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ORIGINAL ARTICLE

T cell receptor gene recombinations in human tumor specimen exome files: detection of T cell receptor-β VDJ recombinations associates with a favorable oncologic outcome for bladder cancer

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Abstract Understanding tumor-resident T cells is important for cancer prognosis and treatment options. Conventional, solid tumor specimen exome files can be searched directly for recombined T cell receptor (TcR)-α segments; RNASeq files can include TcR-β VDJ recombinations. To learn whether there are medically relevant uses of exome-based detection of TcR V(D)J recombinations in the tumor microenvironment, we searched cancer genome atlas and Moffitt Cancer Center, tumor specimen exome files for TcR-β, TcR-γ, and TcR-δ recombinations, for bladder and stomach cancer. We found that bladder cancer exomes with productive TcR-β recombinations had a significant association with No Subsequent Tumors and a positive response to drug treatments, with $p < 0.004$, $p < 0.05$, and $p < 0.004$, depending on the sample sets examined. We also discovered the opportunity to detect productive TcR-γ and TcR-δ recombinations in the tumor microenvironment, via the tumor specimen exome files.

Keywords TCGA · T cell receptor recombination · Exome · Bladder cancer · Stomach cancer · Tumor microenvironment

Abbreviations

BAM	Binary alignment map
BLCA	Bladder cancer
CAR	Chimeric antigen receptor
IMGT	Immuno gene tics
MHC	Major histocompatibility complex
STAD	Stomach adenocarcinoma
TCGA	The cancer genome atlas
USF	University of South Florida
WXS	Exome

Introduction

Tumor immunoscoring has been used for prognosis and for identifying tumor specimens that could yield T cells that can be expanded in vitro for re-administration to the patient. More recently, molecular approaches have been developed that have the promise of complementing histology- and flow cytometry-based immunoscoring [1]. For example, molecular and genomics methods have been developed for exon capture of T cell receptor gene segments to assess VDJ recombinations and usage, in tumor-resident lymphocytes [2] and for assays for MHCII and TcR expression correlations, which may indicate an effective combination of antigen presenting cells and T cells [3, 4]. We have recently determined that the conventional exome (WXS) files generated from tumor specimens, primarily to assess tumor mutations, also include reads representing productive TcR-α VJ recombinations, presumably representing tumor-resident T cells [5]. Recombinations

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representing TcR-β VDJ segments have been detected via RNASeq assays [6]. The number of RNASeq reads representing TcR-β recombinations was comparable to what was observed for the TcR-α detection in the tumor WXS files. However, neither of the preceding studies provided any data as to the potential role of the T cells that were detected by searching tumor specimen WXS files. Also, neither of these studies indicated any medical usefulness of the opportunity to detect TcR recombinations in tumor specimen WXS files.

Given the worldwide, extensive production of tumor specimen WXS for tumor mutation analyses, and the relevance of tumor-resident lymphocytes that may be impacting the tumor independently of TcR expression, we were interested in detecting both productive and unproductive TcR-β, -γ, and -δ V(D)J recombinations in tumor WXS. While TcR-α VJ recombinations are biallelic and common in T cells [7], the recombinations of the remaining three TcR chains are potentially more problematic, owing to their more limited occurrence. To accomplish this task, we employed WXS files from the cancer genome atlas (TCGA) and WXS representing Moffitt Cancer Center patients. The results presented below represent the first indication of the functional and medical relevance of the detection of TcR recombinations using tissue specimen exome files.

Methods

Overview

The methods used for detecting the presence of recombined TcR-β, -γ, -δ genes in the tumor specimen WXS files were closely related to the approach used for detecting the TcR-α VJ recombinations in Gill et al. [5], except the TcR-β, -γ, and -δ V and J segments were substituted for the TcR-α V and J segments in the TcR-α search algorithms. Note, the TcR-α VJ recombinations are also part of the current study. The following text briefly describes the past TcR-α approach and details additional algorithms and code used for determining whether reads represented productive or unproductive V(D)J recombinations.

Obtaining BAM (binary alignment/map) files

BAM files were obtained from the CGHUB repository, following approval of dbGaP project number 6300 and obtaining a key file. The BAM files were obtained using a manifest file and downloaded with *GeneTorrent*. The files were stored in a single folder, using facilities available from USF research computing.

Preliminary subset of candidate V-region reads

The program, "FindV2a," detailed in Ref. [5], was used as a starting point to search all BAM files in a specified folder, using University of South Florida (USF) research computing resources, for all possible, candidate TcR- V-regions. To allow for N-region diversity, only the first ten of the 3′-most fifteen nucleotides were obtained from each V-region used in the search algorithm. All of the TcR-α V-regions, used in the search algorithm, are indicated in Ref. [5]. All of the TcR-β, -γ, and -δ V-regions used as source material in the search algorithm, and for the search code for the V-regions, are in Supplementary table S6. The names of the programs with the TcR-β, -γ, -δ V-region sequences are as follows: "FindV2a_BETA," "FindV2a_GAMMA," and "FindV2a_DELTA." Each BAM file was searched for the V-Regions between chr14:18000000-31000000 in the case of the TcR-α gene and was searched in the analogous chromosomal regions for the TcR-β, -γ, and -δ V-regions. The BAM file was opened to the specific chromosomal regions in each case using *SAMtools*. After the candidate V-region reads were identified by the above-indicated processing step, the reads were dumped into a TSV file and stored in a folder labeled "V_Region." The files have the following name structure: vRegion_[NAME].tsv, where [NAME] is taken from the BAM filename, i.e., including the TCGA barcode (which represents a specific cancer patient).

Obtaining J-Region reads

The program SearchJ searched through the candidate V-Region read files established in a "V_Region" folder for the presence of exact matches to J-Region reads. As in the case of "FindV2a" in Ref. [5], SearchJ obtained the TcR-α J-Regions only, and other variants of SearchJ, e.g., programs termed, SearchJ_BETA, SearchJ_DELTA, obtained the reads representing exact matches for the indicated TcR J-Regions. The SearchJ programs contain the full-length J-Regions, but to account for N-Region diversity, SearchJ truncated each J-Region to 30 nucleotides to search the files with the candidate V-regions. If a J-region was not found, SearchJ shortened the J-region by one nucleotide, repeatedly, until there was a final search for ten J nucleotides. SearchJ repeated the search until either a match was found or the minimum length was reached. SearchJ continued through all J-region sequences. After all the matching J-Region sequences were found for a single vRegion[NAME].tsv file, they were collected and dumped into a TSV file and stored in a folder labeled "J_Region." The files followed the naming structure, jRegion_[NAME].tsv where [NAME] again was taken from the BAM filename and represented the TCGA or Moffitt Cancer Center

barcode. Only the truncated section of the J-Region was recorded in the file.

Batch analysis of a large set of BAM files

An original script, termed TcR_Batch.sh, generated all the above-indicated folders for TcR-α, -β, -γ, -δ, as well as their corresponding V_Region and J_Region folders, and ran the FindV2a and SearchJ programs, along with the above indicated, modified programs for TcR-β, -γ, -δ, for all BAM files located in a specified folder. This script automated the approach for this study, rather than processing one BAM file at a time.

Identifying productive recombinations

An original php program, imgtSearchTCR.php, allowed for identification of productive and nonproductive V(D)J rearrangements by submitting the reads which contained a V- and a J-Region to the online IMGT VQUEST tool (http://www.imgt.org/IMGT_vquest/vquest?livret=0&Option=humanTcR). This program matched jRegion and vRegion TSV's, exploiting their shared names, and extracted all the reads that have J-Regions as specified in the jRegion TSV file. Each read was submitted, and the result as well as the original read was dumped into a TSV file, which followed the above-indicated naming structure: mRegion_[NAME].tsv. Each mRegion TSV file was stored in the TcR folder to which it corresponded (α, β, γ, δ), and each individual file corresponded to a BAM file.

Creating summary files

Using the mRegion TSVs, described above, a summary file, for each TCGA cancer dataset, was created documenting the number of reads representing productive TcR recombinations for each BAM file. All of the above computer code is available from the corresponding author upon request.

Characterizing TCGA barcodes and Moffitt Cancer Center samples with regard to clinical parameters

Clinical data for the BLCA and STAD cancer datasets were downloaded from the TCGA data portal. The clinical follow-up files were used to categorize barcodes into "*New Tumor*" and "*No Subsequent Tumor*" groups based on the new tumor event data. The *No Subsequent Tumor* barcodes were compared to the *New Tumor* barcodes and any *No Subsequent Tumor* barcode appearing in the *New Tumor* group was eliminated from the *No Subsequent Tumor* group. The clinical drug files were used to categorize barcodes based on treatment best response. Barcodes with Clinical Progressive and Stable disease were grouped into the Negative Outcome category, while barcodes with Partial and Complete response were grouped into the Positive Outcome category. Any barcodes appearing in more than one treatment response group were eliminated from further analysis. We then obtained an overlapping set of barcodes as follows. We compared the *No Subsequent Tumor* and Positive Outcome groups and retained any barcode that appeared in both groups. We then compared the *New Tumor* and Negative Outcome barcodes and retained any barcode that appeared both groups. These two sets of barcodes, "*No Subsequent Tumor*/Positive Outcome" and "*New Tumor*/Negative Outcome" comprised the BLCA-55 dataset. Two analogous sets of barcodes were established for the STAD-65 dataset. The BLCA-82 set represented the *New Tumor* category without consideration of drug response information. The BLCA-146 set represented the *No Subsequent Tumor* category also without consideration of drug response information. Thus, these two latter groups represented a lower stringency in terms of patient outcome. The Moffitt Cancer Center samples represented invasive, high-risk bladder cancer. Patients were stratified into groups with and without pathologic lymph node metastases, and exomes were generated, by the Moffitt Cancer Center functional genomics core facility, from primary bladder cancer samples representing these two groups of patients. There was no systemic therapy prior to surgery in the cases used for this study.

Results

Detection of TcR-β V(D)J recombinations

Previous work led to the detection of productive TcR-α VJ recombinations in tumor WXS files [5] and the detection of TcR-β VDJ recombinations in tumor specimen RNASeq files [6]. In the latter case, no specific VDJ recombinations were described. The report represented an overview of the basic detection rates for the TCGA cancer datasets [6]. Thus, we performed searches for TcR-β VDJ recombinations for 55 TCGA BLCA WXS files; 16 Moffitt Cancer Center BLCA WXS files; and 65 TCGA STAD WXS files. Productive TcR-β VDJ recombinations were identified for 7 barcodes in BLCA-55 set; 3 barcodes in Moffitt BLCA-16 set; and 8 barcodes in the STAD-65 set. The specific recombinations and the VDJ usage are indicated in Table 1 (BLCA); in Supplementary table S1 (STAD-65); in Fig. 1 (BLCA); and in Supplementary figure S1 (STAD-65). These results represent the first identification of specific TcR-β V's, D's, and J's appearing as recombinants in tumor specimen WXS files, presumably representing recombinants from tumor-resident T cells at the time of preparation of the tumor sample for DNA extraction and

Table 1 Detection of TcR-β reads representing productive VDJ recombinations, in the TCGA BLCA-55 and Moffitt Cancer Center datasets

TCGA BLCA barcode	Reads	Number of reads detected	Clinical status	V usage	D usage	J usage
TCGA-FD-A5BS	TCCCAGACATCTGTGTACTTCTGTGCCAGCAGTGCCCG GACAGGGAAGTATGGCTACACCTTCGGTTCGGGGACCA	7	No Subsequent Tumor/Positive Outcome	TRBV10-3	TRBD1	TRBJ1-2
TCGA-FD-A5C1	AGCCCCAACCAGACCTCTCTGTACTTCTGTGC CAGCAGTTTATCGGGCGGGGGAGTATACGAGCAG TACTTCGGGC	20	No Subsequent Tumor/Positive Outcome	TRBV27-01	TRBD2	TRBJ2-7
TCGA-FD-A43S	TCTGCGCCAGCAGCCAAGATAGCAGTTACGGGGC CAACGTCCTGACTTTCGGGGCCGGCAGCAGGCTGAC CGTGCT	6	No Subsequent Tumor/Positive Outcome	TRBV10-1/2/3	TRBD2	TRBJ2-6
TCGA-SY-A9G5	CAGCCAGAAGACTCAGCCCTGTATCTCTGCGC CAGCAGCCAATCTGCGGGGGGGGCCGGGGAGACC CAGTACTTCG	2	No Subsequent Tumor/Positive Outcome	TRBV4-1	TRBD2	TRBJ2-5
TCGA-DK-AA6 M	CTTCTGTGCCAGCAGTTTAGCTTCCCTAGTATTCTAC GAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGT CACA	10	No Subsequent Tumor/Positive Outcome	TRBV10-3/ TRBV12-3	TRBD2	TRBJ2-7
TCGA-XF-AAN3	CCATCCTGAAGACAGCAGCTTCTACATCTGCAGTGCTA GACCTACAGGATACCAAGAGACCCAGTACTTCGGGCCA	9	No Subsequent Tumor/Positive Outcome	TRBV20-1	TRBD1	TRBJ2-5
TCGA-XF-AAN5	CATCTGTGTACTTCTGTGCCAGCAGTGATGGACAGTTC CGAGAGACCCAGTACTTCGGGCCAGGCACGCGGCTCCT	6	No Subsequent Tumor/Positive Outcome	TRBV10-3/6-1	TRBD1	TRBJ2-5
Moffitt BLCA-1	GGAGCTTGGTGACTCTGCTGTGTATTTCTGT GCCAGCAGCCAAGGTCGGACTACGACTGAA GCTTTCTTTGGACA	6	(No subsequent metastatic progression)	TRBV3-1/2	TRBD2	TRBJ1-1
	GAGCTGGGGGACTCGGCCCTTTATCTTTGCGCCAGCAGC CAGGAGACAGGGAGCCACGAGCAGTACTTCGGGCCGG	4	(above)	TRBV5-1	TRBD1	TRBJ2-7
Moffitt BLCA-2	TTTATCTTTGCGCCAGCAGCTTGGCCCCGGGCGGAAC TAGCACAGATACGCAGTATTTTGGCCCAGGCACCCG GCT	2	(No subsequent metastatic progression)	TRBV5-1	TRBD1	TRBJ2-3
	CTGTATCTCTGCGCCAGCAGCCAAGAATGGCCCCG GACAGGCTCCTACGAGCAGTACTTCGGGCCGGGCAC CAGGC	4	(above)	TRBV23-1; TRBV4-1/2	TRBD1	TRBJ2-7
Moffitt BLCA-3	CTGCTCCCTCCCAGACATCTGTGTACTTCTGTGC CAGCAGTGAGGTGAACACCGGGGAGCTGTTTTTTG GAGAAGG	8	(lymph node metastasis)	TRBV6-1/5/6	None	TRBJ2-2

The association of detection of productive TcR-β recombinations with the No Subsequent Tumor/Positive Outcome group for this set of samples has a $p < 0.04$

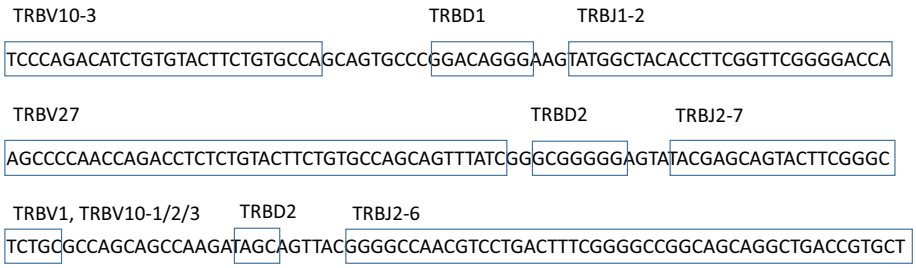

Fig. 1 Example TcR-β recombinations represented by reads recovered from TCGA BLCA WXS files, with V, D, and J usage indicated. *Barcode*, TCGA-FD-A5BS, *top*; TCGA-FD-A5C1, *middle*; TCGA-FD-A43S, *bottom*

Table 2 More frequent detection of TcR-α recombinations in WXS files

	TcR-α	TcR-β
Productive read total for 55 TCGA BLCA barcodes	176	60
Number of barcodes with productive reads	19	11
Average number of reads (per barcode with reads)	9.26	5.45
Number of barcodes with an α/β overlap	4	
Productive read total for 65 TCGA STAD barcodes	192	156
Number of barcodes with productive reads	26	8
Average number of reads (per barcode with reads)	7.38	19.5
Number of barcodes with an α/β overlap	4	

sequencing. These results also represent the first identification of TcR-β N-region nucleotides using tumor specimen WXS files. The results also verified the relatively high rate of detection of TcR-β VDJ for STAD (Supplementary table S1 and Table 2); that is, in the report of detection of TcR-β recombinations in tumor specimen RNASeq files, a relatively high number TcR-β VDJ recombination reads were detected in the STAD dataset, compared with other TCGA RNASeq files [6]. The sample size in the analyses of this study is relatively small, but there was no significant association of TcR-β VDJ usage with mutation rates or specific mutations. Finally, it is worth noting that numerous unproductive TcR-β VDJ recombinations were identified.

Higher frequency of TcR-α versus TcR-β recombinations

Many T cells rearrange both TcR-α alleles [7]; thus, our previous report of TcR-α recombinations represented the lowest stringency search for indications of tissue resident T cells in tumor specimen WXS files [5]. For this report, we conducted an additional search for TcR-α VJ recombination in the above-indicated TCGA BLCA-55 and STAD-65 datasets, using exactly the search algorithm indicated in Ref. [5]. In both the BLCA-55 and STAD-65 datasets, the productive read counts were higher for TcR-α than TcR-β (Table 2; Supplementary table S2), consistent with the previous reports of the more common TcR-α VJ genome recombinations [4, 7].

Detection of γ/δ recombinations

We next determined whether V(D)J recombinations representing γ/δ T cells could be detected in the tumor specimen WXS files. Table 3 describes the productive TcR-γ and TcR-δ rearrangements found in the TCGA BLCA-55 and STAD-65 datasets. No TcR-γ VJ or TcR-δ VDJ recombinations, either productive or unproductive, were detected in the Moffitt Cancer Center BLCA-16 WXS files. Comparisons of read counts for productive TcR-α/β, and both productive and unproductive TcR-γ/δ recombinations, are presented in Fig. 2. Although the numbers are smaller than in the α/β case, more TcR-γ than TcR-δ recombinations were detected (Table 3).

Productive TcR-β in bladder cancer correlates with a favorable oncologic outcome

The BLCA-55 WXS set represents a statistically significant number of barcodes with productive TcR-β recombinations and the combined *No Subsequent Tumor*/Positive Outcome category (see "Methods" section; Table 1). There was no correlation of the tumor specimen, WXS detected TcR-α VJ recombinations with either the *No Subsequent Tumor*/Positive Outcome or *New Tumor*/Negative Outcome groups.

To confirm the initial linkage of the TcR-β VDJ recombinations with the *No Subsequent Tumor*/Positive Outcome group for BLCA, we searched the WXS files of a BLCA-82 set that represented the single parameter category, *New Tumor* and a BLCA-146 set representing *No Subsequent Tumor*. While these two sets represented larger numbers of barcodes, these two sets did not have the stringency of the dual parameter sets, whereby the BLCA barcodes had to meet the condition of being in both the tumor assessment and treatment assessment groups (as indicated in Table 1; "Methods" section). Nevertheless, the percentage of barcodes where TcR-β VDJ recombinations were detectable was significantly higher in the BLCA-146 *No Subsequent Tumor* group than in the BLCA-82 *New Tumor* group (Table 4; Supplementary tables S4, S5).

We noticed that several samples in the BLCA-146 and BLCA-82 sets had an unusually high number of reads representing TcR-β VDJ recombinations (Supplementary

Table 3 Detection of productive TcR-γ/δ recombination reads in TCGA BLCA-55 and STAD-65 WXS files

	Reads	Number of reads detected	Clinical status	V usage	D usage	J usage
TCGA BLCA barcode						
TCGA-BT-A42E	ACCATCAGAGAGAGATGAAGGGTCT TACTACTGTGCCTGTGACACCGTG GGGGGCCACACCGATAAACTCA TCTTT	4	No Subsequent Tumor/ Positive Outcome	TRDV2	TRDD3	TRDJ1
TCGA-DK-A3WY	TGAAAATGATTCTGGGGTCTAT TACTGTGCCACCTGGGACAG GCCCAGTAGAAGGTATTATAA GAAACTCTTTGGC	27	No Subsequent Tumor/ Positive Outcome	TRGV3/5	N/A	TRGJ1
TCGA-FJ-A3Z9	GAAATCTAATTGAAAATGATTCTG GGGTCTATTACTGTGCCACCTG GGACAGGCGAGGGTATAAGAA ACTCTTTGN	9	No Subsequent Tumor/ Positive Outcome	TRGV5	N/A	TRGJ1
TCGA STAD barcode						
TCGA-BR-6457	TAGAGAAACAGGACATAGCTACCTAC TACTGTGCCTTGTGGGAGGGGCCG GGCCATAAGAAACTCTTTGGCA GCGG	2	No Subsequent Tumor/ Positive Outcome	TRGV9	N/A	TRGJ1

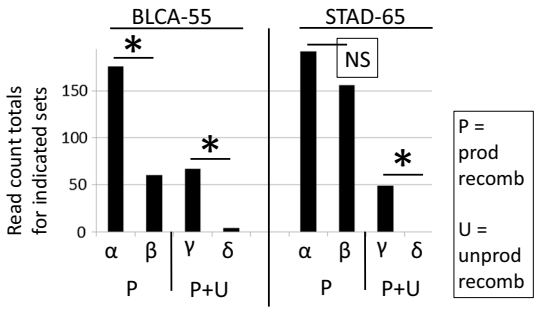

Fig. 2 Comparison of read counts for productive TcR-α and TcR-β, and for both productive and unproductive TcR-γ and TcR-δ, for the BLCA-55 and STAD-65 TCGA sets (Supplementary table S3)

tables S4, S5). We considered the possibility that a high level of inflammation would have a negative effect on outcome, keeping in mind previous reports of associations of chronic inflammation with tumorigenesis [8, 9]. We also considered the fact that we were not able to detect an association of TcR-β VDJ recombinations with a favorable outcome for STAD in this study (compare Table 1 and Supplementary table S1) and that in both this study and in Ref. [6] the STAD read counts for TcR VDJ recombinations are relatively high. Thus, we recalculated the *p* value, eliminating high read-number barcodes from both the BLCA-146 and BLCA-82 sets. For this latter analysis, the association of the *No Subsequent Tumor*, BLCA-146 set with TcR-β VDJ recombinations in the WXS files, revealed a higher degree of statistical significance (Table 4).

Discussion

The above results indicate that tumor specimen WXS files can be used to assess TcR-β, -γ, -δ V(D)J recombinations, presumably representing the recombinations present in tumor-resident T cells. These results indicate there is an opportunity to evaluate the tumor microenvironment using standard, very common WXS files, as opposed to more expensive and less common, highly specialized approaches. Also, more complicated approaches to assessing tumor cell infiltrates may run a risk of artificial alteration of samples with each processing step. For example, in some cases isolation of T cells following tumor disaggregation may lead to a more comprehensive assessment, but in other cases, the additional and more complicated sample manipulations may reduce the opportunities for consistency and reliability; may lead to greater sample degradation, thereby limiting important opportunities for identification of microenvironment components; or may lead to the obscuring of a detection "window of usefulness," due to a hyper-sensitive TcR detection process. However, it is also important to note that the algorithms used above will almost certainly be improved, now that it is clear that tumor microenvironment preparation, followed by exome generation, can lead to medically relevant TcR V(D)J recombination results.

Specifically, the above results indicate that a favorable outcome in bladder cancer is associated with detection of TcR-β sequences in primary sample, bladder cancer WXS files. Keeping in mind the inevitable variations in assembling large databases of clinical information for public access, detecting statistically significant associations as

Table 4 Association of TcR-β VDJ recombinations with the No Subsequent Tumor, BLCA-146 set

	No. Subsequent Tumor, BLCA-146 set	New tumor, BLCA-82 set	*p* value <
No. of barcodes with a productive TcR-β VDJ recombination	30/146	10/82	0.04647
No. of barcodes with a productive TcR-β VDJ recombination (exclude 20 reads and above)	28/146	6/82	0.00356

indicated above (Tables 1, 4) is quite striking. While these results certainly need to be confirmed, it is highly likely that a much more controlled source of clinical information will yield a more dramatic level of statistical significance, for the association of WXS file-detectable TcR-β VDJ recombinations and a favorable oncologic outcome.

The association of TcR-β VDJ recombinations with a favorable outcome supports the idea that the VDJ recombinations detected in the tumor specimen WXS files represent T cells relevant to the progression of the cancer, rather than T cells that are present only adventitiously during the preparation of the tumor specimen. Furthermore, because the TcR-β VDJ recombinations are most closely associated with TcR antigen specificity, i.e., represent the complementarity determining region, the question can now be asked, is the association of the detection of the TcR-β VDJ recombinations in these WXS files due to a T cell response against a bladder cancer tumor antigen? If so, the above approach offers the hope of a highly efficient opportunity to either identify and amplify T cells directed against a specific patient's tumor or generate an immune receptor that can be used to create a specific, effective CAR T cell [10].

The results above indicate a further validation of the approach of using exome files for assessing the immune status of the tumor sample, namely the ratios of α/β chains and γ/δ chains. As would be expected, the recombined α and γ chains are more readily detected, compared with the β and δ chains, respectively, consistent with previous assessments of increased recombination frequencies for the α chain in T cells [7] but also consistent with the probabilistic impact of requiring one recombination event (VJ), rather than two (VDJ), for detection of recombined α and γ genes.

The opportunity to evaluate TcR recombinations in WXS offers new opportunities, again due to the fact that tumor WXS are available in such large numbers. For example, knowledge of the extent of the association of HLA haplotypes with V(D)J usage is promising but remains incomplete [11]. Obviously, having the HLA alleles and V(D)J recombinations in one sample file renders the goal of attempting to solidify the association of HLA alleles and V(D)J usage virtually automatic. Also, it is likely that, with enough tumor specimen, WXS files, there will be opportunities to correlate specific mutations or mutation rates [2, 12–15] with specific TcR VDJ usage.

Finally, while antigen recognition is more often associated with α/β T cells, the above detection of γ/δ T cells may afford the opportunity to better understand the impact of the presence of these cells in terms of cytokine release or other factors that could impact the tumor microenvironment and tumor development [16].

Conclusion

The detection of TcR recombinations in tumor specimen exome files is likely useful for prognosis in bladder cancer.

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Compliance with ethical standards

Conflict of interests All authors declare that they have no conflict of interest.

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