The colorectal cancer microbiome

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Financial interests

Chair of Scientific Advisory Board and consultant for OrigiMed
Research support and previous consulting from Bayer, with patents and patents pending, no royalties yet
Royalties on patents for EGFR mutations in lung cancer diagnosis, licensed to LabCorp
Other patents and patent applications related to cancer, no royalties yet
Previous research support from and consulting for Novartis
Founding advisor to Foundation Medicine, previous consulting, previous equity, previous sale of shares to Roche
Several patent applications related to *Fusobacterium*, none issued
Issued patent on computational subtraction (US patent # 6996477), not yet licensed

I will discuss investigational use of metronidazole in animal models
Technical advances have led to the discovery of chronic disease microbes

Human papillomavirus (HPV) 16 and 18: cervical cancers
   Nucleic acid hybridization methods
   Since found in head and neck, penile, and other cancers
   --zur Hausen: Heidelberg

Helicobacter pylori: gastric ulcers, gastric cancer
   Advances in microbial culture (microaerophilic techniques)
   --Warren and Marshall: Perth

Human herpesvirus 8: Kaposi’s sarcoma
   PCR-based subtraction (representational difference analysis)
   Since found in pleural effusion lymphoma
   --Chang, Moore, and Cesarman: New York
Sequence-based computational subtraction for pathogen discovery

Principle

Infected tissues contain both human and microbial RNA and DNA
Microbial sequences can be identified by subtraction of human sequences

Generate & sequence libraries from human tissue

Summary of novel pathogens discovered by our computational subtraction research program, 2001 to 2009

(Next slide)
Fusobacterium enrichment in human colorectal carcinomas

Alex Kostic, PhD
Former graduate student
Now Assistant Professor of Microbiology, Harvard
**PathSeq**: software to identify or discover microbes by deep sequencing of human tissue

Kostic et al., Nature Biotechnology, 2011
GATK PathSeq: the next generation

Reference preparation

PathSeq pipeline
- Sample BAM
- Quality Filter + Host Subtraction
- Microbe Alignment
- Taxonomic Scoring

Abundance report and sample metrics
Annotated BAM

Host k-mer file
Host ref image
Microbe ref image
Taxonomy file

PathSeqBuildReferenceTaxonomy

RefSeq / Genbank catalogs
Taxonomy datafiles
NCBI FTP Server

Host reference FASTA
Microbial reference FASTA

PathSeqBuildKmers
CreateBwaMemIndexImage

https://gatkforums.broadinstitute.org/gatk/discussion/10913/how-to-run-the-pathseq-pipeline#latest

Walker, Pedamallu et al., Bioinformatics, 2018
Pathogen analysis of 9 colorectal cancer/normal genome pairs

Adam Bass, MD
Former post-doctoral fellow
No

Bass et al., Nature Genetics, 2011
Kostic et al., Genome Research, 2011
**Fusobacterium** enrichment in colorectal tumors compared to adjacent normal tissue

Statistical enrichment in population of tumors

Pairwise enrichment in tumor/normal pairs

Kostic et al., Genome Research, 2011

Castellarin et al., Genome Research, 2011
Fluorescence *in situ* hybridization finds *Fusobacterium* localized to the colonic lamina propria and mucus

Kostic et al., Genome Research, 2011
with Wendy Garrett laboratory
Promotion of intestinal tumorigenesis by *Fusobacterium* in *Apc*(Min) model

Kostic et al., Cell Host and Microbe, 2013

with Wendy Garrett laboratory
Fusobacterium persistence in liver metastases and in patient-derived xenografts

Susan Bullman, PhD
Post-doctoral fellow
Persistence of *Fusobacterium* in liver metastases of colon cancer: culture

*Fusobacterium* species can be cultured from both primary cancers and metastases. The isolates from liver metastases are genetically nearly identical to the primary cancer isolates.

Bullman et al., *Science*, 2017
Persistence of *Fusobacterium* in liver metastases of colon cancer: microbiome

Bullman et al., *Science*, 2017
Localization of *Fusobacterium* to primary and metastatic colon cancer cells: *in situ* hybridization

Bullman et al., *Science*, 2017

Paolo Nuciforo
Persistence of *Fusobacterium* in mouse xenografts: serial sampling

Bullman et al., *Science*, 2017
Persistence of *Fusobacterium* in mouse xenografts: microbiome

Bullman et al., *Science*, 2017
Metronidazole treatment of xenografts: impact on Fusobacterium load and cell proliferation

Bullman et al., Science, 2017
Metronidazole treatment of xenografts: impact on tumor growth

Fusobacterium-negative cell line

Fusobacterium-positive cancer-derived xenograft

Bullman et al., Science, 2017
Conclusions on the colon cancer microbiome

• “Finally, we ask in conclusion, does *Fusobacterium* cause cancer, is it an innocent bystander, or is its presence a consequence of cancer? Our view at present is that *Fusobacterium* could be liable for causing colorectal cancer in a civil suit, which requires a preponderance of the evidence. However, we believe that *Fusobacterium* would currently be found not guilty of causing colorectal cancer in a criminal case, which requires evidence beyond a reasonable doubt.”
Fusobacterium and colorectal cancer: More questions than answers!!
The *Fusobacterium* associated microbiome

- Does the microbiome travel with the original cancer and migrate together with it as it metastasizes?
- Are the different bacterial genera (*Fusobacterium, Bacteroides, Selenomonas*) mutually necessary for one another and reinforcing one another?
- Should antibiotic treatment be directed against the full microbiome rather than against *Fusobacterium*?
Fusobacterium and inflammation

- Where does the expression of inflammatory genes (including interleukin genes) come from?
- Immune/inflammatory cells? The cancer itself?

- Does the inflammation promote the growth of the cancer?
- Or does it suppress it?
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