Characterization of the Immune Landscape of Colorectal Adenomas

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Background

- Colorectal cancer (CRC) accounts for 8.3% of all new cancer cases and 8.4% of all cancer deaths in the United States [1].
- The adenomatous polyp is the precursor lesion of CRC, with 1/3 of the population estimated to develop an adenoma by age 60 [2].
- There is growing support for the role of intratumoral host immune response in the development of CRC [3].

Hypothesis

- Measures of T-cell infiltration and T-cell repertoire diversity differ across adenoma subtypes and polyps with a strong and diverse immune response may be associated with a lower risk of developing CRC.

Objective

- We conducted a pilot study to investigate the immune landscape of colorectal adenomas using 30 tissue samples from 22 patients, including 8 synchronous lesions from a subset of patients (n=4).

Methods

Data collection:

- 13 fresh frozen (FF) and 17 formalin-fixed paraffin-embedded (FFPE) adenoma samples were extracted from 22 Total Cancer Care (TCC)-consented patients diagnosed with at least one colorectal adenoma (Table 1).
- Four patients contributed multiple adenoma samples (three patients had two adenomas each, one patient had six adenomas).

Table 1

22 patients from TCC		
FF	FFPE	Total
13(2)	17(6)	30

Numbers in parentheses indicate the extra adenomas from the same patient

- Histopathological features of adenoma samples such as adenoma infiltrating lymphocyte (AIL) level, lesion size and adenoma subtype were collected.
- Adenomas were classified into low-risk and high-risk groups according to the guidelines from the American Gastroenterological Association [4] (Table 2).

Table 2

Subtype	n (%)	Risk group
Hyperplastic	2 (6.7)	Low risk
Serrated	1 (3.3)	
Tubular	6 (20.0)	
Tubulovillous	17 (56.7)	High risk
Villous	4 (13.3)	

DNA Extraction and Quantification:

- DNA was extracted using Qiagen products (QIAGEN N.V., Venlo, Netherlands) and Qubit were used to measure the quantity and quality of the extracted DNA from both FF and FFPE samples.

T-Cell Receptor Sequencing:

- The immunoSEQ assay (Adaptive Biotechnologies, Seattle, WA) was used for sequencing of the TCRβ complementarity determining region 3 (CDR3), which determines the diversity of T-cell receptors.
- Two main variables were derived from sequencing data:
  - T-cell fraction (measure of T-cell quantity in a sample)
  - Simpson clonality (measure of T-cell diversity in a sample [0=high clonal diversity; 1=no diversity]

Statistical Analysis:

- Univariate linear regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between immune variables and histopathological features of adenoma samples.
- For patients with multiple samples (n=4), unique samples from each patient were randomly selected to ensure independent observations.

Immune T-cell receptor repertoire by sample characteristics

Table 3

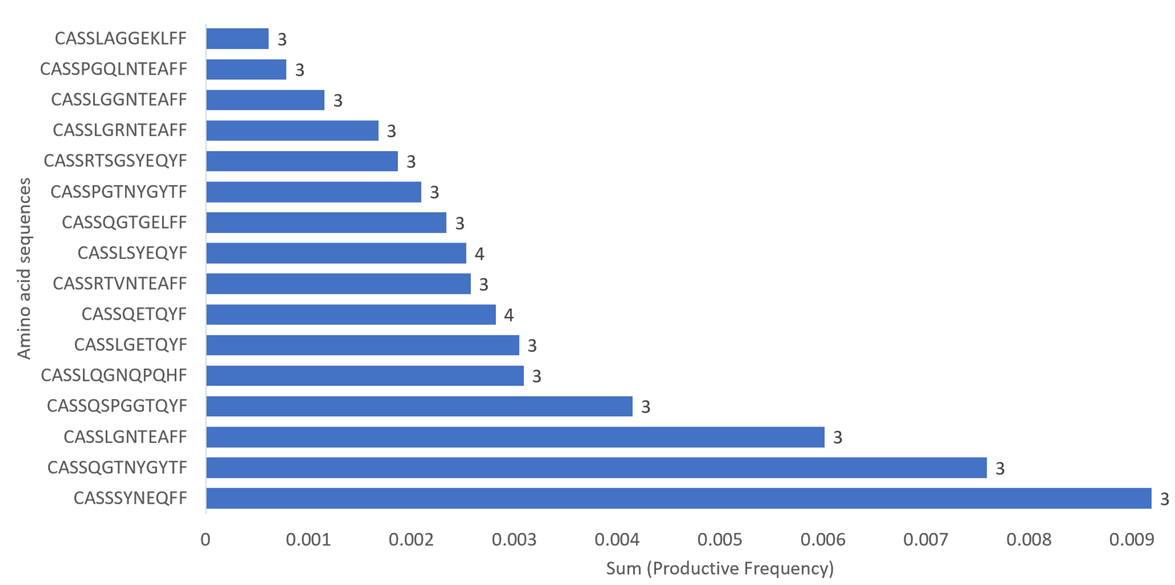
		T Cell Fraction		Simpson Clonality	
	n	OR (95%CI)	p-value	OR (95%CI)	p-value
Size		0.76 (0.19-3.07)	0.7	2.23 (1.22-4.05)	0.016
Subtype					
Low risk	7	Ref		Ref	
High risk	15	0.26 (0.13-0.51)	0.00089	1.74 (1.2-2.53)	0.00838
AIL level					
Low	10	Ref		Ref	
Medium	7	0.81 (0.3-2.19)	0.685	1.52 (0.977-2.38)	0.0789
High	5	0.86 (0.29-2.61)	0.799	1.45 (0.89-2.38)	0.154

- Unique samples from each patient were used for this analysis (n=22).

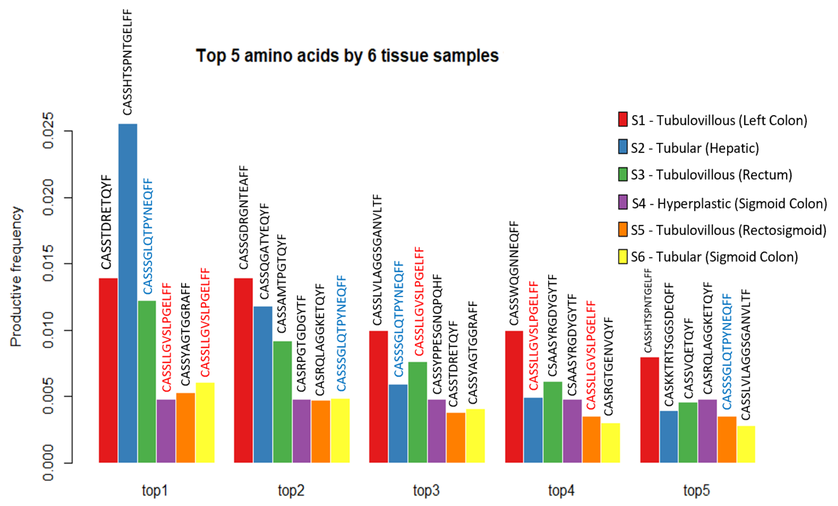
- T-cell fraction was significantly lower in high-risk adenomas vs low-risk adenomas (OR=0.26, 95% CI=0.13-0.51, p-value<0.001) (Table 3).
- Simpson clonality was significantly higher in high-risk adenomas vs low-risk adenomas (OR=1.74, 95% CI=1.2-2.53, p-value<0.01), which translates into a less diverse T-cell repertoire (Table 3).
- The unexpected association between adenoma size and Simpson clonality requires further investigation in a larger study population (Table 3).

CDR3 amino acid diversity across samples

- Figure 1 displays the most frequent CDR3 amino acid sequences identified across all samples (n=30). The numbers to the right of each bar represent the number of samples where the corresponding amino acid was found.



- A preliminary analysis with the VDJdb (https://vdjdb.cdr3.net/) and IEDB (https://www.iedb.org/) neoantigen prediction resources did not identify a potential role of these amino acid sequences or their respective T-cell receptors in cancer-related immune responses.
- Figure 2 shows the five most common CDR3 amino acid sequences identified across the six multiple adenoma samples from the same patient. Two amino acid sequences (CASSLLGVSLPGELFF and CASSSGLQTPYNEQFF) overlapped across five and four out of the six samples, respectively.



Shared Amino Acid Sequences	n
CASSLLGVSLPGELFF	5
CASSSGLQTPYNEQFF	4
CASRQLAGGKETQYF	2
CASSHTSPNTGELFF	2
CASSLVLAGGSGANVLTF	2
CASSTDRETQYF	2
CASSYAGTGGRAFF	2
CSAASYRGDYGYTF	2

- Substantial overlap in the top CDR3 amino acid sequences was also observed for two out of the three patients with two adenoma samples each.

Conclusions

- Our results indicated that low risk adenomas have both a stronger and more diverse T-cell response in comparison to high risk adenomas, which could partially explain the lower chance of their progression into CRC
- The substantial overlap in CDR3 amino acid sequences between multiple adenoma samples derived from the same patients suggest that the tumor-associated T-cell receptor repertoire is fairly consistent throughout the colon for humans

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References

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