Molecular Variances Between Rectal and Left Sided Colon Cancers

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Abstract

Background: Recent analysis of CALGB 80405 showed that left sided colon tumors (LT) respond differently to biologics compared with right-sided tumors, likely due to molecular differences. Molecular variations between LT and rectal tumors remain undefined. Herein, we report our exploration of these variations.

Methods: Tumors with origins clearly defined as splenic flexure to descending colon (SFT), sigmoid colon (SgT), or rectum (RT) were included. Protein expression, gene amplification and NextGen sequencing was tested. Microsatellite instability (MSI) was measured by PCR. Tumor mutational load (TML) was calculated using only somatic nonsynonymous missense mutations. Chi-square tests were used for comparative analyses.

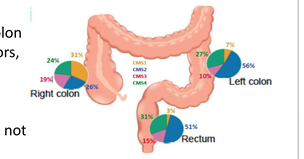
Results: In total, 1,457 primary tumors (SFT 125; SgT 460, and RT 872) were examined. When compared with SFT, RT had a higher frequency of *TP53* (71% vs. 57%, p = 0.03) and *APC* (66% vs. 49%, p = 0.01); a lower frequency of *PIK3CA* (11% vs. 22%, p = 0.02), *BRAF* (3% vs. 15% p = 0.0001), *GNAS* (0.9% vs. 4%, p = 0.04), *HNF1A*(0.7% vs. 5%, p = 0.01), and *CTNNB1*(0.3% vs. 4%, p = 0.003); and a higher expression of TOPO1 (52% vs. 31%, p = 0.0001), ERCC1 (29% vs. 15%, p = 0.03), and MGMT (64% vs. 53%, p = 0.048). When compared with SgT, RT had higher expression of TLE3 (33% vs. 23%, p = 0.007), TOPO1 (52% vs. 35%, p < 0.001), TUBB3 (41% vs. 28%, p = 0.003), and MGMT (64% vs. 54%, p = 0.003). There were no differences between SFT, SgT, and RT in the frequency of PD-L1 expression (5%, 2%, and 2%) on tumor cells, PD-1 expression on tumor-infiltrating lymphocytes (54%, 42%, and 42%), or Her-2 expression (1%, 2%, and 3%) and amplification (3%, 3%, and 5%). MSI was seen in 7% of SFT, 4% of SgT, and 0.7% of RT (total LT vs. RT, p = 0.01). Mean TML was 23, 6.5, and 7 mutations (mut)/MB (332 tumors), and the portion of tumors carrying a TML of > 17mut/MB was 9%, 1.6%, and 4% for SFT, SgT, and RT, respectively. In all 3 cohorts, a TML > 17 mut/MB was highly concordant with MSI. There was a correlation between PD-1 and TML in RT (p = 0.04) but not in SFT or SgT. There were no correlations between PD-L1 and TML.

Conclusions: Tumors arising in the rectum may carry genetic alterations that are distinct from LT. A better understanding of disease biology may help to identify therapeutic targets and advance precision medicine

Background

- CRC was recently classified into four consensus molecular subtypes (CMSs) with distinguishing features[1] and different prognoses
- CM1-4 tumors have different carcinogenic pathways and biology which may influence response to therapy
  - ✓ Defined by pathway activity and cellular processes
  - ✓ No subtype is defined by a single gene
  - ✓ No alteration is limited to a single subtype
- Recent retrospective analysis of CALGB 80405 showed that left sided colon tumors respond differently to biologics compared with right-sided tumors, likely due to molecular differences
- left-sided tumors typically include both colon and rectum tumors as a common entity
- However molecular variations between left colon and rectal tumors are not well defined

CMS1 MSI immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGF-β activation, angiogenesis
Worse survival after relapse			Worse relapse-free and overall survival



Methods

- Retrospective analysis of 10,570 CRC tumors that were profiled by Caris Life Sciences between 2009 and 2016 was performed
- All samples were independently reviewed by at least one pathologist, in addition to the local pathologist
- Only primary tumors were included in the current analysis
- Tumors without clearly defined origins were excluded
- Chi-square test was used for comparison between groups (IBM SPSS Statistics, Version 23) and significance was defined as p < 0.05

Immunohistochemistry (IHC)

ALK	PGP
AR	PR
cMET	PTEN
EGFR	RRM1
ER	TLE3
ERCC1	TOP2A
Her2/Neu	TOPO1
MGMT	TS
PD-1	TUBB3
PD-L1	

Microsatellite Instability fragment analysis (Promega)

- Microsatellite Instability

In-situ hybridization (CISH or FISH)

- Her2; cMET; EGFR

Next-Generation Sequencing

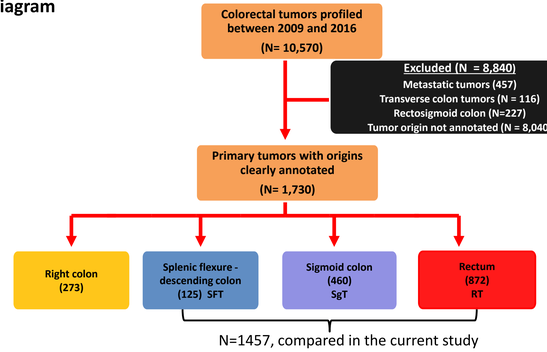
- Illumina MiSeq platform ; Illumina TruSeq Amplicon Cancer Hotspot panel
- All tumor samples micro-dissected
- Average depth of coverage > 1500X
- Analysis of tumor tissue,
- 45 gene panel

ABL1	CSF1R	FGFR2	IDH1	PIK3CA
CDH1	FGFR1	HRAS	cMET	SMARCB1
FBXW7	HNF1A	**KRAS**	PDGFRA	**BRAF**
GNAS	cKIT	**NRAS**	SMAD4	ERBB4
KDR	NPM1	RET	ATM	GNAQ
NOTCH1	RB1	VHL	ERBB2	JAK3
PTPN11	TP53	APC	GNA11	MPL
STK11	ALK	EGFR	JAK2	PTEN
AKT1	CTNNB1	FLT3	MLH1	SMO

- 10% of all tumors were tested with NextSeq platform: Agilent SureSelect XT, 592 gene panel, which were used also to calculate Tumor Mutational Burden (TMB)

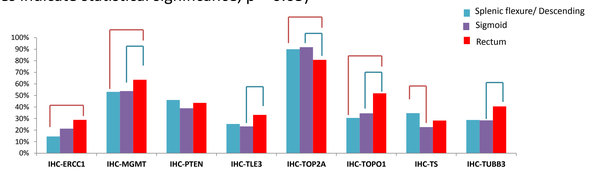
Results

1. Consort Diagram

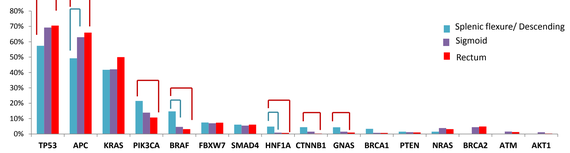


Results

2. Frequency of protein overexpression by immunohistochemistry (connective lines indicate statistical significance, p < 0.05)

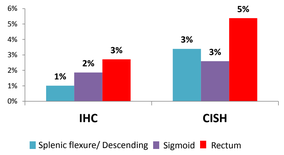


3. Frequency of gene mutations by NextGen SEQ (connective lines indicate statistical significance, p < 0.05)

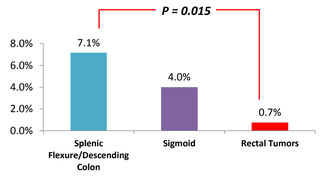


4. Frequency of (A) Her2 overexpression (IHC) and amplification (CISH) (threshold: Her2 IHC: ≥ 3+ and > 10%; Her2 ISH: Her2/Neu:CEP 17 signal ratio of >= 2.0) **(B) MSI in the three cohorts.**

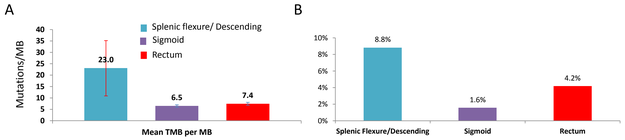
A. Her2 overexpression and amplification



B. Frequency of microsatellite instability



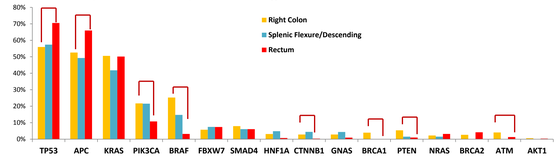
5. Tumor mutation burden (TMB) in the three cohorts. (A) Mean TMB, error bars indicate standard errors; **(B) % of tumors carrying TMB >= 17 mutations/megabase.**

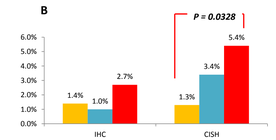


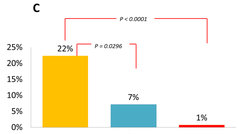
- *TMB was calculated using only somatic nonsynonymous missense mutations sequenced with a 592-gene panel.*
- *TMB was calculated on a separate cohort of 331 tumors tested with 592-gene panel (both primary tumors and metastasis included), Splenic flexure N=34; Sigmoid N=129; Rectum N=168*
- *No significant difference was seen in the three cohorts*

Results

6. Comparison of (A) NextGen SEQ; (B) Her2 overexpression (IHC) and amplification (CISH); and (C) MSI in right colon, splenic flexure/descending, and rectal cohorts (connective line indicates significance when right colon tumors are compared to splenic flexure/descending colon or rectal tumors.







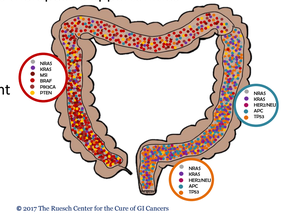
Conclusions

- Rectal cancers carry molecular features that are different from left-sided colon tumors
- CRCs carry a continuous spectrum of genetic alterations from right- sided to rectums, without a clear-cut difference between sides.
- Randomized trials must be stratified based on the location of the primary tumor as well as molecular features
- Low frequency mutations exist in several druggable genes (e.g., MSI, Her2, BRCA1/2, PIK3CA, or PTEN)—this may provide therapeutic opportunities

Future directions

We need:

- More comprehensive molecular testing is needed to better stratify patients, which might enable us to further tailor therapy for our patients (precision medicine).
- To carry out prospective clinical trials of treatments tailored to patients' molecular targets (gene mutations) to show improved patient outcome



- Collaborative efforts involving multiple cancer centers/institutions
- Effective data sharing in the research community to promote advances in molecularly-guided therapy

References

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