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

Hassan Brim, Ph.D. Howard University



NCIBI, University of Michigan, July 2010



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. Clsutering, diffrential methylation vs ummulative??





Diel and Gul I Iora

- Diet's role is unquestionnable: 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 abd 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

	HB01 (Polyp patient)	HB02	HB03 (Polyp patient)	HB04
Bacteroides	31.5%	4.8%	30.2%	10.2%
Clostridia	65%	93.3%	65%	68.5%

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 (Euclidian 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	В	С	D	E	F	G	Н		J	K	L	М	
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 re	597561.3	390553.4	342278.8	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	307008.1	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	265810.3	164876.6	9528.132	505039.1	21881.8	476529.8	160153.2	447287.8	
Lactobacillus catenaformis et	112.6963	106.6845	101.2991	102.4484	105.2726	103.8866	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	14909.51	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	4520.685	13392.09	13627.08	4618.951	19628.66	6370.53	7391.313	8401.939	

pSTAT3 Nuclear Staining of Normal and Colon ADenoma Samples



pSTAT3 Cytoplasmic Staining in Normal and Colon Adenomas 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)

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

H-H Partnership: Research Project