Microbiome & Metabolomics

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Outline

• Microbe rules the world
• Microbial product matters
• Examples of microbiome in health and disease
• Examples of metabolites in health and disease
Microbial infection and systemic disease

Healthy Mouth

Healthy Body

Cardiovascular Complications

Respiratory infection

Pregnancy complication

Colon cancer
Complex microbial communities-dental plaque
Microbes are everywhere

Who are they?
What are they doing?
How is the host responding?
What maintains the equilibrium?
How do we differ?
How can we manipulate microbes?

10 % human cells
90 % microbial cells

Genetic info > 100
The Human Microbiome Project

- Microbial components of the human genetic and metabolic landscape, and how they contribute to health and disease
- The genomes of microbial symbionts provide traits that humans did not need to evolve on their own
- Humans, a composite of microbial and human cells
- Human genetic landscape dictated by the genes in the human genome and the microbiome
- Human metabolic features, a blend of human and microbial traits

A human ‘supraorganism’

The Human Genome Project

The project funded by the US government in 1990, and declared complete in 2003. 
A parallel project by the Celera Genomics in 1998. 
Capacity-3 billion bps 
Major advance in DNA sequencing

versus the Human Microbiome Project
Microbiome Analysis - microbial profiling/genomics

Plaque or saliva

PCR with bar coded primers specific for 16S rDNA region for amplification

Metagenomics
Human Microbiome Project
Transcriptomic Analysis - gene expression profiling

Plaque or saliva

RNA isolation

Add bar coded primers, DNA synthesis

Overlapping ds-cDNA Library

PCR

Amplified ds-cDNA

Removal of primers

Microarray RNA-sequencing

Data extraction and processing

NextSeq 500
Proteomic Analysis-protein profiling

Plaque or saliva

Protein preparation

Quantitative proteomics by spectral counting

peptides

RPLC

LC-MS/MS

spectral counting

MS/MS
Integration of Multi”Omics”

Biological samples

Microbiome

Networking pathways for metabolites
### Microbiomes impact behaviors

<table>
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<th>Microbiome</th>
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<td><strong>Gut microbiota</strong></td>
<td>Diet-specific microbiota influence mating preferences</td>
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<td><strong>Human skin microbiota</strong></td>
<td>Skin microbes of humans influence attraction to mosquitoes</td>
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<td><em>Lactobacillus rhamnosus</em></td>
<td>The probiotic <em>L. rhamnosus</em> decreases anxiety in mice</td>
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**Fighting microbes or Farming microbes?**
Microbiomes impact behaviors

Gut microbiota
Diet-specific microbiota influence mating preferences


*Lactobacillus plantarum* strain IMAU:10272
cuticular hydrocarbon sex pheromones
the hologenome theory of evolution
Microbiomes impact behaviors

Individuals

Distinct microbiome

Various volatile compounds

Highly attractive

Poorly attractive


Do metabolites matter???
Microbiome and Carbohydrate Metabolism

• Carbohydrates: humans and bacterial nutrients
• *Human and mammals*: disaccharides and starches
• *Microbes*: complex polysaccharides by Carbohydrate-active enzymes: glycoside hydrolases, carb esterases, glycosyltransferases and polysaccharide lyases
• Biogeographical distribution of microbiome/genes/pathways such as simple Carb transport PTS small intestine>colon
• Probing microbe altered pathways in the development of metabolic disorders in humans
Microbiome and Atherosclerosis

Flavin monooxygenases (FMOs)

Inhibition of trimethylamine production by microbiome for the treatment of atherosclerosis

Cell. 2015 Dec 17;163(7):1585-95.
Obesity and Cancer: a Microbial Connection

- hepatic stellate cells (HSC)
- hepatocellular carcinoma (HCC)
- senescence-associated secretory phenotype (SASP)

Nature. 2013 Jul 4;499:97-101

deoxycholic acid (DCA)
Obesity and Cancer: a Microbial Connection

1. Fatty food
   - The liver produces digestive chemicals called primary bile acids. These are stored in the gall bladder and then released into the intestines during a meal.

2. Gall bladder
   - Nutrients, bile acids and bacterial by-products, including LPS and DCA, pass into the liver.

3. Intestine
   - When exposed to a high-fat diet, populations of Firmicutes bacteria rise. Many of these bacteria produce LPS, and some of them convert primary bile acids to secondary bile acids, including the toxic DCA.

4. Hepatic portal vein

5. LPS binds to immune receptors called TLRs, leading to inflammation in the liver.
   - Chronically high levels of DCA and LPS can increase cancer risk.

DCA, deoxycholic acid; LPS, lipopolysaccharide; TLR, Toll-like receptor.
Gut Bacteria and the Workings of Our Minds

Of Humans
MRI scans to look at the brains
the types of bacteria in their guts

Of Mice
Changes gut microbiota alter mouse behavior
Bold to timid, brain-derived neurotrophic factor

Probiotics alters brain activity
Gastroenterology, 144, 1394–1401. June 2013
Cell, 155, 1451–63, 19 December 2013
Gut Bacteria & Healthful Chocolate

Cocoa powder (polyphenols and Fibers)

Smaller molecules short fatty chain acids

2014 American Chemical Society meeting
Gut Microbes and Your Weight

Slimming gut microbes?

Microbe transplants from obese humans

*Science*, 6 September 2013: 341(6150)
When do our microbiome established?
Antibiotic use and growth promotion in farming animals
The Use of Antibiotics prescriptions
Obesity Among U.S. Adults by State and Territory

2014
Increased body fat by the use of antibiotics
Increased body fat by the use of antibiotics

Dysbiosis in Gut and Obesity

Cox et al., Cell 158, 705–721, August 14, 2014
Antibiotics in Infancy and Early Childhood Obesity

• 69% of children exposed to antibiotics before end of first year
• Increase in the antibiotics use associated with increased risk to obesity
• Asthma and wheezing also predicted obesity

Bailey et al., *JAMA Pediatr*. Published online September 29, 2014
Antiseptic Mouthwash and Blood Pressure

• Bacteria in the mouth reduce nitrates to nitrites
• Nitrite reduced to NO – relaxes vessels and lowers blood pressure
• Antiseptic mouthwash reduced oral nitrite production by 90% and plasma nitrite levels by 25% ($p<0.001$)
• Systolic and diastolic blood pressure increased by 2–3.5 mm Hg

The nitrate- and nitrite-reducing capacity of oral bacteria

Oral bacteria in systemic conditions

*Fusobacterium nucleatum*
Fusobacterium & Colon Caner

Fusobacterium nucleatum

Kostic AD et. al., Cell Host Microbe 2013, 14:207-15.
Rubinstein MR et. al., Cell Host Microbe. 2013, 14:195-206.
Kostic AD et. al., Genome Res. 2012, 2:292-8.
Whole-genome analysis of the colorectal cancer microbiome

(A) DNA isolated from colon tissues (9 tumor-normal sample pairs) 
Illumina sequencing 
Human whole-genome sequencing data (1.51 billion reads; median per sample) 
PathSeq analysis 
Microbial sequences (101,000 reads; median per sample)

(B) Genomic tree

Fusobacterium
Streptococcus
Aggregatibacter

(C) Whole-genome sequencing

(D) Percent relative abundance of Fusobacterium

Bacterial abundance in colon cancer

**Fusobacterium nucleatum**

Bacteria and Colorectal Cancer

Colon luminal environment
- Fusobacteria
- Model 1
  - Single microbes
- Model 2
  - Microbial community
- Model 3
  - Single microbes interacting with microbial community

Host mucosal environment
- Tumor microenvironment
- Inflammation
- Host genetics

Colorectal Cancer

Cell Host Microbes, 2013
Cell Host Microbes, 2014
New Findings
Bacterial biofilms and cancer connection


Butyrate, a short chain fatty acid, is produced by the gut microbiota and can be absorbed into the bloodstream. It activates the niacin/butyrate receptor GPR109A in the colonic epithelium, leading to downstream effects on adiposity, immunity, blood pressure, cell cycle, and cell proliferation, among others.
Microbial Metabolism Drives Transformation of Colon Epithelial Cells (Msh2-deficient)
Gut microbiota induce colon cancer in MSH2-deficient mice
Reduced dietary carbohydrates decreases polyp frequency in $\text{APC}^{\text{Min}/+}\text{MSH2}^{-/-}$ mice.
Butyrate induces colon cancer in \( \text{APC}^{\text{Min}+/+\text{MSH2}^{-/-}} \) mice.
Complex Interactions
Genetic background matters!

Diet

Microbiota

Metabolites

Host & Host factors
(Health and Disease)
What’s next?

• Presence in the tumor environment
• Progression of tumor and bacterial infection
• Role in tumorigenesis via inflammation mechanisms
• Tumor diagnostics
  • Fusobacterium biomarkers
• Bacterial cancer therapy
Microbiome- and metabolites-targeted Therapies
Microbiome & metabolites......
Microbes rule the world

Cocoa powder (polyphenols and Fibers)

Smaller molecules
short fatty chain acids

2014 American Chemical Society meeting
Thank you!