Original paper

Mast cells influence neoangiogenesis in prostatic cancer independently of ERG status

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A significant proportion of prostatic adenocarcinomas show recurrent translocation leading to ERG expression. Previously we found that ERG+ cases have higher microvessel density than negative ones. One factor influencing angiogenesis in cancer is mast cells. The aim of the present study was to evaluate the relationship between microvessels, mast cells and ERG status.

Tissue microarrays prepared from 113 radical prostatectomy specimens were analyzed with immunohistochemistry for CD31, tryptase and chymase. Vascular profiles and tryptase-positive and chymase-positive cells were counted.

The average number of tryptase-positive cells was 28.93/mm^2 and chymase-positive cells 9.91/mm^2. The average number of CD31+ vascular profiles was 352.66/mm^2. The average number of tryptase-positive cells was 26.35/mm^2 for ERG– cases and 32.12/mm^2 for ERG+ cases. The average number of chymase-positive cells was 8.14/mm^2 for ERG– cases and 12.06/mm^2 for ERG+ cases. The average number of CD31+ vascular profiles was 321.34/mm^2 for ERG– cases and 390.74/mm^2 for ERG+ cases. The number of CD31+ vascular profiles was positively correlated with the number of tryptase-positive and chymase-positive cells ($R = 0.26$ and $R = 0.20$).

In summary, we demonstrated an interrelationship between mast cells, microvascular density and ERG status in prostatic carcinoma.

Key words: mast cell, chymase, tryptase, ERG, prostate cancer, microvessel density, CD31.

Introduction

In the Western world prostatic carcinoma (PC) is the most frequent cancer in males [1, 2]. Its molecular pathogenesis is complex, yet a significant proportion of cases bear recurrent translocation involving ETS family genes; this results in increased expression of ETS transcription factors, most frequently ERG, which may be easily detected by immunohistochemistry [3, 4]. In one of our previous studies, the frequency of ERG positivity in PC was 46%, which is similar to other European populations [5]. We found that ERG-positive PC have a higher number of microvessels [6]. Tumor vascularity is dependent on a number of factors; in some cases mast cells were shown to influence microvessel density [7, 8], while in others no such association was seen [9]. The aim of the study was to analyze the relationship between ERG expression, microvessel density and mast cell count.

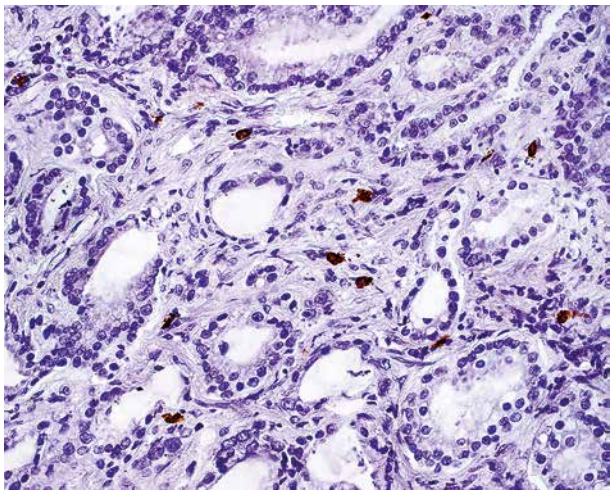

Fig. 1. Tryptase-positive mast cells in prostatic carcinoma. Immunohistochemistry, original magnification 400×

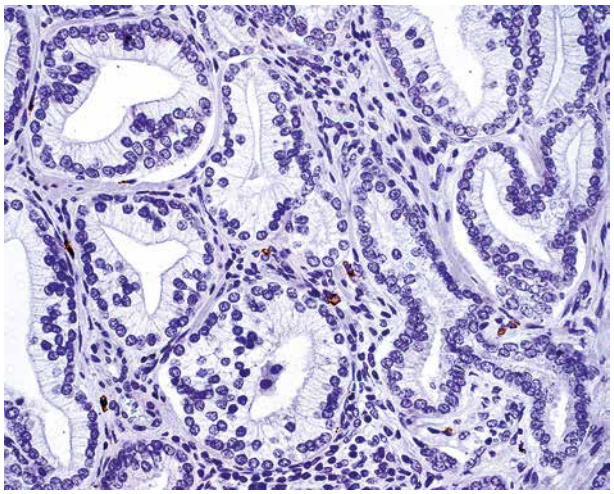

Fig. 2. Chymase-positive mast cells in prostatic carcinoma. Immunohistochemistry, original magnification 400×

Material and methods

The material for the study consisted of unselected radical prostatectomy specimens from the files of the Pathology Department. All prostate specimens were dissected by the standard method, routinely processed and completely embedded in paraffin. The slides were reviewed by a urologic pathologist. The cases were reclassified according to the 2015 revised Gleason system and staged according to current TNM criteria [0, 11, 12]. Lymphovascular invasion and status of surgical margins were also reevaluated. From each case one representative section was chosen. On the slide, the region of interest containing carcinoma infiltrate was marked, then its extent was copied to the surface of the paraffin block. For the tissue microarray (TMA) construction a manual device (Histopathology Inc., Hungary) was used. From the region marked as cancer on each paraffin block, two 2 mm cores were obtained and transferred into a recipient block. The case numbers with respective location in the TMA were noted on an Excel (Microsoft Inc., USA) spreadsheet. The upper-left corner of the TMA was left empty to allow proper orientation of the resulting slides. From the TMA paraffin blocks, 2 mm sections were prepared and stained with hematoxylin-eosin (HE) and immunohistochemistry. HE slides were used to control the quality of tissue. Immunohistochemistry was done in the routine manual manner. Heating in citrate buffer for 30 minutes was used for antigen retrieval. For ERG staining, a rabbit monoclonal antibody (clone EPR3864), produced by Abcam was used in 1 : 200 dilution. Primary anti-tryptase antibody (Leica Biosystems GmbH, Wetzlar, Germany) at 1 : 100 dilution and anti-chymase antibody (Abcam, Cambridge, UK) at 1 : 100 dilution were used. The Lab Vision detection system (Thermo Fisher Scientific, Waltham, USA) was used. 3-amino-9-ethylcarbazole was used as the chromogen. The slides were counterstained with Mayer's hematoxylin (Thermo Fisher Scientific, Waltham, USA) and coverslipped. The results of the ERG staining were scored as positive when unequivocal nuclear staining was present; very faint nuclear as well as any cytoplasmic reaction was ignored, as previously reported [5]. The slides for tryptase (Fig. 1) and chymase (Fig. 2) were examined on an Olympus CH20 optical microscope equipped with a 40× lens; the entire TMA core was visually scanned, and the number of immunopositive cells and number of fields of view were recorded. The results were expressed as the number of positive cells per square millimeter. The data on microvessel density were derived from our previous work [6]. The person performing the counting was not aware of the ERG status or other data under study. The results were collected in an Excel spreadsheet containing the case numbers. Statistics were calculated with Statistica 10 (StatSoft Inc., USA). Student's t test was used. The correlations were assessed by Spearman's method. The significance level was set to 0.05.

Results

The study group consisted of 113 radical prostatectomy specimens. The mean age of the patients was 62.2 years (range 42 to 78, SD 6.40). None of the patients received androgen deprivation therapy.

Distribution of Gleason scores and grade groups is shown in Table I. Extraprostatic extension was seen in 69 cases (61.1%). pT stages are shown in Table II. In 1 case lymph node metastases were present. The surgical margins were positive in 50 cases (44.2%). Lymphovascular invasion was detected in 38 cases (33.6%). Reaction for ERG was negative in 62 cases (54.9%) and positive in 51 cases (45.1%).

Table I. Distribution of grades by modified Gleason score and corresponding ISUP grade groups

Gleason	N	%	grade group	N	%
6 = 3 + 3	47	41.6	1	47	41.6
7 = 3 + 4	41	36.3	2	41	36.3
7 = 4 + 3	13	11.5	3	13	11.5
8 = 4 + 4	3	2.7	4	6	5.3
8 = 3 + 5	2	1.8			
8 = 5 + 3	1	0.9			
9 = 4 + 5	4	3.5	5	6	5.3
9 = 5 + 4	2	1.8			

Table II. Distribution of stages

	N	%
pT2a	7	6.2
pT2b	3	2.7
pT2c	34	30.1
pT3a	52	46.0
pT3b	15	13.3
pT4	2	1.8

The average number of tryptase-positive cells was 28.93/mm^2 (SD 17.06) and of chymase-positive cells 9.91/mm^2 (SD 11.55). The average number of CD31+ vascular profiles was 352.66/mm^2 (SD 170.18). The average number of tryptase-positive cells was 26.35/mm^2 (SD 13.90) for ERG– cases and 32.12/mm^2 (SD 19.99) for ERG+ cases (Fig. 3). This difference was not statistically significant ($p < 0.08$). The average number of chymase-positive cells was 8.14/mm^2 (SD 8.81) for ERG– cases and 12.06/mm^2 (SD 13.98) for ERG+ cases (Fig. 4). This difference was not statistically significant ($p < 0.08$). The average number of CD31+ vascular profiles was 321.34/mm^2 (SD 159.52) for ERG– cases and 390.74/mm^2 (SD 176.45) for ERG+ cases. This difference was statistically significant ($p < 0.037$). The number of CD31+ vascular profiles was positively correlated with the number of tryptase-positive and chymase-positive cells ($R = 0.26$, $p < 0.01$ and $R = 0.20$, $p < 0.05$ respectively). The number of CD31+ vascular profiles showed some reverse correlation with grade group ($R = –0.11$ and $R = –0.13$, respectively) yet this was not statistically significant. The cases without extraprostatic extension showed a somewhat higher number of tryptase-positive cells than cases extending out of the prostate (32.73/mm^2 versus 26.46/mm^2) yet this was not statistically significant. No relationship between number of mast cells and stage, lymph node status, surgical margins status or lymphovascular invasion was detected.

Discussion

Mast cells (MCs) are often disregarded because they constitute usually a minor population of cells in inflammatory infiltrate and they are difficult to observe without specific stains. However, the number of observations concerning their role in both chronic inflammation and cancer is increasing. They have been implicated in promotion of tumor growth, principally by their proangiogenic effect, as well as participation in antitumor immunity [13, 14, 15]. In the last years a number of studies concerning mast cells in the microenvironment of prostatic carcinoma have been published. The issue remains controversial, because cells with different phenotypes and location within different compartments may have contrasting effects on tumor growth [15].

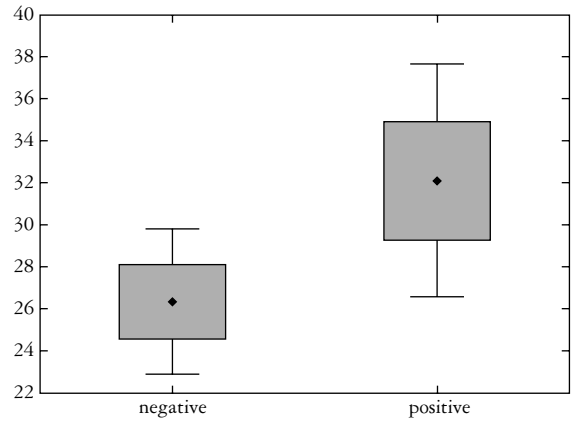

Fig. 3. Tryptase-positive mast cells in ERG-negative and ERG-positive prostatic carcinoma. Central point is mean, box mean ± standard error, whisker mean ± 1.96* standard error

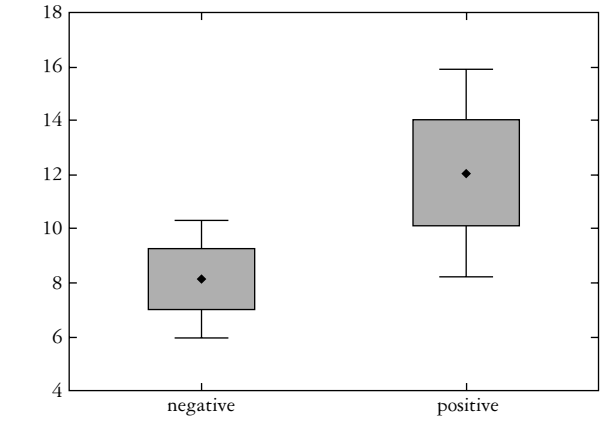

Fig. 4. Tryptase-positive mast cells in ERG-negative and ERG-positive prostatic carcinoma. Central point is mean, box mean ± standard error, whisker mean ± 1.96* standard error

Aydin *et al*. [16] compared the number of mast cells in prostatic hyperplasia and carcinoma. They found a significantly higher number of MCs within the tumor, compared to hyperplasia, while where was no difference between hyperplasia and benign prostatic tissue surrounding carcinoma. There was no association between MC number and Gleason score in cancer cases.

Stawerski *et al*. [17] found a significant correlation between number of mast cells and microvessel density. This correlation was present both in cancer and nonneoplastic prostate. Both mast cell number and microvessel density were correlated with Gleason score, and to a lesser degree with PSA level. In our study we observed a similar relationship with microvessel MC density, while the relationship between MCs and tumor grade was inverse and very weak. The inverse correlation between Gleason score and MC density could be expected from other studies [18, 19], while others reported results similar to Stawerski *et al*. [20].

Some studies use traditional toluidine blue staining for MCs, which may be of limited sensitivity and specificity yet facilitates appreciation of the degranulation status of MC [19]. Other studies ignore the phenotype of mast cells in the prostate, using tryptase as the only marker, and others, such as Globa *et al.* [21], evaluated the phenotype of mast cells. They analyzed prostatic carcinoma in comparison to normal or hyperplastic prostate and found two populations of MCs, namely tryptase+/CD117+/chymase– and tryptase–/chymase+/CD117+ in benign prostate, while in cancer cases intratumoral cells were tryptase+/chymase+/CD117+, and in the peritumoral areas three different populations of MCs, namely tryptase+/ chymase+/CD117–, tryptase+/CD117+/chymase– and chymase+/CD117+/tryptase–, were detected. In our study we used only tryptase and chymase stain, and analyzed only intratumoral population of MCs.

Johansson *et al*. [22] found that mast cells have a stimulatory effect on tumor angiogenesis in PC. Forced degranulation of mast cells increases tumor growth in an experimental PC model parallel to increased microvessel volume density, whereas stabilization of mast cells inhibited both tumor growth and vessel formation. Importantly, these effects were not observed in tumor-cells-only *in vitro* models. It was hypothesized that the tumor inhibitory effect mediated by mast cell reduction may be obtained by imatinib, which opens the way to clinical application of mast cell research. Obviously, this is not the only mechanism whereby imatinib may act on cancer cells. One mechanism by which mast cells induce angiogenesis could be production of FGF-2, although FGF-2 may be secreted by other cells, such as macrophages, fibroblasts and possibly epithelial cells. In human subjects two contrasting effects of mast cell infiltrate were seen. The number of mast cells within nonneoplastic prostate was not correlated with microvessel density; nevertheless high mast cell number was a strong and independent predictor of poor prognosis. Conversely, the patients with a low intratumoral mast cell number fared significantly better; also this effect was independent of other recognized prognostic factors. Castration therapy increased the number of peritumoral but not intratumoral mast cells.

Colombo *et al*. [18] suggested divergent effects of mast cells on prostate cancer depending on the degree of differentiation of the tumor. The low grade cancers with clear-cut glandular differentiation were significantly more proliferative in the presence of mast cells and in animal models could in fact be mast cell dependent, while this effect was lost in poorly differentiated areas. High mast cell number could indeed be protective against appearance of neuroendocrine differentiation during progression of the tumor.

Pittoni *et al*. [19] in an animal model observed the number of MCs increasing in the sequence: normal → PIN → well-differentiated adenocarcinoma. However, this decreased in poorly differentiated areas. In areas of well-differentiated adenocarcinoma MCs were also degranulated, indicating their functional role. The presence of MCs was correlated with the ability of tumor cells to produce CSF, the ligand of C-KIT receptor constitutively expressed by MCs. The presence of functional MCs was necessary for the growth of well-differentiated PC; this could be abolished by blockage of MC degranulation. In MC-deficient mice, tumor growth was inhibited and this inhibition was reversed by MC restoration. This MC dependence could be explained by the inability of cancer cells to produce MMP9, which was produced and supplied by the MCs. The long term anti-MC treatment in PC-prone TRAMP mice caused the appearance of a rapidly growing tumor with neuroendocrine features. Similar tumors developed in bioengineered mice combining TRAMP and C-KIT-negative phenotypes.

Lu *et al.* [23] found that *in vitro* antiandrogenic agents recruit increased numbers of mast cells. They found higher numbers of mast cells *in vivo* in PC than in adjacent non-neoplastic prostate as well as increased ability to recruit mast cells *in vitro* of prostate cancer cell lines compared to non-neoplastic prostate cell lines. Co-culture of stimulated mast cells with prostate cancer cells increased invasiveness of the latter. These effects could depend on downregulation of the AR pathway and finally de-suppression of MMP9. Mast cell/PC cell co-culture also resulted in increased numbers of tumor stem cells. AR pathway modulation could lead to increased neuroendocrine differentiation of PC cells [24]. Effects on the AR pathway and MMP9 were more pronounced in CD133+ than

in CD133– cells. In mice, adding MCs to injected PC cells increased the metastasis formation rate from 20 to 70% of cases. Similarly to *in vitro* studies, MC supplemented cases showed reduced AR and increased MMP9 expression [23].

Some papers have focused attention on the specific location of mast cells, notably intra- and peritumoral; analysis of these were not possible in the present study because of the TMA experiments' design, similar to e.g. Fleischmann *et al*. [25]. Nonomura *et al*. [20] analyzed mast cells in prostatic biopsy material. They found MCs only in the immediate vicinity of cancer infiltrate. They found a significant correlation of MC number with the stage of the tumor as well as Gleason grade. Also, the patients with biochemical relapse had higher MC counts compared with the rest of the study group, and survival of patients with a low MC number was significantly higher than the high MC group. There was however no correlation between MC number and preoperative PSA level or patients' age. The prognostic effects of MCs were observed both in radically treated (surgical radiation) patients and in patients on androgen deprivation only and were present also in multivariate analysis.

Xie *et al*. [26] observed significantly higher recruitment of MC by PC cell lines in comparison to normal prostatic cell lines. Also, after MC recruitment, cancer cell lines became resistant to the chemotherapeutic agent docetaxel; this resistance was due to decreased apoptosis caused by activating p38/p53/p21 signaling, and could be reversed by its inhibition. Similar results could be obtained by mice *in vivo* models. Also, PC cell lines co-cultured with MC showed higher resistance to radiation and less radiation-induced DNA damage. The increased radioresistance was due to increase in ATM phosphorylation.

Fleischmann *et al.* [25] analyzed a large cohort of PC patients for MC status using TMAs. They found an inverse correlation between the number of mast cells and PSA level, Gleason score and tumor stage. The patients with the highest mast cell count also had better survival; this effect disappeared however if the Gleason score was taken into account. It is of note that the method of assessment (TMA) allows only counting of intratumoral mast cells, whose prognostic significance is different from peritumoral mast cells.

In summary, we demonstrated an interrelationship between mast cells, microvascular density and ERG status in prostatic carcinoma. These results need to be confirmed in larger material, and the nature of the link elucidated. It will also be important to determine whether and how this interacts with the prognosis.

The authors declare no conflict of interest.

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