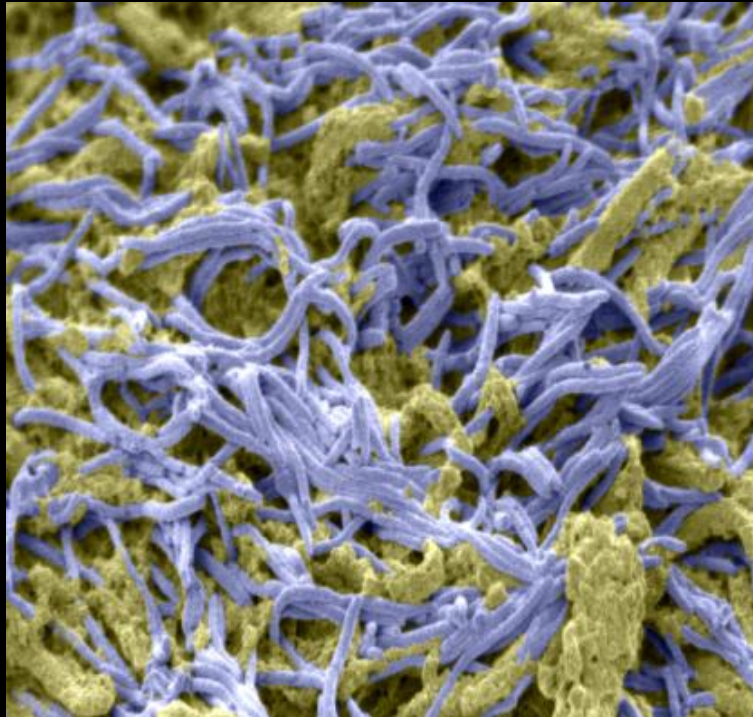


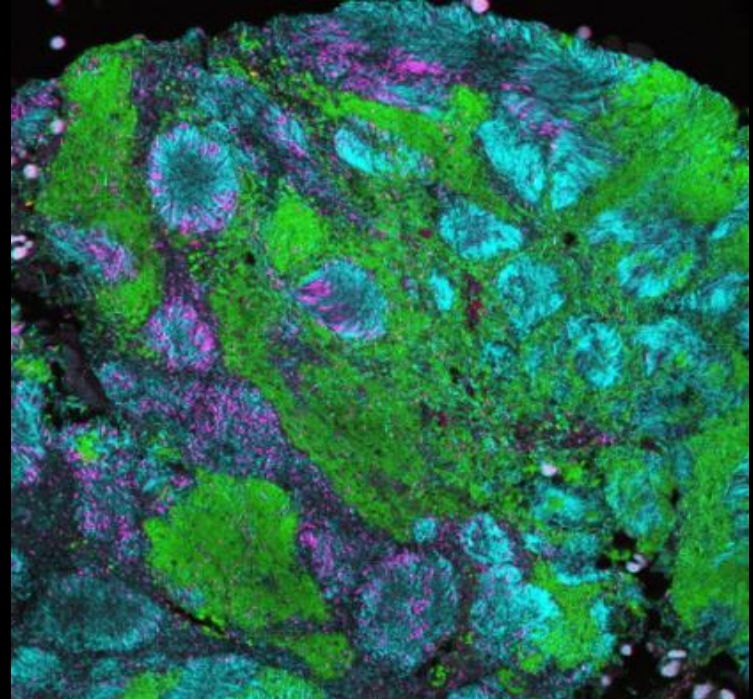
Using biofilms (and metagenomics) to diagnose colon cancer

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2 μ m



First International Conference on Clinical Metagenomics
October 14, 2016

Microbial:Colon Cancer Disease Paradigms

Single species

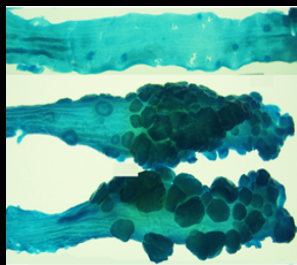
ETBF

Escherichia coli (pks island)
Fusobacterium nucleatum

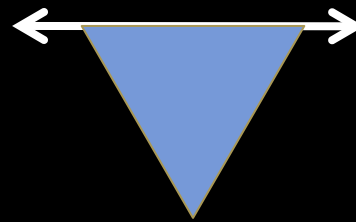
Control

ETBF

ETBF



BFT, IL-17-mediated



Keystone
Alpha-Bugs
&
Consorts
(Driver:Passenger)

Microbiome



Microbiota

Germ
free

ETBF = Enterotoxigenic *Bacteroides fragilis*

Sears CL, Pardoll DM
J Infect Dis 203:306, 2011
Sears CL, Garrett WS
Cell Host Microbe 15:317, 2014

Hypothesis

The microbiota and likely specific members of the microbiota are important to the initiation and progression of colon cancer.

Microbiota organization is a distinct feature of proximal colorectal cancers

Christine M. Dejea^a, Elizabeth C. Wick^b, Elizabeth M. Hechenbleikner^b, James R. White^{c,1}, Jessica L. Mark Welch^d, Blair J. Rossetti^d, Scott N. Peterson^{e,2}, Erik C. Snestrud^{e,3}, Gary G. Borisy^d, Mark Lazarev^f, Ellen Stein^f, Jamuna Vadivelu^g, April C. Roslani^h, Ausuma A. Malik^h, Jane W. Wanyiri^f, Khean L. Gohⁱ, Iyadorai Thevambiga^g, Kai Fu^j, Fengyi Wan^{j,k}, Nicolas Llosa^l, Franck Housseau^k, Katharine Romans^{m,n}, XinQun Wu^f, Florencia M. McAllister^k, Shaoguang Wu^f, Bert Vogelstein^{m,n}, Kenneth W. Kinzler^{m,n}, Drew M. Pardoll^{f,k}, and Cynthia L. Sears^{a,f,k,4}

PNAS | December 23, 2014 | vol. 111 | no. 51 | 18321–18326

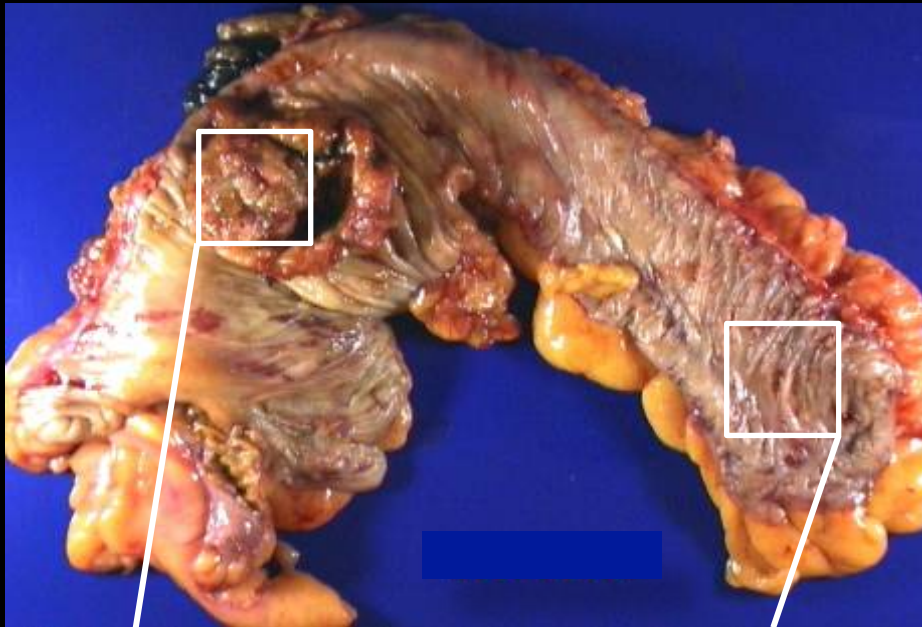


Christine Dejea

Johns Hopkins
School of Medicine

Human Sample Collection

Individuals with colon cancer

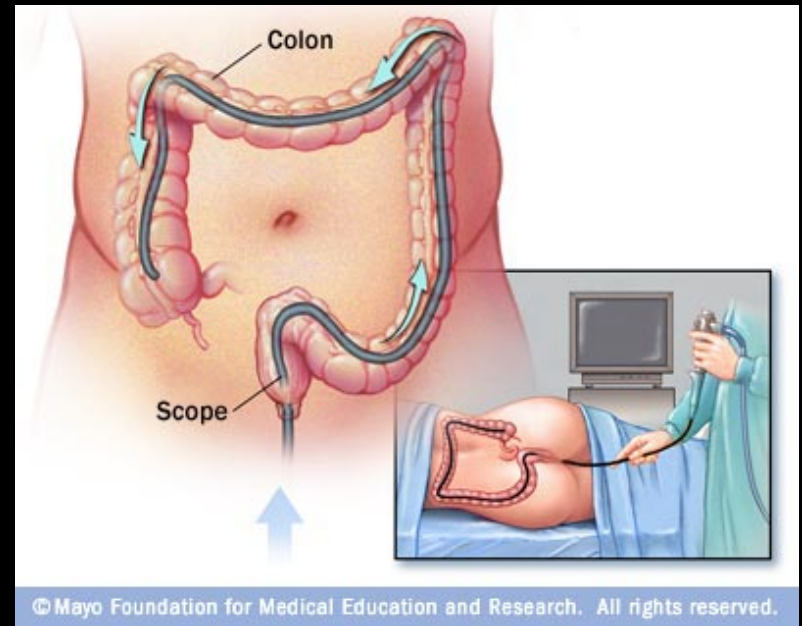


Tumor Mucosa

Paired normal mucosa

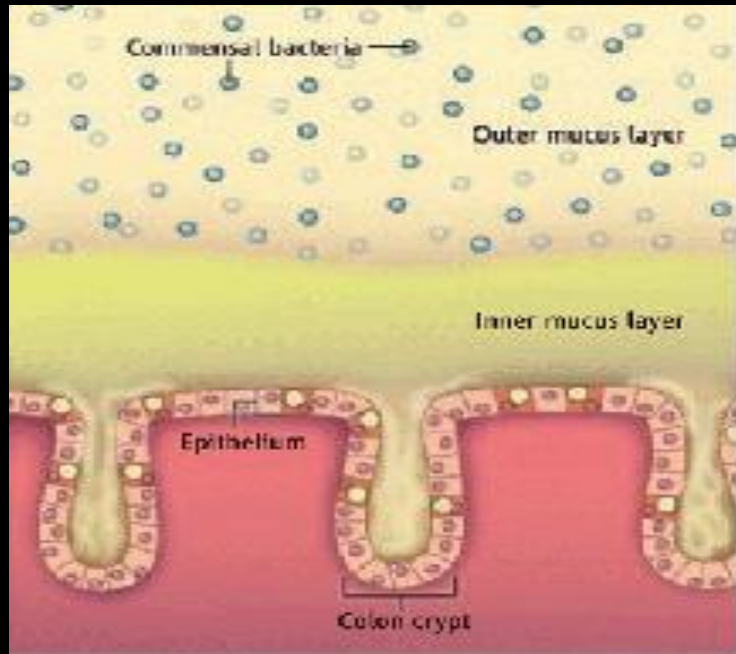
Fix tissues in Carnoy's

Colonoscopy control subjects
without colon cancer



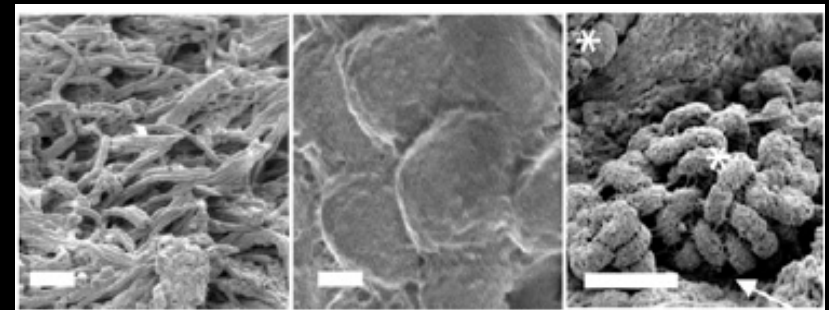
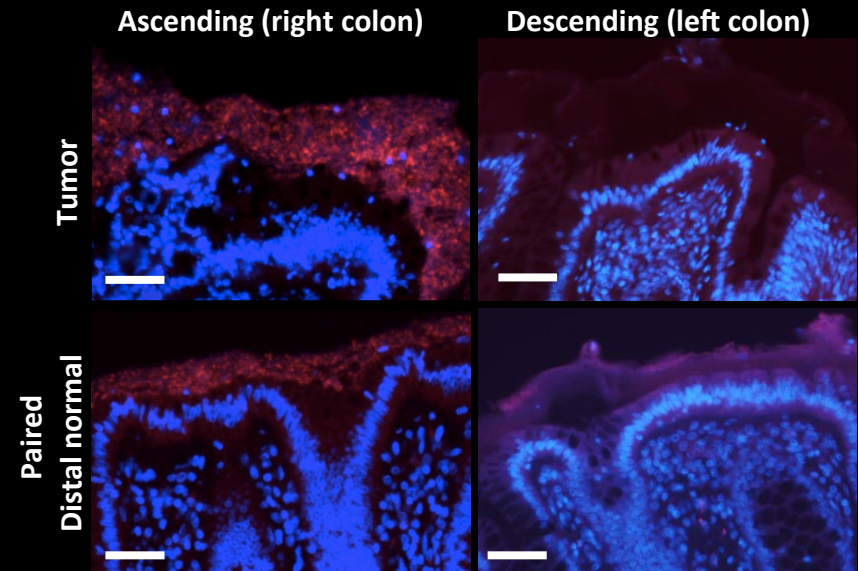
Vogelstein group
Pathology Suite
Surgery Department, Dr. Liza Wick
Colonoscopy Suite, Drs. Ellen Stein and Mark Lazarev

Spatial mucosal microbiota geography: defining CRC bacterial biofilms



Gunnar Hansson

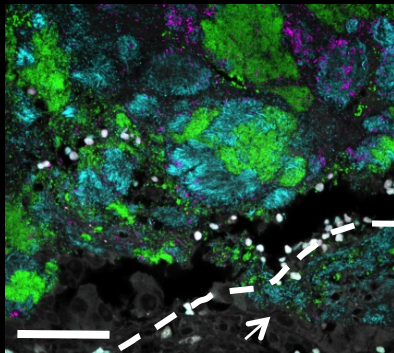
Colon Cancer Host Universal Bacterial 16S FISH



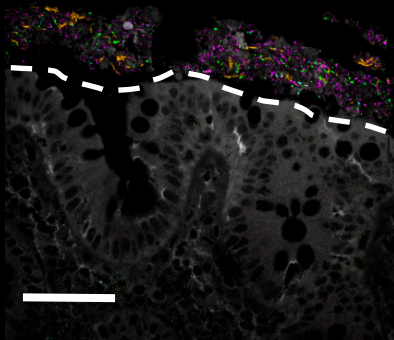
Dejea et al. PNAS, December 2014

Bacterial biofilms in CRC or colonoscopy hosts are polymicrobial & in CRC, mucosa invasive.

Colon Cancer Host



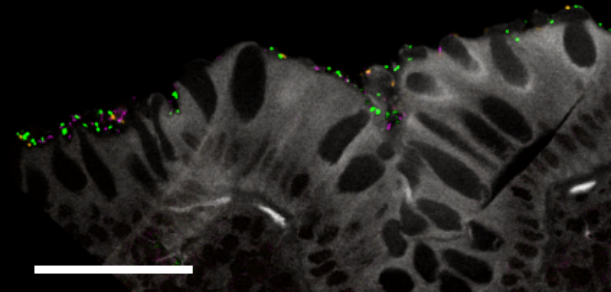
Right tumor
polymicrobial
bacterial
invasion
(100%)



Paired normal
polymicrobial
bacterial
invasion
(50%)

Healthy Colonoscopy Host

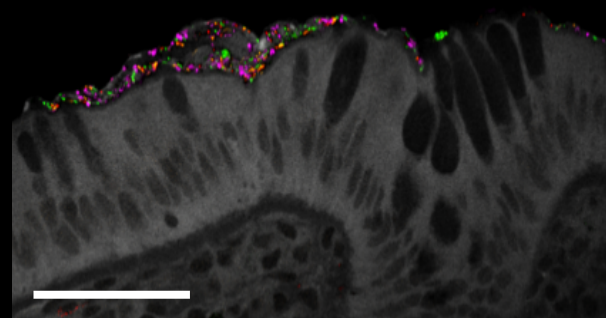
Right colonoscopy bx



Throughout
colon

~15% of
healthy
hosts bf+

Left colonoscopy bx



Bacterial
invasion
not seen

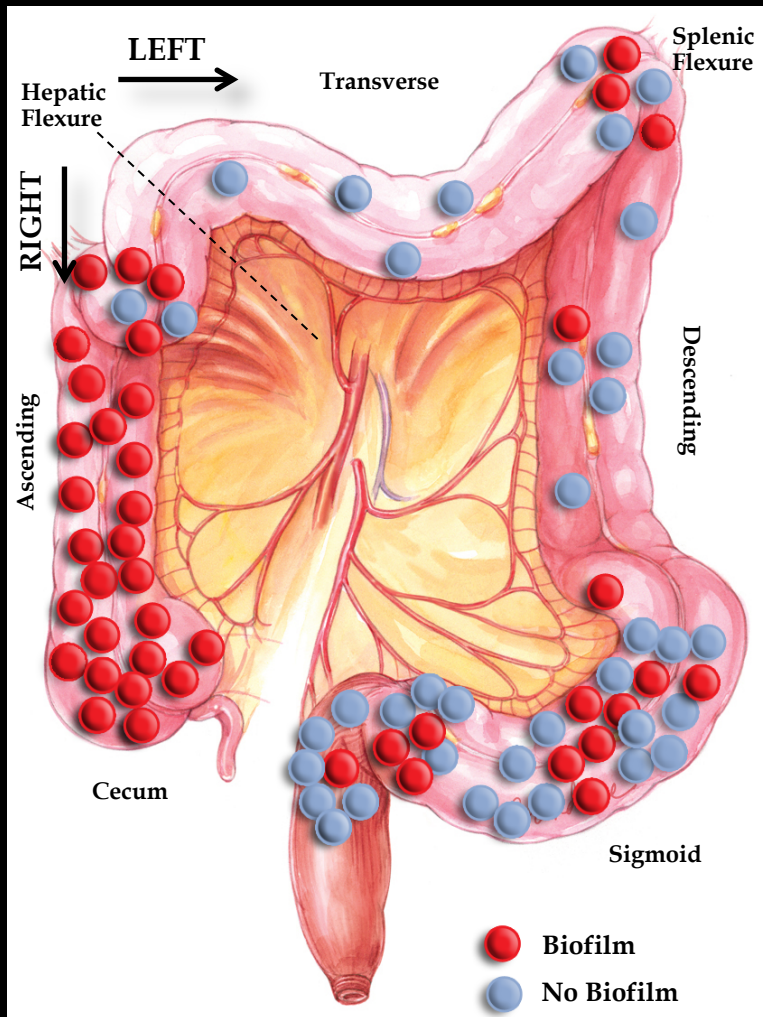
N=142

Sporadic right colon tumors are defined by bacterial biofilms

Johns Hopkins & University of Malaya cohorts



Julia Drewes



When biofilms are present, CRC and normal tissues are nearly always 100% concordant for biofilms.

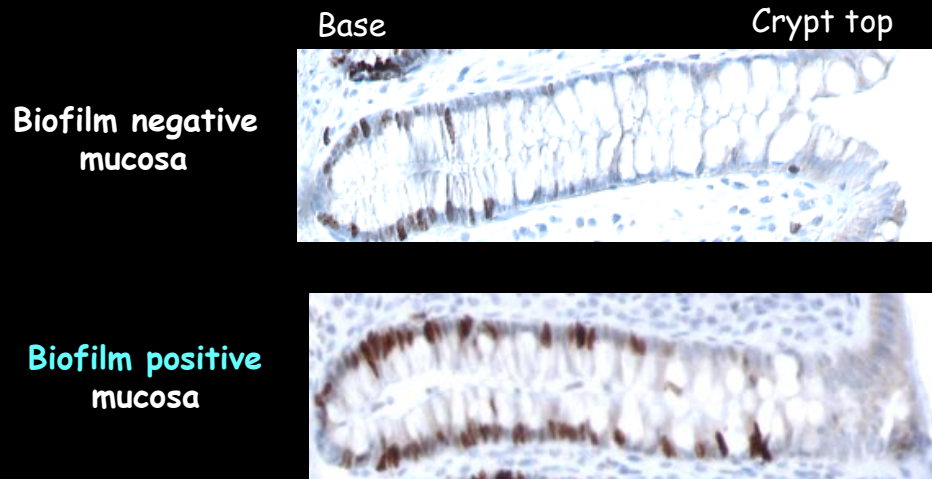
Diet, colon prep, other demographics do not correlate with findings

Dejea et al. PNAS, December 2014

Dr. Jamuna Vadivelu

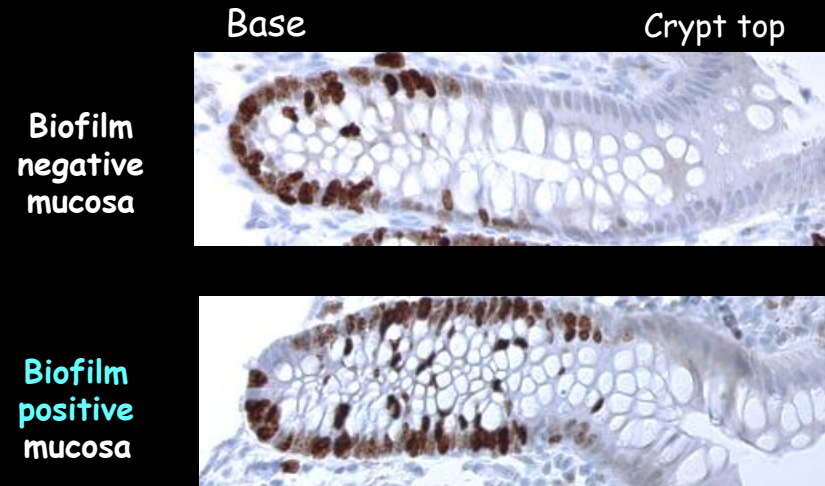
Biofilms alter normal colon epithelial biology: colonic epithelial cell proliferation (Ki67)

Colon Cancer Patient Distal Normal



$P < 0.0001$

Colonoscopy Control Biopsy



$P < 0.01$

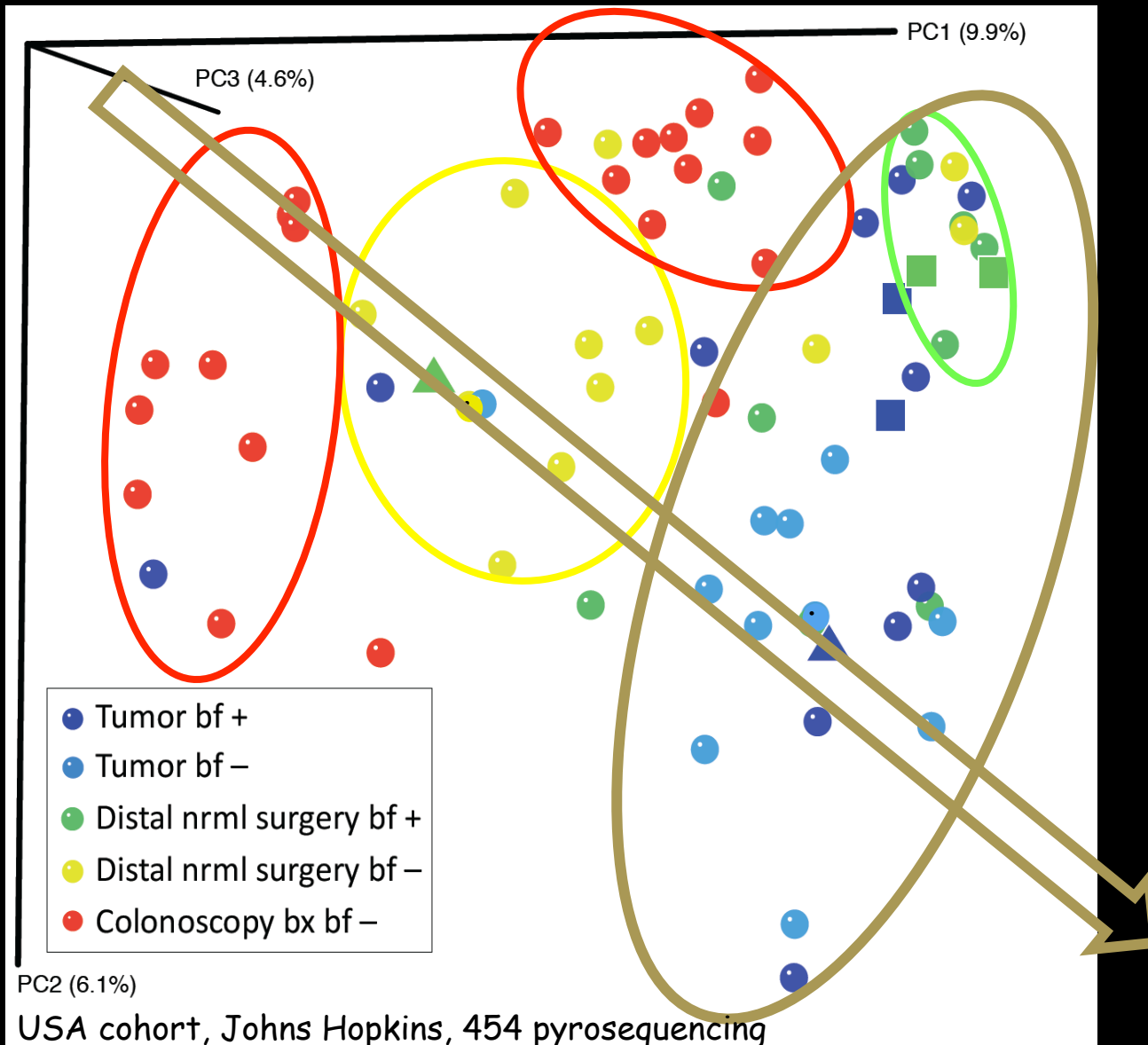
Also changes E-cadherin, IL-6, pStat3

All left samples

Dejea et al. PNAS, December 2014

Principle coordinates analysis of bacterial clustering

PNAS, December, 2014

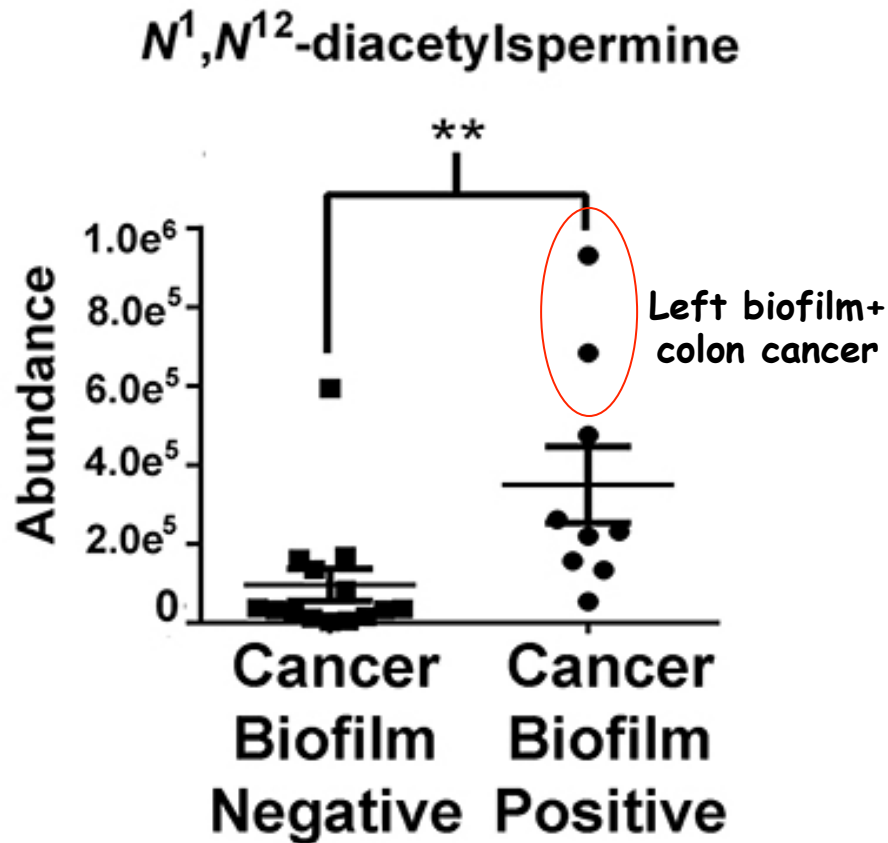


16S rRNA sequencing
Hopkins cohort

Limited number specific
species identified as
differentially abundant
between biofilm+ and
biofilm- tissues.

**Decreased
diversity
of microbiome**

Metabolomics: Biofilm presence correlates with increased N^1, N^{12} -diacetylspermine (untargeted \rightarrow targeted mass spectroscopy pipeline)



N^1, N^{12} -diacetylspermine
also increased in biofilm+
normal tissues.

More detailed studies
suggest produced both
by biofilm & host cells



Caroline Johnson



Gary Siuzdak

Scripps Institute

Cell Metab 21:891, 2015

Hypothesis

Biofilms in healthy individuals increase the risk of developing adenomas or colon cancer

Biofilm formation



Decreased barrier function



Immune activation (IL-6)



Activation (phosphorylation) Stat3



CEC proliferation

Polyamine metabolism



Carcinogenesis

Working Postulates

Sporadic Colon Cancer

Biofilm+ colon cancer is driven by carcinogenic mechanisms induced by invasive polymicrobial bacterial biofilms.

Biofilm negative colon cancer: work in progress

Hereditary Colon Cancer

Work in progress

Goal

By defining the putative microbial drivers of colon carcinogenesis, we can identify microbial-based biomarkers to test as new approaches to the prevention of human colon cancer.

Acknowledgements



Sears Laboratory

Ki-Jong Rhee
Shervin Rabidazeh
Emilia Albesiano
Florence McAllister
Augusto Franco

Microbiology

Brandon Ellis
Karen Carroll

Casero Laboratory

Christina Destefano-Shields

Baylin Laboratory

Heather O'Hagan

JHU/SKCCC

David Huso
Bert Vogelstein
Kathy Romans-Judge
University of Malaya
Jane Wanyiri
Jamuna Vadivelu
April Roslani
Ausama Malik
Khean Goh
Ambiga Iyadorai



Funding: NIH (NIDDK, NCI), Institut Mérieux