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Mast cells as a tumor microenvironment factor associated with the aggressiveness of prostate cancer

Taras ZADVORNYI[1,*], Natalia LUKIANOVA[1,*], Tetiana BORIKUN[1,*], Andrii TYMOSHENKO[1,2], Oleksandr MUSHII[1], Olena VORONINA[1], Iurii VITRUK[2], Eduard STAKHOVSKYI[2], Vasyl CHEKHUN[1]

[1]R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine; [2]National Cancer Institute of the Ministry of Health of Ukraine, Kyiv, Ukraine

**Correspondence: tito132007@ukr.net; nataluk10@gmail.com; tborikun@gmail.com*

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We aimed to investigate the relationship between the degree of mast cells' (MCs) infiltration and clinicopathological features of prostate cancer (PCa) malignancy and to find out the possible mechanisms of the involvement of these cells in the formation of the aggressive course of the PCa development. The study was conducted on the clinical material of 60 patients with PCa of stages II-III. MCs in the PCa tissue were determined by a histochemical method using toluidine blue. The expression of osteopontin (OPN) was studied by the immunohistochemical method. The expression of miRNA-21, -126, -146a, -181a, and -221 was investigated by quantitative real-time PCR. Statistical processing of the results was performed using the GraphPad Prism 8 program. Our results demonstrated that the increased level of infiltration and degranulation of MCs in the PCa tissue was associated with such indices of the malignancy of the tumor process as the Gleason score and the preoperative PSA level in the blood serum of patients. A high level of MCs infiltration of the PCa tissue was associated with a significant decrease in the two-year recurrence-free survival rates of the patients by 23.3% (p=0.0455). A high degree of MCs infiltration of the PCa tissue was associated with 1.2 times (p=0.0347) higher level of OPN expression and 1.7 (p=0.0051) and 1.65 (p=0.0087) times lower levels of miR-126 and miR-181a expression, respectively. The obtained results indicate the participation of MCs as a factor of the tumor microenvironment in the PCa progression.

Key words: prostate cancer, mast cells, osteopontin, tumor microenvironment, miRNA-126, miRNA-181a

Prostate cancer (PCa) is one of the most frequently diagnosed cancers in men worldwide [1]. The clinical picture of PCa is quite variable and can be characterized by a localized indolent or a rapidly progressing metastatic tumor process. It has been established that the features of the PCa growth and the intensity of metastasis are due to the morphological and molecular intertumoral and intratumoral heterogeneity of neoplasms. This creates diagnostic problems and has serious implications for planning a treatment strategy [2].

Understanding the clinical polymorphism of PCa is one of the biggest challenges faced by urologists and oncologists worldwide [3]. That is why the search for factors that would allow predicting the aggressive potential of PCa, taking into account the biological features of tumor cells, is highly relevant.

It is known that the tumor microenvironment (TME) plays a key role in the progression of many neoplasms, including PCa [4, 5]. TME is a complex dynamic system consisting of an extracellular matrix, stromal cells (fibroblasts, mesenchymal stromal cells, pericytes, adipocytes), and immune cells (including T- and B-lymphocytes, natural killer cells, tumor-associated macrophages, etc.), as well as networks of blood and lymphatic vessels [4, 6].

If the role of tumor-associated fibroblasts, which participate in the formation of the extracellular matrix by the secretion of type I and III collagens, and are also the main source of growth factors and cytokines, is well characterized and studied, then the importance of mast cells (MCs) in these processes remains insufficiently studied yet [7].

It is known that in some cancer types MCs can perform a tumor suppressor function, while in others they are characterized by oncogenic properties, stimulation of neovascularization and TME remodeling, and modulation of the immune microenvironment. This is primarily due to the wide range of effector molecules produced by MCs, which are usually divided into three categories. The first category includes effector molecules stored in granules, such as serotonin, histamine, heparin, tryptase, and chymase. The

second category includes lipid mediators (platelet-activating factor (PAF)), prostaglandins (PDG2), and leukotrienes (LTB4, LTD4) synthesized *de novo* during MCs stimulation. The third category includes various cytokines (IL-2, -3, -4, -5, -6, -8, -10, -11, -12, -13, 15, -33, TNF-α, IFN-γ), chemokines, growth factors (GM-CSF, TGF-β, VEGF, FGF-2, stem cell factor (SCF), nerve growth factor (NGF)), metalloproteinases (MMP-1, -2, -3, -9), and others [5, 8].

Infiltration of the tumor by MCs is observed in the tissue of patients with breast cancer [9], gastric cancer [10], ovarian cancer [11], colorectal cancer [12, 13], etc. Since the literature provides conflicting information about the importance of MCs in the development and progression of PCa, the aim of this study was to investigate the relationship between the degree of MCs infiltration and clinicopathological features of PCa and to find out the possible mechanisms of the participation of these cells in the formation of the aggressive course of the PCa development.

Patients and methods

The work is based on the analysis of the results of the examination and treatment of 60 patients with PCa of stages II–III who were treated at the National Cancer Institute of the Ministry of Health of Ukraine during 2015–2017. The patients were examined in accordance with the standards of diagnosis and treatment of patients approved by the orders of the Ministry of Health of Ukraine. The clinical diagnosis was established on the basis of the determination of the level of prostate-specific antigen (PSA) in blood serum, digital rectal examination, computed tomography of the pelvic organs, and/or transrectal ultrasound examination of the prostate gland and abdominal organs, osteoscintigraphy, radiography of the chest cavity. In all patients, the diagnosis was verified after transrectal multifocal prostate biopsy under ultrasound control. The patients with PCa were not treated with neoadjuvant therapy.

For morphological research, the material of surgically removed tumors was fixed in a 10% neutral formalin solution and further processing was carried out in accordance with the generally accepted principles of histological technique. 5 μm thick sections were prepared from paraffin blocks of the surgical material, stained with hematoxylin and eosin, and the morphological features of the tumor structure were studied using light microscopy.

After surgical treatment, the patients underwent follow-up for 2 years to identify the possible development of biochemical relapse, which was determined when the PSA level increased >0.2 ng/ml during two consecutive examinations.

The clinical characteristics of patients with PCa, whose average age was 61.8±6.5 years (in a range from 42 to 80 years) are shown in Table 1.

In 70.0% of patients, neoplasms were localized within the prostate gland (T2 category), in 30.0% of patients, the tumors spread beyond the capsule of the organ (T3 category). Morphological examination allowed to establish that all examined tumors were prostate adenocarcinomas, among which 53.3% were moderately differentiated adenocarcinomas – Gleason score ≤7, and 47.7% were poorly differentiated adenocarcinomas (Gleason score >7). According to PSA level, patients were divided into 2 groups: group I – PSA level up to 10 ng/ml (51.7%), and group II – higher than 10.0 ng/ml (48.3%). Biochemical recurrence of the disease was established in 30.8% of patients.

All procedures performed in the current study were approved by the Institutional Review Board and Research Ethics Committee of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine (protocol No 3 of 24.09.2021) in accordance with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

Histochemical method. In order to identify MCs, a method based on metachromatic staining of granules inside the cells with toluidine blue (Sigma-Aldrich, Burlington, VT, USA) was used. The preparations were examined using a Primo Star light microscope (Carl Zeiss, Oberkochen, Germany) [14].

Localization of MCs was considered intratumoral in cases when they were identified in the parenchymal component of the PCa or were located at the border of the stromal and parenchymal components of the tumor tissue. The stromal localization of MCs was considered when the location of MCs in the stromal component of the PCa had no visual contact with cancer cells.

The number of MCs was evaluated in 20 fields of view at a magnification of ×200 and presented as M ± m/unit area (1 mm^2), where M is the arithmetic mean and m is the

Table 1. Clinicopathological characteristics of patients with PCa.

Index	Number of patients	
	N	%
Total number of patients	60	100
Age of patients (years)		
Average	61.8±6.5	
Range	42–80	
Category T by TNM		
T2	42	70.0
T3	18	30.0
Gleason score		
≤7 points	32	53.3
>7 points	28	47.7
PSA level		
<10 ng/ml	31	51.7
>10 ng/ml	29	48.3
Biochemical relapse		
Established	21	35.0
Not found	39	65.0

standard error of the mean. The degree of MC degranulation was determined at ×1000 magnification. The MC degranulation index (DI) was calculated according to Lindner's formula [15]:

$$DI=(A\times0 + B\times1 + C\times2 + D\times3)/n,$$

where A are inactive MCs (granules are densely located in the cytoplasm, the nucleus is not is visualized), B – weakly degranulating MCs (the nucleus is well visualized, the granules are located inside the cell and do not go beyond the cytoplasmic membrane), C – moderately degranulating MCs (granules partially go beyond the boundaries of the intact cytoplasm), D – strongly degranulating MCs (completely degranulated MCs with a ruptured cytoplasmic membrane), n is the total number of analyzed mast cells. The obtained DI values were presented in arbitrary units (a.u.).

Immunohistochemical method. The study of the expression of osteopontin (OPN) in tumor cells was performed on 5 μm thick paraffin sections. Monoclonal antibodies specific to OPN (clone 441; Thermo Scientific, Waltham, MA, USA) were used as primary antibodies. To visualize the results of the reaction, a Mouse/Rabbit PolyVue Plus HRP/DAB Detection System reagents kit was used (Diagnostic BioSystems, Pleasanton, CA, USA) in accordance with the manufacturer's recommendations; sections were stained with Mayer's hematoxylin (Thermo Scientific Richard-Allan, Kalamazoo, Michigan, USA).

The analysis of the results of immunohistochemical studies was carried out by counting immunopositive cells using a Primo Star light microscope (Carl Zeiss, Oberkochen, Germany), at a magnification of ×400. In order to evaluate the expression of the studied molecules quantitatively, in immunocytochemical studies, the H-Score method was used by the formula:

$$S = 0^*N_0\ (\%) + 3\times N_1\ (\%) + 2\times N_2\ (\%) + 1\times N_3\ (\%),$$

where S is the "H-Score" index; N_0 – the number of cells with no expression; N_1, N_2, and N_3 – the number of cells with low, medium, and high expression, respectively. The final result of the calculation was expressed in points: from 1 to 100 points – low, from 101 to 200 – medium, and from 201 to 300 – high level of expression [16, 17].

Quantitative real-time polymerase chain reaction (PCR). The expression of miRNA-21, -126, -146a, -181a, and -221 was investigated using quantitative real-time PCR with the use of a QuantStudio 5 Dx Real-Time PCR System (Thermo Scientific, Waltham, MA, USA).

"RNeasy FFPE Kit" (QIAGEN, Hilden, Germany) was used to isolate total RNA from paraffin blocks of surgical material. The amount of isolated RNA was determined on a spectrophotometer "NanoDrop 2000c Spectrophotometer" (Thermo Scientific, Waltham, MA, USA). Single-stranded DNA was synthesized from 100 ng of total RNA using a standard method for reverse transcription. To perform PCR with reverse transcriptase (RT-PCR), we used the ready-made TaqMan™ MicroRNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA) according to the manufacturer's instructions, using a set of primers specific to the studied microRNA (Table 2).

Primer sequences for RT-PCR and real-time PCR were determined using the resource http://genomics.dote.hu:8080/mirnadesigntool/ and synthesized by Metabion, Germany. According to the Stem-loop primer sequences, for microRNA-125b and -181a the standard reverse primer 5'-GTGCAGGGTCCGAGGT-3' was used, as well as forward primers (Table 2).

The cDNA obtained was used for quantitative PCR, which was performed in triplicate using Maxima SYBRGreen/ROX qPCR MasterMix (Thermo Scientific, Waltham, MA, USA) in 96-well plates (Applied Biosystems, Waltham, MA, USA). RNU48 miRNA was used as an endogenous control to normalize miRNA expression indices. The sequences of RNU48 microRNA primers were taken from the resource https://www.ncbi.nlm.nih.gov: primer for cDNA synthesis: 5'-CTCTGACC-3', Forward 5'-AGTGATGATGACCCCAG-GTAACTC-3', Reverse 5'-CTGCGGTGATGGCATCAG-3'. Relative miRNA expression was calculated according to the method of K. Livak (2008). The average cycle threshold (Ct) value of the miRNA under study was normalized relative to the Ct of the endogenous control (expressed in a.u.). The

Table 2. Primer sequences for determining miRNA expression.

miRNA ID	Sequence of mature miRNA 5'→3'	Stem-loop primer for cDNA synthesis 5'→3'	Forward primer 5'→3'
hsa-miR-21-5p	UAGCUUAUCAGACUGAUGUUGA	GTTGGCTCTGGTGCAGGGTCCGAGG-TATTCGCACCAGAGCCAACTCAACA	GTTTGGTAGCTTATCAGACTGA
hsa-miR-126-3p	UCGUACCGUGAGUAAUAAUGCG	GTTGGCTCTGGTGCAGGGTCCGAGG-TATTCGCACCAGAGCCAACCGCATT	GGGTCGTACCGTGAGTAAT
hsa-miR-146a-5p	UGAGAACUGAAUUCCAUGGGUU	GTTGGCTCTGGTGCAGGGTCCGAGG-TATTCGCACCAGAGCCAACAACCCA	UGAGAACUGAAUUCCAUGGGUU
hsa-miR-181a-5p	AACAUUCAACGCUGUCGGUGAGU	GTTGGCTCTGGTGCAGGGTCCGAGG-TATTCGCACCAGAGCCAACACTCAC	GGAACATTCAACGCTGTCG
hsa-miR-221-3p	AGCUACAUUGUCUGCUGGGUUUC	GTTGGCTCTGGTGCAGGGTCCGAGG-TATTCGCACCAGAGCCAACGAAACC	GGAGCTACATTGTCTGCTG

change in miRNA expression compared to the control was calculated according to the formula $2^{-\Delta Ct}$ [18].

Statistical analysis. Statistical analysis was performed using the software package GraphPad Prism v. 8.00 (GraphPad Software Inc., San Diego, CA, USA). The Mann-Whitney U-test was used to quantitatively compare two independent groups. Analyzed data are presented as M ± m, where M is the arithmetic mean and m is the standard error of the mean. Patient survival was analyzed using the Kaplan-Meier method, and reliability between curves was analyzed using the log-rank test. The critical level of statistical significance was taken as equal to 0.05.

Results

After histochemical staining of paraffin sections with toluidine blue, tumor cells and normal epitheliocytes of the prostate gland acquired a light blue color while the connective tissue remained practically unstained (Figures 1A, 1B). In MCs, a metachromatic purple coloration of the granules was observed, while the nuclei and basophilic structures of the cytoplasm of the cells acquired a blue color. In the PCa tissue, MCs were localized mainly in the connective tissue stroma adjacent to neoplastic interacinar structures or located near vessels. The study of the quantitative indices of MCs infiltration of the PCa tissue established the average value of 5.79±0.57 cells/mm² with individual fluctuations from 0.95 cells/mm² to 25.0 cells/mm² (Figures 1C), the median level of MCs infiltration was 4.07 cells/mm². The analysis of the MCs infiltration of the PCa tissue has shown that this index was 1.3 times (p=0.0089) higher in the stromal component compared to the intratumoral component. It is also worth noting that the average value of MCs DI in the PCa tissue, as one of the main indicators of the functional activity of these cells, was 1.02±0.24 a.u. with individual fluctuations from 0.5 a.u. up to 2.2 a.u.

Next, the relationship between the degree of MCs infiltration of neoplasms and the clinicopathological parameters of PCa was analyzed. As shown in Table 3, in patients with neoplasms that spread beyond the organ capsule (T3 category), the number of MCs localized in the intratumoral region was 1.6 times (p=0.0320) higher compared to the group of patients with tumors localized in the prostate gland (T2 category). There was no significant difference in the total levels of MCs infiltration and the degree of infiltration of the stromal component depending on the category T by TNM.

It was established that the level of MCs infiltration in the PCa tissue was 1.5 times (p=0.0204) higher in the tumors with a Gleason score >7, compared to tumors with a Gleason score ≤7. It is also worth noting that in tumors with a high Gleason score, the level of MCs infiltration of intratumoral zones was 2.1 times higher (p=0.0041) compared to a similar index in tumors with a Gleason score ≤7. The level of MCs infiltration of the stromal component did not depend on the PCa differentiation grade (p>0.05).

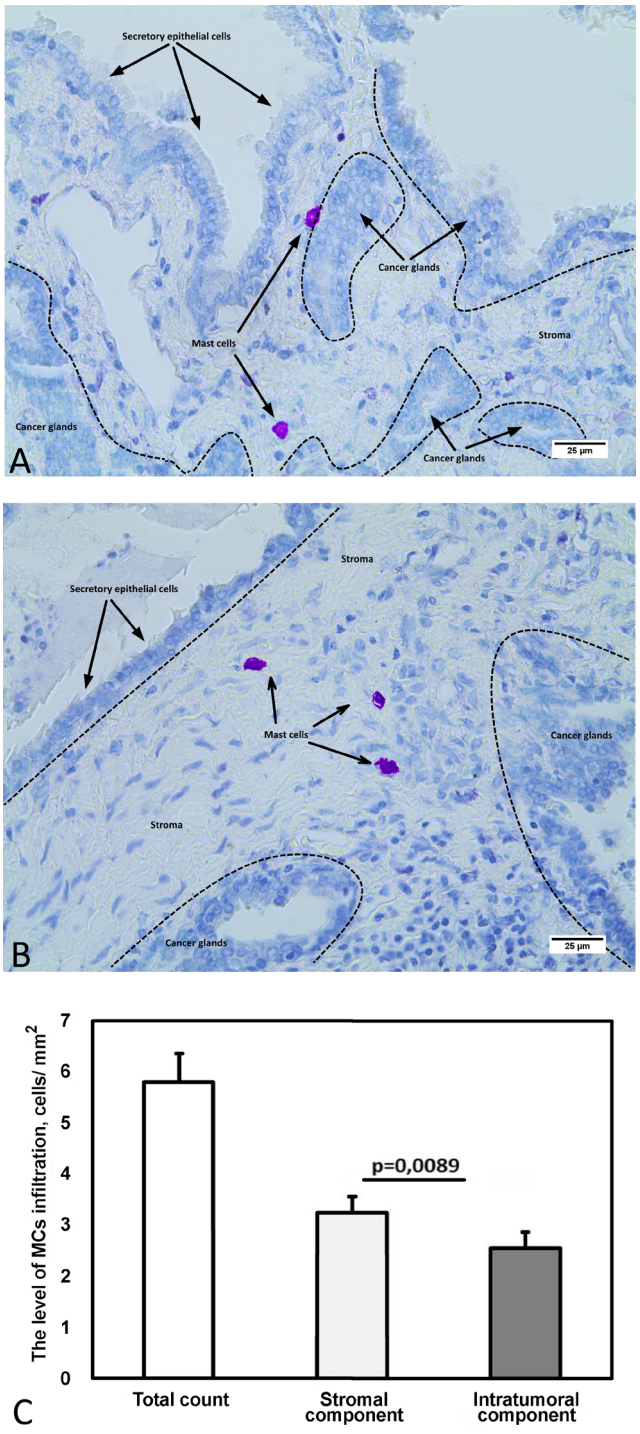

Figure 1. Features of MCs' infiltration of PCa tissue: A, B) Representative photos of PCa tissue after staining with toluidine blue; C) Quantitative indices of MCs infiltration of PCa tissue. Mast cells can be identified by the presence of metachromatic purple coloration of the granules after metachromatic staining with toluidine blue. In our study, MCs were localized mainly in the connective tissue stroma adjacent to neoplastic interacinar structures or located near vessels. The study of the quantitative indices of MCs infiltration of the PCa tissue established that the infiltration index was 1.3 times (p=0.0089) higher in the stromal component compared to the intratumoral component.

We have revealed that in patients with a PSA level higher than 10 ng/ml the total level of MCs infiltration was 1.3 times (p=0.0371) higher compared to the group of patients with a PSA level lower than 10 ng/ml. There was no significant difference in the infiltration rates of MCs of the stromal and intratumoral components depending on the preoperative PSA level in blood serum.

When analyzing the DI of MCs depending on the clinical and pathological features of the tumor process, the highest indices were recorded in the group of patients whose tumors spread beyond the organ capsule (1.28±0.17 a.u., p=0.0463) and in tumors of a high Gleason score ≥8 points (1.20 ± 0.11 a.u., p=0.0405) compared to neoplasms of the T2 category and tumors with Gleason score ≤7 points (0.95±0.05 a.u., and 1.20±0.11 a.u., respectively).

So, we have demonstrated that the increase in the level of infiltration and degranulation of MCs in the PCa tissue is associated with a Gleason score and the preoperative PSA level in the blood serum of patients. The growth of neoplasms outside the capsule of the organ is accompanied by an increased count of MCs localized in the intratumoral region.

In order to find out the prognostic value of MCs in the PCa tissue, we conducted a study of the recurrence-free survival of patients taking into account the value of the median level of MCs infiltration. As can be seen from the data presented in Figure 2, a significant decrease in the two-year recurrence-free survival rate by 23.3% was found in patients with a high level of MCs infiltration of the PCa tissue (p=0.0455). Thus, the presence of a high MCs count in the tumor tissue is associated with an unfavorable course of PCa, which is characterized by a high degree of malignancy according to clinical and pathological indices.

In order to elucidate the mechanisms causing the association of MCs with the degree of PCa malignancy and progression, we analyzed the relationship of OPN expression depending on the level of MC infiltration. According to the literature, OPN is one of the chemoattractants of MCs, which stimulates their migratory activity, and is involved in the regulation of IgE-mediated degranulation [19]. We revealed that a high degree of MCs infiltration (>Me) of the PCa tissue was associated with a 1.2-fold increase (p=0.0347) in the level of OPN expression in tumor cells compared to the PCa tissue with a low level of MCs infiltration (Figure 3). The OPN level was also correlated with the topology of MCs infiltration of PCa tissue: the highest indices of OPN expression (p=0.0442) were recorded in the PCa tissue with a predominance of intratumoral MCs localization, in contrast to similar indices with MC localization mainly in the stromal component (Figure 3).

So, the results obtained by us indicated a direct relationship between the degree and topology of MCs' infiltration with the level of OPN expression in the PCa cells and confirmed the participation of this protein in the regulation of the functional activity of MCs.

Table 3. The relationship between infiltration rates and functional activity of MCs with clinical and pathological characteristics of patients with PCa.

Index	The infiltration rates of MCs, cells/mm²			DI, a.u.
	Total	Localization of MCs		
		Stromal	Intratumoral	
Category T by TNM				
T2	4.20±0.26	2.50±0.17	1.69±0.13	0.95±0.05
T3	5.54±0.88	2.84±0.32	2.69±0.56[1]	1.28±0.17[1]
Gleason score				
≤7	4.28±0.27	2.72±0.16	1.56±0.18	0.95±0.06
>7	6.31±0.94[2]	3.00±0.45	3.30±0.67[2]	1.20±0.11[2]
PSA level				
<10 ng/ml	4.14±0.43	2.36±0.20	1.78±0.29	0.85±0.15
>10 ng/ml	5.55±0.68[3]	3.28±0.36	2.28±0.45	1.17±0.08

Notes: [1]p<0.05 in comparison with the corresponding indices in patients with PCa of T2 category by TNM; [2]p<0.05 in comparison with the corresponding indices in patients with PCa of Gleason score ≤7; [3]p<0.05 in comparison with the corresponding indices in patients with a PSA level <10 ng/ml

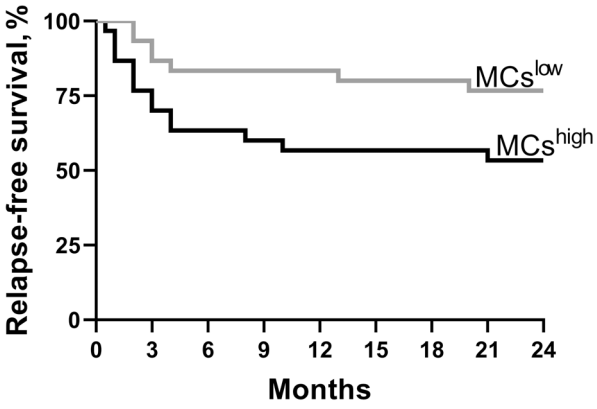

Figure 2. Relapse-free survival of patients depending on the MCs infiltration rate of PCa tissue (the Kaplan-Meier method, log-rank test). We observed a significant decrease in the two-year recurrence-free survival rate by 23.3% in patients with a high level of MCs infiltration of PCa tissue (p = 0.0455). Thus, the presence of a high MCs count in the tumor tissue is associated with an unfavorable course of PCa, which is characterized by a high degree of malignancy according to clinical and pathological indices.

With the aim of an in-depth study of the epigenetic component in the mechanisms of MC functioning, we conducted a study of microRNA expression in the PCa tissue. Based on literature data, as well as the results of the bioinformatic analysis, we selected 5 miRNAs – hsa-mir-21-5p, hsa-mir-126-5p, hsa-mir-146a-5p, hsa-mir-181a-5p, and hsa-mir-221-5p (Figure 4), involved in regulation of MCs and *SPP1* (OPN) gene expression.

The analysis of the obtained data made it possible to establish (Figure 4) that a high degree of MCs infiltration of the PCa tissue was associated with a decreased expression of miR-126 and miR-181a in the tumor sample. In particular, the level of miR-126 and miR-181a in the PCa tissue with a

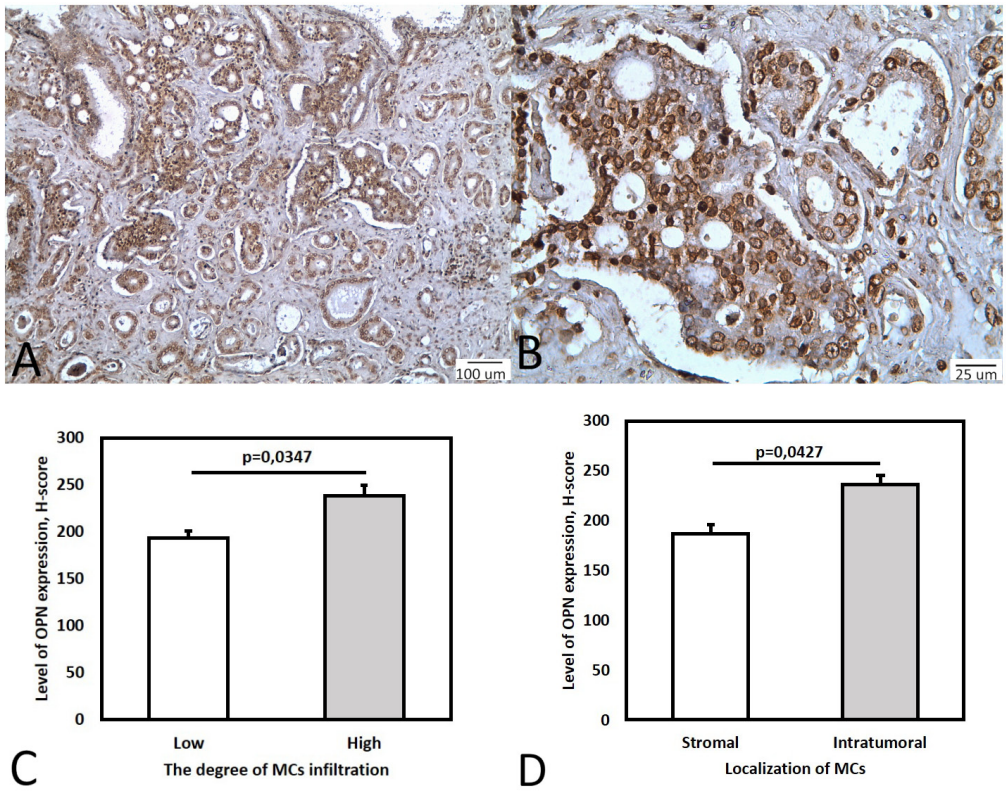

Figure 3. Expression of OPN in the PCa tissue: A, B) Expression of OPN in the PCa tissue. Immunohistochemistry, chromogen 3-diaminobenzidine tetrachloride. Staining with Mayer's hematoxylin (A 100×; B 400×); C) levels of OPN expression in the PCa tissue depending on the degree of MCs infiltration; D) the expression levels of OPN in the PCa tissue depending on the localization of MCs. A high degree of MCs infiltration of the PCa tissue was associated with a 1.2-fold increase (p=0.0347) in the level of OPN expression in tumor cells compared to the PCa tissue with a low level of MCs infiltration. The OPN level also correlates with the topology of MCs infiltration of PCa tissue: the highest indices of OPN expression (p=0.0442) were recorded in the PCa tissue with a predominance of intratumoral MCs localization, in contrast to similar indices with MC localization mainly in the stromal component.

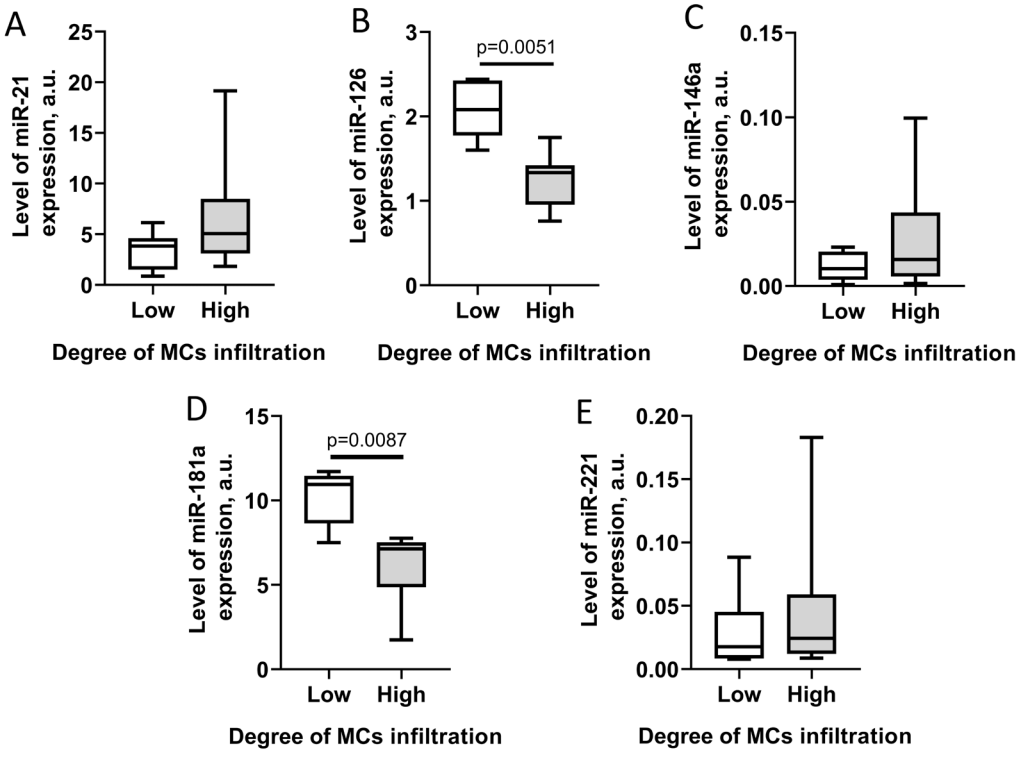

Figure 4. The relationship between miRNA expression indices and the degree of MC infiltration of the PCa tissue. A high degree of MCs infiltration of the PCa tissue was associated with a decreased expression of miR-126 and miR-181a in a tumor sample. In particular, the level of miR-126 and miR-181a in the PCa tissue with a low degree of MCs infiltration was 1.7 (p=0.0051) and 1.65 (p=0.0087) times higher compared to neoplasms with a level of MCs infiltration lower than median level (4.07 cells/mm^2). No correlation was found between the level of MCs infiltration in the PCa tissue and the expression levels of miR-21, miR-146a, and miR-221 (p>0.05).

low degree of MCs infiltration was 1.7 (p=0.0051) and 1.65 (p=0.0087) times higher compared to neoplasms with a level of MCs infiltration lower than Me. No correlation was found between the level of MCs infiltration in the PCa tissue and the expression levels of miR-21, miR-146a, and miR-221 (p>0.05).

Thus, the results obtained by us indicated the possible participation of miR-126 and miR-181a in the regulation of the functional activity of MCs in the PCa tissue.

Discussion

The role and significance of MCs in the occurrence and progression of PCa remains debatable, which may be due to different methodological approaches (the use of experimental or clinical materials and different markers and methods), as well as the heterogeneity of the MCs population [20–22]. Moreover, there are data indicating the dependence of MCs functions on their microenvironment [23, 24]. That is why we conducted a study of the relationship between the degree of infiltration and the topology of MCs in the PCa tissue and indices of the aggressiveness of the tumor process.

According to the results of our study, MCs were localized mainly in the stromal component of the prostate tumors. At the same time, the degree of MCs infiltration of the PCa tissue was significantly higher in poorly differentiated neoplasms (Gleason score >7) and in patients with a preoperative PSA level >10 ng/ml, and was associated with low rates of patients' survival. This confirms the previously published results of Nonomura et al. (2007), who demonstrated a higher density of MCs in poorly differentiated adenocarcinomas of the prostate gland (Gleason score >8) in patients with an unfavorable course of the tumor process and low rates of recurrence-free survival [23].

According to the data obtained, the high degranulation activity of MCs was a characteristic feature of low-differentiated adenocarcinomas that spread beyond the prostate capsule. The mentioned facts confirmed the dysfunction of MCs during the PCa progression. In our opinion, this can be explained by the secretion of IL-8 and histamine by MCs, which act as chemotactic factors for immune cells and as tumor mitogens. Along with this, MCs produce various matrix metalloproteinases (for example, MMP-9) and proteases (tryptases and chymases) regulating the proteolysis of extracellular matrix proteins and disrupting the physiological communication between the stroma and epithelium, in such a way contributing to the detachment of tumor cells, their migration and invasion [24].

In general, the increase in the degree of MCs infiltration in neoplasms of various genesis, including PCa, is due to the production of various chemotactic factors by malignantly transformed cells, as well as TME cells. These include SCF,

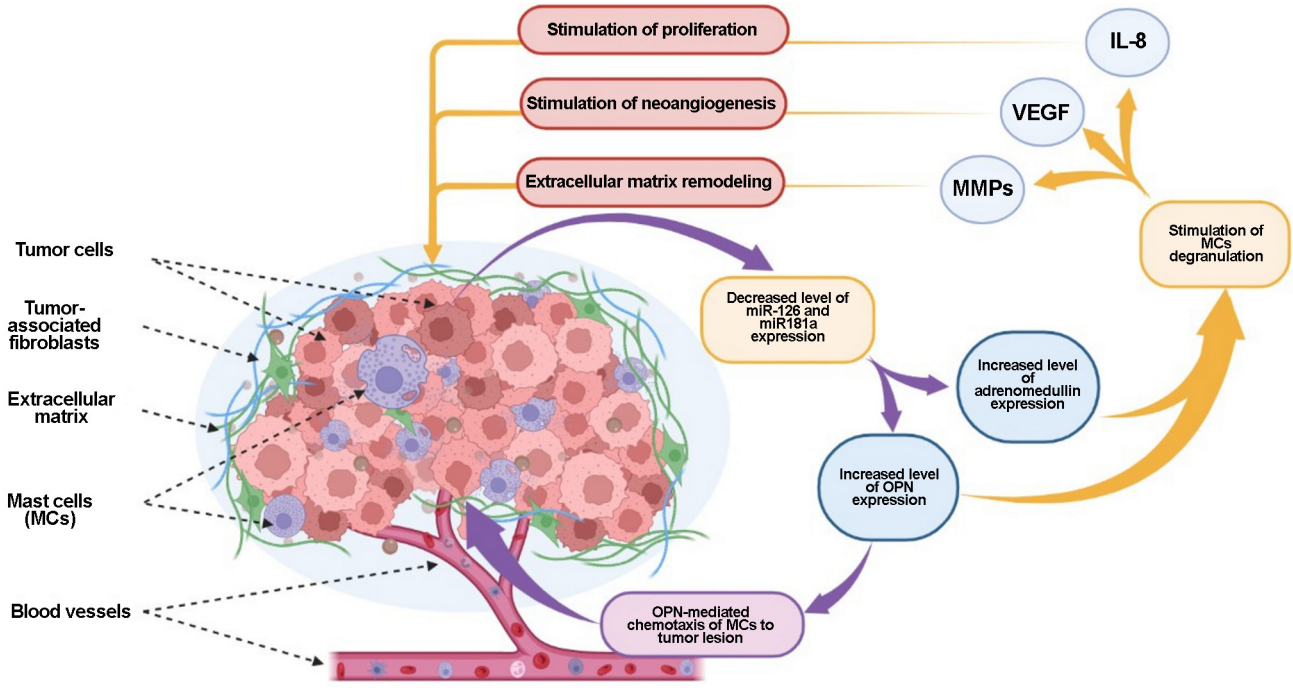

Figure 5. The role of MCs in the progression of PCa. Decreased levels of miRNA-126 and miRNA-181a expression induce an increase in the expression of OPN and adrenomedullin in tumor cells, which promotes the migration of MCs progenitor cells to the tumor center where their maturation and activation occurs. At the next stage, under the influence of these factors, stimulation of MCs degranulation and release of heparin, IL-8, MMP-9, and VEGF occurs. This leads to the activation of the proliferation of tumor cells, remodeling of the extracellular matrix, and neoangiogenesis, which, finally, can lead to PCa progression.

monocyte chemotactic protein-1 (MCP-1), VEGF, angiopoietin 1 (Ang1), IL-8, CCL2, CXCL1, CXCL10, and OPN, which, in addition to recruiting MCs progenitor cells, are also capable of inducing their maturation [25, 26]. The given data confirm the results obtained by us, which indicate a direct relationship between the degree of MCs infiltration, the features of their intratumoral localization, and the level of OPN expression in PCa cells. According to the results of Nagasaka et al. (2008), OPN can stimulate MCs' degranulation and, as a result, contribute to PCa progression. Moreover, OPN is expressed in MCs as well, which indicates its possible autocrine regulation of MCs functions [19]. In this context, it should also be mentioned that according to our previous studies carried out *in vitro*, high levels of OPN expression are characteristic of human PCa cell lines of high malignancy grade [27].

Another protein that is expressed by tumor cells and can stimulate MCs degranulation through the PI3K-AKT signaling pathway is adrenomedullin [28]. It has been established that high levels of this protein inhibit the proliferation of PC-3 and LNCaP cells (i.e., human PCa cell lines), however, this effect is not observed in DU-145 cells with a high degree of malignancy. These facts once again point to the possible involvement of MCs in the PCa progression [29].

In the study of epigenetic disorders of the regulation of MCs in PCa tissue, we recorded high levels of expression of miR-126 and miR-181a in neoplasms with a low level of MCs' infiltration, which was characteristic of adenocarcinoma of high and moderate differentiation grades and patients with a PSA level of less than 10 ng/ml. These results are consistent with the report of Hua et al. (2018) and Song et al. (2016) on the tumor suppressor properties of miR-126 and miR-181a [30, 31]. It is worth noting that miR-126 can also stimulate the proliferation of MCs and their IgE-mediated degranulation [32]. In addition, according to the data of Lukianova et al. (2022), miR-126 and miR-181a are directly involved in the regulation of OPN expression, which may indicate the involvement of these miRNAs in the regulation of MC recruitment to the tumor site [27].

Based on the results of our research, as well as the information available in the scientific literature, we proposed a concept that reflects the role of MCs in the progression of PCa (Figure 5).

In particular, decreased levels of miRNA-126 and miRNA-181a expression induce an increase in the expression of OPN and adrenomedullin [33] in tumor cells, which promotes the migration of MCs progenitor cells to the tumor center where their maturation and activation occurs. At the next stage, under the influence of these factors, stimulation of MCs degranulation and release of heparin, IL-8, MMP-9, and VEGF occurs. This leads to the activation of the proliferation of tumor cells, remodeling of the extracellular matrix, and neoangiogenesis, which, finally, can lead to PCa progression.

In conclusion, the obtained results indicate the participation of MCs as a factor of the tumor microenvironment in PCa progression, which indicates the need for further research focused on their potential use for predicting the aggressiveness of the course of the tumor process.

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