Assessing Microenvironment Immunogenicity Using Tumor Specimen Exomes: Co-detection of TcR-α/β V(D)J Recombinations Correlates with PD-1Expression

Yaping N. Tu 1 , Wei Lue Tong 1 , Mohammad D. Samy 1 , John M. Yavorski 1 , Minjung Kim 2 , and George Blanck 1,3,*.
1 Department of Molecular Medicine, Morsani College of Medicine, University of South Florida; and 2 Department of Molecular Oncology; and 3 Immunology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida

Abstract

T-cell receptor (TcR) recombinations can be recovered from tumor specimen, whole exome (WXS) files. However, it is not yet clear how these recombinations represent lymphocytes or an anti-tumor immune response. Here we report the identification of productive TcR-β recombinations in WXS files representing primary and metastatic melanoma. The recombinations are identifiable in about 20% of the cancer genome atlas melanoma samples. This frequency of detection is lower than the frequency of TcR-α VJ recombinations, consistent with the occurrence of biallelic TcR-α recombinations and possibly consistent with fact that only one junctional recombination is required for TcR-α whereas two recombinations are required to form a TcR-β gene. Nevertheless, the ratio of productive TcR-β to unproductive TcR-β samples, in comparison to the ratio of productive to unproductive TcR-α or TcR-γ positive-samples, is very high. This result indicates that detection of a productive TcR-β VDJ recombination represents a comparatively high standard for potential antigen binding capacity, when employing a tumor specimen exome file for the assessment. Additionally, PD-1 expression and antigen presentation functions correlated with the co-detection of TcR-α and -β recombinations (e.g., $p < 0.0004$), suggesting that co-detection of TcR-α and -β recombinations represents an anti-melanoma response that has been blunted by the advent of PD-1 expression. We further show that the algorithm for detecting the TcR-β VDJ recombinations is applicable to exome files generated from mouse tissue, thus providing for opportunities to develop empirical paradigms for interpreting the identification of TcR V(D)J recombinations in tissue resident lymphocytes.

Methods

- Tumor specimen whole exome sequence files were downloaded directly from NIH websites.
- Files were searched for sequence reads representing recombination of the TCR (αβγδ), following procedures and utilizing softwares developed in a previous publication (PubMed ID: 26966347)
- TCR reads were then verified using a web tool hosted by the IMmune GeneTic website
- Data is tabulated in the indicated displays for this poster
- RNA Sequencing z-scores (representing standard deviations from the average) were obtained from cBioPortal.com

Results

Table 1. TCGA barcode count for productive and unproductive recombinations identified for TcR (α, β, γ, δ) genes in primary and metastatic TCGA SKCM WXS files

TcR gene	99 primary samples				307 metastatic samples			
	Productive		Unproductive		Productive		Unproductive	
α	22	22%	16	15.84%	113	37%	102	33.22%
β	19	19%	3	3%	55	24.4%	14	4.6%
γ	3	3%	7	7%	14	4.6%	31	10%
δ	0	0%	0	0%	3	0.98%	1	0.33%

Figure 1: Ratio of Productive to Unproductive TcR Recombinations in Primary and Metastatic TCGA SCKM WXS Files.

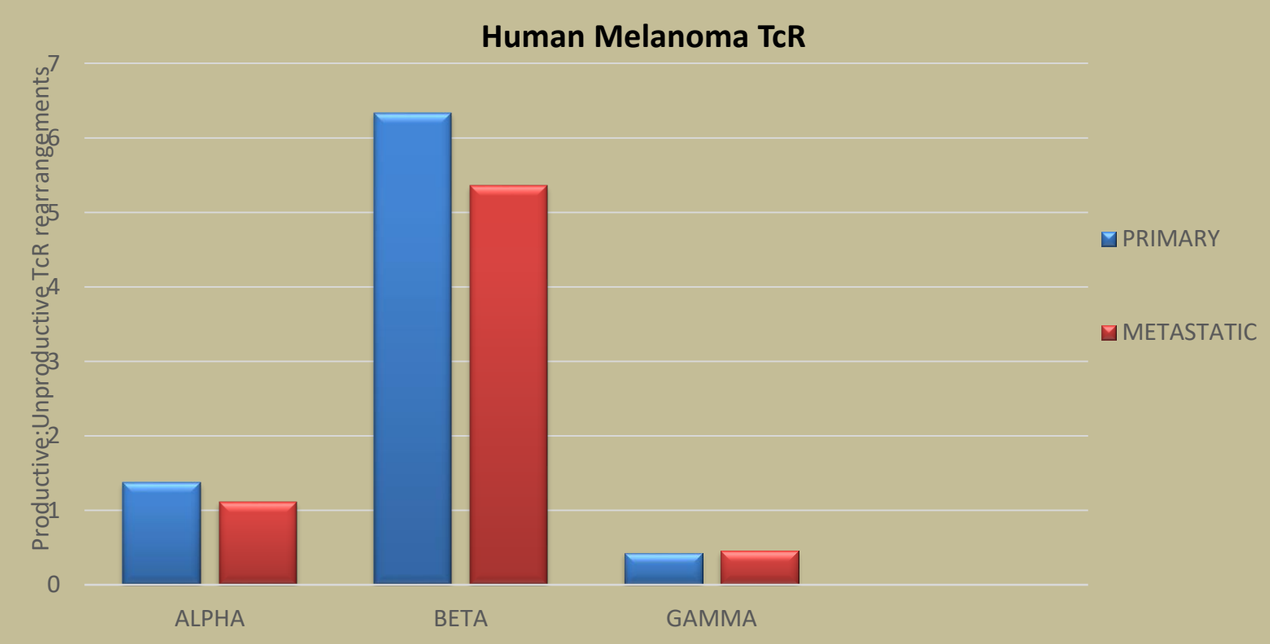

Figure 2: Examples of Primary TcR-β Productive Read from WXS SKCM files

TRBV3-1/2 TRBD1 TRBJ1-4

TGACTCTGCTGTGTATTTCTGTGCCAGCAGCC CC GGGACA TCCAGTGCAACTAATGAAA AACTGTTTTTTGGCAGT

(Barcode ID: TCGA-BF-A1PX)

Conclusion

- As expected there are significantly more TcR-α than TcR-β, TcR-γ than TcR-δ. This is consistent with more frequent recombination for the TcR-α and TcR-γ chains in both primary and metastatic tumor samples (Table 1).
- The high level of TcR-α recombinations detected could indicate the presence of T-cells in the tumor which impact the tumor without reacting to antigens.
- Co-detection of productive TcR-α and TcR-β correlates with high expression of the immune check point protein PD-1, possibly explaining the immune responses which are not leaving these patients free of melanoma (Table 6).
- Verification of this detection method was also completed in mouse tumor exomes (Table 4).

Table 6. RNASeq z-scores for immune function genes, for TCGA SKCM barcodes representing co-detection of TcR α and β V(D)J recombinations in the WXS files.

p-values < 0.05 in bold. RNASeq z-scores for each barcode, for each gene are in the ESM.

Row number for matching p-value comparisons	SKCM barcode sets and set comparisons	PDCD1 (PD-1)	CIITA	CD33	CD68	ITGAX	CTSS	IFNG
1 (test set)	productive α and productive β	0.88	0.87	0.26	0.58	0.42	0.83	0.70
2 (control set)	productive α but no detection of productive β	0.02	0.11	-0.01	0.00	-0.04	0.09	0.18
1 vs. 2	p-values for comparisons of rows 1 and 2.	**0.0004**	**0.0015**	**0.0472**	**0.0107**	**0.004**	**0.0015**	0.054
4 (control set)	productive β but no detection of α	-0.13	0.07	-0.22	0.06	0.25	-0.03	-0.01
1 vs. 4	p-values for comparisons of rows 1 and 4.	**7.47 E-05**	**0.0025**	**0.0039**	**0.0461**	0.5023	**0.0043**	**0.0148**
5 (control set)	unproductive α but no detection of productive α or productive β	-0.022	0.105	-0.031	0.006	0.100	0.056	0.056
1 vs. 5	p-values for comparisons of rows 1 and 5.	**0.0001**	**0.0012**	**0.0327**	**0.0155**	0.0772	**0.0009**	**0.0117**

Results above indicated a strong association of expression of the T-cell, programmed cell death receptor gene, PDCD1 (12, 14), and co-detection of productive TcR-α and productive TcR-β recombinations. A strong association of expression of the MHCII transcriptional coactivator, CIITA (15), with co-detection of productive TcR-α and productive TcR-β recombinations. There was also a correlation of the neutrophil marker, CD33 (16), and the macrophage marker, CD68 (17), along with the antigen processing protease CTSS (18-20), with co-detection of productive TcR-α and productive TcR-β recombinations. No correlation with the other genes were found.

Table 4. Productive and unproductive TcR recombinations recombinations found for TcR (α, β, γ, δ) genes in mouse normal tissue and melanoma WXS files

TcR gene	3 normal tissue samples		3 melanoma samples	
	Productive	Unproductive	Productive	Unproductive
α	0	1	0	3
β	1	0	0	0
γ	2	1	0	3
δ	0	0	0	0