PMCID: PMC3075033
[32] Eisermann K, Broderick CJ, Bazarov A, Moazam MM, Fraizer GC. Androgen up-regulates vascular endothelial growth factor expression in prostate cancer cells *via* an Sp1 binding site. Mol Cancer, 2013, 12:7. https://doi.org/10.1186/1476-4598-12-7 PMID: 23369005 PMCID: PMC3616929
[33] Jia J, Zhang H, Zhang H, Du H, Liu W, Shu M. Activated androgen receptor accelerates angiogenesis in cutaneous neurofibroma by regulating VEGFA transcription. Int J Oncol, 2019, 55(1):157–166. https://doi.org/10.3892/ijo.2019.4797 PMID: 31059067
[34] Tretiakova MS, Wei W, Boyer HD, Newcomb LF, Hawley S, Auman H, Vakar-Lopez F, McKenney JK, Fazli L, Simko J, Troyer DA, Hurtado-Coll A, Thompson IM Jr, Carroll PR, Ellis WJ, Gleave ME, Nelson PS, Lin DW, True LD, Feng Z, Brooks JD. Prognostic value of Ki67 in localized prostate carcinoma: a multi-institutional study of >1000 prostatectomies. Prostate Cancer Prostatic Dis, 2016, 19(3):264–270. https://doi.org/10.1038/pcan.2016.12 PMID: 27136741 PMCID: PMC5536893
[35] Fantony JJ, Howard LE, Csizmadi I, Armstrong AJ, Lark AL, Galet C, Aronson WJ, Freedland SJ. Is Ki67 prognostic for aggressive prostate cancer? A multicenter real-world study. Biomark Med, 2018, 12(7):727–736. https://doi.org/10.2217/bmm-2017-0322 PMID: 29902938 PMCID: PMC6219443
[36] Hammarsten P, Josefsson A, Thysell E, Lundholm M, Hägglöf C, Iglesias-Gato D, Flores-Morales A, Stattin P, Egevad L, Granfors T, Wikström P, Bergh A. Immunoreactivity for prostate specific antigen and Ki67 differentiates subgroups of prostate cancer related to outcome. Mod Pathol, 2019, 32(9):1310–1319. https://doi.org/10.1038/s41379-019-0260-6 PMID: 30980038 PMCID: PMC6760646
[37] Mesko S, Kupelian P, Demanes DJ, Huang J, Wang PC, Kamrava M. Quantifying the Ki-67 heterogeneity profile in prostate cancer. Prostate Cancer, 2013, 2013:717080. https://doi.org/10.1155/2013/717080 PMID: 24222860 PMCID: PMC3816071

Corresponding authors

Paul Ioan Tomescu, Associate Professor, MD, PhD, Department of Urology, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone/Fax +40251–426 688, e-mail: paul.tomescu@yahoo.com

Petru Octavian Drăgoescu, Lecturer, MD, PhD, Department of Urology, University of Medicine and Pharmacy of Craiova, 2 Petru Rareş Street, 200349 Craiova, Dolj County, Romania; Phone/Fax +40251–426 688, e-mail: pdragoescu@yahoo.com

Received: November 2, 2020 *Accepted: March 23, 2021*