Comparative molecular analyses of colon versus rectal tumors.

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Abstract #3552

Background: Colorectal cancer (CRC) is a heterogeneous disease. There is limited data on colon and rectal tumor molecular differences.

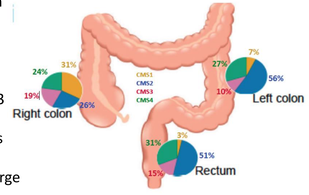
Methods: A total of 8174 CRC tumors submitted to Caris Life Sciences for IHC (protein expression), ISH (gene amplification), and NGS sequencing between 2009 and 2015 were studied. Only tumors with origins that were clearly defined as ascending, transverse, or descending colon or rectum were included in this study. We excluded any cases without clear designation. Chi-square tests determined molecular differences between colon and rectal tumors.

Results: A total of 2,010 tumors met our inclusion criteria and were examined. We compared primary (1°) colon (n = 502) with 1° rectal (n = 872) tumors; the colon 1°s had higher rates of ATM (7.5% vs. 3.2%, p = 0.01), BRAF (24% vs. 3.4%, p < 0.01), CTNNB1 (4% vs. 0.3%, p = 0.003), IDH1 (2% vs. 0%, p = 0.01), GNAS (3.3% vs. 1%, p = 0.04), HNF1A (15% vs. 9%, p = 0.01), JAK3 (8% vs. 2.2%, p = 0.002), PIK3CA (24% vs. 12%, p = < 0.01), RB1 (1.7% vs. 0%, p = 0.02), and SMAD4 (17% vs. 12%, p = 0.05). However, mutations in APC and TP53, and amplification of HER2, were higher in rectal tumors (67% vs. 54% and 72% vs. 60% [both p < 0.01], and 5.4 % vs. 1.5% [p < 0.01]). Colon 1° tumors had higher MSI (17% vs. 0.7%, p < 0.01), EGFR expression (64% vs. 42%, p < 0.01), PD-1 (55% vs. 44%, p = 0.01), and TS (36% vs. 28%, p < 0.01). In contrast, rectal 1otumors had higher expression of MGMT (64% vs. 52%, p = 0.002), TLE3 (33% vs. 26%, p = 0.03), TOPO1 (52% vs. 36%, p < 0.01), and TUBB3 (41% vs. 30%, p < 0.01). When we excluded MSI+ tumors and analyzed only the MSI- tumor differences, colon tumors still had a greater frequency of BRAF (13% vs. 4.5%, p = 0.003) and PIK3CA (22% vs. 12%, p = 0.009), and higher EGFR expression (63% vs. 51%, p = 0.047) than rectal tumors, whereas, PTEN (56% vs. 41%, p = 0.004), TLE3 (43% vs. 29%, p = 0.004) and TOPO1 (50% vs. 38%, p = 0.03) were overexpressed in rectal tumors.

Conclusions: Molecular profile differences between colon and rectal tumors suggest different carcinogenic pathways and biology that may influence response to therapy. Low frequency mutations in several druggable genes, or protein overexpression (e.g., HER-2, PIK3CA, TOPO1) provide therapeutic opportunities. Our findings are consistent with the consensus molecular subtyping of CRC.

Background

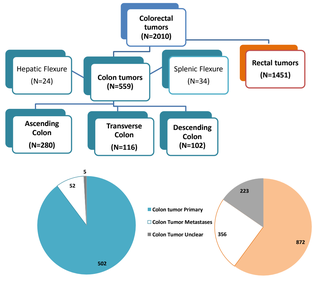
While cancers arising from the colon and rectum are often categorized as colorectal cancer in clinical studies, clinical behavior and management of these two cancer types are distinctly different. Recently the consensus molecular subtyping of colorectal cancer classified CRCs into CMS1 (microsatellite instability immune), CM2 (canonical), CMS3 (metabolic) and CMS4 (mesenchymal) groups. Differential distribution of the four classes in various anatomic regions suggests differences in colon and rectal tumors. We investigated the proteomic and genetic aberrations of a large cohort of clinical CRC samples to further delineate the molecular differences.



Adopted from Guinney, J, 2015, Nature Medicine

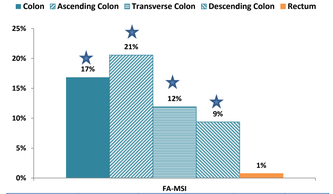
Results

Figure 1: Tumor characteristics. Left: Composition of the tumor cohort analyzed. Shown are the tumor origins. For colon tumors, any cases without clear designation of a specific colon region (ascending, descending, etc) were excluded. **Right: Patient age and gender.** Patients with tumors of the colon are significantly older than patients with rectal tumors; patients with primary tumors profiled are older than patients with metastatic tumors profiled for both colon and rectal tumors. Males are more prevalent in rectal cancer than colon cancer.



	Colon Tumor	Rectum Tumors	P values
Average Age (yrs)	62.6	58.9	1.20748E-07
Ascending Colon	64.1		
Transverse Colon	62.3		
Descending Colon	60.1		
Metastatic Tumors	58.5	57.4	p=0.02 (colon)
Primary Tumors	63	59.1	p=0.01 (rectum)
Gender (Male %)	46.9%	62%	<0.0001
Ascending Colon	42.5%		
Transverse Colon	50.9%		
Descending Colon	49.0%		
Metastatic Tumors	44.2%	63.0%	
Primary Tumors	47.4%	60.4%	p=ns

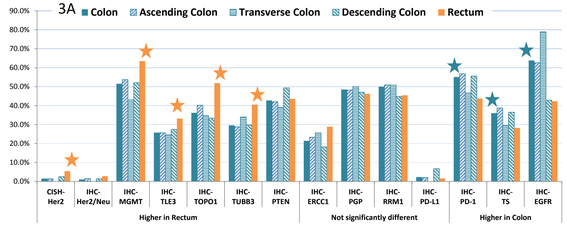
Figure 2: Microsatellite instability is significantly higher in colon tumors than in rectal tumors, and the frequency decreases significantly from ascending, transverse to descending colon. Stars indicate significantly higher MSI frequency than rectal tumors (p<0.05).



	All Primary Colon Tumors	Ascending Colon	Transverse Colon	Descending Colon	Rectum
MSI H	33	22	5	3	1
Cases Tested	196	107	42	32	134
Percent	16.8%	20.6%	11.9%	9.4%	0.7%

Figure 3: Protein expression (IHC) and Her2 amplification (CISH) differences in colon and rectal tumors taken from the primary sites. 3A shows the comparison in all tumors taken from primary sites while 3B shows comparisons in tumors with a confirmed negative MSI status. Stars indicate statistical significance between the two groups. Frequencies in ascending, transverse and descending primary colons are also displayed.

- TOPO1 and TLE3 expression are significantly higher in rectal tumors while EGFR expression is higher in colon tumors in both comparisons.



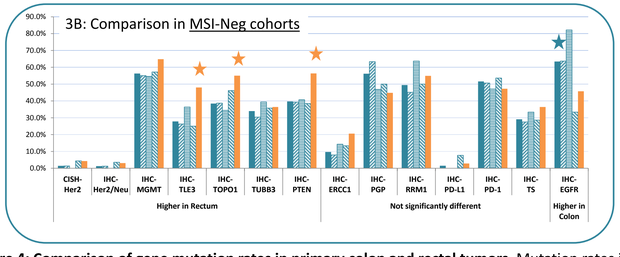
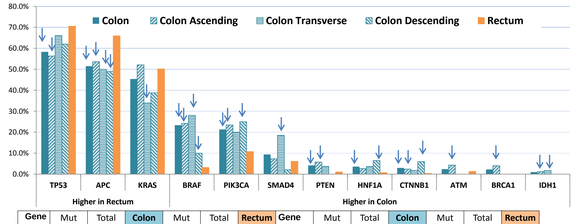


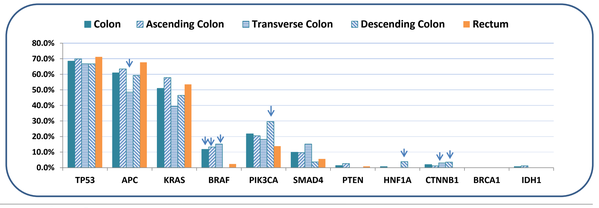
Figure 4: Comparison of gene mutation rates in primary colon and rectal tumors. Mutation rates in ascending, transverse and descending colon are also displayed. Only pathogenic and presumed pathogenic mutations were included. An arrow indicates statistical significance (p<0.05) when compared to rectal tumors. The table shows additional mutations (pathogenic or presumed pathogenic).



Gene	Mut	Total	Colon	Mut	Total	Rectum	Gene	Mut	Total	Colon	Mut	Total	Rectum
FBXW7	21	298	7.0%	23	312	7.4%	SMO	1	260	0.4%	0	268	0.0%
MEK1	2	32	6.3%	0	21	0.0%	STK11	1	290	0.3%	0	304	0.0%
NF1	2	32	6.3%	0	20	0.0%	AKT1	1	301	0.3%	1	314	0.3%
ERBB3	1	31	3.2%	0	21	0.0%	c-KIT	1	301	0.3%	0	314	0.0%
PTCH1	1	32	3.1%	0	20	0.0%	EGFR	1	302	0.3%	0	316	0.0%
WT1	1	32	3.1%	0	20	0.0%	MLH1	1	303	0.3%	0	317	0.0%
GNAS	9	304	3.0%	3	318	0.9%	VHL	0	278	0.0%	1	286	0.3%
NRAS	6	303	2.0%	10	313	3.2%	JAK3	0	299	0.0%	1	314	0.3%
BRCA2	2	138	1.4%	6	143	4.2%	PTPN11	0	303	0.0%	1	315	0.3%
ERBB2	2	293	0.7%	3	309	1.0%							

Figure 5: Comparison of gene mutation rates in primary colon and rectal tumors with confirmed negative MSI status. Mutation rates in ascending, transverse and descending colon are also displayed. Only pathogenic and presumed pathogenic mutations were included. An arrow indicates statistical significance (p<0.05) when compared to rectal tumors.

- BRAF mutation rate is significantly lower in the MSI negative cohort. Mutation rates of PIK3CA, HNF1A, CTNNB1 are significantly higher in various regions of colon tumors compared to rectal tumors.



Conclusions

- In this large cohort of CRC tumors, patients with colon and rectal tumors showed distinct clinical features, including older age and lower male prevalence in colon tumors.
- Differences in molecular profiles were observed in colon vs. rectal tumors.

1. Microsatellite instability decreases significantly from ascending to transverse to descending colon primary sites and were seen at extremely low frequency in rectal tumors.

2. Dramatically different mutation rates of BRAF (24% vs. 3%) were seen between colon and rectal cancer, and the differences were seen when only MSI-negative tumors were considered. The BRAF frequency in colon contrasts previously reported 5-10%, suggesting colon and rectal tumors were grouped together in previous studies. BRAF mutation rate was seen at higher rate in the right colon than the left, potentially underlying the worse prognosis and poorer response to cetuximab observed in the C80405 study for tumors originated on the right, and warrants further investigation.

3. Increased expression of Her2, TOPO1 and TLE3 in rectal tumors compared to colon tumors suggests therapeutic opportunities for rectal tumors.

- Consistent with consensus molecular subtyping of CRC, our observation of molecular differences in colon vs. rectal tumors from a large cohort of clinical samples confirms the molecular heterogeneity of this cancer type and an uneven distribution of molecular subtypes in various anatomic regions. These results hold importance in designing prospective clinical trials for personalized treatments of colorectal cancer.

References

1. Guinney, J., S. Tejpar (2015) "The consensus molecular subtypes of colorectal cancer", Nature Medicine 21 (11): 1350
2. Cancer Genome Atlas Network, (2012) "Comprehensive molecular characterization of human colon and rectal cancer", Nature 487(47407): 330-7