Colorectal cancer: impact of primary tumor location on genetic alterations

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Abstract

Background: Recent data show that patients with left-sided colon tumors (LT) have better survival and respond differently to biologics compared to patients with right-sided tumors (RT), likely due to molecular differences. We sought to examine these differences.

Methods: Primary colorectal tumors (n = 1730) with origins clearly defined as RT (cecum to hepatic flexure; n = 273), LT (splenic flexure to sigmoid colon; n = 585), or rectal (RC; n = 872) were examined by NextGen sequencing, protein expression, and gene amplification. Tumor mutational load (TML) was calculated in 1001 of these tumors using only somatic nonsynonymous missense mutations. Chi-square test was used for comparison.

Results: When compared to LT, RT carried a significantly higher rate of BRAF (25% vs. 7%; p < 0.0001), PTEN (5.4% vs. 1.3%; p = 0.008), and ATM (4% vs. 1%; p = 0.04) mutations. RT were likely to have more MSI-high tumors (22% vs. 5%; p < 0.0001) and PD-1 overexpression (58% vs. 44%; p = 0.01). There were no differences in the rate of KRAS (50% vs. 42%; p = 0.07) or NRAS mutations (2.2% vs. 3.4%; p = 0.4). When compared to RC, RT had a higher rate of BRAF (25% vs. 3%; p = $7E^{-07}$), PIK3CA (22% vs. 11%; p = 0.001), CTNNB1 (3% vs. 0.3%; p = 0.02); ATM (3% vs. 1%; p = 0.04), PTEN (5% vs. 1%; p = 0.004), and BRCA1 mutations (4% vs. 0%; p = 0.02). However, a lower rate of TP53 (56% vs. 71%; p = 0.001) and APC (53% vs. 66%; p = 0.003) mutations were observed in RT than RC. When compared to RC, LT showed higher rates of BRAF (6.7% vs. 3.2%; p = 0.04) mutations, CTNNB1 (2.1% vs. 0.3%; p = 0.04) mutations, and MSI-high tumors (4.6% vs. 0.7%; p = 0.04); however RC had a higher rate of KRAS mutations (50% vs. 42%; p = 0.04). There were no differences between RT, LT, and RC for the frequency of PD-L1 (2%, 2%, and 1%) or Her-2 (1%, 2%, and 3%) overexpression, although Her-2 amplification was significantly different (1%, 3%, and 5%, RT vs. RC; p = 0.03). Mean TML was 12, 11, and 8 mutations/megabase for RT, LT, and RC (RT vs. RC; p = 0.01), respectively. There was a correlation between TML and PD-L1 expression (p = 0.04), and TML and PD-1 expression (p = 0.01).

Conclusions: Tumors arising in the right colon carry genetic alterations that are different from LT as well as RC. However, it appears that CRCs carry a continuum of molecular alterations from the right to the left side, rather than displaying sharp, clear-cut differences.

Background

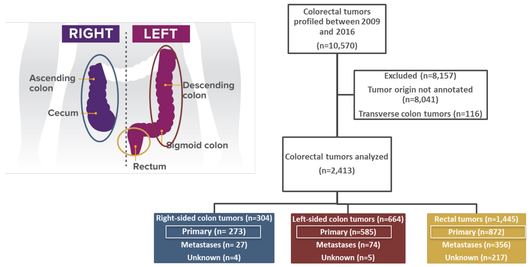
- Left-sided and right-sided colorectal tumors differ in their embryonic origin, luminal content, and bacteria flora.
- Clinically, right-sided colorectal tumors carry significantly inferior prognosis compared to left-sided tumors, irrespective of treatment.
- Retrospective-prospective studies of clinical colorectal cancer trials—including CALGB/SWOG 80405, FIRE-3, and CRYSTAL—unanimously show a significant effect of tumor location on response to therapy. Thus, a lack of cetuximab treatment-benefit to patients with right-sided RAS wild type tumors is suggested, resulting in a possible treatment paradigm shift, whereby treatment decisions for patients with colorectal cancer must be based not only on their tumor RAS status but also on their tumor location.
- Recognizing that tumor sidedness is a surrogate of tumor biology, we aimed to investigate different molecular alterations underlying left-sided colon, right-sided colon, and rectal tumors.

Methods

- Immunohistochemistry (IHC) was performed on full formalin-fixed paraffin-embedded (FFPE) sections on glass slides. A board-certified pathologist evaluated all IHC results independently.
- Microsatellite instability was tested with MIA (Microsatellite Instability Analysis) fragment analysis.
- Next-generation sequencing (NGS) was performed on genomic DNA isolated from FFPE tumor samples using either the MiSeq platform or the NextSeq platform (Illumina, Inc., San Diego, CA); no matched normal tissue was sequenced. All variants were detected with > 99% confidence based on allele frequency and depth of coverage with an average sequencing depth of coverage of > 500 and an analytical sensitivity of 5%

Results

Figure 1: patient characteristics



Patient characteristics	Right Colon	Left Colon	Rectum
Age (median, interquartile range in years)	64(54-74)	59(50-68)	59(51-67)
	Right vs. Left: p=6.2E-08, Left vs. Rectum: p=0.79, Right vs. Rectum: p=1.0E-08		
Gender (% female)	56%	46%	38%
	Right vs. Left: p=0.0046, Left vs. Rectum: p=0.001, Right vs. Rectum: p<0.0001		

Figure 2: Gene mutation rates in primary tumors of right-sided colon, left-sided colon, and rectal origin. * = p < 0.05, ** = p < 0.01, *** = p < 0.001 by Chi-square tests. A. Comparison in all primary tumors; B. Comparison in MSS tumors.

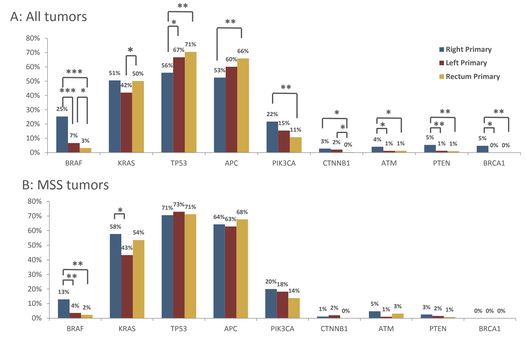


Figure 3: Protein expression rates in primary tumors of the right colon, left colon, and rectum. * = p < 0.05, ** = p < 0.01, *** = p < 0.001 by Chi-square tests. A. Comparison in all primary tumors; B. Comparison in MSS tumors.

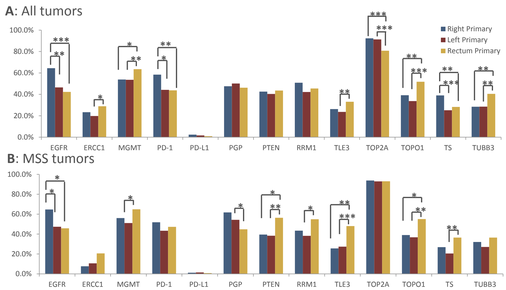


Figure 4: Box plots of mutational load seen in each tumor, and correlation with MSI status and tumor location. **A**: Mutational load per tumor (number of mutations per megabase) in MSI-high and MSS tumors. **B**. Mutational load comparison in primary tumors of the right colon, left colon, and rectum. **C**. Mutational load comparison in MSS primary tumors of the right colon, left colon, and rectum.

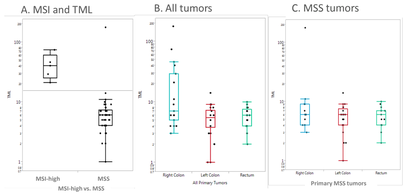
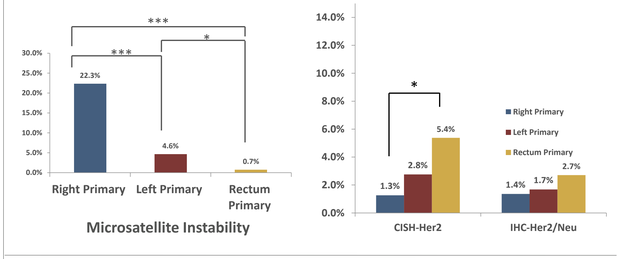
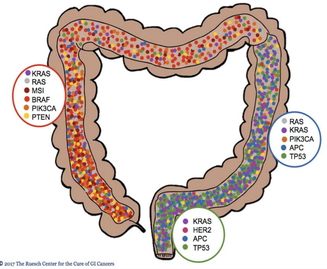


Figure 5: Left: MSI-high frequency in right-sided colon cancers, left-sided colon cancers, and rectal cancers. * = p < 0.05, *** = p < 0.001 by Chi-square tests. **Right:** Her2/neu amplification and overexpression in primary tumors of the right colon, left colon, and rectum.



Conclusions

- The incidence of BRAF mutations and MSI-high status decrease consecutively from right-sided colon to left-sided colon to rectal tumors. BRAF mutations are associated with poor prognosis and MSI-high status is associated with poor survival rate after relapse. Both BRAF mutation and MSI-high status contribute to the poor prognosis seen in patients with right-sided tumors.
- Molecular alterations that have been associated with cetuximab/panitumumab resistance, including PIK3CA and PTEN mutations, are seen in decreasing frequency from right to left, also contributing to the poorer responses seen in patients with right-sided tumors.
- Differential expressions of Her2, TOPO1, TS and ERCC1 in different tumor locations were observed, suggesting that different therapeutic strategies may be necessary.
- Our data suggest that CRCs carry a continuum of molecular alterations from right to left, rather than having a sharp, clear-cut distinction



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