The A, B, C's of Bowel Flora

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49 yo woman, 6 months vague/variable abd. pain
T3N2 adenoCa, 4.1 cm tumor

42 yo woman, hx similar, asymptomatic anemia
T3N1M1 adenoCa

32 yo male, hx similar, difficulty passing stool
T3N1 adenoCa

31 yo female, post-partum abd. symptoms
T4N0 adenoCa

29 yo female, vague/variable abd. pain
T4N0 adenoCa

Colorectal Surgery Division
Dept of Surgery, JHH
2010-2011
CRC
Gross Pathology
(T3N2)
Progression to CRC

Problem

\[ ? \rightarrow \{\]

Colon epithelial cell mutations
Colon epithelial cell hyperplasia

\[ \downarrow \]

Colon cancer

Vogelgram
We are a 'colony of creatures'.

90% of the cells in our body are not our own but rather the microbes, mostly bacteria, that live in and on us.

The Microbiome
The Microbiota
Not a new concept........

Joshua Lederberg, Ph.D.
Nobel Prize, 1958, bacterial genetics

Coined the term ‘microbiome’ or the collective genome of our indigenous microbes.

Comprehensive view of human genetics and physiology is a composite of human and microbial genetics.
Dynamic Partnerships: Beneficial bacteria and their hosts

We Get By with a Little Help from Our (Little) Friends.

Ruby et al
Science 303:1305, 2004
Diversity of the Human Intestinal Microbial Flora

Paul B. Eckburg,1* Elisabeth M. Bik,2 Charles N. Bernstein,3
Elizabeth Purdom,4 Les Dethlefsen,2 Michael Sargent,3
Steven R. Gill,5 Karen E. Nelson,5 David A. Relman1,2,6*  

Science 308:1635, 2005  
N = 3 adults

Metagenomic Analysis of the Human Distal Gut Microbiome

Steven R. Gill,1‡ Mihai Pop,3‡ Robert T. DeBoy,1 Paul B. Eckburg,2,3,4
Peter J. Turnbaugh,3 Buck S. Samuel,3 Jeffrey I. Gordon,5 David A. Relman,2,3,4
Claire M. Fraser-Liggett,1,6 Karen E. Nelson1

Science 312:1355, 2006  
N = 2 adults

~60% flora novel  
~80% flora uncultivatable  
Most cultured flora is Firmicutes and Bacteroidetes  
500-1000 bacterial species  
Differs person to person  
Differs mucosal to luminal

Microbiome is enriched compared to human genome in metabolic pathways such as vitamin synthesis. Humans are superorganisms whose metabolism represents an amalgamation of microbial & human attributes.
Key Goal: Baseline to empower future clinical studies.

Assess microbial diversity of 250 healthy individuals at 5 sites (gut, nasal, oral, vaginal and skin)

- 16s rRNA sequencing, defining who is in the microbial population
- Sequence bacterial reference genomes
- Metagenomics, predicting the function of a microbial population by sequencing its genome content without genus/species assignment

NIH Roadmap for Medical Research
Human Microbiome Project (HMP), Sept. 2007
5 years, $115 million
Ecology: Site-specific distributions of bacterial phyla in healthy humans

The Bacterial Kingdom
Phyla 50 on earth, 4 humans
Genera
Species 3%, all individuals
Strains

Concepts:
Interpersonal variability with relative intrapersonal stability.
Microbial mutualism essential to health (nutrition/metabolism, development, immune system).

Pie distributions = phyla detected
Size of circles = number species detected

Concept: Interpersonal Variability, Intrapersonal Stability

N=161 subjects aged 65 or older

N=27 sites, 7-9 subjects, 4 times

- **Bacteroidetes** 3-92% (Phyla)
- **Clostridium cluster IV** 2-34% (Genera)
- **Ruminococcus** Faecalibacterium <1-16% (Species)
**Nutritional Impact of Colon Microbiome**

**Metagenomics:** Bacteroides thetaiotaomicron genome is dedicated to polysaccharide utilization, containing capacities not in the human genome and directs induction of monosaccharide transporters. *Science* 299:2074, 2003

**Biology:** Colonic microbiota induces fat storage. *PNAS* 101:15817, 2004

Colonization of GF mice with normal colonic flora produces a 60% increase in body fat and insulin resistance within 14 days despite reduced (30%) food intake. Leptin, insulin, glucose & adipocyte factor levels increase significantly.

Adipocyte factor = angiopoietin-like protein 4
Modifies fat storage.
**Developmental Impact of Colon Microbiome**

**Mucosal IECs:** Colonic bacteria or B. theta alone induce IEC fucose-containing glycoconjugates.

Brown = fucose-containing glycoconjugates

*Trends in Microbiology* 12:129, 2004

**Mucosa development:** Colonic bacteria or B. theta alone induce intestinal angiogenesis.

*PNAS* 99:15451, 2002

**Upper third of distal small intestinal villi**

Germ free mouse

Mouse with conventional flora for 10 days

Mouse colonized with B. theta for 10 days

Bacteroides thetaiotaomicron
Symbiotic bacteria induce IEC RegIIIγ, an antimicrobial protein binding peptidoglycan.

Science 313:1126, 2006
Science 334:255, 2011
Microbiome-induced Diseases?

Gingivitis, Peridontitis, Halitosis
OM, Sinusitis
Chemorx & RT-induced mucositis

Psoriasis, Acne, Dermatitis

IBD, IBS, Colon Ca

Systemic Diseases: Obesity/Malnutrition
Diabetes/Metabolic syndrome
HIV progression
Atherosclerosis
Hepatitis, cirrhosis, liver Ca
Atherosclerosis
Rheumatoid Arthritis
Pancreatic Ca

Asthma, COPD, CF

Esophagitis, Barrett’s, Esophageal Ca

Gastritis, PUD

Vaginitis

Gut

Oral
Microbiome-inducing Disease Paradigms

Single species  Microbiome

Single gene  Complex
Mendelian diseases  multigene diseases

Systems biology
Integrated Microbiome-inducing Disease Paradigm

- Alpha Bugs & Their Consorts
- Environmental Science Keystone species

Disease Induction

Host cell & tissue responses

Paradigm 1
Single microbiome members are responsible for disease.

~20-30% epithelial cancers globally are associated with single microbial infections

- **Gastric cancer**: *Helicobacter pylori*
- **Liver cancer**: HBV, HCV
- **Biliary tree cancer**: *Clonorchis sinensis*, *Opisthorchis viverrini*
- **Cervical cancer**: HPV
- **Urinary bladder cancer**: *Schistosoma hematobium*

>2 million cancers globally per year
Paradigm 2
The microbiome complex is required for disease.

Mucosal disease: IBD  
PNAS 104:13780, 2007

IBS  
Gastroenterology 141:1782, 1792, 2011; NEJM 364:1, 2011

Systemic disease: Obesity
Diabetes/Metabolic syndrome

N=12 obese patients & 5 lean controls
Promoted a simple ratio as predictive of obesity.

A luminal, microbe only view

TLR5 KO (flagellin) induced obesity, hypertension, hyperlipidemia & insulin resistance

The Microbes Made Me Eat It
Science 328: 179, 2010

Implicates the microbiome & host innate immune responses as key to pathogenesis

Nature 444:1022, 2006

Science 328: 228, 2010
Paradigm 3
Dominant microbiota members (Alpha Bugs) are aided by select microbiome members (Consorts).

Colon cancer
Fusobacterium
Structural microbiology of human CRC & controls
Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma

Mauro Castellanin,1,2,6 René L. Warren,1,6 J. Douglas Freeman,1 Lisa Dreolini, Martin Krzywinski,3 Jaclyn Strauss,3 Rebecca Barnes,4 Peter Watson,4 Emma Allen-Vercoe,3 Richard A. Moore,3,5 and Robert A. Holt1,2,7

Genomic analysis identifies association of Fusobacterium with colorectal carcinoma

Aleksandar D. Kostic,1,2 Dirk Gevers,3 Chandra Sekhar Pedamallu,1,3 Monia Michaud,4 Fujiko Duke,1,3 Ashlee M. Earl,1 Akinyemi I. Ojesina,1,3 Joonil Jung,1 Adam J. Bass,1,3 Josep Tabernero,5 Jose Baselga,5 Chen Liu,6 Ramesh A. Shvidarasani,3 Shuji Ogino,2,3,7 Bruce W. Birren,3 Curtis Huttenhower,1,8 Wendy S. Garrett,1,3,4 and Matthew Meyerson1,2,3,9

Relative abundance Fusobacterium (T:C)

N=99

Normal

N=95

Tumor