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Urologic Oncology: Seminars and Original Investigations 37 (2019) 810.e1–810.e5

UROLOGIC ONCOLOGY

Laboratory-Bladder cancer

Human urothelial bladder cancer generates a clonal immune response: The results of T-cell receptor sequencing

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Received 1 March 2019; received in revised form 22 March 2019; accepted 12 April 2019

Abstract

Background: High T-cell receptor (TCR) repertoire clonality is associated with clinical response to immune checkpoint blockade in bladder cancer.

Objective: To determine if TCR repertoire is more clonal in tumors than in benign inflammation.

Methods: We prospectively identified 12 patients with bladder lesions undergoing transurethral resection. Specimens were collected at time of transurethral resection and stored at −80C. DNA was extracted and high throughput DNA sequencing of the CDR3 region of the TCR beta chain using the immunoSEQ assay (Adaptive Biotechnologies) was performed. T-cell fraction, clonal dominance, and maximum frequency of TCR clone were assessed.

Results: Of the 12 bladder lesions resected, 3 of 12 were cT0, 3 of 12 were cTa, 3 of 12 were cT1, and 3 of 12 were cT2 or greater. The median number of T cells in urothelial carcinoma specimens (UC+) and benign (UC−) specimens was 5,569 and 25,872, respectively. The number of unique TCRs sequenced in UC+ and UC− specimens was 3,069 and 9,680, respectively. The median tumor infiltrating lymphocyte percentage in UC+ and UC− specimens was 2% and 12%, respectively. The UC+ specimens demonstrated clonality as evidenced by identification of a specific T-cell clone being present in up to 17% of the total tumor infiltrating lymphocyte pool, in contrast to 2% among UC− specimens.

Conclusions: Primary urothelial tumors contain clonally expanded T-cell populations. These data support the hypothesis that bladder tumors induce an antigen-driven immunogenic host response, in contrast to the benign inflammatory response, which does not appear to demonstrate any T-cell clonal dominance.

Keywords: Urinary bladder neoplasm; Immunotherapy; T-cell receptor

1. Introduction

Recent discoveries in the field of immuno-oncology have focused attention on tumor-host interactions in urothelial carcinoma. Tumor specific antigens, also known as neoantigens, arise from somatic mutations in malignant cells. They are identified by the host immune system, and can elicit a tumor-specific response [1,2]. Expression of certain detectable neoantigen signatures in melanoma leads to enhanced immune-mediated tumor killing which can be augmented by the administration of systemic immunotherapy [3]. Predicting how these neoantigens are identified by the host has prognostic and therapeutic implications.

T-cell receptor (TCR) sequencing of tumor infiltrating lymphocytes (TILs) is useful for predicting neoantigen signatures that can, in turn, help characterize tumor immunity [4]. Prior studies have suggested that TCR repertoire clonality is associated with clinical outcomes [5].

Since a cancer-directed immune response is dependent on the identification of a distinct tumor neoantigen, we hypothesized that T-cell repertoire is more clonal in

Funding: Montefiore Medical Center and Albert Einstein College of Medicine, Department of Urology.

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https://doi.org/10.1016/j.urolonc.2019.04.011
1078-1439/

primary urothelial tumors than in nonmalignant inflammatory bladder tissue. We sought to confirm this hypothesis by identifying dominant T-cell clones present in urothelial tumors through TCR sequencing of TILs obtained from transurethral resection (TUR) specimens.

2. Materials and methods

2.1. Patient selection and clinical characteristics

All patients had bladder lesions and were treated by a single surgeon at Montefiore Medical Center. Clinicopathologic variables were collected from electronic patient medical records. Collected variables include age at time of TUR, gender, tumor grade, American Joint Committee on Cancer TNM stage (clinical stage). Lesions were classified as cT0 if no urothelial carcinoma was present, regardless of the patients' bladder cancer history.

2.2. Tumor specimen acquisition and processing

After obtaining informed consent, patients were prospectively enrolled onto the study and tissue was collected according to an IRB-approved biospecimen collection protocol. Tumor specimens used for sequencing were collected at time of TUR and snap frozen and stored at −80C. The presence of malignant tissue in the specimens being sequenced was confirmed by examination of a hematoxylin and eosin-stained representative slide by a genitourinary pathologist.

2.3. DNA extraction and sequencing

Genomic DNA was extracted from specimens using the Qiagen DNeasy extraction kit. The CDR3 region of TCR beta chains was amplified and high throughput DNA sequencing was performed using the immunoSEQ assay (Adaptive Biotechnologies, Seattle, WA) as previously described [6]. To summarize, V and J gene primers were used to amplify the rearranged V(D)J segments at 20× coverage. Variable chain segments were sequenced and then annotated according to the International ImMunoGeneTics Collaboration [7]. Immunological endpoints including T-cell percentage, clonality, and maximum frequency of TCR clone were assessed through previously described methods [6]. TIL percentage in specimens was calculated by amplifying several housekeeping genes and quantifying their template count to determine the quantity of DNA usable for CDR3 region sequencing.

2.4. Statistical analysis

Due to a limited sample size, all data reported are observational and no formal statistical analysis was performed.

3. Results

3.1. Clinicopathologic characteristics

All tumors were histologically confirmed urothelial carcinoma. Nine of 12 (75%) patients were male and median age at time of TUR was 72. There was an even distribution of specimens across all pathologic stages: of 12 specimens, 3 (25%) were cT0, 3 (25%) were cTa, 3 (25%) were cT1, and 3 (25%) were cT2 or greater. Of the 3 cT0 patients, 2 (67%) had a history of prior urothelial carcinoma. Of the 9 malignant specimens, 7 (78%) were high grade and 2 (22%) were low grade.

3.2. DNA sequencing

Individual TCR transcripts across all specimens were isolated and sequenced. A TCR transcript was defined as a productive rearrangement if it was in-frame, did not contain a stop codon, and was capable of producing a functional peptide receptor. There were 272,763 productive rearrangements sequenced across all specimens. The median number of productive TCR rearrangements sequenced in each sample in urothelial carcinoma specimens (UC+) and benign (UC−) specimens was 5,569 and 25,872, respectively (Table 1).

Of the 272,763 productive rearrangements sequenced, 71,924 were unique T-cell clones across all specimens. Nine hundred and fifty-one unique clones were present in 2 or more patients, including 120 clones in 3 or more patients, 27 clones in 4 or more patients, and 4 clones in 5 patients. The median number of unique TCRs sequenced in UC+ and UC− specimens was 3,069 and 9,680, respectively.

The UC+ specimens demonstrated higher clonality as evidenced by identification of a specific T-cell clone being present in up to 17% of the total TIL pool; in contrast, the frequency of the max T-cell clone was 2% in UC− specimens (Figs. 1 and 2)

3.3. Tumor microenvironment

The median TIL percentage was approximated from sequencing data by calculating the relative abundance of CDR3 sequences in the library of total PCR products. This method enabled us to estimate the ratio of T cells to total nucleated cells in each specimen. TIL percentage across all specimens ranged from 0.3% to 38%. The median TIL

Table 1
Characteristics of immune microenvironment

	UC+ $n=9$	UC− $n=3$
Total productive TCR rearrangements (median)	5,569	25,872
Unique productive TCR rearrangements (median)	3,069	9,680
TIL percentage (median)	2	12

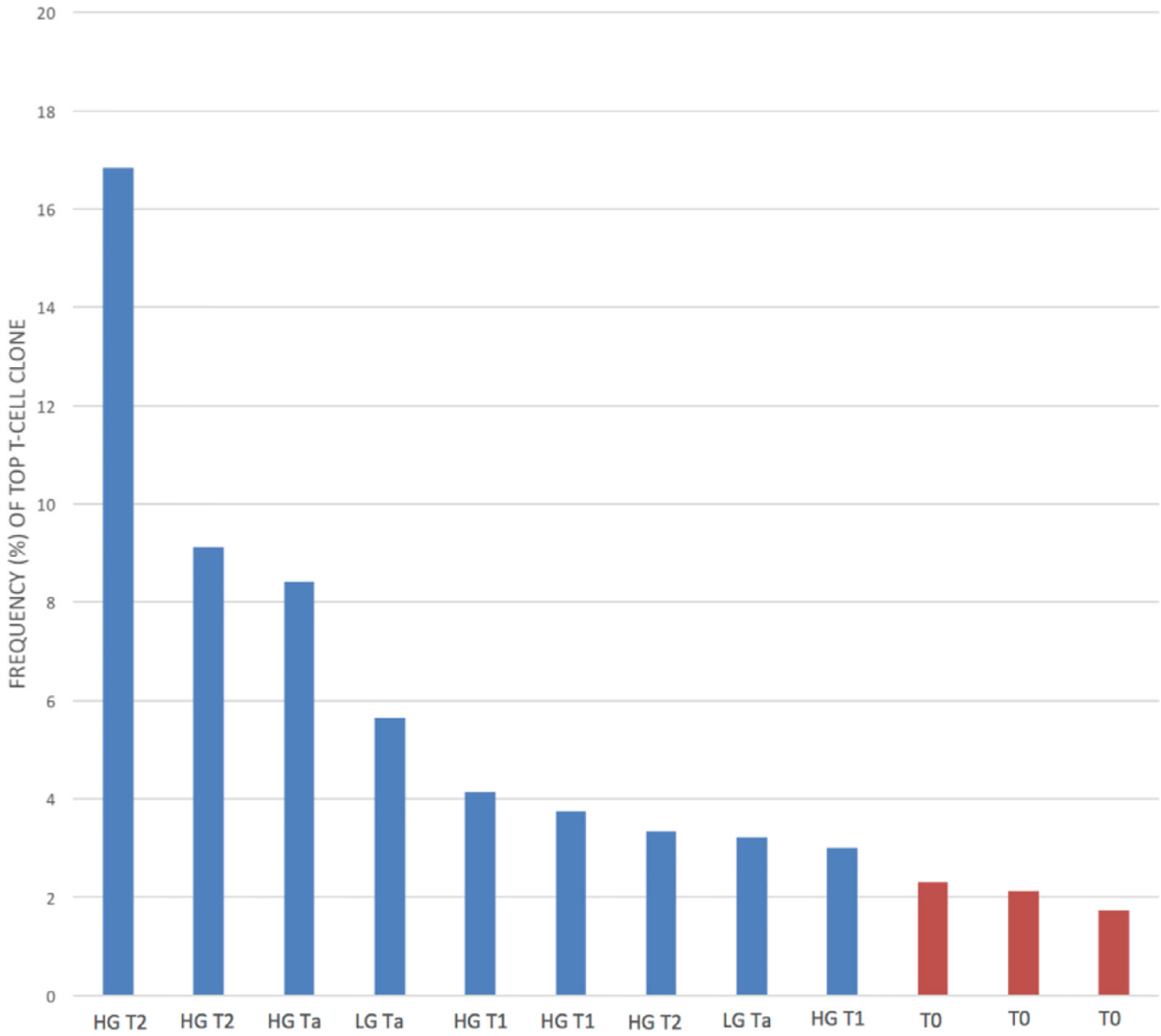

Fig. 1. Frequency of the most dominant T-cell clone present in each lesion. Each bar represents 1 lesion and is annotated by pathologic stage. Blue bars represent malignant lesions (Ta, T1, T2). Red bars represent benign lesions (T0). Lesions were classified as T0 if no urothelial carcinoma was present, regardless of the patients' bladder cancer history. Color version of figure is available online.

percentage in UC+ and UC− specimens was 2% and 12%, respectively.

4. Discussion

Neoantigens appear to play a critical role in the genesis of the human immune response to cancer; however, the mechanism whereby specific neoantigens are identified by the immune system remains to be elucidated. High throughput sequencing of expressed TCR transcripts is a newly described technique for identifying the T-cell repertoire present in the tumor microenvironment [8]. In our study, TCR repertoire clonality was higher in malignant tissue compared to benign tissue, suggesting there is an enhanced, tailored immune response after tumor neoantigen identification. In concordance with these findings, 2 other recent studies have suggested that clonal expansion of certain populations of T cells in the primary tumor and blood are associated with clinical benefit in patients with urothelial carcinoma.

The first study by Chowdury et al. revealed that patients with bladder tumors containing clonally expanded T cells have superior clinical outcomes [9]. The authors investigated the immunologic landscape of the primary tumors on the surgical specimens of patients who underwent radical cystectomy for muscle invasive bladder cancer. After sequencing the TCR of all TILs, they assigned a diversity score to characterize the heterogeneity of T-cell clones present. Tumors with lower diversity score were associated with longer recurrence-free survival, independent of pathologic stage, and receipt of chemotherapy.

The second study by Snyder et al. identified that peripheral blood expansion of tumor associated T-cell clones after treatment with systemic immunotherapy is associated with

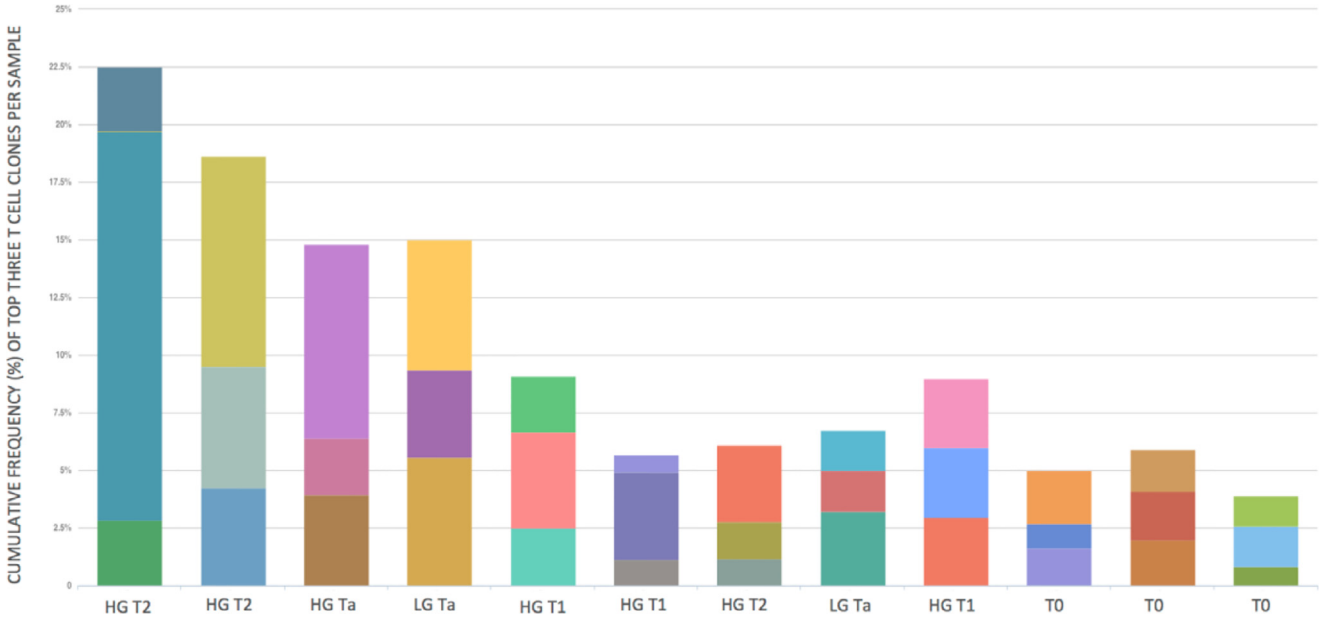

Fig. 2. Frequency of the 3 most prevalent T-cell clones present in each lesion. Each bar represents 1 lesion and is annotated by pathologic stage. Each color represents a unique clone. None of the clones represented in the figure were present in more than 1 patient. Color version of figure is available online.

durable remission in advanced urothelial carcinoma [5]. The authors examined the immune features of the primary tumor and peripheral blood of patients with metastatic urothelial carcinoma treated with PD-L1 blockade. By sequencing the TCR of TILs and corresponding blood, they discovered that clinical responders to immunotherapy had a diverse repertoire of T cells in their blood and an increase in tumor associated T-cell clones after starting treatment. These findings suggest that TCR sequencing can predict patients who are able to generate systemic immune response against tumor cells and who may benefit from a durable clinical remission after treatment with immunotherapy.

TCR sequencing of urothelial tumors have several potential applications. Firstly, it has potential to be a novel biomarker of host response. Secondly, it might enable identification of T-cell clones in circulation that have potential to identify bladder tumor-specific neoantigens at regional and distant sites. Lastly, it is a novel technique for studying tumor immune evasion pathways and developing therapeutic strategies to enhance immune-mediated tumor destruction.

This is the first study to our knowledge demonstrating a clonal immune response in nonmuscle invasive bladder tumors. There was a max clonal frequency ranging from 3% to 8% seen in the Ta/T1 tumors in our study. Expansion of tumor-identifying T-cell clones may be a critical early step in eliciting an appropriate anti-tumor response with intravesical immunotherapy such as BCG. These findings provide a rationale for continued research into T-cell clonality and response to immunotherapy in nonmuscle invasive bladder cancer. Incorporating correlative studies utilizing TCR sequencing should be considered by investigators designing clinical trials for immunotherapy trials in nonmuscle invasive bladder cancer.

Our study is limited by its small sample size and lack of robust statistical analysis. The results are observational and hypothesis generating. Future studies should be conducted with larger sample sizes to validate our findings and to identify which tumor derived antigens are of most clinical significance.

Bladder tumors induce an antigen driven immunogenic host response, in contrast to the benign inflammatory response, which does not appear to demonstrate any T-cell clonal dominance. Inflammatory lesions demonstrated a higher proportion of TILs as well as a larger total number of rearranged TCRs, suggesting that oligoclonal T-cell expansion in primary tumors may be a novel mechanism of host immune response to urothelial tumors.

Conflict of interest

None.

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