Characterization of Sideness-related Differentiated Genetic Alterations in Stage I-IV Colorectal Cancer Patients by using Whole Exome Sequencing

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Danyi Wang[1], Lee McDaniel[2], Sean Boyle[2], Giuseppe Locatelli[3],Juergen Scheuenpflug[3], Zheng Feng [1#],

1. Clinical Genomics, Global Clinical Biomarkers & Companion Diagnostics , Translational Medicine | Biometrics & Precision Medicine , EMD Serono , A business of Merck KGaA, Darmstadt, Germany
2. Personalis, Inc. 1330 O' Brien Dr, Menlo Park, CA., 94025
3. Global Clinical Biomarkers and Companion Diagnostics, Translational Medicine, Global Development, Merck KGaA, Darmstadt, Germany

Corresponding author

BACKGROUND

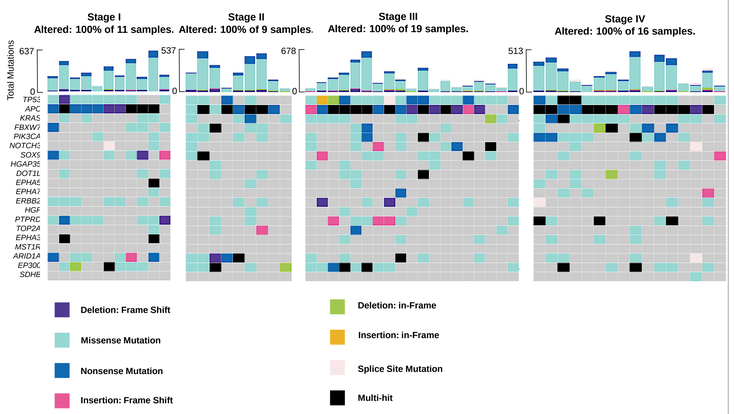
Although constituting a single organ, the right colon and left colon arise from midgut and hindgut embryonic precursors respectively. In the context of these embryonic origins, tumor laterality has been associated with differential microsatellite instability (MSI), aneuploidic karyotype, and loss of heterozygosity between the left and right colon. Differential genetic mutation profiles are not fully characterized in the context of left vs right laterality colorectal cancer (CRC). Therefore, we investigated associations between CRC anatomical site and somatic gene mutation patterns using whole exome sequencing.

METHODS

- Whole exome sequencing data were collected from 55 FFPE samples from different stages (stage I: 11, II: 9; III: 19, IV: 16), treatment-naive CRC patients using the Personalis® tumor-only ImmunoID NeXT Platform®[1,2], which captures somatic mutations (SNVs), copy number variants (CNVs), percent MSI in the exome, oncovirus detection, etc.
- The R package maftools was used to identify differential rates of mutation between samples resected from the rectum, left, and right colon respectively. Maftools-derived mutated gene lists, filtered to p<0.5 and odds-ratio>1, were assessed for pathway enrichment with Enrichr.

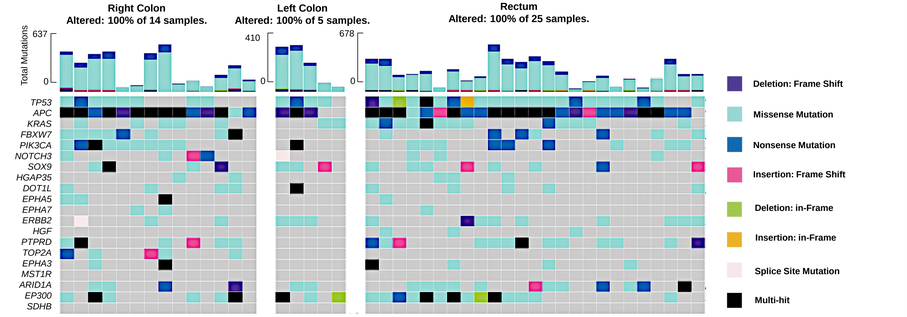
RESULTS

Gene-level mutations stratified by stage



APC and TP53 were the most commonly mutated genes in the entire cohort, with mutations seen in >90% (APC) and >75% (TP53) of samples respectively.

Gene-level mutations stratified by tumor laterality

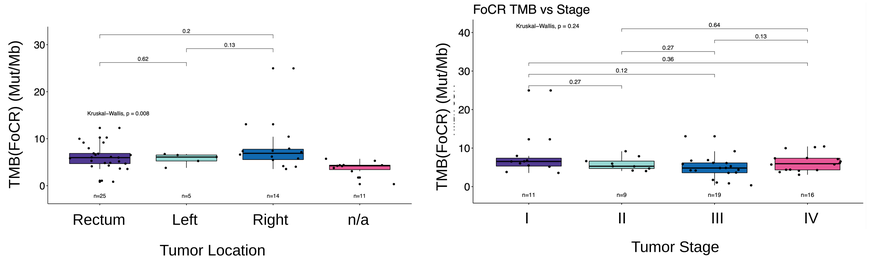


- Mutation frequency was compared between tumor locations via Fisher's Exact Test with the following trends in mutation frequency identified:
  - Left side vs Right Side : R-sided CRC has higher trend for *FBXW7*, *APC* mutations
  - Left side vs Rectal: Rectal has higher trend for *TRRAP, AHNAK2, PTPRD, APC* mutations
  - Right side vs Rectal : R-sided CRC has higher trend for *TOP2A, MORC4* mutations
- Individual gene mutation frequency was not significantly different among the primary tumor locations (left colon; right colon; rectum) after Bonferroni multiple test correction, with *FBXW7*, *APC*, trending towards differential mutation between right colon tumors and left/rectal tumors.

	right	left	rectum	n/a
I	2	2	7	0
II	4	1	4	0
III	3	1	5	10
IV	5	1	5	5

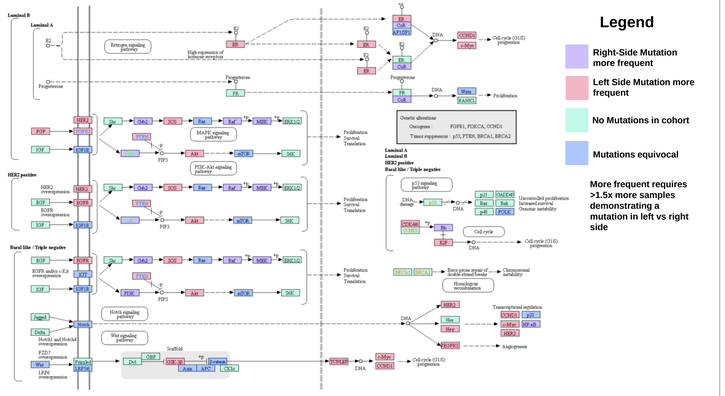
No bias between stage and tumor site (chi-squared, p=0.052) likely driven by "na" (non-annotated tumor site status)

TMB comparable between CRC locations



Tumor Mutation Burden (TMB) was calculated according to Friends of Cancer Research (FoCR) phase I mutation inclusion criteria as part of the Personalis ImmunoID NeXT platform, which allows for assessment of tumor mutations across the entire human exome, conformant with the FoCR standard. Continuous quantification of TMB demonstrates a trend for higher TMB in the right colon, with all-group significance on tumor laterality (p=0.008, Kruskal-Wallis)

Estrogen signaling in cancer (KEGG)



- Maftools-derived mutated gene lists, filtered to p<0.5 and odds-ratio>1, were processed with Enrichr, identifying the following pathways when comparing right-sided vs rectal tumors:
  - **Estrogen-related mutation events** (n=81 genes; Bioplanet 2019; q=5x10-4; WikiPathway 2021 Human; q<5x10-6), shown above.
  - **PI3K/AKT signaling mutations** were more enriched (n=106 genes; Kyoto Encyclopedia of Genes and Genomes 2021; q<4x10-6; MSigDB Hallmark 2020; q=9x10-3), not shown

CONCLUSIONS

- A statistically significant increase in frequencies of estrogen pathway gene mutations on right-sided tumors, and on PI3K/AKT mutations on rectal tumors, were identified. These are consistent with prior expression-based findings indicating the association of estrogen signaling with right-sided tumors, and increased AKT expression with left-sided CRCs.
- The whole exome sequencing-based laterality associated genetic mutation results provided clinically applicable evidence that patients diagnosed with left versus right CRC tumors may benefit differential treatment therapies, and further investigation is ongoing to explore the potential correlation between tumor laterality and stage.

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