High Extratumoral Mast Cell Counts Are Associated with a Higher Risk of Adverse Prostate Cancer Outcomes

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ABSTRACT

Background: Given our previous findings that low intratumoral and high extratumoral mast cell numbers are associated with higher risk of biochemical recurrence after radical prostatectomy, we now assessed this relationship with race and the development of metastases.

Methods: We stained for mast cell tryptase via IHC and fluorescent immunolabeling in 885 men across multiple tissue microarray sets designed to assess biomarkers in association with race and prostate cancer outcomes (median follow-up, 7.0 years).

Results: Intratumoral and extratumoral mast cell counts were significantly lower in tissues from African-American compared with European-American men, but not within strata of cancer grade. There was no association between mast cell counts and ERG positivity, PTEN loss, or *TP53* missense mutation. Higher minimum extratumoral mast cells were associated with an increased risk of biochemical recurrence [comparing highest with lowest tertiles: HR, 1.61; 95% confidence interval (CI), 1.12–2.29; P trend = 0.01]; this pattern was similar among European-American and African-American men and by grade of disease. There was no significant association between minimum intratumoral mast cell count and biochemical recurrence, overall or within strata of race and grade. Finally, high minimum number of extratumoral mast cells was associated with prostate cancer metastases (comparing highest with lowest tertiles: HR, 2.12; 95% CI, 1.24–3.63; P trend = 0.01).

Conclusions: High extratumoral mast cell numbers are associated with biochemical recurrence and the development of metastases after radical prostatectomy.

Impact: Higher numbers of benign tissue mast cells are associated with a higher risk of adverse outcomes after radical prostatectomy, including metastatic prostate cancer.

Introduction

Chronic inflammation is frequently observed upon histologic examination of prostate biopsies, radical prostatectomy specimens, and autopsy prostate specimens (1), and is hypothesized to contribute to prostate cancer development and progression (2–4). Inflammation is also proposed to drive the development of a putative precursor lesion, proliferative inflammatory atrophy, which may develop into prostatic intraepithelial neoplasia and/or to adenocarcinoma (5, 6). Importantly, the presence of inflammation in benign tissues was previously shown to be associated with higher grade prostate cancer (7). Furthermore, inflammatory cells and mechanisms of immune modulation and tolerance have been suggested to play key roles in the development of castration resistance in advanced prostate cancer (8–13).

Whereas much of the current attention in cancer immunology is focused on lymphocyte-mediated adaptive immunity given the success of immune checkpoint blockade as a cancer therapy, increasing evidence points to an important role for cells of the myeloid lineage in both tumor initiation and promotion, as well as in mediating therapeutic response (14–16). Much of this attention has been placed on macrophages, dendritic cells, granulocytes, and myeloid-derived suppressor cells (MDSC, including both monocytic and polymorphonuclear subsets; ref. 16). For example, in prostate cancer, tumor-infiltrating MDSC secretion of IL23 has been shown to promote castration resistance (11). Likewise, a progressive increase in the number of potentially immune-suppressive CD206-positive M2 macrophages in primary, hormone-sensitive metastatic, and castration-resistant metastatic prostate cancer has been previously reported (17). Comparatively, little attention has been given to mast cells, which comprise a separate subset of the myeloid lineage.

Mast cells are immune cells that are resident in most tissues of the body, including the prostate, and secrete effector molecules such as IL4, IL13, histamine, VEGF, and matrix metallopeptidase 9 (MMP9; refs. 18, 19). In addition, C-X-C Motif Chemokine Receptor 4 (CXCR4) and C-C Motif Chemokine Receptor 5 (CCR5) mediate mast cell progenitor chemotaxis (18). Mast cells potentially contribute to angiogenesis, invasion, immune modulation, and tumor cell proliferation, and are highly proinflammatory (19–26). Mast cell numbers are increased in areas of prostate cancer versus benign prostate tissues, and mast cells numbers are higher in density in lower Gleason grade than in higher Gleason grade cancers (20, 26–29). Previous studies have reported that high intratumoral mast cell numbers are associated with favorable prognosis (20, 29, 30), yet others report that low intratumoral mast cell numbers are associated with favorable prognosis (31). We previously analyzed mast cell density in relation to biochemical recurrence (PSA progression) after radical prostatectomy in a cohort of patients who underwent radical prostatectomy using tissue microarrays (TMA) that sampled both tumor and matched

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benign tissue (30). Our findings indicated a reciprocal relationship where higher intratumoral mast cell density was associated with a lower risk of biochemical recurrence, whereas higher benign tissue (extratumoral) mast cell density was associated with a higher risk of biochemical recurrence (30).

Herein, we further investigated the prognostic significance of mast cell counts in the development of metastases after radical prostatectomy. We also examined mast cell distribution by race, as African-American men have an increased prevalence of inflammation in prostate biopsies free of prostate cancer compared with European-American men (32), and microarray studies identified differential inflammatory gene expression profiles in prostate tumors from African-American men compared with European-American men (33–36). Many of the genes identified as being more highly expressed in tumors of African-American men, namely IL4, IL13, CXCR4, C-C Motif Chemokine Ligand 5/CCR5, and MMP9, are associated with chemotaxis and function of mast cells (18, 37). We utilized five different TMA cohorts containing tumor and benign prostate tissues from 885 African-American and European-American men to compare mast cell counts by race and in relation to risk of biochemical recurrence and the development of metastases after radical prostatectomy. Finally, we examined the presence of mast cells in distant metastases from tissue collected at autopsy.

Materials and Methods

Patients and tissue samples

All samples were obtained and analyzed under an Institutional Review Board–approved study with waiver of consent. Five separate TMA sets of prostatectomy specimens were used in this study: Prostate Cancer Biorepository Network (PCBN) Low Grade, PCBN High Grade, Race Disparity (38, 39), High Grade African-American (39), and Intermediate/High Risk (40). To our knowledge, no men included on the TMA sets had received neoadjuvant hormonal therapy, which would have been rare in this setting, at the time of prostatectomy. Two TMA sets were obtained via the PCBN (http://prostatebiorepository.org). The "PCBN Low Grade" TMA set contains cancer and benign tissue from a radical prostatectomy cohort of 77 African-American men matched to 75 European-American men on age $\pm$ 3 years, grade, and stage and contains only Gleason score 6 and 7 cancers. The "PCBN High Grade" TMA set contains radical prostatectomy cancer and benign tissue from 60 African-American men matched to 60 European-American men on age $\pm$ 3 years, grade, and stage and is enriched for cases with Gleason score $\geq$ 8. The "Race Disparity" TMA set contains cancer and benign tissue from a radical prostatectomy cohort of 169 African-American men matched to 169 European-American men selected via stratified random sampling among Gleason score groupings (3+3, 3+4, 4+3, 8, 9–10) as previously described (38, 39); and 269 men with complete mast cell, demographic and pathologic information available were included in the analyses. The "High Grade African-American" TMA set contains cancer and matched benign radical prostatectomy tissues from 96 African-American men with high grade cancer (Gleason 4+3 = 7 and higher) enriched for adverse oncologic outcomes as previously described (39). The "Intermediate/High Risk" TMA set contains cancer and benign tissues from a radical prostatectomy cohort of primarily European-American men, retrospective case-cohort design of 356 men with intermediate- or high-risk disease that received no additional treatment until the time of metastases as previously described (40); and 253 men in the subcohort with complete mast cell, demographic, and pathologic information available were included in the analyses. All men undergoing prostatectomy at Johns Hopkins Hospital (JHH), including those on these TMA sets, are prospectively followed for oncologic outcomes by the Johns Hopkins University prostate cancer database team. Follow-up for all men who undergo radical prostatectomy at JHH is captured in our master radical prostatectomy database at 3, 6, 9, 12, 18, and 24 months after radical prostatectomy, and then annually thereafter. For patients who do not return to JHH for follow-up, follow-up is assessed by contacting the patient by phone or mail annually. Queries are also sent to ObituaryData.Com and the National Death Index. For men who are diagnosed with metastasis by providers in their community rather than at JHH, the method of diagnosis is requested. Men are followed for biochemical recurrence, defined as postoperative serum PSA $\geq$0.2 ng/mL subsequently confirmed by a second test, and metastatic disease, defined as radiologic evidence of at least one metastatic lesion in a nonregional lymph node, bone, or other site ($\geq$M1a). There were 37 men who were included on more than one TMA set; therefore, we randomly selected the observation to include in the analyses and excluded the other. Thus, there were 885 men included in the analysis; Supplementary Table S1 provides the demographic and pathologic characteristics of the men by TMA set.

Finally, formalin-fixed paraffin-embedded tissue sections from 3 prostates (e.g., the man had not undergone radical prostatectomy) and 11 metastases (including liver, lung, and bone) from three prostate cancer rapid autopsies were assessed.

IHC staining

For the PCBN Low Grade, PCBN High Grade, and Race Disparity TMA sets and autopsy tissues, slides containing the TMA or whole tissue sections were deparaffinized in xylene and rehydrated through a series of graded ethanol, followed by water and 1% tween in water. The slides were then steamed for 45 minutes in antigen retrieval solution (Cat. No. S1699, Agilent) and treated with a dual peroxidase block for 5 minutes. Slides were then incubated at room temperature with 1:32,000 dilution of mouse anti-tryptase antibody (clone AA1, Abcam) and a 1:800 dilution of rabbit anti-cytokeratin 8 (clone EP1628Y, Abcam) for 45 minutes, followed by a cocktail of alkaline phosphatase–conjugated anti-mouse secondary and horseradish peroxidase–conjugated anti-rabbit secondary antibody at room temperature for 30 minutes. The slides were then treated with 3,3′-Diaminobenzidine and/or Vector Red (tryptase-positive cells, red) for 20 minutes each for visualization. IHC and scoring for ERG, PTEN, and TP53 were performed as previously reported in these TMA sets (39, 41).

Scanning and analysis with PIP software

PIP software was used to measure the number of mast cells per TMA spot (mast cell count) and mast cell density (mast cell count/tissue area) as previously described (30).

Immunofluorescence staining

TMAs were treated with antigen retrieval followed by incubation with a mouse monoclonal anti-tryptase antibody (Abcam Ab2378, 1:2,000). Antigen detection was accomplished with chicken-anti-mouse IgG Alexa Fluor 647 secondary (A21463, 1:100, ThermoFisher), as well as treatment with DAPI for nuclear visualization. The PCBN High Grade, Intermediate High Risk, and High Grade African-American sets were stained with immunofluorescence (IF).

Scanning and analysis with TissueGnostics software

The stained IF slides were imaged with a 20X objective using the TissueFAXS Plus (TissueGnostics) automated microscopy workstation equipped with a Zeiss Z2 Axioimager microscope. All TMAs were

scanned using identical exposure times—35 ms for Alexa Fluor 647 (tryptase) and 10 ms for DAPI (nuclear counterstain). The digitized fluorescent images were then quantified using the TissueQuest 6.0 software module. Tryptase-positive cells were segmented and identified using the Cy5 channel. Finally, the number of mast cells per TMA spot (mast cell count) was determined.

Comparison of mast cell count by IHC and IF staining

IHC and IF were performed on adjacent cuts of the PCBN High Grade set to determine if the methods are comparable. Because different cuts were used, we expected similar, but not identical values between methods. We calculated the minimum, maximum, mean, median, and SD of mast cell count for each man in the set separately for tumor and benign tissue using each method. We then compared the median value for mast cell measures by method and compared them using the Wilcoxon signed-rank test. For tumor tissue, there was no significant difference in minimum, maximum, or SD between methods; the IHC method yielded higher mean and minimum values than the IF method (Supplementary Table S2). For benign tissue, there was no significant difference in minimum, maximum, mean, median, or SD of mast cells between methods. Given the similarity of findings from both methods, particularly for the minimum value, which we have previously shown to be associated with biochemical recurrence (30), the results from sets stained with IHC and IF were pooled in the analysis.

Collection and preparation of rapid autopsy tissues

Metastatic tissues were obtained at autopsy, fixed in 10% buffered formalin for 48 hours, and then paraffin embedded and sectioned. Bone metastatic samples were decalcified before formalin fixation and embedding.

Statistical analyses

We calculated the pooled medians (continuous) and proportions (categorical) of demographic and clinical factors and the follow-up time for all men in all TMA sets (**Table 1**). Demographic and clinical factors and follow-up time by TMA set are provided in Supplementary Table S1. We calculated the minimum mast cell count for each man for tumor (intratumoral) and benign (extratumoral) tissue. To determine if minimum mast cell count differed by race (African American vs. European American) or molecular alterations (PTEN intact vs. PTEN loss, ERG negative vs. positive, and P53 negative vs. positive) independent of clinical factors, we estimated the adjusted minimum mast cell count in tumor by race and molecular alterations using negative-binomial regression, overall and stratified by grade (Gleason $\leq$3+4, Gleason $\geq$4+3) for the race analysis.

In our previous study (30), the minimum number of mast cells counted among a man's TMA spots in tumor and benign tissue was the most robustly different between PSA recurrence cases and controls. Therefore, similar to our previous analysis, all men were categorized into tertiles of minimum mast cell count. We evaluated the cross-sectional association between mast cell tertile and molecular alterations (PTEN loss vs. PTEN intact, ERG positive vs. negative, and P53 positive vs. negative) using adjusted logistic regression. To evaluate the association between mast cell tertiles and oncologic outcomes after surgery, we used Cox proportional hazards regression to estimate the HR and 95% CI of biochemical recurrence and distant metastases by tertile of minimum mast cell count. Men were followed from the date of prostatectomy until the event of interest, death, or end of follow-up, which was through 2012 for the "Intermediate/High Risk" TMA and through 2018 for all other TMA sets (Supplementary Table S1). Analyses were conducted separately by tissue type (tumor, benign) overall and stratified by grade (Gleason $\leq$3+4, Gleason $\geq$4+3) and race (African American vs. European American). We also estimated adjusted differences in 10-year restricted mean survival time to biochemical recurrence and to metastases by tertile of mast cells among tumor and benign tissues overall. All analyses were adjusted for TMA set, age, race (African American vs. European American; except in race stratified analyses), grade (prostatectomy Gleason sum $\leq$6, 3+4, 4+3, $\geq$8), stage ($\leq$T2 vs. >T2), PSA (continuous), body mass index (BMI; kg/m^2, <25, $\geq$25 and <30, $\geq$30), and number of TMA spots per man (continuous). *P* for trend was estimated by entering a continuous variable for each tertile in the model. For our evaluations of the association between mast cell tertiles and oncologic outcomes after surgery, we confirmed the proportional hazards assumption was met in all models by adding a time-dependent interaction term of the tertile of minimum mast cell count and the log of survival time to the multivariate Cox models; the *P* value for the interaction term was not statistically significant ($P > 0.05$) in any model. All tests were two-sided, with $P < 0.05$ considered to be statistically significant. In all models, tertiles were based on the pooled distribution of all men; use of TMA-specific tertiles did not change inferences (Supplementary Table S3). All were conducted using SAS 9.4 (SAS Institute) and R (R Foundation for Statistical Computing), 'survRM2' package (https://cran.r-project.org/web/packages/survRM2/index.html).

Table 1. Demographic and pathologic characteristics of men and tertiles of minimum mast cell count in combined TMA sets.

	***N* = 885**
Median age (years)	60
Race, %	
European American	57.6
African American	41.7
Other	0.7
Gleason, %	
$\leq$6 (Grade Group 1)	19.8
3+4 (Grade Group 2)	30.4
4+3 (Grade Group 3)	23.5
$\geq$8 (Grade Groups 4–5)	26.3
Stage	
T2 or below	46.3
T3 or above	51.3
Minimum mast cell count (tertile range)	
Tumor	
T1	0–7
T2	8–14
T3	15–72
Benign	
T1	0–5
T2	6–11
T3	12–56
Median follow-up time (years), interquartile range	7.0, 3.0–12.0

Results

Mast cell counts differ by race

African-American men had significantly lower minimum mast cell counts in the tumor tissue TMA spots (adjusted median 13.7) compared with European-American men (adjusted median 15.3, $P = 0.03$),

Table 2. Adjusted pooled minimum mast cell count by race overall and stratified by grade[a].

	Tumor			Benign		
	European American	African American	*P* value	European American	African American	*P* value
All	*N* = 499	*N* = 358		*N* = 505	*N* = 366	
	15.3	13.7	0.03	13.0	11.1	0.03
Low grade (3+4 or lower)	*N* = 262	*N* = 167		*N* = 263	*N* = 171	
	12.7	11.2	0.1	9.6	8.5	0.1
High grade (4+3 or higher)	*N* = 237	*N* = 191		*N* = 242	*N* = 195	
	15.2	14.4	0.7	12.7	11.0	0.2

[a]Minimum mast cell count estimated using negative binomial regression and adjusting for TMA set, age (continuous), grade (prostatectomy Gleason sum ≤6, 3+4, 4+3, ≥8), stage (≤T2 vs. >T2), BMI (<25, ≤25 and <30, ≥30), and number of TMA spots per man (continuous).

and in benign tissue TMA spots (African American = 11.1, European American = 13.0, $P = 0.03$). However, when stratified by grade, adjusted minimum mast cell count in tumor and in benign tissue did not differ between European-American and African-American men within either strata of low-grade or high-grade disease (**Table 2**).

Mast cell counts and molecular alterations

We previously reported increased tumor-infiltrating lymphocyte density in correlation with ERG positivity, PTEN loss, and *TP53* missense mutation (39, 41). We therefore examined these molecular alterations in association with mast cell counts, but did not find any association between any of the molecular alterations and tertile of minimum mast cell count (**Table 3**), or the median or adjusted mean minimum mast cell count (Supplementary Table S4).

Mast cell counts in tumor and oncologic outcomes

There were no significant associations between minimum mast cell counts for tumor tissue and biochemical recurrence or metastases overall, by race, or by grade among all men (**Table 4**). Similarly, adjusted 10-year restricted mean survival time to biochemical recurrence and to metastases did not differ by tertile of minimum mast cell count (Supplementary Table S5).

Mast cell counts in benign tissue and oncologic outcomes

Men in the second and third tertiles of minimum mast cell count for benign tissue had a significantly increased risk of prostate cancer recurrence as compared with men in the first tertile (P trend = 0.01, **Table 4**). When stratified by race, the patterns of association were similar though only statistically significant among European-American men. When stratified by grade, men with low-grade disease in the second and third tertiles of minimum mast cell count for benign tissue had a greater than 2-fold increased risk of prostate cancer recurrence as compared with men in the first tertile (P trend = 0.01, **Table 4**). Men with high-grade disease in the second and third tertiles of minimum mast cell count for benign tissue had an increased risk of prostate cancer recurrence as compared with men in the first tertile, though the association was only statistically significant for the second tertile. In addition, when compared with men in the first tertile, adjusted 10-year restricted mean survival time to biochemical recurrence was significantly less among men in second and third tertiles (Supplementary Table S5).

Men in the third tertile of minimum mast cell count for benign tissue had a greater than 2-fold increased risk of metastases as compared with men in the first tertile (P trend = 0.01, **Table 4**). When stratified by race, the patterns of association were similar though only statistically significant among European-American men. European-American men in the third tertile of minimum mast cell count for benign tissue had a greater than 3-fold increased risk of metastases as compared with European-American men in the first tertile (P trend = 0.0003, **Table 4**). Among African-American men, this association was positive for the second tertile, but not as strong, and not statistically significant. When stratified by grade, the patterns of association were similar and statistically significant among both men with low-grade and men with high-grade disease (Low-grade P trend = 0.03; High-grade P trend = 0.04, **Table 4**). In addition, when compared with men in the first tertile, adjusted 10-year restricted mean survival time to metastases was significantly less among men in second and third tertiles (Supplementary Table S5).

Table 3. ORs (95% CI) for molecular alterations by tertile of mast cell minimum count[a].

	N (Loss/Intact)	PTEN loss[b] OR (95% CI)	*N* (Positive/Negative)	ERG positive OR (95% CI)	*N* (Positive/Negative)	P53 positive[b] OR (95% CI)
Tumor						
T1(0–7)	59/173	Ref	89/178	Ref	16/216	Ref
T2(8–14)	54/150	1.28 (0.80–2.04)	99/154	1.31 (0.88–1.93)	10/194	0.91 (0.37–2.25)
T3(15–79)	53/163	1.04 (0.65–1.68)	105/171	1.12 (0.75–1.65)	10/206	0.79 (0.31–2.00)
Benign						
T1(0–5)	58/173	Ref	107/171	Ref	14/217	Ref
T2(6–11)	56/148	1.28 (0.80–2.03)	81/172	0.71 (0.48–1.05)	9/195	0.66 (0.25–1.72)
T3(12–56)	56/170	0.99 (0.62–1.59)	106/164	1.04 (0.72–1.52)	13/213	0.87 (0.36–2.13)

[a]OR and 95% confidence intervals (CI) estimated from logistic regression adjusting for TMA set, age (continuous), grade (prostatectomy Gleason sum ≤6, 3+4, 4+3, ≥8), stage (≤T2 vs. >T2), BMI (<25, ≥25 and <30, ≥30), and number of TMA spots per man (continuous).
[b]Excludes men from PCBN Low Grade TMA set, as PTEN and P53 staining were not performed.

Table 4. Association between tertile of minimum mast cell count and biochemical recurrence and prostate cancer metastases in combined TMA sets.

	Biochemical recurrence			Metastasis		
Tertile of minimum mast cell count[a]	**Cases**	**Person-years**	**HR[b] (95% CI)**	**Cases**	**Person-years**	**HR[b] (95% CI)**
Tumor						
T1 (lowest)	89	1,441	Reference	45	1,766	Reference
T2	66	1,308	0.96 (0.69–1.35)	25	1,621	1.11 (0.65–1.89)
T3 (highest)	77	1,515	0.99 (0.71–1.35)	28	1,817	1.19 (0.70–2.01)
P trend[c]			0.9			0.5
Tumor, European American						
T1 (lowest)	64	1,135	Reference	37	1,373	Reference
T2	36	910	1.15 (0.74–1.79)	14	1,100	0.95 (0.49–1.83)
T3 (highest)	48	1,090	1.01 (0.67–1.51)	22	1,283	1.14 (0.64–2.03)
P trend[c]			0.9			0.7
Tumor, African American						
T1 (lowest)	24	290	Reference	7	372	Reference
T2	29	369	0.96 (0.54–1.73)	10	490	1.74 (0.46–6.55)
T3 (highest)	29	425	1.00 (0.55–1.84)	6	534	1.10 (0.23–5.30)
P trend[c]			0.9			0.9
Tumor, low grade						
T1 (lowest)	19	780	Reference	4	882	Reference
T2	11	843	0.58 (0.26–1.31)	4	920	0.83 (0.15–4.46)
T3 (highest)	22	983	1.36 (0.64–2.89)	5	1,098	3.50 (0.73–16.92)
P trend[c]			0.4			0.2
Tumor, high grade						
T1 (lowest)	70	661	Reference	41	884	Reference
T2	55	465	1.16 (0.79–1.70)	21	701	1.06 (0.60–1.89)
T3 (highest)	54	512	0.95 (0.65–1.39)	23	695	1.02 (0.58–1.80)
P trend[c]			0.8			0.9
Benign						
T1 (lowest)	62	1,654	Reference	30	1,950	Reference
T2	78	1,312	2.01 (1.42–2.86)	29	1,603	1.66 (0.97–2.86)
T3 (highest)	92	1,328	1.61 (1.12–2.29)	40	1,664	2.12 (1.24–3.63)
P trend[c]			0.01			0.01
Benign, European American						
T1 (lowest)	40	1,237	Reference	21	1,429	Reference
T2	53	994	2.10 (1.37–3.23)	23	1,232	1.93 (1.04–3.58)
T3 (highest)	56	916	2.01 (1.28–3.16)	30	1,094	3.09 (1.67–5.73)
P trend[c]			0.002			0.0003
Benign, African American						
T1 (lowest)	20	391	Reference	7	488	Reference
T2	25	299	1.82 (0.96–3.44)	6	352	2.13 (0.49–9.28)
T3 (highest)	36	412	1.08 (0.58–2.01)	10	570	0.87 (0.25–3.12)
P trend[c]			0.9			0.7
Benign, low grade						
T1 (lowest)	12	1,017	Reference	3	1,108	Reference
T2	18	791	3.53 (1.65–7.56)	4	884	4.15 (0.73–23.68)
T3 (highest)	21	801	2.85 (1.30–6.26)	6	906	9.35 (1.25–69.72)
P trend[c]			0.01			0.03
Benign, high grade						
T1 (lowest)	50	637	Reference	27	842	Reference
T2	60	516	1.64 (1.10–2.45)	25	714	1.42 (0.79–2.55)
T3 (highest)	70	512	1.31 (0.87–1.97)	34	739	1.82 (1.02–3.26)
P trend[c]			0.2			0.04

[a]Tertiles were defined based on the distribution of minimum mast cell count among all men in all TMA sets.
[b]Pooled HR and 95% confidence intervals (CI) were estimated from Cox hazard regression model adjusting for TMA set, age (continuous), grade (prostatectomy Gleason sum $\leq$6, 3+4, 4+3, $\geq$8), stage ($\leq$T2 vs. >T2), BMI (<25, $\geq$25 and <30, $\geq$30), and number of TMA spots per man (continuous).
[c]*P* trend estimated by modeling tertile of mast cell count as a continuous variable in the regression model.

Mast cells in distant metastases

In prostate tissues evaluated at time of autopsy (e.g., the patients, $n = 3$, had not undergone radical prostatectomy for their prostate cancer), no intratumoral, but many extratumoral, mast cells were observed (**Fig. 1A** and **B**). Likewise, in metastases observed in liver and lung, no intratumoral mast cells were observed infiltrating the tumor, but mast cells were present in extratumoral regions (**Fig. 1C** and **D**). In contrast, infiltrating intratumoral mast cells were present in bone metastases (**Fig. 1E** and **F**).

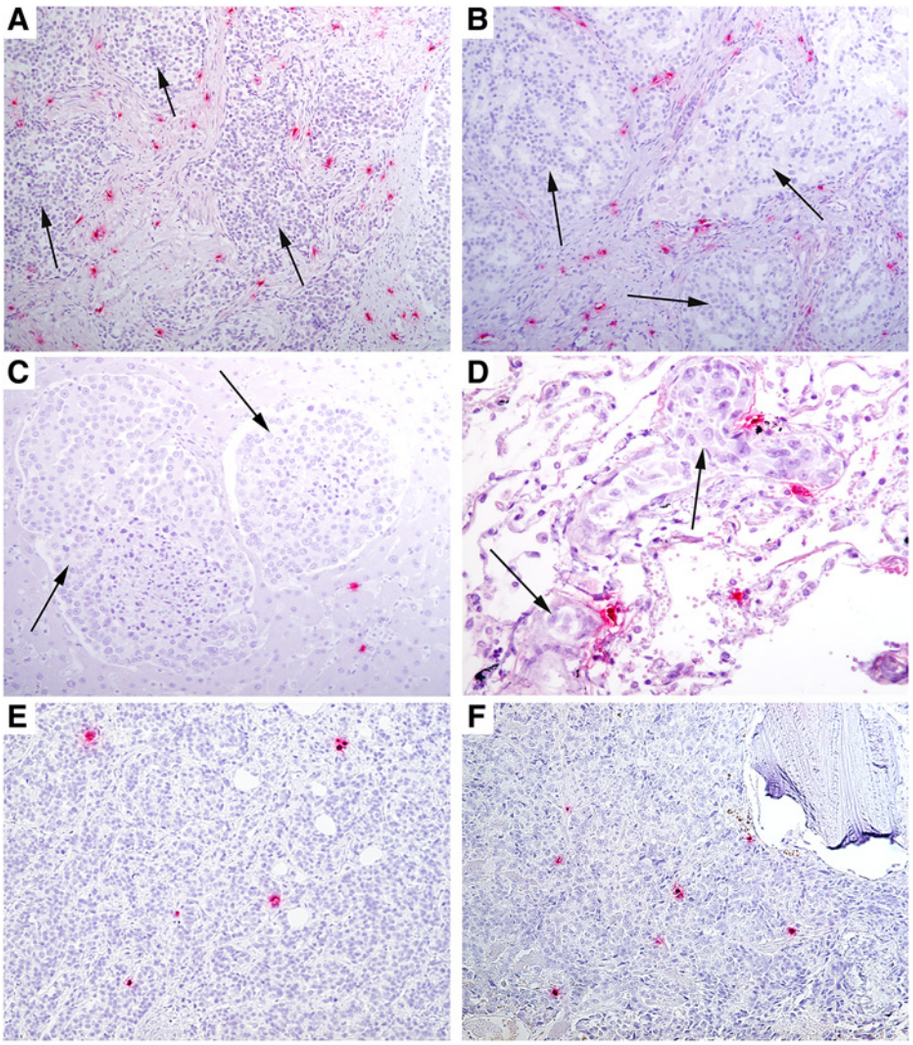

Figure 1.
IHC for mast cell tryptase in prostate cancer metastases. **A** and **B,** Prostate cancer in the prostate in two separate autopsy cases (100× original magnification). No intratumoral mast cells (red staining cells) are observed within tumor foci (arrows), but many extratumoral mast cells are observed in stromal regions. **C,** Prostate cancer liver metastases (arrows, 100× original magnification) with no intratumoral mast cells. Mast cells are observed in unaffected liver (red staining cells). **D,** Prostate cancer lung metastases (arrows, 200× original magnification) with no intratumoral mast cells but several mast cells immediately adjacent to tumor. **E** and **F,** Prostate cancer bone metastases in two separate autopsy cases (100× original magnification) with infiltrating intratumoral mast cells (red staining cells).

Discussion

Given our previous results (30), we postulated that mast cells numbers may serve as a biomarker of cancer aggressiveness and/or recurrence after radical prostatectomy. As African-American men have been shown to be more likely to develop aggressive prostate cancer and to recur at a higher rate than European-American men, as well as present with higher levels of inflammation, there was rationale to study the role of mast cells in racial disparities in prostate cancer (32, 42). We first aimed to determine if tumor and benign tissues from African-American men contain similar numbers and density of mast cells inside and outside the tumor compared with European-American men. Secondly, we aimed to determine if mast cells have a similar relationship with risk of biochemical recurrence (PSA progression) in African-American men as we previously observed; specifically, that low numbers of intratumoral but high numbers of extratumoral mast cells correlate with higher risk of recurrence.

We found that tumor and benign tissues from African-American men had significantly lower mast cells numbers compared with tumor and benign tissues of European-American men, which was unexpected given the prior reported higher prevalence of inflammation-related genes in the prostate microenvironment of African-American men (33–36). However, when stratified by grade, there were no significant differences in mast cells numbers among African-American and European-American men within strata of lower Gleason grade (Grade 6 or 3+4 = 7) cancer or higher Gleason grade (Grade 4+3 = 7 or higher) cancer. Mast cell numbers were similarly associated with risk of biochemical recurrence and metastases in African-American and European-American men. It is possible that the expression of inflammation-related genes in the tumor microenvironment of African-American men is driven by cell types other than mast cells, such as macrophages or cells of the adaptive immune system. This will certainly be the focus of future studies.

Similar to our prior study (30) and a prior study by Johansson and colleagues (29), we found that higher minimum extratumoral mast cell was associated with an increased risk of biochemical recurrence, and this pattern was similar among European-American and African-American men, and by grade of disease. Unlike our prior study and the studies by Fleischmann and colleagues (20) and Johansson and colleagues (29), there was no significant association between minimum intratumoral mast cell count and biochemical recurrence, overall or within strata of race and grade. Nonomura and colleagues performed a study in prostate needle biopsies and reported that high numbers of intratumoral

mast cells are associated with biochemical recurrence after prostatectomy, radiotherapy, or androgen deprivation therapy (31). Here, mast cell counts were limited to mast cells immediately adjacent to cancer epithelium (31), which differs from ours and other studies that used TMAs, included mast cells present in tumor-associated stromal regions, and found the opposite association (20, 29, 30). As compared with our current study, our prior study was enriched for European-American men and men with lower-grade disease overall (30). These differences in the study populations could have contributed to the differences in our findings.

The strengths of our study include the use of a large number of patient samples across well-annotated TMA sets that are linked to clinical outcomes data. Our study further includes a large proportion of African-American men and the ability to look at potential differences across race. One key limitation to our study and the studies by Fleischmann and colleagues (20) and Johansson and colleagues (29) is the use of TMAs that only sample a small portion/region of the cancer, and therefore may not fully characterize the heterogeneity of the tumor. As such, additional research is needed to better characterize the relationship between decreased intratumoral mast cells and biochemical recurrence.

We additionally report that high number of extratumoral mast cells is associated with the development of metastases after radical prostatectomy. These patterns of association were consistent by race and grade; although the numbers of events were too low for stable estimates within strata. These results were also intriguing in light of our results in a limited number of metastatic lesions, where in all tissues except for bone, mast cells were not seen to be infiltrating the tumor, but were found to be present in extratumoral regions. Additional studies are needed to confirm our finding in metastases and to determine if the higher numbers of mast cells observed in extratumoral regions in prostatectomy specimens are a surrogate for the presence of chronic inflammation in these regions, or whether mast cells are increased in the absence of other immune cells types. Indeed, mast cells have been demonstrated to increase in number in areas of chronic inflammation in the prostate (2), and the presence of chronic inflammation in benign tissues was previously shown to be associated with higher grade prostate cancer (7).

In conclusion, our study is consistent with our previous finding that high extratumoral mast cell numbers may predict biochemical recurrence and additionally identified a strong association with high benign tissue mast cells counts and the development of metastases after radical prostatectomy. Future studies will seek to define the mechanistic basis for this phenomenon in assessing the differential role of tumor versus benign tissue mast cells in the prostate tumor microenvironment.

Disclosure of Potential Conflicts of Interest

T.L. Lotan reports receiving a commercial research grant from Ventana/Roche. No potential conflicts of interest were disclosed by the other authors.

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