The gut microbiome in health and CRC

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Disclosures

- Research funding from Janssen Pharmaceuticals, Prodigest, Aphea.bio, Cargill, Beneo
- Past consulting for Merck, Janssen, Prodigest, Aphea.bio, Biofortis, GSK, Takeda
The promise of the microbiome field: diagnostics & cure for all major diseases on the planet.
Reality check:
We don’t even know what a healthy flora means!

Microbiome sequencing state-of-the-art:
MetaHIT, HMP + specific lab studies combined have sequenced ±3000 healthy individuals world-wide, still biased cut of the population

Variation in clinically relevant population = largely unknown
Temporal variation & stability of biomarkers = largely unknown
Factors influencing gut flora composition = largely unknown
Effect environment = largely unknown

Clinical end points for functional foods, pre-/pro/synbiotics, pharma-/nutriceutical interventions etc are unknown
Flemish Gut Flora Project
N=3400 population microbiome survey

- Collection of faecal, blood (GP) and saliva samples
- Questionnaires:
  - Self-reported health
  - Detailed health (GP)
  - Diet (incl probiotics, drugs)
  - Wellbeing/QoL
  - Hygiene
  - Bowel habit/Bristol scale
  - Travel, Stress etc
- Blood analysis: metabolic (e.g. glucose, HDL/LDL, triglycerides, insulin,...) and immunological/inflammatory readouts (cell counts, interleukins, CRP,...)
- Secured database, patient encoding
- 2018: host genotyping, metabolon data added

Logistics tricks & tips: Vandeputte et al FEMS Micr Rev 2017

Identification of 69 factors associated with microbiota variation


92% of comparable factors replicate in LLDeep
Microbiota-drug associations as primary confounder category

A

<table>
<thead>
<tr>
<th>Medication type</th>
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Community composition changes | Observed richness decreases | Pelou evenness decreases | Fisher diversity decreases | Higher proportion of core genera | Abundance differences | Abundance differences |

B

Drugs interacting with cofactor-associations

See also: Forslund*, Hildebrand*, Nielsen*, Falony* Nature 2015
Microbiota members associated with cancer immunotherapy response

Fig. 3 | Existing evidence for the role of the gut microbiota in cancer development and treatment response. Circos plots illustrating the relationships of unique microbial taxa response to cancer immunotherapy agents (anti-CTLA-4 and/or anti-PD-1). The blue ribbons represent the microbial taxa that are enriched in the context of a beneficial therapeutic response. The red ribbons represent the microbial taxa that are enriched in nonresponders to therapy. These reported associations should be interpreted with caution as they are correlative in nature, and causality has yet to be established. Different taxa are further grouped and colored by their phyla as indicated by the legend. Numbers in parentheses indicate the references that demonstrate these relationships: (1) Chaput et al.; (2) Frankel et al.; (3) Gopalakrishnan et al.; (4) Matson et al.; (5) Rount et al. Credit: Debbie Maizels/Springer Nature.

Note: skin/lung/kidney cancers

Helmink et al Nature Medicine 2019
Model for microbiota involvement in CRC

Bacteria: the good and the bad

Vivarelli et al
Cancers 2019
Towards microbiome diagnostics for crc?

Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma

Aleksandar D. Kostic,1,2 Dirk Gevers,1 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 Taberner,5 José Baselga,5 Chen Liu,6 Ramesh A. Shivdasani,3 Shuji Ogino,2,3,7 Bruce W. Birren,1 Curtis Huttenhower,1,8 Wendy S. Garrett,1,3,4 and Matthew Meyerson1,2,3,9

**Figure 2.** 16S rDNA sequencing analysis of the colorectal cancer microbiome. (A) Schematic of experimental and computational 16S rDNA sequencing analysis workflow. (B) Beta diversity distances calculated using phylotype relative abundance measurements between all pairs of samples demonstrate that the microbial composition of tumor/normal pairs within individuals is more highly correlated than tumor/tumor pairs, normal/normal pairs, or tumor/normal pairs from different individuals. (C) Linear discriminant analysis (LDA) coupled with effect size measurements identifies *Fusobacterium* as the most differentially abundant taxon in colon tumor versus normal specimens by 16S rDNA sequencing in 95 individuals. Tumor enriched taxa are indicated with a positive LDA score (black), and taxa enriched in normal tissue have a negative score (gray). Only taxa meeting an LDA significant threshold of 4.2 are shown. (D) A cladogram representation of data in C. (Red) Tumor-enriched taxa; (blue) taxa enriched in normal tissue. The brightness of each dot is proportional to its effect size.
Fecal microbiome biomarkers outperform FOBT test, combination works best

Table 1. Summary of study population F, G, and H.

<table>
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<th>Study population</th>
<th>Healthy</th>
<th>Adenoma</th>
<th>Colorectal cancer</th>
<th>Country of residence</th>
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<td>Early stages$^c$</td>
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<td>0</td>
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<td>Early stages$^b$</td>
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<td>H (N = 297)$^a$</td>
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<td>0</td>
<td>0</td>
<td>Denmark, Spain, Germany</td>
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<td>Early stages$^b$</td>
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<td>Early stages$^c$</td>
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</table>

Zeller et al MSB 2014
Meta-analysis identifies core crc-associated bacteria

Wirbel et al
Nat Med 2019
Core bacteria cluster in co-abundance groups linked to patient subgroups

Wirbel et al
Nat Med 2019
Generalization of classifiers based on meta-analysis-identified markers seems possible

Wirbel et al
Nat Med 2019

But: study variability >> crc variability!!! Emphasizes importance of confounder control and standardized approaches
Mucosal signals: sometimes they do replicate across studies....

Figure 2 | Validations of metacommunity markers in independent cohorts. (a,b) Fold-change analyses in paired carcinoma and carcinoma-adjacent samples in two additional cohorts demonstrated significant agreement with our discovery cohort: (a) Kostic et al.\textsuperscript{7} data set (n = 74) and (b) Zeller et al.\textsuperscript{20} data set (n = 48). Shown are adjusted $R^2$ and P values for goodness of fit from multiple linear regression models. (c) Real-time PCR amplifications of

Data: 47 paired samples of adenoma and adenoma-adjacent mucosae, 52 paired samples of carcinoma and carcinoma-adjacent mucosae and 61 healthy controls, Hong Kong

Nakatsu et al 2015 Nature Comms 6:8727
...and sometimes they don’t!

Tumor vs adjacent tissues, compared across cohorts (US, Spain)

- Eikenella overrepresented in US tumors (P=0.03)
- Fusobacterium (P<0.0001), Bulleida (P=0.02), Gemella (P=0.03), Parvimonas (P=0.03), Campylobacter (P=0.047), and Streptococcus (P=0.05) significantly over represented in Spanish tumors.

22 US adenocarcinomas + adjacent
67 Spanish adenocarcinomas, 1 carcinoma + adjacent

CRC μbiome project

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Janssen Pharmaceuticals

CRC μbiome

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Dr. L. Bijnens

Prof. J. Raes
Dr. R. Tito
C. Verspecht
Dr. L. Lahti

Janssen Pharmaceutical Companies of Johnson & Johnson
IWT
**Aims:**

- Improved diagnostics, investigation of polyp to carcinoma transition
- Mechanistic insights at gut mucosa interface
DNA/RNA Extraction kit choice has important effect on colonic mucosa profiling
Biopsy storage conditions (Flash Frozen vs RNALater) affects recovered microbiota community composition.
All fixation procedures (Carnoy/Methacarn/FFPE) introduce bacteria contaminants, Carnoy and Methacarn modifies the microbial profile to a greater degree than formalin.
From Relative to Quantitative profiling

Vandeputte et al., Nature 2017
Discovery of a low-microbial load enterotype associated with multiple inflammatory conditions

Vieira-Silva et al., *Nature Microbiology* 2019
QMP allows disentangling effects behind specific biomarkers
Bile acid vs inflammatory signal in IBD/PSC

Vieira-Silva et al., Nature Microbiology 2019
Disentangling effects behind specific biomarkers
Bile acid vs inflammatory signal in Primary Sclerosing Cholangitis & IBD

Inflammatory markers, but in different pathologies: Fuso & Veillonella as communicating vessels

Vieira-Silva et al., *Nature Microbiology* 2019
QMP, B2 etc in CRC development?

Collab S. Tejpar, E. Dekkers, J. Reumers
Lessons learned

• Definition of healthy microbiota variation important to identify confounders and provide background
• Clear potential for microbiome diagnostics/prognostics in crc
• A wide range of targets & mechanistic insights appearing, some of which replicate; relative importance unclear
• Quantitative profiling & confounder control can help disentangling signals and prioritize targets
• Coherence in methods (sampling, extraction, amplification, contamination control) is essential for biopsies/sections
Acknowledgments & collaborators

Youssef Darzi
Falk Hildebrand
Gwen Falany
Gipsi Lima-Mendez
Samuel Chaffron
Karoline Faust
Sara Vieira-Silva
Joao Sabino
Clara Caenepeel
Marie Joossens
Leen Rymenans
Chloe Verspecht
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Thanks for your attention

Open PhD/Postdoc positions
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