Molecular Variances Between Rectal and Left-Sided Colon Cancers

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Disclosure

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• Consultant for Genentech, Bayer and Taiho
• Speaker’s Bureau Member: Genentech, Bayer and Taiho
Background

- Colorectal cancer (CRC) is a heterogeneous disease with different genetic alterations and clinical behavior.
- CRC was recently classified into four consensus molecular subtypes (CMSs) with distinguishing features.
- CMS 1-4 tumors have different carcinogenic pathways and genomic patterns.
Background

- Recent retrospective analysis of CALGB 80405 showed that left-sided colon tumors respond differently to biologics compared to right-sided colon tumors\(^1\), likely due to molecular differences.
- In the CALGB 80405 analysis, rectal cancers were included as part of the “left-sided” tumors.
- However, molecular variations between rectal and left-sided colon tumors are not well defined.

\(^1\) Venook AP et al. Clin Oncol. 2016;34 (suppl; abstr 3504)
Objective

- To identify the molecular variations among left-sided CRC tumors:
  - Rectal cancers
  - Sigmoid colon cancers
  - Descending colon cancers (plus splenic flexure)
Methods

- Retrospective analysis of 1,730 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 $p < 0.05$. 

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CONSORT Diagram

Colorectal tumors profiled between 2009 and 2016 (N = 10,570)

Excluded (N = 8,840)
- Metastatic tumors (457)
  - Rectosigmoid tumors (227)
  - Transverse colon tumors (116)
  - Tumor origin not confirmed (8,040)

Primary tumors with clearly defined origins (N = 1,730)

Right colon (N = 273)
Splenic flexure - descending colon (N = 125)
Sigmoid colon (N = 460)
Rectum (N = 872)

N = 1457, compared in the current study
Multi-platform profiling

- **Immunohistochemistry (IHC):**
  - ALK
  - AR
  - cMET
  - EGFR
  - ER
  - ERCC1
  - ER2/Neu
  - MGMT
  - PD-1
  - PD-L1
  - PGP
  - PR
  - PTEN
  - RRM1
  - TLE3
  - TOP2A
  - TOP2B
  - TS
  - TUBB3
  - PD-L1 antibody clone used: SP142

- **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
  - CDH1
  - FBXW7
  - GNAS
  - KDR
  - NOTCH1
  - PTPN11
  - STK11
  - AKT1
  - CSF1R
  - FGFR1
  - HRAS
  - KRAS
  - cMET
  - NRAS
  - PDK1
  - SMAD4
  - GNAQ
  - RET
  - VHL
  - ERBB2
  - APC
  - GNA11
  - JAK3
  - AKT2
  - FLT3
  - MPL
  - PTEN
  - PIK3CA
  - SMARCB1
  - ATM
  - GNAQ
  - ERBB4
  - 10% of tumors were tested with NextSeq platform: Agilent SureSelect XT, 592 gene panel, which were used to calculate tumor mutation load

Testing was performed under accreditation from CLIA, CAP and ISO 15189:2017

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Results
<table>
<thead>
<tr>
<th>Primary tumor location</th>
<th>Splenic flexure - descending colon (N=125)</th>
<th>Sigmoid colon (N=460)</th>
<th>Rectum (N=872)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age (yr.)</td>
<td>62</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>50%</td>
<td>44%</td>
<td>37%</td>
</tr>
<tr>
<td>Male</td>
<td>50%</td>
<td>56%</td>
<td>63%</td>
</tr>
</tbody>
</table>
Next-Generation Sequencing
Mutation Frequency Comparison Between Rectal and Descending Colon Tumors

- TP53
- APC
- KRAS
- PIK3CA
- BRAF
- FBXW7
- SMAD4
- HNF1A
- CTNBB1
- GNAS
- BRCA1
- PTEN
- NRAS
- BRCA2
- ATM
- AKT1

* indicates a significant difference between rectal and descending colon tumors (p < 0.05)
Mutation Frequency Comparison Between Rectal and Sigmoid Colon Tumors

No significant differences were found between rectal and sigmoid colon tumors.
Mutation Frequency Comparison Between Sigmoid Colon and Descending Colon Tumors

- Red: Rectum
- Purple: Sigmoid
- Green: Descending

Star indicates a significant difference between Sigmoid Colon and Descending colon tumors (p < 0.05)

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Frequency of microsatellite instability in left-sided CRC

P = 0.015

Microsatellite instability was tested with Microsatellite Instability fragment analysis (Promega)
Tumor Mutation Load (TML)

% of cases with TML ≥ 17 mutation/megabase

- TML was calculated using only somatic nonsynonymous missense mutations sequenced with a 592-gene panel.
- On a separate cohort of 331 tumors tested with 592-gene panel (both primary tumors and metastasis included). Descending colon, N = 34; Sigmoid colon, N = 129; Rectum, N = 168
- No significant difference was seen between the three cohorts
Correlation of MSI with TML

Salem et al. Comparative molecular analyses of left-sided colon, right-sided colon, and rectal cancers. Unpublished data

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Her2/Neu: Overexpression and Amplification

- There were no significant differences in Her2 overexpression or amplification between rectal, sigmoid colon and descending colon cancers.

- Threshold for positivity:
  - Her2 IHC: ≥ 3+ and > 10%
  - Her2 ISH: Her2/Neu:CEP 17 signal ratio of ≥ 2.0
IHC - Protein Overexpression

- ERCC1
- MGMT
- PTEN
- TLE3
- TOP2A
- TOPO1
- TS
- TUBB3

★ indicates a significant difference between rectal and descending colon tumors (p < 0.05)
★☆ indicates a significant difference between rectal and sigmoid colon tumors (p < 0.05)
Rectal vs. Descending Colon vs. Right-Sided Colon Cancers

- RIGHT
  - Ascending colon
  - Transverse colon
- LEFT
  - Descending colon
  - Sigmoid colon
  - Rectum

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**Mutation Frequency Comparison Between Rectal and Right-sided Colon Cancers**

- **TP53**: Rectum (70%), Descending (50%), Right (30%)
- **APC**: Rectum (60%), Descending (45%), Right (25%)
- **KRAS**: Rectum (55%), Descending (40%), Right (20%)
- **PIK3CA**: Rectum (65%), Descending (50%), Right (35%)
- **BRAF**: Rectum (40%), Descending (30%), Right (10%)
- **FBXW7**: Rectum (50%), Descending (40%), Right (30%)
- **SMAD4**: Rectum (45%), Descending (30%), Right (15%)
- **HNF1A**: Rectum (40%), Descending (30%), Right (20%)
- **CTNNB1**: Rectum (35%), Descending (25%), Right (10%)
- **GNAS**: Rectum (20%), Descending (15%), Right (5%)
- **BRCA1**: Rectum (10%), Descending (5%), Right (0%)
- **PTEN**: Rectum (5%), Descending (0%), Right (0%)
- **NRAS**: Rectum (5%), Descending (0%), Right (0%)
- **BRCA2**: Rectum (0%), Descending (0%), Right (0%)
- **ATM**: Rectum (0%), Descending (0%), Right (0%)
- **AKT1**: Rectum (0%), Descending (0%), Right (0%)

*Indicates a significant difference between rectal cancers vs. right-sided colon tumors (p < 0.05)*
Frequency of Microsatellite Instability

- **Rectum**: P < 0.0001
- **Descending Colon**: P = 0.0296
- **Right Colon**: P = 0.0152

### Microsatellite Instability

<table>
<thead>
<tr>
<th>MSI</th>
<th>Right Colon (N = 112)</th>
<th>Descending Colon (N = 42)</th>
<th>Rectum (N = 134)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient</td>
<td>25</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Proficient</td>
<td>87</td>
<td>39</td>
<td>133</td>
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</table>
Her2/Neu: Overexpression and Amplification

<table>
<thead>
<tr>
<th>IHC-Her2/Neu</th>
<th>Right Colon</th>
<th>Descending Colon</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive N</td>
<td>3</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Negative N</td>
<td>218</td>
<td>98</td>
<td>574</td>
</tr>
<tr>
<td>Total N</td>
<td>221</td>
<td>99</td>
<td>590</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left vs. Right</td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>Left vs. Rectum</td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>Right vs. Rectum</td>
<td></td>
<td></td>
<td>ns</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CISH-Her2/Neu</th>
<th>Right Colon</th>
<th>Descending Colon</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive N</td>
<td>2</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Negative N</td>
<td>156</td>
<td>57</td>
<td>264</td>
</tr>
<tr>
<td>Total N</td>
<td>158</td>
<td>50</td>
<td>279</td>
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<td>p value</td>
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<tr>
<td>Left vs. Right</td>
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<td></td>
<td>ns</td>
</tr>
<tr>
<td>Left vs. Rectum</td>
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<td></td>
<td>ns</td>
</tr>
<tr>
<td>Right vs. Rectum</td>
<td></td>
<td></td>
<td>0.0328</td>
</tr>
</tbody>
</table>

P = 0.0328

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IHC - Protein Overexpression

- Indicated a significant difference between right-sided colon vs. rectal tumors (p < 0.05)

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Conclusions

- CRCs carry a continuum of molecular alterations from right to left, rather than having a sharp, clear-cut distinction
Limitations

- This was a retrospective analysis
- Potential effects of treatments including chemoradiation are unknown
- Limited clinical information was available for analyzed tumors
- A large number of samples were excluded due to a lack of definitive tumor location information
Conclusions

- Rectal cancers have molecular features that are different from left-sided colon tumors
- Clinical trials should stratify patients based on the location of the primary tumor (right vs. left) as well as molecular features
- Better understanding of disease biology may help to identify therapeutic targets and advance precision medicine