ONCOIMMUNOLOGY
2022, VOL. 11, NO. 1, e2054757 (6 pages)
https://doi.org/10.1080/2162402X.2022.2054757

Taylor & Francis
Taylor & Francis Group

ORIGINAL RESEARCH

Intratumoral T-cell repertoires in DNA mismatch repair-proficient and -deficient colon tumors containing high or low numbers of tumor-infiltrating lymphocytes

Jin K. Kim[a], Chin-Tung Chen[a], Ajaratu Keshinro[a], Asama Khan[a], Canan Firat[b], Chad Vanderbilt[b], Neil Segal[c], Zsofia Stadler[c], Jinru Shia[b], Vinod P. Balachandran[a,d], and Martin R. Weiser[a]

[a]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, New York, USA; [b]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York, USA; [c]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York, USA; [d]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, New York, USA

ABSTRACT

Colon tumors with deficient DNA mismatch repair (dMMR) are generally infiltrated by T cells more densely than tumors with proficient mismatch repair (pMMR). However, high numbers of tumor-infiltrating lymphocytes (TILs) are found in select pMMR tumors, and low numbers of TILs are seen in select dMMR tumors. In this study, we compared T-cell repertoires in 20 pMMR and 27 dMMR colon tumors with high and low TIL counts. We found that T cells in dMMR tumors are more clonal and their repertoire is less rich compared with T cells in pMMR tumors. In the dMMR group, T cells in TIL-high tumors were more clonal and their repertoire was less rich compared with T cells in TIL-low tumors, but in the pMMR group, T-cell diversity in TIL-high tumors was comparable to T-cell diversity in TIL-low tumors. These findings suggest that T cells clonally expand in dMMR tumors, possibly in response to MMR deficiency-induced tumor neoantigens.

ARTICLE HISTORY
Received 30 December 2021
Revised 14 March 2022
Accepted 15 March 2022

KEYWORDS
Colon cancer; tumor-infiltrating-lymphocytes; T-cell repertoire; tumor mutational burden; mismatch repair deficiency

Introduction

Characterization of the colorectal tumor immune microenvironment has become an important area of research, as abundance of tumor-infiltrating lymphocytes (TILs), which are thought to represent an active host immune response to tumors,[1] has been shown to be associated with better outcomes.[2–6] Colorectal tumors with deficiency in DNA mismatch repair (dMMR), in particular, have high infiltration of TILs.[7,8] The impaired DNA damage repair pathways in these tumors are thought to lead to a high tumor mutational burden (TMB) and high expression of neoantigens, increasing the immunogenicity of the tumor and thereby attracting T cells to the tumor microenvironment.[7,9] TMB has been shown to be prognostic in patients receiving immunotherapy;[10] however, whether TMB truly captures tumor immunogenicity is debatable.[11]

High immunogenicity and abundance of TILs have been associated with response to chemotherapy and immunotherapy.[12–17] Immunotherapy achieves better outcomes in patients with dMMR colon cancer than chemotherapy[9,12,15] and is now the first-line treatment for metastatic disease. Not all dMMR tumors are enriched in TILs,[18–20] and some MMR-proficient (pMMR) tumors have high levels of TILs, which appear to be associated with the underlying genomics.[18,19] pMMR tumors with high levels of TILs have been shown to respond well to immunotherapy,[15,17,21] and this finding has led to a clinical trial (NCT04262687) evaluating the effectiveness of immune checkpoint blockade in patients with these tumors.

Given that the immunologic background and clinical outcomes of patients with dMMR tumors differ from those of patients with pMMR tumors[22,23] and that TILs have the potential to prognosticate outcomes and guide therapy, a better understanding of the difference in the role of TILs in dMMR and pMMR tumors is needed. In this study, we compared the T-cell repertoires of pMMR and dMMR colon tumors by performing targeted sequencing of the CDR3 region of the β chain of the T-cell receptor (TCR) as a means of assessing T-cell clonality and diversity.[24]

Materials and methods

Patients and samples

With approval from the institutional review board, an institutional database was queried for patients who underwent curative surgery for nonmetastatic colon adenocarcinoma at Memorial Sloan Kettering Cancer Center between February 2007 and December 2014. Patients who received neoadjuvant therapy or underwent palliative resection were excluded.

Twenty slides of 10-μm thickness were cut per formalin-fixed paraffin-embedded tissue block. A pathologist reviewed the hematoxylin-eosin-stained slides of all the sections to annotate the boundaries of the tumor. Macrodissection of tumor tissues from unstained slides was guided by the markings of the stained slides. DNA was extracted using QIAamp DNA FFPE kits (Qiagen Inc., Valencia, CA).

CONTACT Martin R. Weiser weiser1@mskcc.org Colorectal Service, Department of Surgery, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065, USA

Supplemental data for this article can be accessed on the publisher's website

Sequencing of the TCR β chain

CDR3 regions of the TCR β chain were sequenced using the ImmunoSEQ Assay (Adaptive Biotechnologies, Seattle, WA). Extracted genomic DNA was amplified in a bias-controlled multiplex PCR, followed by high-throughput sequencing. Sequences were collapsed and filtered in order to identify and quantitate the absolute abundance of each unique TCRβ CDR3 region for further analysis as previously described.[24–26] The fraction of cells represented by T cells was calculated by normalizing TCRβ template counts to the total amount of DNA usable for TCR sequencing.

Pathological and genomic analyses

Tumors were staged according to the guidelines of the American Joint Committee on Cancer and categorized as right sided (cecum, ascending colon, or transverse colon) or left sided (descending colon or rectosigmoid). Features associated with high risk of recurrence, such as venous invasion, lymphovascular invasion, or perineural invasion, were annotated. TIL count within the tumor was quantified as previously described, by quantifying lymphocytes in five high-power fields in an area determined to have the highest concentration of TIL.[18] A tumor was classified as TIL-high if the mean number of TILs per high-power field (HPF) was ≥4, as previously described.[19]

Tumor DNA was analyzed with pooled normal DNA using MSK-IMPACT, a next-generation sequencing platform that captures mutations for 410–468 oncogenes.[27] TMB was quantified as mutations per megabase. Immunohistochemical staining for MMR proteins MLH1, MSH2, MSH6, and PMS2 was performed as previously described.[28]

Statistical analyses

Simpson clonality was calculated for productive rearrangements with p_i as the proportional abundance of rearrangement i and N as the total number of rearrangements. Clonality values range from 0 to 1 and describe the shape of the frequency distribution: clonality values approaching 0 indicate a very even distribution of frequencies, whereas values approaching 1 indicate an increasingly asymmetric distribution in which a few clones are present at high frequencies. Sample richness was calculated as the number of unique productive rearrangements in a sample after computationally downsampling to a common number of T cells to control for variation in sample depth. Repertoires were randomly sampled without replacement five times, and richness is reported as the mean number of unique rearrangements downsampled to 2,150 templates. Categorical variables were evaluated with the Fisher exact test (two-sided). Continuous variables were compared by using the Mann-Whitney two-sided test. Spearman's rank correlation (two-sided) was used to assess relationships between two continuous variables. P-values < 0.05 were considered statistically significant. Analyses were performed using Prism 8.0 (GraphPad, La Jolla, CA).

Data availability

The data generated in this study are available within the article and its supplement.

Results

Sample characteristics

A total of 443 samples from patients with stage I, II, or III colon cancer were profiled for MMR status and histologically examined to quantify TILs.[18,19] Supplementary Table S1 lists the clinicopathological characteristics of this cohort by MMR status (340 pMMR, 103 dMMR). In our study set, patients with dMMR tumors were older, with tumors located predominately in the right colon, and less likely to have lymph node metastases or vascular or perineural invasive features (Supplementary Table S1). The mean TIL count in dMMR tumors was 10.3 versus 2.9 in pMMR tumors (P < .001). Seventy-eight (76%) of the 103 dMMR samples were classified as TIL-high, whereas 93 (27%) of the 340 pMMR samples were classified as TIL-high. To better balance the two cohorts, we selected a subset of samples in each MMR group (20 pMMR tumors and 27 dMMR tumors) by taking specimens with the highest and lowest TIL counts to widely sample the tumor immune microenvironment for TCR and MSK-IMPACT sequencing. The clinicopathological characteristics of these 47 samples are listed in Table 1. The mean TIL counts per HPF for pMMR and dMMR tumors (7.2 and 8.1, respectively) did not differ significantly (P = .5). All 27 dMMR tumors were located in the right colon, whereas 12 of the 20 pMMR tumors were located in the left colon. TMB was significantly higher in dMMR tumors (87.7 versus 48.8; P < .001). Patient age and sex, pT or pN classification, and features associated with high

Table 1. Clinicopathological characteristics of pMMR and dMMR tumors.

Characteristic	No. of patients (%) pMMR (n = 20)	dMMR (n = 27)	P[a]
TIL count/HPF[b]	7.2 ± 9.3	8.1 ± 9.7	0.6
TIL classification			0.6
TIL-high	11 (55)	12 (44)	
TIL-low	9 (45)	15 (56)	
Age, years[b]	60.0 ± 13.5	69.8 ± 10.4	0.02
Sex			>0.9
Male	8 (40)	11 (41)	
Female	12 (60)	16 (59)	
Tumor site			<0.001
Right	8 (40)	27 (100)	
Left	12 (60)	0	
pT			0.5
T1/2	5 (25)	4 (15)	
T3/4	15 (75)	23 (85)	
pN			0.5
N0	13 (65)	20 (74)	
N+	7 (35)	7 (26)	
VELPI			0.6
Yes	7 (35)	12 (44)	
No	13 (65)	15 (56)	
TMB[b,c]	48.8 ± 42.2	87.7 ± 24.3	<0.001

VELPI, venous invasion, lymphovascular invasion, or perineural invasion.
[a]Mann-Whitney test or Fisher exact test.
[b]Mean and standard deviation.
[c]Mutations per megabase.

risk of recurrence (venous invasion, lymphovascular invasion, or perineural invasion) did not differ significantly between the two groups.

T-cell composition in dMMR vs. pMMR tumors

TIL count based on histological quantification correlated positively with the total number of T cells measured by TCR sequencing (Spearman rho = 0.391; P = .007) (Figure 1a). Total numbers of T cells in pMMR and dMMR tumors did not differ significantly (P = .5) (Figure 1b). The median T-cell densities (calculated by dividing the total number of productive T-cell templates by the total number of cells) also did not differ significantly (P = .8; Figure 1c). Computation of the Simpson clonality index identified a trend toward higher T-cell clonality in dMMR tumors (P = .09) (Figure 1d), with significantly lower repertoire richness than in pMMR tumors (P = .030; Figure 1e). TMB did not correlate strongly with TIL count per HPF, T-cell density, Simpson clonality index, or richness of the TIL repertoire (Supplementary Fig. S1).

Higher clonality and lower repertoire richness of T cells in TIL-high dMMR tumors compared with TIL-low dMMR tumors

The mean TIL counts per HPF for TIL-high and TIL-low dMMR tumors were 16.3 (range, 4–37) and 1.6 (range, 0–3), respectively (P < .001; Figure 2a). Patients with TIL-high dMMR tumors and patients with TIL-low dMMR tumors did not differ significantly in age, sex, pT, pN, high-risk features, or TMB (Figure 2b,c and Supplementary Table S2). Compared with T cells in TIL-low dMMR tumors, T cells in TIL-high dMMR tumors had higher density (P = .037), higher clonality (P = .016), and a less rich repertoire (P = .014) (Figure 2d-f).

Similar clonality and similar repertoire richness of T cells in TIL-high pMMR tumors and TIL-low pMMR tumors

The mean TIL counts per HPF for TIL-high pMMR tumors and TIL-low pMMR tumors were 12.8 (range, 6–37) and 0.3 (range, 0–1), respectively (P < .001; Figure 3a). TIL-low pMMR tumors were generally more advanced (higher pT category) than TIL-high pMMR tumors (P = .038), but patient age, sex, tumor site, pN, high-risk features, and TMB did not differ significantly between TIL-high and TIL-low pMMR tumors (Figure 3b,c and Supplementary Table S3). Median T-cell density was significantly higher in the TIL-high group (P = .038), but T-cell clonality and repertoire richness did not differ significantly between the two groups (Figure 3d-f).

Discussion

Our study found that dMMR and pMMR colon tumors differ in T-cell repertoires. In TIL-high dMMR tumors, TILs were more clonal and repertoire richness was lower than in TIL-low dMMR tumors, but no such differences

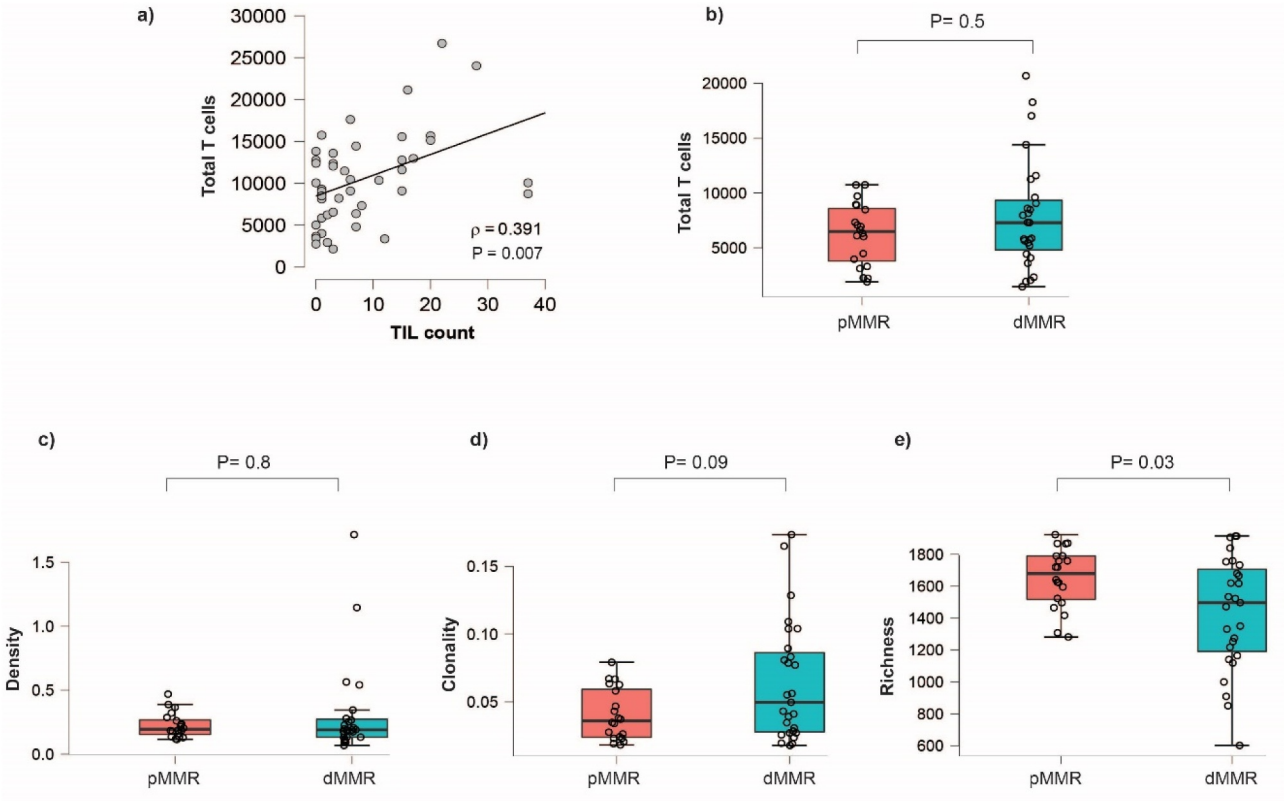

Figure 1. T-cell characteristics of pMMR (n = 20) and dMMR (n = 27) tumors. (a) Spearman correlation of TIL count and total number of T cells (TCR sequencing) for all 47 tumors. **(b to e)** Comparison of pMMR and dMMR tumors in terms of total numbers of T cells (b), T-cell density (c), Simpson clonality (d), and repertoire richness (e). Medians and quartiles are indicated.

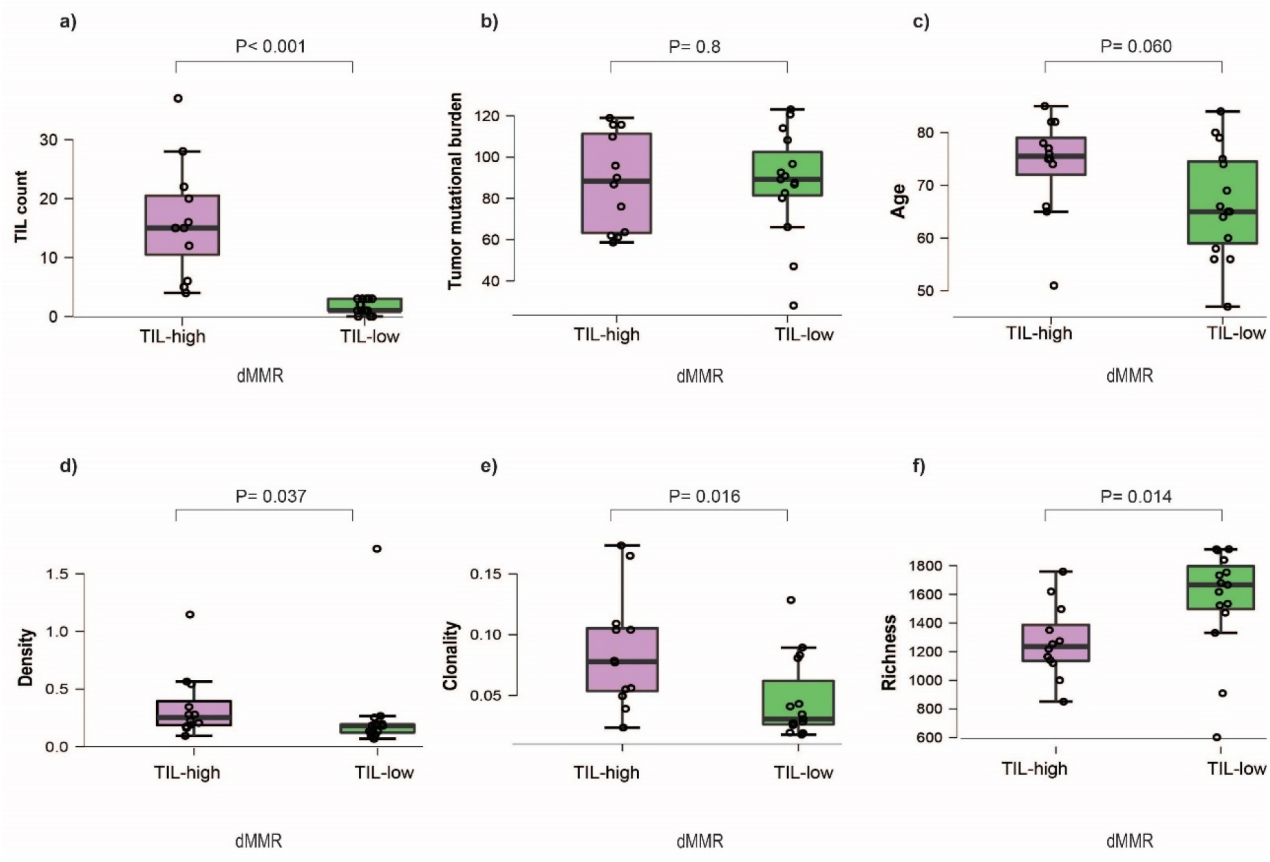

Figure 2. Higher clonality and lower richness in TIL-high dMMR tumors ($n = 12$) compared with TIL-low dMMR tumors ($n = 15$). (a) TIL count; (b) TMB; (c) patient age; (d) T-cell density; (e) Simpson clonality; (f) repertoire richness. Medians and quartiles are indicated.

were found between TIL-high and TIL-low pMMR tumors. T-cell density measured by TCR sequencing correlated strongly with histological TIL count, indicating that our method of quantifying TILs provides a good representation of the actual intratumoral T-cell population. TMB did not correlate with T-cell density, clonality, or repertoire richness. Our findings indicate that a comprehensive measure including TIL count and assessment of the T-cell repertoire may be needed to reliably identify patients who can benefit from immunotherapy.

The results of our study are consistent with single-cell RNA sequencing data showing that Th1 cell clonality is higher in dMMR colon tumors.[29] This may be reflective of the immunoediting mechanism present in dMMR tumors due to the expression of relatively abundant neoantigens that result in a negative selection of highly antigenic mutations.[30] And despite having a dense infiltration of lymphocytes, TILs in dMMR tumors express high levels of checkpoint inhibitors,[31] which may explain why dMMR tumors respond well to immune checkpoint blockade therapy. In malignant melanoma, tumors with higher TIL clonality have been associated with better outcomes after immune checkpoint blockade therapy.[32,33] While the underlying biology is not fully understood, the higher T-cell clonality in TIL-high dMMR colon tumors may explain why these tumors respond particularly well to immune checkpoint blockade.[15] Conversely, high T-cell density with a diverse T-cell repertoire has been reported to be associated with better outcomes in patients with pMMR tumors.[34] While TIL density has important implications in both pMMR and dMMR tumors, our findings suggest that the composition of T cells in colon tumors also varies with MMR status.

Our analyses were subject to limitations inherent to small retrospective studies. MMR profiling was based on clinical practice patterns, and TIL count was not available in all cases; however, no clinical bias was evident for why a sample wasn't assessed for TIL count. TMB may be overestimated in our analysis, as we normalized the data by a pooled set of normal DNA instead of using the patient's matched DNA to call mutations. Although we compared the T cells by TCR sequencing, it's been reported that T cells with the same TCR can be functionally distinct.[35] And while we analyzed T cell clones in untreated samples in this study, the composition of T cells can change in response to treatment.[36]

In conclusion, our data indicate that T cells infiltrate dMMR and pMMR colon tumors differently, possibly due to differences in the tumor immunoediting mechanism. Further investigation of why some dMMR tumors respond well to immunotherapy may help make the benefits of immunotherapy available to a subset of patients with pMMR colon tumors.

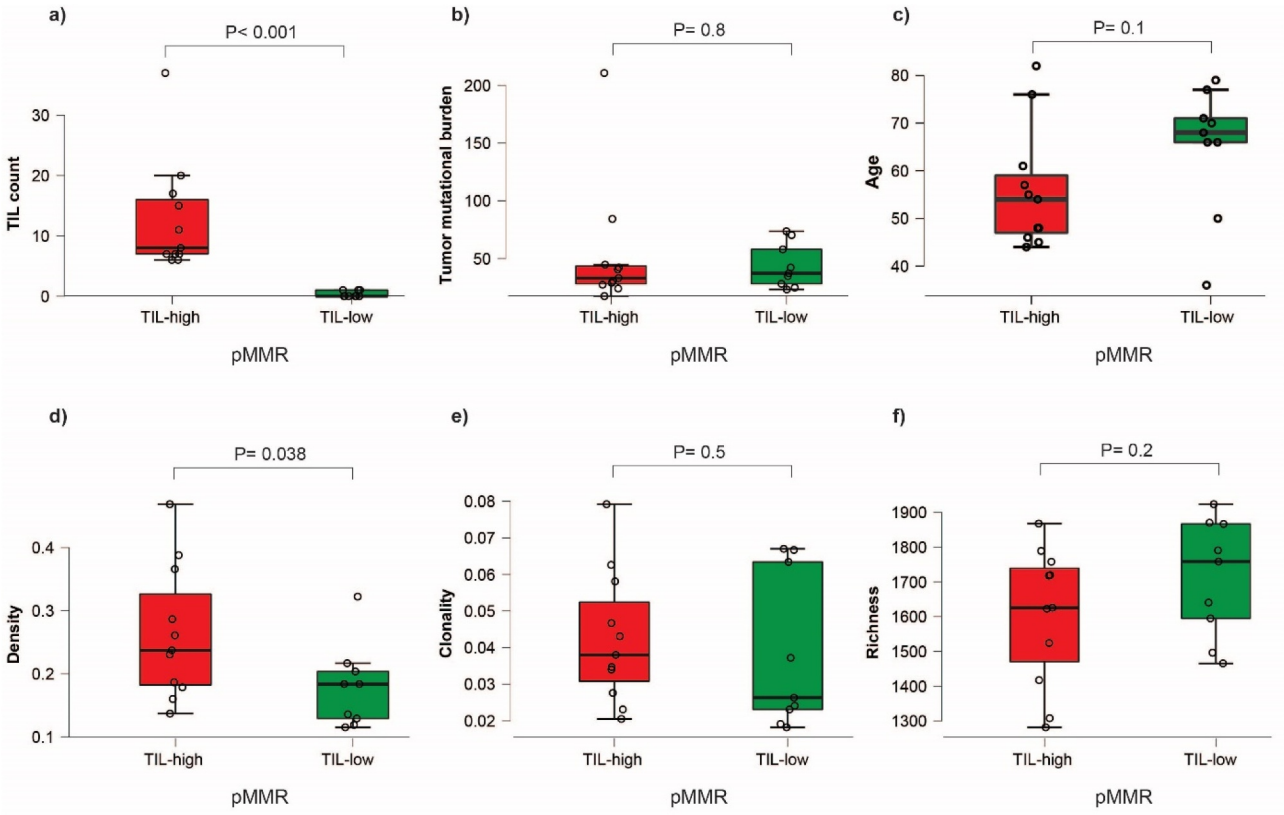

Figure 3. Similarity of T-cell repertoires in TIL-high (n = 11) and TIL-low (n = 9) pMMR tumors. (a) TIL count; (b) TMB; (c) patient age; (d) T-cell density; (e) Simpson clonality; (f) repertoire richness. Medians and quartiles are indicated.

Acknowledgments

We gratefully acknowledge Memorial Sloan Kettering's Integrated Genomics Operation Core and Marie-Josée and Henry R. Kravis Center for Molecular Oncology for support with the genomic analyses, Sarah Kaewert from Adaptive Biotechnologies for support with the TCR sequencing analysis, and Arthur Gelmis for editing the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

National Cancer Institute grants T32CA009501 and P30CA008748 and the John and Michelle Martello Research Fund.

ORCID

Jin K. Kim http://orcid.org/0000-0002-4373-1431
Jinru Shia http://orcid.org/0000-0002-4351-2511

References

1. Sherwood AM, Emerson RO, Scherer D, Habermann N, Buck K, Staffa J, Desmarais C, Halama N, Jaeger D, Schirmacher P, *et al.* Tumor-infiltrating lymphocytes in colorectal tumors display a diversity of T cell receptor sequences that differ from the T cells in adjacent mucosal tissue. Cancer Immunol Immunother. 2013;62(9):1453–1461. doi:10.1007/s00262-013-1446-2.
2. Angell HK, Bruni D, Barrett JC, Herbst R, Galon J. The Immunoscore: colon cancer and beyond. Clin Cancer Res. 2020;26(2):332–339. doi:10.1158/1078-0432.CCR-18-1851.
3. Galon J, Fridman WH, Pagès F. The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. Cancer Res. 2007;67:1883–1886. doi:10.1158/0008-5472.CAN-06-4806.
4. Pagès F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damota D, *et al.* Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med. 2005;353:2654–2666. doi:10.1056/NEJMoa051424.
5. Pagès F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C, Lugli A, Zlobec I, Rau T, Berger M, *et al.* International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet. 2018;391:2128–2139. doi:10.1016/S0140-6736(18)30789-X.
6. Ropponen KM, Eskelinen MJ, Lipponen PK, Alhava E, Kosma VM. Prognostic value of tumour-infiltrating lymphocytes (TILs) in colorectal cancer. J Pathol. 1997;182:318–324. doi:10.1002/(SICI)1096-9896(199707)182:3<318::AID-PATH862>3.0.CO;2-6.
7. Giannakis M, Mu XJ, Shukla SA, Qian ZR, Cohen O, Nishihara R, Bahl S, Cao Y, Amin-Mansour Ali, Yamauchi M, *et al.* Genomic correlates of immune-cell infiltrates in colorectal carcinoma. Cell Rep. 2016;15:857–865. doi:10.1016/j.celrep.2016.03.075.
8. Tougeron D, Fauquembergue E, Rouquette A, Le Pessot F, Sesboüé R, Laurent M, Berthet P, Mauillon J, Di Fiore F, Sabourin J, *et al.* Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. Mod Pathol. 2009;22:1186–1195. doi:10.1038/modpathol.2009.80.
9. Xiao Y, Freeman GJ. The microsatellite instable subset of colorectal cancer is a particularly good candidate for checkpoint blockade immunotherapy. Cancer Discov. 2015;5:16–18. doi:10.1158/2159-8290.CD-14-1397.

10. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, Barron DA, Zehir A, Jordan EJ, Omuro A, *et al*. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet. 2019;51(2):202–206. doi:10.1038/s41588-018-0312-8.
11. Budczies J, Seidel A, Christopoulos P, Endris V, Kloor M, Győrffy B, Seliger B, Schirmacher P, Stenzinger A, Denkert C, *et al*. Integrated analysis of the immunological and genetic status in and across cancer types: impact of mutational signatures beyond tumor mutational burden. Oncoimmunology. 2018;7(12): e1526613. doi:10.1080/2162402X.2018.1526613.
12. André T, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, Smith D, Garcia-Carbonero R, Benavides M, Gibbs P, *et al*. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer. N Engl J Med. 2020;383(23):2207–2218. doi:10.1056/NEJMoa2017699.
13. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber W-J, *et al*. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature. 2014;515(7528):577–581. doi:10.1038/nature13988.
14. Halama N, Michel S, Kloor M, Zoernig I, Benner A, Spille A, Pommerencke T, von Knebel DM, Folprecht G, Luber B, *et al*. Localization and density of immune cells in the invasive margin of human colorectal cancer liver metastases are prognostic for response to chemotherapy. Cancer Res. 2011;71(17):5670–5677. doi:10.1158/0008-5472.CAN-11-0268.
15. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, *et al*. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372 (26):2509–2520. doi:10.1056/NEJMoa1500596.
16. Pagès F, André T, Taieb J, Vernerey D, Henriques J, Borg C, Marliot F, Ben Jannet R, Louvet C, Mineur L, *et al*. Prognostic and predictive value of the Immunoscore in stage III colon cancer patients treated with oxaliplatin in the prospective IDEA France PRODIGE-GERCOR cohort study. Ann Oncol. 2020;31 (7):921–929. doi:10.1016/j.annonc.2020.03.310.
17. Turksma AW, Coupé VM, Shamier MC, Lam KL, de Weger VA, Belien JA, van den Eertwegh AJ, Meijer GA, Meijer CJLM, Hooijberg E, *et al*. Extent and location of tumor-infiltrating lymphocytes in microsatellite-stable colon cancer predict outcome to adjuvant active specific immunotherapy. Clin Cancer Res. 2016;22 (2):346–356. doi:10.1158/1078-0432.CCR-13-2462.
18. Jimenez-Rodriguez RM, Patil S, Keshinro A, Shia J, Vakiani E, Stadler Z, Segal NH, Yaeger R, Konishi T, Shimada Y, *et al*. Quantitative assessment of tumor-infiltrating lymphocytes in mismatch repair proficient colon cancer. Oncoimmunology. 2020;9 (1):1841948. doi:10.1080/2162402X.2020.1841948.
19. Keshinro A, Vanderbilt C, Kim JK, Firat C, Chen CT, Yaeger R, Ganesh K, Segal NH, Gonen M, Shia J, *et al*. Tumor-infiltrating lymphocytes, tumor mutational burden, and genetic alterations in microsatellite unstable, microsatellite stable, or mutant POLE/POLD1 colon cancer. JCO Precis Oncol. 2021;5. doi:10.1200/PO.20.00456
20. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. Cancer. 2001;91(12):2417–2422. doi:10.1002/1097-0142(20010615)91:12<2417::AID-CNCR1276>3.0.CO;2-U.
21. de Weger VA, Turksma AW, Voorham QJ, Euler Z, Bril H, van den Eertwegh AJ, Bloemena E, Pinedo HM, Vermorken JB, van Tinteren H, *et al*. Clinical effects of adjuvant active specific immunotherapy differ between patients with microsatellite-stable and microsatellite-instable colon cancer. Clin Cancer Res. 2012;18 (3):882–889. doi:10.1158/1078-0432.CCR-11-1716.
22. Ogino S, Nosho K, Kirkner GJ, Kawasaki T, Meyerhardt JA, Loda M, Giovannucci EL, Fuchs CS. CpG Island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. Gut. 2009;58:90–96. doi:10.1136/gut.2008.155473.
23. Yaeger R, Chatila WK, Lipsyc MD, Hechtman JF, Cercek A, Sanchez-Vega F, Jayakumaran G, Middha S, Zehir A, Donoghue MTA, *et al*. Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. Cancer Cell. 2018;33 (1):125–36.e3. doi:10.1016/j.ccell.2017.12.004.
24. Robins HS, Campregher PV, Srivastava SK, Wacher A, Turtle CJ, Kahsai O, Riddell SR, Warren EH, Carlson CS. Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. Blood. 2009;114:4099–4107. doi:10.1182/blood-2009-04-217604.
25. Carlson CS, Emerson RO, Sherwood AM, Desmarais C, Chung MW, Parsons JM, Steen MS, LaMadrid-Herrmannsfeldt MA, Williamson DW, Livingston RJ, *et al*. Using synthetic templates to design an unbiased multiplex PCR assay. Nat Commun. 2013;4(1):2680. doi:10.1038/ncomms3680.
26. Robins H, Desmarais C, Matthis J, Livingston R, Andriesen J, Reijonen H, Carlson C, Nepom G, Yee C, Cerosaletti K, *et al*. Ultra-sensitive detection of rare T cell clones. J Immunol Methods. 2012;375(1–2):14–19. doi:10.1016/j.jim.2011.09.001.
27. Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, Chandramohan R, Liu ZY, Won HH, Scott SN, *et al*. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn. 2015;17(3):251–264. doi:10.1016/j.jmoldx.2014.12.006.
28. Shia J, Black D, Hummer AJ, Boyd J, Soslow RA. Routinely assessed morphological features correlate with microsatellite instability status in endometrial cancer. Hum Pathol. 2008;39:116–125. doi:10.1016/j.humpath.2007.05.022.
29. Zhang L, Yu X, Zheng L, Zhang Y, Li Y, Fang Q, Gao R, Kang B, Zhang Q, Huang JY, *et al*. Lineage tracking reveals dynamic relationships of T cells in colorectal cancer. Nature. 2018;564 (7735):268–272. doi:10.1038/s41586-018-0694-x.
30. Ballhausen A, Przybilla MJ, Jendrusch M, Haupt S, Pfaffendorf E, Seidler F, Witt J, Hernandez Sanchez A, Urban K, Draxlbauer M, *et al*. The shared frameshift mutation landscape of microsatellite-unstable cancers suggests immunoediting during tumor evolution. Nat Commun. 2020;11:4740. doi:10.1038/s41467-020-18514-5.
31. Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, Blosser RL, Fan H, Wang H, Luber BS, *et al*. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov. 2015;5(1):43–51. doi:10.1158/2159-8290.CD-14-0863.
32. Roh W, Chen PL, Reuben A, Spencer CN, Prieto PA, Miller JP, Gopalakrishnan V, Wang F, Cooper Z, Reddy S, *et al*. Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade reveals markers of response and resistance. Sci Transl Med. 2017;9. doi:10.1126/scitranslmed.aah3560
33. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, *et al*. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568–571. doi:10.1038/nature13954.
34. Sanz-Pamplona R, Melas M, Maoz A, Schmit SL, Rennert H, Lejbkowicz F, Greenson J, Sanjuan X, Lopez-Zambrano M, Alonso MH, *et al*. Lymphocytic infiltration in stage II microsatellite stable colorectal tumors: a retrospective prognosis biomarker analysis. PLoS Med. 2020;17:e1003292. doi:10.1371/journal.pmed.1003292.
35. Krogsgaard M, Davis MM. How T cells 'see' antigen. Nat Immunol. 2005;6:239–245. doi:10.1038/ni1173.
36. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, *et al*. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357(6349):409–413. doi:10.1126/science.aan6733.