Immune Cell Infiltration in Bladder Cancer

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Abstract

Background Bladder cancer is one of the most common malignant diseases with high recurrence rates worldwide. Although immunotherapy has been applied in bladder cancer for a long period of time, the tumor-infiltrating immune cells (TIICs) in bladder cancer has not been systematical investigated. Methods CIBERSORT, a versatile computational method for quantifying cell fractions from bulk tissue gene expression profiles (GEPs), was applied to calculate the TIICs fraction proportion in normal bladder tissues and in bladder cancer tissues with the TCGA data. Results compared to normal bladder tissue, B cells naïve, T cells CD4 memory resting and Mast cells resting fractions proportion decreased, and NK resting, macrophages M0 and macrophages M1 increased. In BLCAs tissue, pro-tumorigenic related immune cells were negatively correlated with anti-tumor immune cells. New tumor with locoregional had high fraction proportion of macrophages M0 and macrophages M1. Dendritic cells activated, Monocytes, macrophages M1, Tregs and T cells follicular helper significantly increased in low grade BLCAs. Tregs had a high proportion in BLCAs patients accepted low radiation dose. Conclusions This study indicates that macrophages M2 and Tregs could be the promising immunotherapy targets combined radiotherapy in BLCAs. The result provides valuable information to understand the immunity status in BLCAs.

Background

Bladder cancer (BLCA) is one of the most common cancers worldwide. In 2019, there will be more than 80,000 new BLCA cases diagnosed and 17,000 deaths in the USA[1]. The BLCA incidence in men is about four times higher than in women. Hormonal profiles partly contributed to this disparity in gender[1, 2]. Non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC) are generally considered as two forms BLCAs[3]. NMIBC could be divided into low grade non-muscle invasive bladder cancer (LG-NMIBC) and high grade non-muscle invasive bladder cancer (HG-NMIBC) according to the oncological natural histories difference. More than 70% BLCA patients are newly diagnosed with NMIBC. Radiation therapy, chemotherapy, immunotherapy and surgery are the main therapeutic measures. Although there are variety of treatments options available for NMIBC currently, high recurrence and progression rates are the main obstacles to the NMIBC treatment. Treatment options for MIBC are limited. Therefore, the development of more specific and efficient targeting strategies in BLCA treatment is urgent.

[Tumor infiltrating immune cells \(TIICs\) is closely associated with clinical outcome in BLCA \[4, 5\].](#)

Therefore, immunotherapy has been applied to treat NMIBC for more than 40 years for Morales et al first used Bacillus Calmette-Guerin (BCG) to treat bladder cancer patients in 1976[6]. Now, intravesical BCG instillation has been one of the most effective treatments for NMIBC and one of the most successful biotherapies for cancer[7]. Unfortunately, BCG immunotherapy still fails in about 40% of NMIBC patients. Recently research results partly ascribe BCG instillation failure to TIICs. M2 macrophages and Treg cells, which secrete immunosuppressive cytokines have been discovered associating with BCG immunotherapy failure[8-10]. Also, radiotherapy contributes to recruiting tumor associate macrophages and Tregs to the tumor environment [11, 12]. Therefore, further characterization of the TIICs in BLCA treatment is essential to improve the clinical outcome[13]. Herein, we systematically analyzed the TIICs in

normal tissue and in BLCAs tissue by the means of CIBERSORT, a useful tool to estimate the proportions of 22 immune cell types [14]. This work may helpful to identify new therapeutic targets for BLCA treatment.

Methods

Bladder cancer datasets and normal controls datasets acquisition

Gene expression data of bladder cancer patients and normal controls was collected from TCGA database by the TCGA GDC transfer tool. 414 bladder cancer samples and 19 normal control samples were included. The mRNA expression profiles matrix was obtained by merged each sample mRNA expression data. Then non-protein coding genes were filtered. The retained gene name was annotated to get gene expression matrix.

Collection of Clinicopathological Data

Clinical information was acquired and processed by TCGA-Assembler Module A and Module B. The information on age at diagnosis, gender, tumor grade, stage status and metastasis pathologic was extracted for further analysis.

Estimated Immune Cell Type Fractions

LM22 signature matrix, which contains 547 genes expression information from 22 human leukocytes, including seven T-cell types, three macrophage types, three B cell types, natural killer (NK) cells, and myeloid subsets, was downloaded from the CIBERSORT web portal (<https://cibersort.stanford.edu/index.php>). The gene expression matrix with annotation was used to calculate the LM22 fractions by the algorithm method with 100 permutations. The inferred fractions of immune cell populations produced by CIBERSORT were summed up to one matrix and be interpreted for comparison.

Statistical analysis

R software (version 3.5.3) and origin 8.0 were applied for all statistical analyses. LM22 correlation index was calculated with corplot Package. Two-tailed Student t tests or One-way- ANOVA was performed for statistical analysis. The p values less than 0.05 were considered as statistically significant.

Results

Data Set Containing 414 Bladder Cancer Samples and 19 Normal Bladder Tissue Samples

In this study, a summary of available patient characteristics were presented in Table 1. Bladder cancer samples were classified by age at diagnosis, gender, tumor grade, stage status and metastasis pathologic.

Immune composition differs in Bladder Cancer Tissues and Normal Bladder Tissues

The gene expression matrix was obtained from the TCGA download datasets. Then CIBERSORT algorithm was applied to define the immune cell fraction proportion of BLCA tissues and normal bladder tissues. Figure 1 showed the immune cell fraction proportion in bladder cancer tissues and normal bladder tissues. Compared with normal bladder tissue, the B cells naïve, T cells CD4 memory resting, Mast cells resting fractions in bladder cancer tissue were generally lower, whereas the fractions of NK cells resting, Macrophages M0 and M1 cells were higher in bladder cancer tissue (table 2 and figure 1).

Correlation with 22 immune cell types in bladder cancer tissues

The degree of tumor immune cell infiltration is a crucial factor in cancer development. Interactions between tumor infiltrating leukocytes (TILs) existed in tumor microenvironment [15-17]. However, the TILs interaction in bladder cancer was rarely reported. To further elucidate the interdependence between the degree of immune cell fraction and immune cell composition in bladder cancer tissues—we calculated the coefficient of association of 22 immune cell types in bladder cancer tissues with the corplot package. Our results showed that macrophages M2, which was much abundant in BLCA—negative correlated with numerous anti-tumor immunity cells, including NK resting—T cells CD8, T cells CD4 naïve—mast cells activated, plasma cells—B cells naïve, B cells memory, T cells follicular helper and Dendritic cells activated. Tregs—a subpopulation of T cells that are immunosuppressive, negative correlated with Macrophages M1 cells ($r=-0.21$) and T cells CD4 memory activated ($r=-0.24$). T cells CD8 positively correlated with T cells CD4 memory activated ($r=0.46$) and Tfh ($r=0.2$), negative correlated with Macrophage M2 ($r=-0.17$), Macrophage M0 ($r=-0.33$) and T cells CD4 memory resting ($r=-0.4$). T cells CD4 memory activated positively correlated with macrophages M1 ($r=0.33$), T cells CD8 ($r=0.46$) and NK resting ($r=0.29$), negative correlated with T cells CD4 memory resting ($r=-0.36$) and T cells regulatory ($r=-0.24$) (figure 2).

Composition of immune cells in new tumor

The new tumor event type and site had important prognostic value in BLCA and other cancers [18]. To investigate the composition of immune cells in new tumor, we analysed the immune cells fraction change in new type or site tumor. Results showed that new Primary Tumor had a high fraction of T cells CD4 memory resting, T cells naïve, T cells follicular helper and had a lower fraction of plasma cells, macrophages M2, macrophages M0, compared to locoregional, distant metastasis type tumor.

When we analyzed the immune cells fraction in different types of bladder cancer tissues, It was found dendritic cells and resting macrophages M0 maybe correlate with tumor metastasis (figure3). compared to metastasized to other sites, when cancer cells metastasized to the lymph node only—macrophages M0 content has a great change—which implied high content of Macrophages M0 may be a risk factor for metastasis. When bladder cancer cell transfer to bone, fraction high content of the dendritic cells resting was found in the bladder cancer tissue.

Immune cells fraction change in high and low grade BLCA

To explore the relationship between tumor immune cell infiltration and the bladder cancer grade, we found T cells CD4 memory activated, Macrophages M0 and macrophages M1 fraction proportion increased in high grade BLCA tissues (fig.4a, d, e). However, regulatory T cell-based immunosuppressive effect was weak in high grade BLCA cancer tissue for Treg is much less abundant in high grade BLCA tissue than in low grade BLCA tissue (fig.4b). Monocytes decreased in high grade BLCA tissues (fig.4d). Dendritic cells activation was inhibited in high grade BLCA tissue for the activated Dendritic cells decreased significantly and Dendritic cells resting fraction increased significantly compared to in low grade BLCA tissue (fig.4f, g).

Tumor immune cell infiltration and radiation in bladder cancer

Radiotherapy was one of the most common treatments in the management of various types of cancer. Over half of people who were treated for cancer had accepted with radiotherapy [19, 20]. However, radiotherapy could contribute to the anti-immunogenic effect by recruiting TAMs and MDSCs in tumor microenvironment[21, 22]. The tumor immune cell infiltration in bladder cancer which accepted with radiotherapy remains unclear. Our work demonstrated that radiation dose could cause the tumor immune cell infiltration change (figure5). In low radiation dose group, Tregs increased. In high radiation dose group, macrophages M2 increased. When studying TIICs fraction proportion in BLCA patient with different radiotherapy sites, we found macrophages M2 had a high fraction proportion in local recurrence, T cells CD4 memory resting and Macrophages M0 had a high fraction proportion in distant recurrence, macrophages M2 and T cells CD8 fraction proportion increased in local recurrence.

Discussion

Tumor microenvironment acts as an important role in the development and progression of cancer [23, 24]. In this work, we aim to clarify the immune microenvironment in BLCA. As far as we know, this is the first time to comprehensively assess the immune infiltration in BLCA based on the deconvolution of bulk gene expression data.

Human T cells and B cells could regulate acquired immune system by secreting cytokines, creating antibodies, etc. The anti-tumor ability was impaired in tumor microenvironment of T cells and B cells[25]. Our results showed that the fraction of B cells and T cells CD4 memory resting decreased in BLCA. It may mean B cells and T cells origination exhaustion in BLCA. This could contribute to the impaired anti-tumor activity of B cells and T cells. Furthermore, we observed that effector T cells and B cells, including T cells CD8/ T cells CD4 memory activated / T cells follicular helper/plasma cells, negatively correlated with pro-tumorigenic related immune cells, such as Tregs/macrophages M2, etc.

Macrophages, which could mediate the antigen presentation process, are major components in cancer immune infiltration[26]. It is also characterized by plasticity and heterogeneity. Macrophages M0 could differentiate into macrophages M1 or macrophages M2 under different cytokines stimulation. The

disturbance of macrophages M1/M2 balance could alter antitumor effects. In human bladder cancer, increased infiltration of tumor associated macrophages has been showed to be associated with the poor clinical prognosis [27]. Some researcher suggested that targeting macrophage may be a promising strategy for anticancer therapy [28, 29]. Our analyses revealed that a high level of macrophages M0 and M1 existed in BLCA. Although macrophages M0 and macrophages M1 fraction increased in BLCA, compared to normal, the M1/M2 balance and M1/total macrophages balance did not shift.

Mast cells contain large granules, which could release the immunomodulatory and vasoactive molecules. Mast cells infiltration has been showed involved in several types of cancer physiological and pathological conditions[30]. Previous studies showed that mast cells influence BLCA patient outcomes [31, 32]. Mast cells could enhance bladder cancer metastasis by increasing CCL2/CCR2/EMT/MMP9 signals axis. In this study, we discovered that total mast cells and mast cells resting decreased in BLCA. Mast cells negative correlated with several types of immune cells, such as macrophages M1/T cells CD8/T cells CD4 memory activated/T cells follicular helper, etc. the result consists with its pro-tumorigenic role in BLCA[31].

More interesting, our data firstly exposed the TIICs fraction in different types of new tumor event type and site. New primary tumor seemed had a T cells pool, T cells CD4 memory resting and T cells naïve, compared to locoregional and distant metastasis BLCA. It suggested that activation of T cells may be a useful strategy to BLCA patients with new primary tumor. Furthermore, TIICs fraction difference also existed in different type of metastasis BLCAs. These results could be helpful to improve BLCAs treatment.

TIICs have been reported in association with tumor grade [10, 33]. Herein, the association between TIICs and tumor grade was also investigated. A high estimated Tregs fraction proportion was observed in low grade BLCA. A high estimated Dendritic cells resting fraction proportion was observed in high grade BLCA. It suggested that Regulatory T Cell-Based Immunosuppressive Effect may play an important role in low grade BLCA and inhibition the Tregs function in low grade BLCA may be a useful immunotherapy in treating low grade BLCA. Moreover, Dendritic cells resting activation may be another therapy strategy for high grade BLCA[34].

Radiotherapy is generally applied to the cancerous tumor for its cytotoxicity. However, radiotherapy could not only cause damage to cancer cells, but also leading to immunologic cells death indiscriminately [35, 36].

We found TIICs fraction proportion are different in BLCA patients who accepted different radiation dose or accepted with different radiotherapy sites. Interestingly, results showed that Tregs are more sensitive to high radiation dose. It determined that combining immunotherapy by inhibiting of Tregs activity with radiotherapy provided an improved treatment for BLCA rather than single low radiation dose [37]. Our data further showed the TIICs fraction proportion in patients with different radiotherapy sites. Results implied that macrophages M2 may act as a vital role in local recurrence and in local recurrence and radiation therapy combined M2 inhibition may be more useful to these patients. In distant recurrence patients, T cells CD4 memory resting and Macrophages M0 had a high fraction proportion. Activation T

cells CD4 memory resting and Macrophages M0 may be a promising strategy to improved radiotherapy effect to distant recurrence patients.

Conclusions

This is the first time to explore the TIICs in BLCA by using the accurately estimate tumor-infiltrating leukocytes analysis tool, CIBERSORT. The complex relationship between different kinds of TIICs was further revealed. Furthermore, we studied the TIICs fraction proportion in BLCAs patients in high or low grade, and the relationship between TIICs fraction proportion and radiotherapy. More importantly, we highlighted the promising immunotherapy value of Tregs, macrophages M2, etc. Our work advances the understanding of TIICs and provides valuable resources for research to improve immunotherapy.

Abbreviations

BLCA: bladder cancer

TIICs: tumor-infiltrating immune cells

TILs: tumor infiltrating leukocytes

TCGA: The Cancer Genome Atlas

GEPs: gene expression profiles

BCG: Bacillus Calmette-Guerin

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by the author.

Consent for publication

Not applicable.

Availability of data and materials

Data on gene expression and corresponding clinical information were downloaded from The Cancer Genome Atlas (TCGA, <https://cancergenome.nih.gov/>) prostate cohort through the R package “TCGA-Assembler (06/04/2018)” in January 2019[38, 39].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SL and LL contributed to the study design. WH, SW, JL and YT performed the data collection and analysis. JZ, SF and ZW prepared the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Baseline patient and primary tumor characteristics

Variable	No. of samples	%	Valid %
age at diagnosis, y			
≥60	324	78.26	78.64
<60	88	21.26	21.36
Missing	2	0.48	
gender			
male	304	73.43	73.79
female	108	26.09	26.21
Missing	2	0.48	
tumor grade			
low grade	21	5.07	5.13
high grade	388	93.72	94.87
Missing	5	1.21	
tumor status			
with tumor	137	33.09	36.83
tumor free	235	56.76	63.17
Missing	42	10.14	
metastasis pathologic			
M0	196	47.34	94.69
M1	11	2.66	5.31
Missing	207	50	

Table2. 22 THCs proportion in normal tissue and in BLCAs tissue.

Immune cell type	CIBERSORT fraction in % of all infiltrating immune cells		
	Normal	BLCA	p value
B cells naive	0.076±0.016	0.035±0.003	0.021
B cells memory	0.008±0.004	0.007±0.002	0.888
Plasma cells	0.035±0.010	0.056±0.003	0.169
T cells CD8	0.128±0.022	0.107±0.004	0.311
T cells CD4 memory resting	0.194±0.020	0.125±0.004	0.003
T cells CD4 memory activated	0.020±0.006	0.030±0.002	0.368
T cells follicular helper	0.026±0.006	0.044±0.002	0.054
T cells regulatory (Tregs)	0.029±0.009	0.046±0.002	0.086
T cells gamma delta	0.015±0.009	0.003±0.001	0.229
NK cells resting	0.004±0.003	0.017±0.001	0.000
NK cells activated	0.039±0.007	0.0030±0.002	0.239
Monocytes	0.021±0.005	0.015±0.001	0.364
Macrophages M0	0.010±0.004	0.126±0.006	0.000
Macrophages M1	0.027±0.009	0.063±0.003	0.002
Macrophages M2	0.139±0.019	0.130±0.004	0.693
Dendritic cells resting	0.037±0.010	0.042±0.003	0.708
Dendritic cells activated	0.021±0.006	0.048±0.003	0.063
Mast cells resting	0.119±0.026	0.043±0.002	0.010
Mast cells activated	0.040±0.021	0.015±0.002	0.259
Eosinophils	0.006±0.002	0.004±0.001	0.329
Neutrophils	0.009±0.004	0.008±0.001	0.775

Figures

Figure 1

TILs proportions in normal bladder tissues and BLCA tissues. CIBERSORT immune cell fractions were determined for each patient. TILs proportion were estimated and showed in the heatmap.

Figure 2

Correlation matrix of 22 immune cell types in bladder cancer tissues and the correlation index was calculated with corplot Package.

Figure 3

TIICs fraction proportions in BLCAs patients with different event types (a) and different sites (b) new tumor.

Figure 4

TIICs fraction proportions in low grade or high grade BLCAs patients. T cells CD4 memory activated (a), T cells regulatory, (b), Monocytes (c), Macrophages M0 (d), Macrophages M1 (e), Dendritic cells resting (f) and Dendritic cells activated (g) were calculated in low grade BLCAs or high grade BLCAs.

Figure 5

TIICs fraction proportions in BLCAs patients with radiotherapy. The 22 immune cells proportion in BLCAs patients accepted with different radiation dose (a) and in BLCAs patients with different radiotherapy sites