

MAST CELL PHENOTYPE IN BENIGN AND MALIGNANT TUMORS OF THE PROSTATE

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The molecular phenotypic heterogeneity of mast cells (MCs) makes them attractive as potential therapeutic targets in anti-cancer adjuvant therapy. Mast cell aggregations observed in tumors suggested their involvement in tumor pathogenesis. Despite several studies using mast cell tryptase, MCs' involvement in the progression of prostate tumors has not been demonstrated. The aim of our study was to identify and quantify the phenotypic heterogeneity of MCs in prostate lesions. Our study included 7 cases of normal prostate, 25 cases of benign epithelial hyperplasia and 64 cases of prostate carcinoma. MCs were immunohistochemically assessed using three markers: tryptase, chymase and CD117. Two immunophenotypes of MCs were identified in benign lesions: tryptase+/CD117+/chymase- and tryptase-/chymase+/CD117+, located in peritumoral areas. Intratumoral MC phenotype of malignant lesions was characterized by tryptase+/chymase+/CD117+, while in the peritumoral areas three different MCs phenotypes were identified: tryptase+/chymase+/CD117-, tryptase+/CD117+/chymase- and chymase+/CD117+/tryptase-. Our results suggest the correlation of chymase positive MCs of the peritumoral areas and CD117 positive MCs of the intratumoral areas with tumor grade.

Key words: tryptase positive mast cells, chymase, CD117 (c-kit), prostate cancer, prostatic benign hyperplasia.

Introduction

Prostate pathology is a common problem in men over 50 years. According to the World Health Organization (WHO), life expectancy will significantly increase in the next decades, and therefore it is expected that the number of patients with prostate tumor processes will increase also [1]. Prostate carcinoma (PCa) and benign prostatic hyperplasia (BPH) are the most common diseases affecting the prostate, and constitute over 90% of all prostate diseases. Although many tissue markers with diagnostic and therapeutic impact have been analyzed in the last decades, the prognosis of prostate diseases remains elusive.

Mast cells (MCs) are present in several organs, including the lung, skin, heart, and digestive system. Multiple evidence sustains MCs in normal and especially in malignancy of various organs as having various phenotypes, mediators content and different response to various stimuli [2-5]. MCs are investigated for their involvement in angiogenesis, tissue remodeling and stroma immunomodulation of human cancers, but the role of mast cells remains controversial. MCs can exert pro- or anti-tumor effects depending on tumor type and tumor microenvironment [6].

The morphologic and functional complexity of MCs could explain the discrepancy between data from different studies showing the possible influence

of MCs in prognosis and survival of patients with various malignancies [7]. In this context, prostate cancer is not an exception. Several studies have attempted to solve this problem [8-12], but there are no certified and convincing data about the actual role of MCs as promoters or suppressors of cancer and/or benign lesions of the prostate.

Based on the previous stated facts and controversies, we aim to study MCs' role in benign and malignant lesions of the prostate, by a qualitative and quantitative analysis of MCs, regarding distribution and immunophenotypes of MCs in normal, benign and malignant conditions of the prostate. Our study proposed to use three different antibodies to better characterize phenotype and mast cell distribution heterogeneity suggested by previous studies for prostate pathology.

Material and methods

89 biopsies were collected from patients with prostate pathology, including 25 cases of BPH and 64 cases of PCa. Specimens were fixed in buffer formalin and paraffin embedded. Three-micrometer thick step sections were performed for each case. One slide from each case was stained with hematoxylin-eosin, for pathologic diagnosis, Gleason score assessment and case selection for immunohistochemistry. The control group was represented by seven autopsy specimens taken within 24 hours after death from persons with no certified history of prostate pathology.

Immunohistochemistry included primary antibodies as mast cell tryptase, mast cell chymase and CD117. Before incubation with primary antibodies, we performed heat-induced epitope retrieval with pH 6.0 citrate solution (Novocastra, Newcastle upon Tyne, UK) for 30 minutes. Endogenous peroxidase blocking was performed with 3% hydrogen peroxide for 5 minutes. This step was followed by 30 minutes incubation with primary antibodies as mast cell tryptase (Dako Glostrup Denmark, dilution 1 : 300, clone AA1), mast cell chymase (NeoMarkers Fremont, CA, ready to use, clone CC1) and CD117 (Novocastra, Newcastle upon Tyne, UK, ready to use, clone T595). Bond Polymer Refine Detection System (Leica Biosystems, Newcastle upon Tyne, UK) was used and 3,3 diamino-benzidine dihydrochloride was applied as chromogen followed by hematoxylin counterstaining. All immunohistochemistry steps were performed with Leica Bond-Max (Leica Biosystems, Newcastle upon Tyne, UK) autostainer. Image acquisition and data analysis were performed using a Nikon Eclipse E 600 microscope and Lucia G software for microscopic image analysis. The local research ethics committee approved the protocol of the study and patients' informed consent was obtained from all subjects according to the World Medical Association Declara-

tion of Helsinki. Quantification of mast cell density (MCD) was made by the hot-spot method. Areas with the highest MC density were chosen by scanning the entire specimen section at low magnification (40× or 100×) followed by selection of three fields (inter-glandular/intratymoral and peri-glandular/peritymoral). The individual mast cells were counted at 200× magnification. MC counting was manually done by calculating the average number for the three selected fields of the inter-glandular/intratymoral and periglandular/peritymoral areas. Each microscope field corresponded to an area of 0.74 mm². The images were made with the optical microscope Nikon Eclipse E600 camera. Statistical analysis was performed using SPSS13.0 and Microsoft Excel 2010 software.

Results

Distribution of mast cells in normal prostate

For normal tissue specimens, MCs were found distributed in all three main histological zones of the prostate. Differences in their morphology were observed depending on their different location. Often, these cells showed a more elongated shape, found near the basal membrane of glandular epithelium. In addition, MCs were present in the axis of the glandular fold. MCs were predominantly located in the periglandular areas, close to blood vessels of prostate stroma. MCs of the periglandular areas showed a high degree of degranulation compared with those from inter-glandular areas. The density of MCs positive for tryptase in the periglandular stroma was 20.4 ± 1.3 compared with interglandular stroma, where this density was 11.7 ± 0.7 . Chymase-positive MCs have been characterized as much smaller than tryptase-positive MCs and are most commonly spread in the periglandular stroma, usually arranged in groups or nests. Mast cell density (MCD) of chymase-positive cells per area is much lower than for tryptase-positive MCs; thus in the inter-glandular areas the average was 3.9 ± 1.3 and in the periglandular areas it was 10.2 ± 1.2 . Higher density of c-kit positive MCs was observed in the periglandular stroma (13.9 ± 1.8) than in the inter-glandular stroma (9.8 ± 0.9).

The results found for normal prostate tissue suggested the existence in normal prostate of three MC types having distinct phenotypes. A particular observation was that MCs have the highest density inside peri-glandular stroma compared with inter-glandular stroma.

The intra-glandular quantification of tryptase, chymase and CD117 in the normal prostate showed a statistically significant correlation ($p = 0.030$) between the expression of tryptase and chymase. Thus,

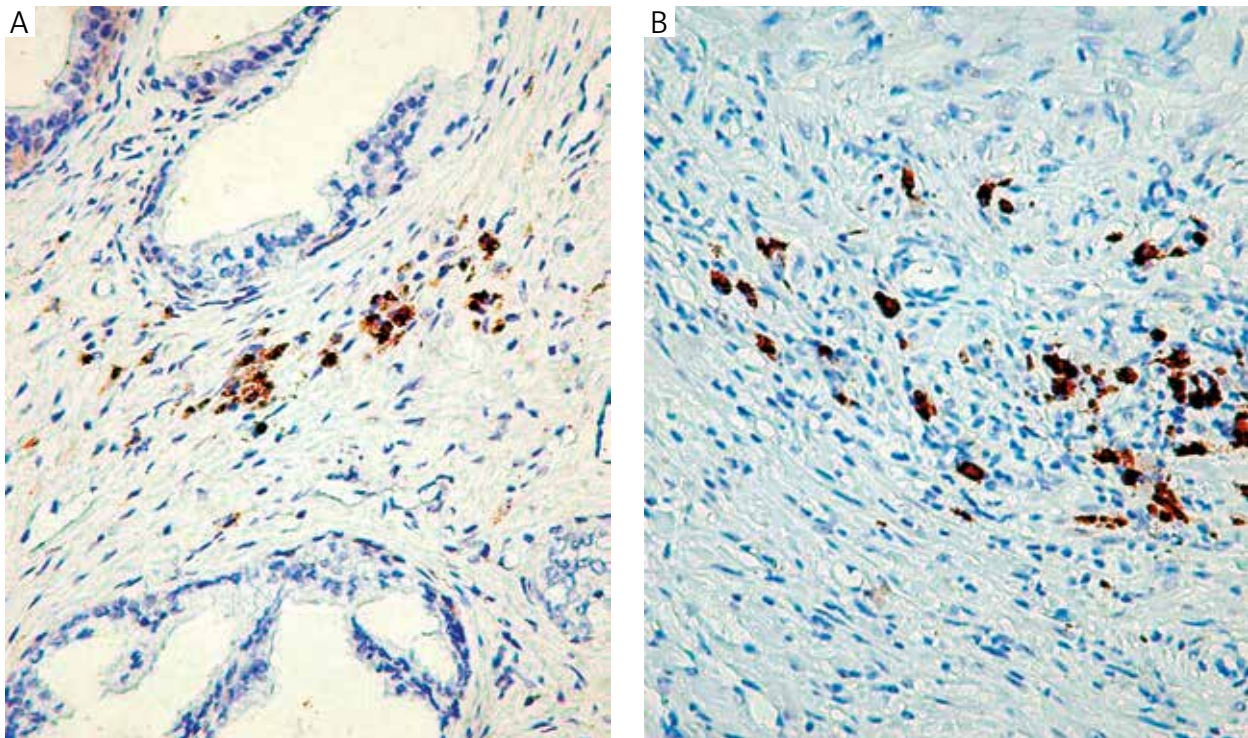


Fig. 1. Immunohistochemical staining for CD117 in benign proliferative lesions. Localization of c-kit positive MCs, forming cords (A) or cluster (B). Original magnification 100×

a unique tryptase+, chymase+, CD117-intraglandular phenotype was observed.

For the periglandular area, mast cell phenotype tryptase+, chymase-, CD117+ was quantified.

Distribution of mast cells in benign proliferative lesions

The distribution of tryptase positive MCs in the BPH did not differ from the results obtained for normal prostate tissue. MCs were found along the blood vessel grouped in small cell clusters. Peritumoral mast cell shape was round or oval, and for intratumoral areas elongated and often located in the glandular fold. MC density in intratumoral areas increased. C-kit (CD117) positive MCs had a particular distribution. The distribution of MCs was not uniform, most often forming clusters (groups) of cells or being distributed in cords. This distribution was characteristic for both peritumoral and intratumoral stromal areas (Fig. 1).

A higher density of CD117-positive MCs accompanied inflammatory infiltrates from some BPH specimens. Distribution of chymase-positive MCs did not differ from results obtained in normal prostate. Tryptase-positive MCs are distributed uniformly inside the inflammatory zone. The c-kit positive MCs have a predilection for the peripheral zone of inflammatory infiltrate. The density of MCs in BPH was in the normal range, maintaining a higher distribution in peritumoral areas. MCD data, depending on the type of the marker, are recorded in Table I.

The intratumoral and peritumoral ratios of three MC types were different; thus the intratumoral and peritumoral expression for each marker showed a statistically significant correlation for tryptase ($p = 0.001$), CD117 ($p = 0.008$) and chymase ($p = 0.033$). There are no significant statistical correlations between tryptase-positive MCs and chymase-positive cells, tryptase and CD117, as well as chymase and CD117 in the intratumoral zones. This fact suggest the existence of a heterogeneous MC population. Quantification of MCs in the peritumoral area, using three types of markers, showed a significant correlation between tryptase and CD117 ($p = 0.011$) and between chymase and CD117 ($p = 0.022$). These results highlight the presence of two immunophenotypes of MCs: tryptase+CD117+ chymase- and chymase+CD117+ tryptase-.

Mast cells in malignant proliferative lesions

Tryptase-positive MC density decreased in the intratumoral versus peritumoral areas. Intratumor MCs were distributed among isolated tumor cells. We noted that in 13 cases (20.3%) intratu-

Table I. Mean density of MCs in peritumoral and intratumoral areas for the three markers

	TRYPTASE	CHYMASE	CD117
Peritumoral	26 ± 0.9	26.9 ± 1.3	23.6 ± 1.6
Intratumoral	23.4 ± 0.9	3.8 ± 0.4	7.1 ± 0.6

moral density of tryptase-positive MCs was higher than in the peritumoral areas. The same observation was noted just in 7 cases (10.9%) of PCa for CD117-positive MCs and in only 1 case (1.6%) for chymase-positive MCs. These differences do not correlate with the histologic type of PCa or Gleason score. The overall density of MCs in investigated areas was for tryptase-positive MCs: intratumoral – 13.7 ± 1.1 and peritumoral – 16.3 ± 0.7 , for CD117-positive MCs: intratumoral – 6.3 ± 0.9 and peritumoral – 11.8 ± 0.7 ; and for chymase-positive MCs: intratumoral – 3.1 ± 0.3 and peritumoral – 8.6 ± 0.6 .

The MCs are arranged around the epithelial tumor cell area and rarely intraepithelially, as well as in close vicinity to blood vessels. Intravascular MCs or MCs inserted between endothelial cells of blood vessels were observed for some undifferentiated tumors (Fig. 2). Intravascular localization of MCs was more obvious for peritumoral stromal areas. Most MCs from peritumoral areas showed a high degranulation rate. This feature is common for all MC phenotypes (tryptase+, chymase+, as well as the CD117+). In 12 cases of prostate carcinoma (18.7%) we found tumor cells' cytoplasmic expression of tryptase. Also, we observed cytoplasmic CD117 expression in tumor cells in 15 cases (23.4%) that did not correspond to the cases of tryptase expression (Fig. 3). The total average of MCs for all three markers in normal stroma was higher than in tumor stroma of malignant lesions.

Tryptase expression in malignant lesions was significantly correlated with intratumoral and peritumoral MCs ($p = 0.001$). The same correlation was demonstrated for CD117 ($p = 0.001$). For chymase a partial significant correlation was obtained (Kendall = 0.024, Spearman = 0.017). The peritumoral tryptase positive MCs were significantly correlated with both chymase ($p = 0.001$)

and CD117 ($p = 0.001$), but not intratumoral correlation of chymase with CD117 was observed. These results suggested the presence inside the tumor areas of two distinct MCs phenotypes: chymase+/tryptase+/CD117– and chymase–/tryptase+/CD117+.

Peritumoral areas were characterized by statistically significant correlations between all three immunophenotypes, compared to the intratumoral phenotype of the MCs. Peritumorally, there was demonstrated the presence of one more particular phenotype: chymase+/tryptase+/CD117+.

The prognostic impact of MC phenotype was determined by correlating the total number of MCs and Gleason score, based on the results described above in the malignant lesions. Relationships between Gleason score and studied markers (tryptase, chymase and CD117) were highly heterogeneous. Peritumoral tryptase-positive MCs were partially correlated with Gleason score (Kendall = 0.037, Spearman = 0.038, but not Pearson), as well as peritumoral chymase-expressing MCs (Kendall = 0.044, Spearman = 0.033, nor for Pearson).

In the intratumoral areas there was no statistically significant correlation for tryptase and chymase with Gleason score. A particular aspect was the significant partial correlation between CD117 expression in MCs and Gleason score, given that the other two types of MCs (tryptase and chymase) were not correlated with Gleason score.

The above data, as well as CD117 expression by tumor cells in some prostate tumors, suggest the involvement of c-kit positive MCs in the initiation and maintenance of the malignant process.

Discussion

Prostate cancer is the most common male cancer and the second leading cause of cancer death after

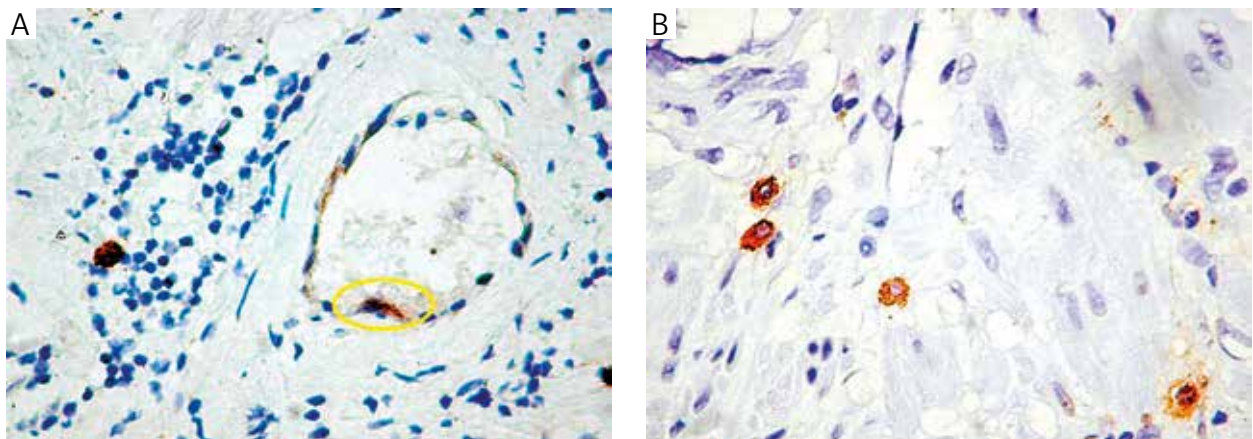


Fig. 2. CD117+ MCs inserted between endothelial cells of one blood vessel from prostate carcinoma (A, magnification 200 \times) and tryptase+ MCs trapped between epithelial tumor cells from prostate carcinoma (B, magnification 400 \times)

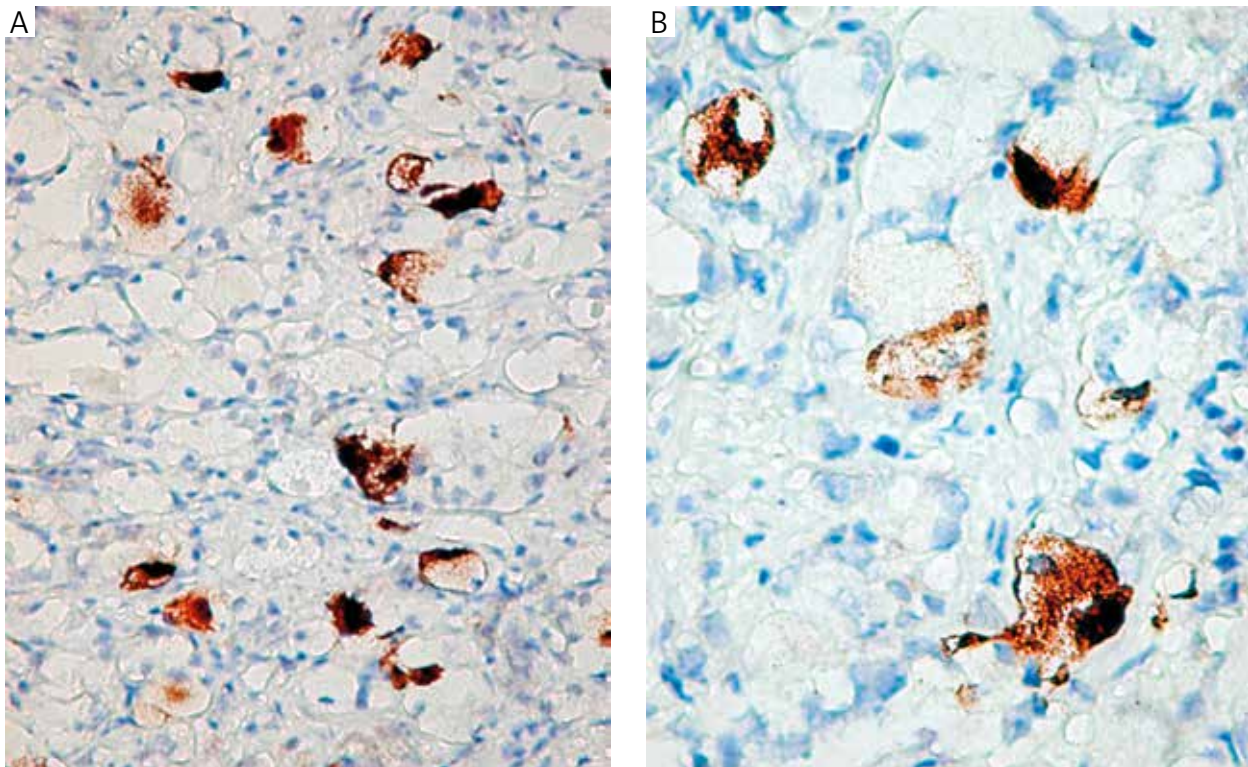


Fig. 3. Immunohistochemical staining for CD117 in prostatic carcinoma. Cytoplasmic expression of CD117 by epithelial tumor cells. Original magnification: A) 100 \times ; B) 200 \times

lung malignancies. Changes in the stroma can initiate the development of BPH and epithelial-stromal interactions may play a role in malignant progression [13-17].

The role of prostate stromal cells is little studied, both in normal stroma and in malignant and benign pathology of the prostate. Activation of the stromal microenvironment is believed to be an important element in the growth and progression of adenocarcinoma. The first step in understanding the stromal-tumor interaction is to define the role of each stromal cell at one moment of the development of benign and malignant prostate lesions. Reactive stroma is a mixture of fibroblasts, endothelial cells, myofibroblasts, MCs and other immune cells. The mast cell is one of the most controversial stromal cells, their role being proved to be complex and thus incompletely elucidated at this moment. Several studies have suggested the potential involvement of MCs in the pathogenesis of various tumors as melanocytic skin lesions [18] or squamous cell carcinomas [19] but others showed the relationship between MCs and tumors due to their cytotoxic action on tumor cells [20, 21] or their ability to deliver products with an anti-tumor effect [22]. Multiple conflicting results about accumulation of MCs in tumor conditions have been reported [23]. It is not known whether MCs are designed to stimulate or inhibit tumor cells' spread [24], but a correlation

between MCs and survival in various cancers was observed [25].

Little attention has been paid to the role of MCs in patients with malignant tumors of the prostate, MCs being more studied regarding their influence on angiogenesis than on tumor biology [26, 27]. The reports are controversial, some showing an increase in the number of MCs in PCa [8, 10], while others state that the number of such cells is not changed or is decreased [11]. The lack of information about the role of mast cells in benign and malignant prostate transformations derives from incomplete immunophenotype characteristic of MCs not only in benign or malignant lesions, but also in normal stroma. Thus, in our study, we used three markers – anti-mast cell tryptase, chymase and CD117 – which allow a complete characterization of MC immunophenotypes. C-kit, tyrosine kinase receptor, in general, has been shown to be important for tumor growth and progression in many types of cancer [28], and mutation of c-kit has a central pathogenic role, for example, in gastrointestinal stromal tumors [29]. The present study demonstrated a high density of MCs in the stroma of normal prostate. Our results suggested different mast cell phenotypes in normal prostate stroma, which could explain the dual role of MCs in different tumors.

Using the same panel of markers in the benign and malignant lesions, we demonstrated mast cells'

heterogeneous immunophenotypes for normal, benign and malignant conditions. Attempts to highlight the level of MCs in the prostate or other organs have been made, but lacking differential immunophenotype assessment.

A correlation between mast cell infiltration and prognosis has been described in various human tumors [30, 31]. Recently, in prostate cancer, MCs were identified as a new independent prognostic marker, and have been suggested as a therapy associated with castration [32]. However, previous studies on prostate biopsies demonstrated that a high mast cell density is associated with favorable tumor characteristics and good prognosis [10]. The differences in data can be explained by the fact that prostate cancer is a multifocal disease and each tumor usually is characterized by neoplastic foci with multiple heterogeneous characteristics.

Expression of c-kit in cases of BPH may suggest autocrine signaling for tumor cells. Recent reports describe the alteration of pattern for c-kit and KL-ligand expression in BPH and PCa. It seems that KL induces proliferation and maturation of MCs with release of proteases. This can explain the accumulation of MCs in tumors, a phenomenon that was not observed in normal prostate [9]. Expression of c-kit by tumor cells is suggested as a competition between tumor cells and MCs for ligand KL-ligand. It is suggested that MCs could have an unexpected role in the control of prostate homeostasis, entering into the competition with prostate stem cells [33].

The significant correlation between tryptase and chymase in peritumoral areas of malignant lesions suggests a new mast cell phenotype, tryptase+ chymase+ CD117+, compared to the mast cell phenotype of benign lesions, from peritumoral areas: tryptase+ CD117+ chymase-. Chymase involvement in malignant transformation is supported by the existence of partial correlations with Gleason score and chymase-positive MCs located peritumorally.

Our results suggest the existence of a specific mast cell phenotypic heterogeneity for prostate lesions. Recently, Manninka *et al.* suggested mast cell phenotypic heterogeneity despite their origin from a common precursor [34]. Numerous studies support the phenotypic heterogeneity of MCs, which could be used as diagnostic markers and targeted therapy [35, 36].

Our paper is the first report of the expression of mast cell heterogeneity in prostate cancer and its benign lesions. The changes of mast cell phenotype described in the present paper support MCs' involvement in the pathogenesis of prostate cancer biology, probably due to their differential and dual role in progression from benign to malignant lesions. Apart from the correlation with tumor grade, further studies will be necessary to demonstrate differential involvement of MCs in different histopathologic types

of prostate cancer. By their phenotypic heterogeneity and high density in benign and malignant lesions of the prostate, MCs could be used as potential therapeutic targets for adjuvant therapies.

The authors declare no conflict of interest.

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