Do the gut flora play a role in African Americans with colon cancer?

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Colon Cancer in African Americans

- High incidence and high mortality
- Very aggressive and usually detected at late stages
- Different factors might be responsible
- Analysis of known TSG and oncogenes
- A high rate of MSI
Ways to understand the causes

• High throughput experiments: genomics and epigenomics
• Genomics: aCGH study of 30 AA CRC
• Chromosome X amplification and implications
• Tumors clustering, maximum likelihood vs Parsimony??, Cloud computing as a solution?
• Epigenomics: 12 AA CRC and 12 AA adenoma study. Clustering, differential methylation vs cumulative??
Diet and Gut Flora

• Diet’s role is unquestionable: patterns of colon cancer in western world, Japanese Americans vs Japanese, African Americans vs Africans

• SLC5A8 study: 82% of AA colon adenomas show methylation of this gene, a butyrate transporter
From single bacteria to microbiomics

- *Enterococcus faecalis*: induce polyp formation in Mi mice and genomic polyploidy (Dr. Huycke, OSU)

- Enterotoxigenic *Bacteroides fragilis*: induces polyp formation in Min mice through a pSTAT3 pathway (Dr. Sears, JHU)

- *Helicobacter pylori*: Macrophages/Nitric oxide/DNA methylation, Our study found a correlation with high size polyps.
Gut Flora Analysis

DNA was extracted from 4 stool samples, PCR amplified using universal 16S rDNA primers and cloned. 384 clones were sequenced from each stool sample DNA and identified using a rDNA database.

<table>
<thead>
<tr>
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<th>HB01 (Polyp patient)</th>
<th>HB02</th>
<th>HB03 (Polyp patient)</th>
<th>HB04</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides</td>
<td>31.5%</td>
<td>4.8%</td>
<td>30.2%</td>
<td>10.2%</td>
</tr>
<tr>
<td>Clostridia</td>
<td>65%</td>
<td>93.3%</td>
<td>65%</td>
<td>68.5%</td>
</tr>
</tbody>
</table>

H-H Partnership: Research Project
HIT Chip Analysis of DNA Stool Samples from Healthy and Adenoma Patients

Stool DNA from 12 patients (6 healthy AFR001-006 and 6 with adenoma AFR007-012) were used for 16 S rDNA amplification. The resulting PCR products were fragmented and fluorescently labeled before the hybridization to a Human Intestinal Tract (HIT) Chip.

Hierarchical clustering of probe profiles was carried out by calculating a distance matrix between the samples based on the squared difference between each pair of profiles (Euclidean distance). The distance matrix was used in the hclust implementation in R of a hierarchical clustering algorithm (Ward’s minimum variance method).
Probe intensity for some bacterial groups (out of 132 detected)

| A                        | B      | C      | D      | E      | F      | G      | H      | I      | J      | K      | L      | M      |
|--------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| **level 2**              | AFR-001| AFR-002| AFR-003| AFR-004| AFR-005| AFR-006| AFR-007| AFR-008| AFR-009| AFR-010| AFR-011| AFR-012|
| **Bacteroides fragilis et rel.** | 404205.6| 643882.7| 279045.9| 344295.2| 593990.9| 354290.9| 409941.2| 508590.7| 17219.98| 648996.1| 647354.6| 286232.5|
| **Bacteroides intestinalis et rel.** | 193203| 157558.9| 250593.8| 143651.3| 257435.8| 102455.1| 106446.3| 206688.7| 9880.207| 219780.9| 73808.98| 260120.5|
| **Bacteroides ovatus et rel.** | 471307.5| 864606.4| 468877.2| 819250| 354856.3| 299033| 65077.28| 691299.1| 10565.32| 466667.5| 261818.3| 452455.7|
| **Bacteroides plebeius et rel.** | 623001| 122350.1| 270979.8| 199864.1| 460078.3| 374274.5| 186989.3| 345312.1| 26183.09| 494751.8| 238098| 337568|
| **Bacteroides splachnicus et rel.** | 597561.3| 390553.4| 342273.8| 139262.6| 323585.6| 489087.3| 252925.7| 513793.5| 17970.27| 724021.6| 580856.8| 364748.6|
| **Bacteroides stercoris et rel.** | 242057.7| 183823.8| 309207.1| 284248.7| 307006.1| 227163.5| 22509.96| 169518.5| 5813.912| 124600.3| 42035.03| 428795.1|
| **Bacteroides uniformis et rel.** | 476185.9| 73727.67| 219603.4| 29108.56| 546778.6| 299590.2| 196847.6| 122945.7| 25421.92| 420628.6| 317231.4| 300954|
| **Bacteroides vulgatus et rel.** | 323517.9| 168155.5| 479625.5| 267107.4| 266810.3| 164876.6| 9528.132| 505039.1| 21888.1| 476529.8| 160153.2| 447287.8|
| **Lactobacillus cateniformis et rel.** | 112.6963| 106.6845| 101.2991| 102.4484| 105.2726| 103.8666| 106.757| 103.744| 104.124| 108.8697| 133.7207| 121.5423|
| **Lactobacillus gasseri et rel.** | 7842.931| 10838.51| 11154.83| 12025.65| 5961.903| 14509.51| 15670.2| 7247.912| 20713.78| 7842.039| 8877.059| 10585.6|
| **Lactobacillus plantarum et rel.** | 2642.92| 2724.514| 2808.287| 2753.393| 2498.765| 2515.937| 2572.69| 2433.368| 2553.681| 2499.832| 2526.579| 2586.053|
| **Lactobacillus salivarius et rel.** | 5754.146| 8437.914| 6038.057| 10073.73| 4906.737| 12699.75| 12884.76| 4449.382| 17776.77| 5782.809| 6800.149| 7827.27|
| **Helicobacter** | 1049.767| 1016.317| 915.1542| 971.8583| 941.3072| 947.4253| 977.9615| 930.4036| 955.7731| 940.3715| 944.2713| 918.7219|
| **Enterococcus** | 6399.066| 9593.229| 6403.768| 11176.7| 4520.635| 13392.09| 13627.08| 4618.951| 19628.65| 6370.53| 7391.313| 8401.939|
pSTAT3 Nuclear Staining of Normal and Colon Adenoma Samples

pSTAT3 Cytoplasmic Staining in Normal and Colon Adenomas Samples

pSTAT3 Stromal Staining in Normal and Colon Adenoma Samples
bft Detection in C. diff (CD) and Adenoma (CA) Stool Samples

- Stool DNA was used in a Touchdown PCR with 16S universal primers and primers specific for *Bacteroides, B. fragilis* and BFT gene
- Out of 82 samples (34 CD and 48 CA), only 7 had unamplifiable DNA.
- Positive for *B. fragilis* were 45
- Positives for BFT were 2 (one CD and one CA samples)

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EIA

• Detection of anti-BFT as evidence of ETBF exposure

• Preliminary data:
  Bangladesh, acute diarrhea
  96% (64/67) patients with diarrhea anti-BFT+, day 7
  12.5% (2/16) endemic control patients anti-BFT+
EIA Results with African American Serum Samples

• EIA experiments for the detection of anti-BFT antibodies involving 87 serum samples from colon adenoma patients and 180 healthy individuals are underway.

• A similar experiment for the detection of anti- *H. pylori* antibodies is also being pursued.

• Metagenomic analysis

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