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ORIGINAL ARTICLE

CD163+ macrophages predict a poor prognosis in patients with primary T1 high-grade urothelial carcinoma of the bladder

Guoliang Yang[1] · Lianhua Zhang[1] · Mengyao Liu[2] · Qiang liu[3] · Xuehui Duan[1] · Juanjie Bo[1]

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Abstract

Purpose Macrophages are a major cell type that can infiltrate solid tumors and exhibit distinct phenotypes in different tumor microenvironments. This study investigates the prognostic value of tumor-infiltrated CD163+ macrophages in patients with T1 high-grade (T1HG) bladder cancer.

Methods CD163+ macrophages were assessed by immunohistochemistry in 94 T1HG bladder cancer samples. Kaplan–Meier analyses and Cox proportional hazards' regression models were applied to evaluate recurrence-free survival, progression-free survival and disease-specific survival.

Results With a median follow-up of 60 months, 37 (39.4%) patients experienced disease recurrence, 14 (14.9%) progression, 11 (11.7%) disease-specific mortality. High CD163+ macrophages were associated with higher risk of disease recurrence and progression ($P < 0.05$, for both). In multivariate Cox proportional hazards regression analysis, high CD163+ macrophages were a significant negative predictor of recurrence-free survival, progression-free survival and disease-specific survival ($P < 0.05$ for all).

Conclusion CD163+ macrophages are a poor prognostic factor in T1HG bladder cancer. This finding provide the ground for further testing it in predicting the outcome of this challenging disease.

Keywords Bladder cancer · T1 high grade · CD163+ macrophage · Prognosis

Introduction

Bladder cancer is the second most common genitourinary malignancy after prostate cancer in the USA. In the year 2018, the number of estimated new cases is 81,190 and the number of estimated deaths from bladder cancer is 17,240 [1]. Bladder cancer can be categorized as non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC). Seventy-five percentage of cases at presentation are NMIBC and 20% (86,000 cases per year) of new bladder cancer cases are T1 high-grade (T1HG) [2]. T1 high-grade bladder cancer not only has a high rate of recurrence and progression, but also a non-negligible risk of metastasis. Long-term progression rates of T1HG range from 21 to 53%, with disease-specific mortality rates of approximately 14–34% [3]. Risk stratification guides selection of T1HG patients for immediate radical cystectomy versus bladder preservation remains an issue of much debate [4, 5].The utilization of biomarkers, which can accurately predict progression in patients with T1HG bladder cancer and help identify patients who need closer surveillance or aggressive treatment, would be very useful in the clinic.

Immune-regulated pathways influence several aspects of malignant development, and cancers present a highly immunosuppressive microenvironment that promotes tumor progression [6]. In that context, tumor-associated macrophages (TAMs) have been identified as key regulators of tumor immunosuppression, angiogenesis, migration, metastasis

Guoliang Yang and Lianhua Zhang have contributed equally to this work.

✉ Juanjie Bo
bojuanjie12@126.com

[1] Department of Urology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[2] Clinical Stem Cell Research Center, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[3] Department of Pathology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

and treatment evasion [7, 8]. Macrophages are divided into two subgroups, the classically activated M1-type and the alternatively activated M2-type [9, 10]. The macrophages present in tumors are known as tumor-associated macrophages (TAMs). They have an M2 phenotype and express CD68 and CD163 [10]. CD163 is a transmembrane scavenger receptor for haptoglobin/hemoglobin complexes that is predominantly regarded as a specific monocyte/macrophage marker for M2 macrophages [8, 11, 12]. Many clinical studies have also suggested a positive correlation between the number of TAMs and/or M2 profiles in a tumor and increased tumor angiogenesis, metastasis and poor prognosis in cancer patients [13–18]. However, the prognostic value of CD163+ macrophages in patients with T1HG bladder cancer remains unknown. In the present study, we examined the prognostic value of CD163+ macrophages in T1HG bladder cancer samples from 94 patients.

Patients and methods

Patients

For the immunohistochemical assay, a total of 94 paraffin-embedded samples of T1HG bladder cancer were collected from our hospital between January 2005 and December 2010. The criteria for study enrollment were (1) pathologic T1 HG confirmed after first transurethral resection of bladder tumor (TURBT), (2) a repeat TURBT performed with 4–6 weeks after complete first TURBT, (3) histopathological diagnosis of transitional cell carcinoma of the bladder, newly diagnosed and untreated, and the potential to follow-up. Clinical information about the samples is described in detail in Table 1. Pathological staging and grading of each tumor were determined according to the International Society of Urological Pathology 1998/World Health Organization 2004 classification [19]. The present study was approved by the Institutional Review Board of Ren Ji Hospital and all patients provided written informed consent before participation.

Follow-up consisted of urine cytology and cystoscopy every 3 months for the first 2 years, every 6 months for the third year, then yearly. Radiographic evaluation of the upper urinary tract was generally done at diagnosis and then every second year. Adjuvant intravesical mitomycin C(MMC) or BCG immunotherapy was administered in 50 and 44 patients, respectively. Drug for intravesical treatment included BCG, mitomycin (MMC), doxorubicin, pirarubicin and epirubicin for NMIBC. BCG has also been shown to be superior to MMC, doxorubicin, and epirubicin [20]. However, BCG has not been approved by Chinese FDA until late 2015 and is still difficult to acquire in most of Chinese Medical institutions due to low production and consummation. So in our study, for the patients with BCG therapy, they buy the BCG from HongKong and other countries.

Table 1 Correlation between CD163+ macrophage and clinicopathologic features of the patients with T1HG bladder cancer

Parameter	Case	CD163+ macrophage		*P* value
		Low	High	
Gender				0.067
Female		14	12	
Male		50	18	
Age (years)				0.791
≤65		36	16	
>65		28	14	
Tumor size (cm)				0.073
≤3		48	17	
>3		16	13	
Tumor number				0.478
Unifocal		40	21	
Multifocal		24	9	
CIS				0.341
Yes		8	6	
No		56	24	
Recurrence				0.005
Yes		19	18	
No		45	12	
Progression				0.005
Yes		5	9	
No		59	21	

Tumor recurrence (Ta or T1) was defined as pathological evidence of disease by TURBT. Progression was defined as the development of local invasion or metastatic disease.

Immunohistochemistry

Formalin-fixed and paraffin embedded tissue sections (5 mm) were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide for 10 min. Slides were incubated overnight at 48° in a humidified chamber with CD163 antibody (1:100 dilution; AbDSerotec, Oxford, UK). Biotinylated anti-rabbit link was used as secondary antibody for 30 min. Slides were then incubated with a streptavidin-horse-radish peroxidase complex. Diaminobenzidine (DAB) was used as chromogen and the sections were counterstained with hematoxylin.

Evaluation of immunohistochemistry

The immunoreactivity of CD163 in cancer cells was evaluated using the same method which was described in the previous study [17]. CD163+ macrophages were quantified by two pathologists who were blinded to the clinical

patient data. They detected each section at low magnification (100×) and identified the areas with the highest macrophage density from which the number of macrophages was counted in ten random high-power fields (HPFs, 400×). The mean values for the number of CD163+ macrophages were 8/HPF, the patients were divided into high (≥ 8/HPF) and low (< 8/HPF) groups based on the mean value.

Statistical analysis

The significance of the relationships between CD163+ macrophages and clinicopathological parameters was evaluated using Chi-squared tests. Recurrence-free survival (RFS), progression-free survival (PFS) and disease-specific survival (DSS) curves were calculated using the Kaplan–Meier method and compared by log-rank test. The significance of various variables for RFS, PFS and DSS was analyzed by the Cox proportional hazards model in the multivariate analysis. SPSS 24.0 software (SPSS, IBM Corporation, New York, USA) was used for statistical analysis. A value of $P < 0.05$ was considered statistically significant.

Results

The clinical relevance of CD163+ macrophage

The patients included 68 males and 26 females from 35 to 79 years (mean age, 60.7 years). The median follow-up time was 60 months for patients and ranged from 9 to 118 months. To investigate the expression of CD163 in bladder cancer tissues, immunohistochemistry was performed in 94 paraffin-embedded T1HG samples. CD163+ macrophages were present in both the tumor stroma and tumor cells (Fig. 1). A high number of CD163+ macrophages were detected in 34% of samples, of which 30 cases and 64 cases showed relatively high and low CD163+ macrophages. CD163+ macrophages were associated with tumor recurrence and progression (Table 1).

CD163+ macrophages in primary T1HG bladder cancer and correlation with clinical outcome.

With a median follow-up of 60 months, 37 (39.4%) patients experienced disease recurrence, 14 (14.9%) progression, 11(11.7%) disease-specific mortality. Using Kaplan–Meier analysis method, we observed that high CD163+ macrophages in T1HG bladder cancer were significantly correlated RFS, PFS and DSS. The log-rank test further demonstrated that the survival time was significantly different between groups with high and low CD163+ macrophages indicating that high CD163+ macrophages were tightly correlated with a shorter survival (Fig. 2). Multivariate analysis was also performed with the Cox proportional hazards model including gender, age, tumor size, tumor number, CIS and CD163+ macrophages. The results showed that high CD163+ macrophages had a significant correlation with prognosis and found to be an independent prognostic factor of outcomes in patients with T1HG bladder cancer ($P < 0.05$ for all, Table 2).

Discussion

In the present study, patients with high CD163+ macrophages in their primary tumors suffered significantly worse clinical outcome compared with patients with low CD163+ macrophage infiltration. This observation was independent of clinicopathological factors with a predictive role in bladder cancer. These results are relevant in patients with T1HG bladder cancer. T1HG tumors are characterized by a higher risk of progression. Identifying patients who

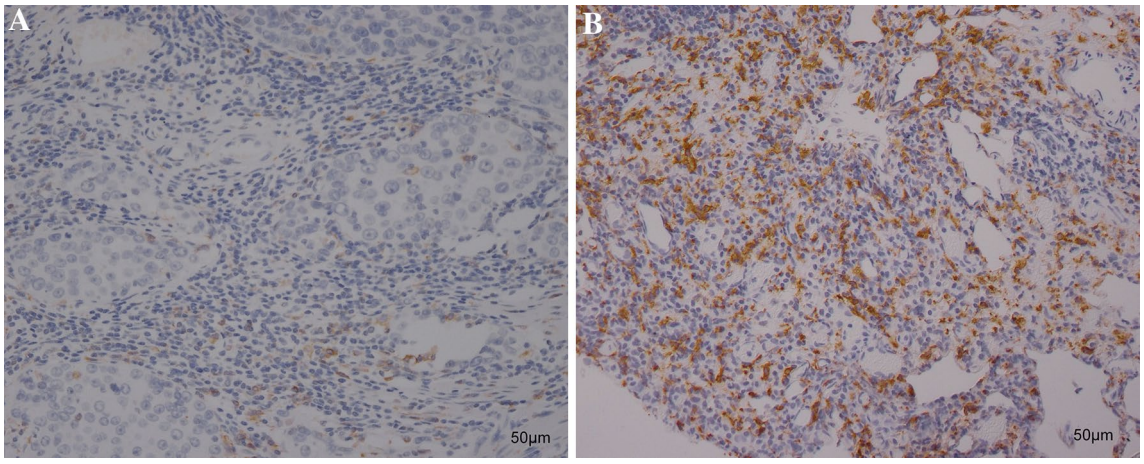

Fig. 1 Representative staining CD163+ macrophages detected by IHC in T1HG bladder cancer. Low CD163+ macrophages (**a** original magnification ×200) and high CD163+ macrophages (**b** original magnification ×200)

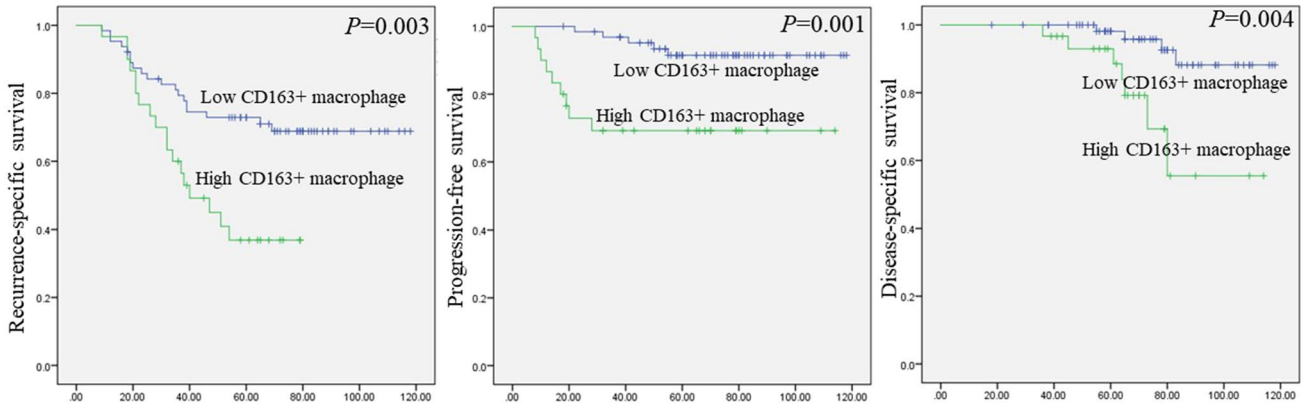

Fig. 2 Kaplan–Meier survival analysis of recurrence-free survival, progression-free survival and disease-specific survival in T1HG bladder cancer patients

Table 2 Multivariable Cox regression analyses predicting recurrence-free survival, progression-free survival and disease-specific survival of 94 patients with primary T1 HG bladder cancer

Case	Recurrence-free survival		Progression-free survival		Disease-specific survival	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Gender		0.887		0.544		0.938
Female versus > Male	1.055 (0.500–2.229)		1.752 (0.286–10.743)		0.924 (0.127–6.733)	
Age (years)		0.194		0.465		0.359
≤ 65 versus > 65	2.046 (0.695–6.026)		2.091 (0.289–15.143)		2.573 (0.341–19.422)	
Tumor number		0.366		0.306		0.125
Unifocal versus mutlifocal	1.377 (0.688–2.757)		2.209 (0.484–10.086)		3.886 (0.685–22.042)	
Tumor size		0.418		0.913		0.310
≤ 3 cm versus > 3 cm	1.336 (0.663–2.691)		0.929 (0.247–3.495)		0.428 (0.083–2.200)	
CIS		0.375		0.002		0.022
Yes versus No	0.617 (0.212–1.794)		13.721 (2.521–74.691)		11.080 (1.420–86.466)	
CD163+ macrophage		0.038		0.002		0.015
Low versus high	2.110 (1.041–4.276)		6.686 (1.987–22.503)		5.859 (1.412–24.302)	
Adjuvant therapy		0.1444		0.100		0.109
MMC versus BCG	2.218 (0.762–6.456)		0.159 (0.018–1.419)		0.140 (0.013–1.549)	

CI confidence interval, *HR* hazard ratio

should be treated conservatively or who should be treated aggressively remains difficult. Several biomarkers were previously reported to be useful as predictive markers for non-muscle invasive bladder cancer, such as p53, FGFR3, Survivin [21–23]. However, none has been translated to patient treatment.

In line with our findings, several studies have shown a protumorigenic role for TAMs [7] and demonstrated a significant association between high levels of TAMs and unfavorable prognosis in patients with various malignancies [13–18]. With regard to T1HG bladder cancer, only few reports on the prognostic relevance of TAMs have been published so far. Previous studies have confirmed that the TAMs include the M2 phenotype and CD163+ status is regarded as a specific marker of M2 macrophages. The present study showed that high CD163+ macrophages both in tumor nest and stroma significantly correlated with poor outcome.

Researches focused on molecular factors for T1HG bladder cancer prognosis have attracted increasing attention. In this study, CD163+ macrophages were an independent prognostic factor. Given the fact that tumor biology is often dictated by several essential cellular and microenvironmental alterations, it may be naive to think that a single factor could be a prognostic factor in cancer [24]. Solutions are now being explored by analyzing multiple factors with tissue microarrays, which has been emerged as an important tool in the discovery and validation of new biomarkers [25].

Based on our results, we proposed a new prognostic model that combines pathological, cellular and molecular features.

Limitations of our study should be acknowledged. The retrospective study can lead to selection bias. Second, relevant prognostic factors such as Lymphovascular invasion and variant histology were not assessed. Third, because all the patients enrolled in our research are from China, the result of this study needs to be validated in other populations and larger cohorts.

Conclusion

In patients with T1HG bladder cancer, high CD163+ macrophages are associated with higher risk of disease recurrence, progression, and disease-specific mortality. This study emphasizes the importance of translating laboratory findings to the clinical setting to provide more individualized management.

Authors' contribution GY, JB: Protocol/project development. GY, LZ, ML: Data collection or management. QL, XD: Data analysis. GY, JB: Manuscript writing/editing.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research ethics/informed consent The study was performed according to the Helsinki Declaration and approved by the Ethics Committee of Renji hospital, school of medicine, Shanghai Jiaotong University, China; Number: 335/16). This was a retrospective study evaluating perioperative data. A signed informed consent was obtained from the patients.

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