Molecular Variances Between Rectal and Left-Sided Colon Cancers

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Disclosure

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

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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
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Colorectal tumors profiled between 2009 and 2016 (N = 10,570)
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Excluded (N = 8,840)
- Metastatic tumors (457)
  - Rectosigmoid tumors (227)
  - Transverse colon tumors (116)
  - Tumor origin not confirmed (8,040)
Colorectal tumors profiled between 2009 and 2016 (N = 10,570)

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

Rectum (N = 872)
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Primary tumors with clearly defined origins (N = 1,730)

Sigmoid colon (N = 460)

Rectum (N = 872)
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- Metastatic tumors (457)
- + Rectosigmoid tumors (227)
- + Transverse colon tumors (116)
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Primary tumors with clearly defined origins (N = 1,730)

- Splenic flexure - descending colon (N = 125)
- Sigmoid colon (N = 460)
- Rectum (N = 872)
Primary tumors with clearly defined origins (N = 1,730)

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

N = 1,457, compared in the current study
Multi-platform profiling

- **Immunohistochemistry (IHC):**
  - ALK
  - PGP
  - AR
  - PR
  - cMET
  - PTEN
  - EGFR
  - RRM1
  - ER
  - TLE3
  - ERCC1
  - TOP2A
  - HER2/Neu
  - TOPO1
  - MGMT
  - TS
  - PD-L1
  - TUBB3
  - PD-L1 antibody clone used: SP142

- **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
  - 10% of tumors were tested with NextSeq platform: Agilent SureSelect XT, 592 gene panel, which were used to calculate tumor mutation load

- **Microsatellite Instability fragment analysis (Promega)**
  - Microsatellite Instability

- **In-situ hybridization (CISH or FISH)**
  - Her2
  - cMET
  - EGFR

Testing was performed under accreditation from CLIA, CAP and ISO 15189:2012
Results
## Patient characteristics

<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>Female 50%</td>
<td>44%</td>
<td>37%</td>
</tr>
<tr>
<td></td>
<td>Male 50%</td>
<td>56%</td>
<td>63%</td>
</tr>
</tbody>
</table>
Next-Generation Sequencing
Mutation Frequency Comparison Between Rectal and Descending Colon Tumors

indicates a significant difference between rectal and descending colon tumors (p < 0.05)
No significant differences were found between rectal and sigmoid colon tumors.
Mutation Frequency Comparison Between Sigmoid Colon and Descending Colon Tumors

indicates a significant difference between Sigmoid Colon and Descending colon tumors (p < 0.05)
Frequency of microsatellite instability in left-sided CRC

Microsatellite instability was tested with Microsatellite Instability fragment analysis (Promega)

P = 0.015
Tumor Mutation Load (TML)

- 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

% of cases with TML ≥ 17 mutation/megabase

- Splenic Flexure/Descending: 8.8%
- Sigmoid: 1.6%
- Rectum: 4.2%
Correlation of MSI with TML

Salem et al. Comparative molecular analyses of left-sided colon, right-sided colon, and rectal cancers. Unpublished data
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
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)
Mutation Frequency Comparison Between Rectal and Right-sided Colon Cancers

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

- Right Colon (N = 112): Deficient 25, Proficient 87
- Descending Colon (N = 42): Deficient 3, Proficient 39
- Rectum (N = 134): Deficient 1, Proficient 133

P-values:
- Right Colon vs. Descending Colon: P < 0.0001
- Right Colon vs. Rectum: P = 0.0296
- Descending Colon vs. Rectum: P = 0.0152
Her2/Neu : Overexpression and Amplification

**IHC**

<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>
</tbody>
</table>

**CISH**

<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>59</td>
<td>279</td>
</tr>
</tbody>
</table>

**p value**

- Left vs. Right: ns
- Left vs. Rectum: ns
- Right vs. Rectum: 0.0328

*P = 0.0328*
IHC - Protein Overexpression

indicates a significant difference between right-sided colon vs. rectal tumors (p < 0.05)
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
Primary Site Effects (left colon vs. rectal)

Figure 1. Overall survival among all pts

OS of all pts. Left-sided colon pts had similar OS as rectal pts
Median OS 18.7 mos left-sided colon versus 18.1 mos rectal
Adjusted HR 1.02 (0.95-1.10) p=0.559
Conclusions

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