Association between Fusobacterium nucleatum and Colorectal Cancer (CRC)

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Joint Graduate Seminar

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Outline of Content

A) Brief on human microbiota and cancer.

- Human Microbiome
- What is microbiota?
- Microbiota-related diseases
- Mechanism of microbiome carcinogenesis

B) Fusobacterium nucleatum (F. nucleatum) and colorectal cancer (CRC)

- Microbiota in CRC
- *Fusobacteria* as biomarker in CRC
- Mechanism and immunity
- Detection and therapeutic planning

- **Brief on human microbiota and cancer**
- **Fusobacterium and Colorectal Cancer (CRC)**

The Importance of the MICROBIOME by the Numbers

90%
Up to 90% of all disease can be raced in some way back to the gut and health of the microbiome

10–100 trillion
Number of symbiotic microbial cells harbored by each person, primarily bacteria in the gut, that make up the human microbiota

>10,000
Number of different microbe species researchers have identified living in the human body

10X
There are 10 times as many outside organisms as there are human cells in the human body

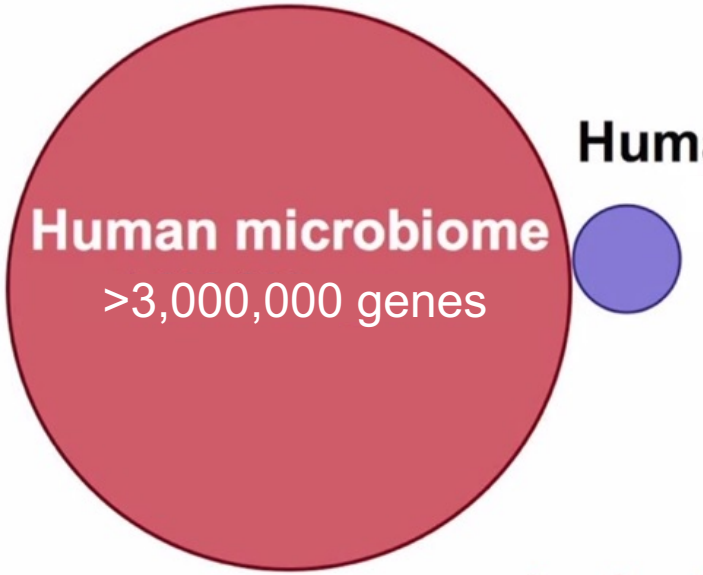
100 to 1
The genes in our microbiome outnumber the genes in our genome by about 100 to 1

3.3 million
Number of non-redundant genes in the human gut microbiome

22,000
Approximate number genes in the human gene catalog

99.9%
Percentage individual humans are identical to one another in terms of host genome

80%–90%
Percentage individual humans are different from one another in terms of the microbiome



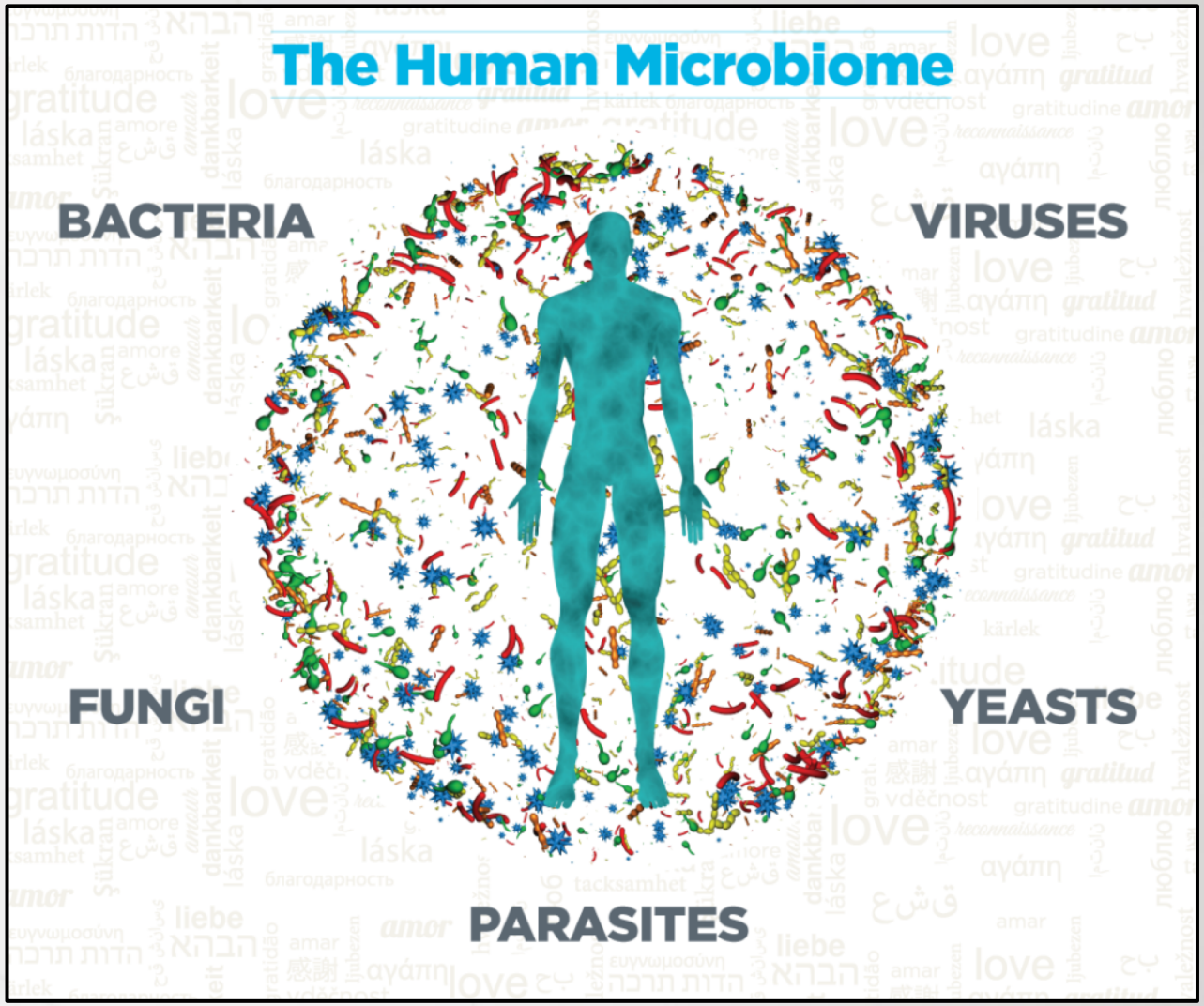
Your Body Has 10 Times As Many Microbe Cells As Human Cells

Inclusion of Microbiome Will Radically Change Medicine

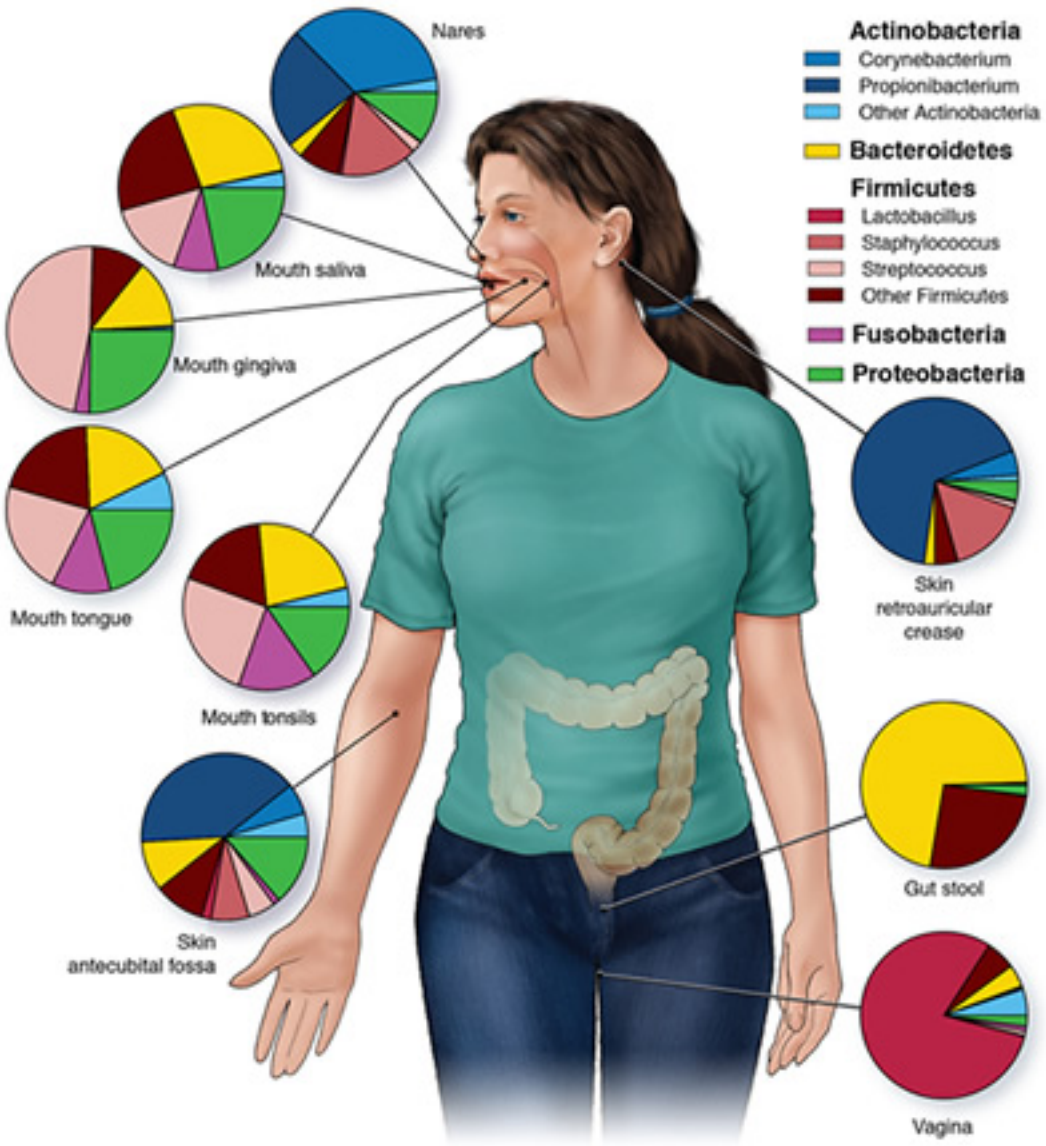
What Is the Human MICROBIOME?

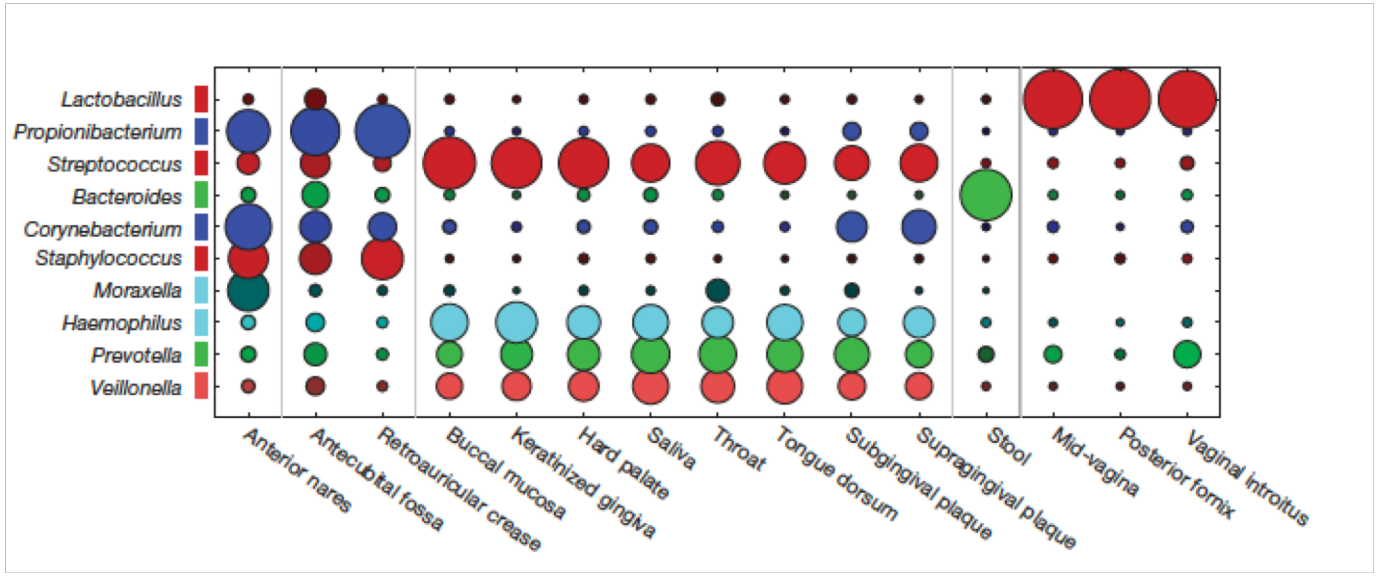
What is microbiota?

- Microbiota is an **ecological community** of microorganisms.
- bacteria, viruses, yeasts, parasites and fungi.
- immunologic, hormonal and metabolic **homeostasis** of hosts
- Potential carcinogenesis



Abundance of human microbiota

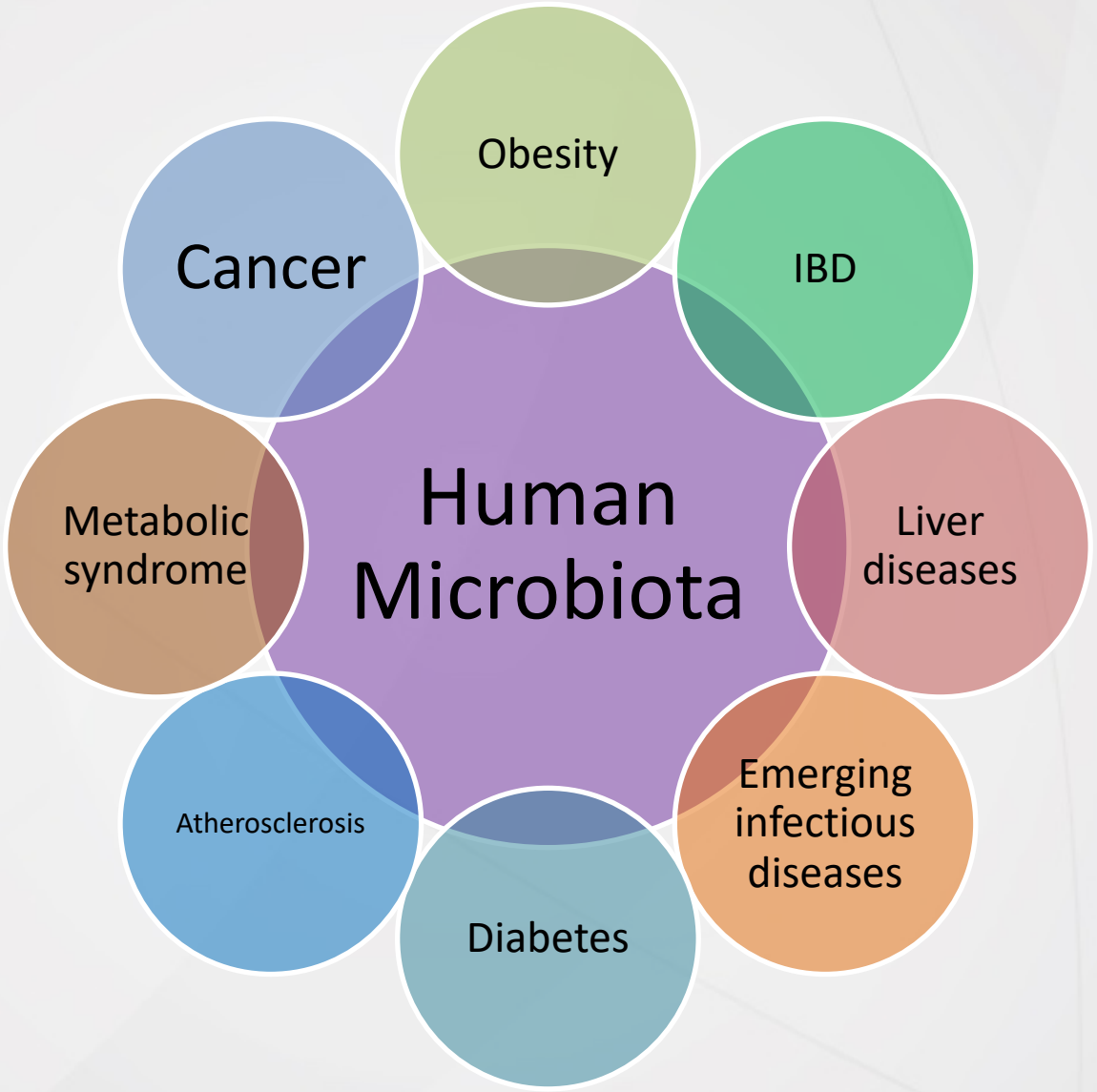




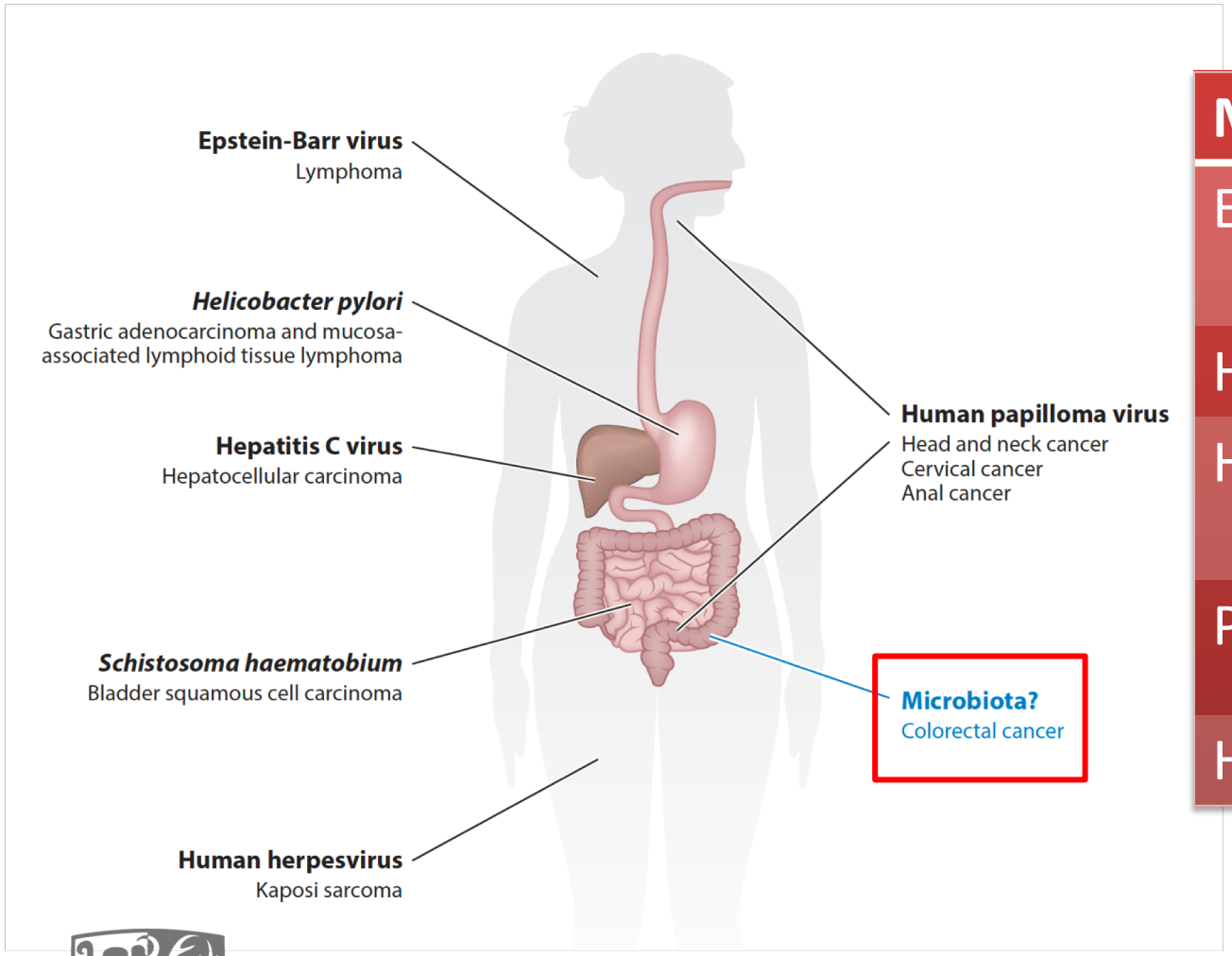
- The microbiome of each organ is **distinct**
- The effects on diseases are **organ specific**

Human Microbiome Project, C. (2012). "Structure, function and diversity of the healthy human microbiome." Nature **486**(7402): 207-214

Microbiota-related diseases



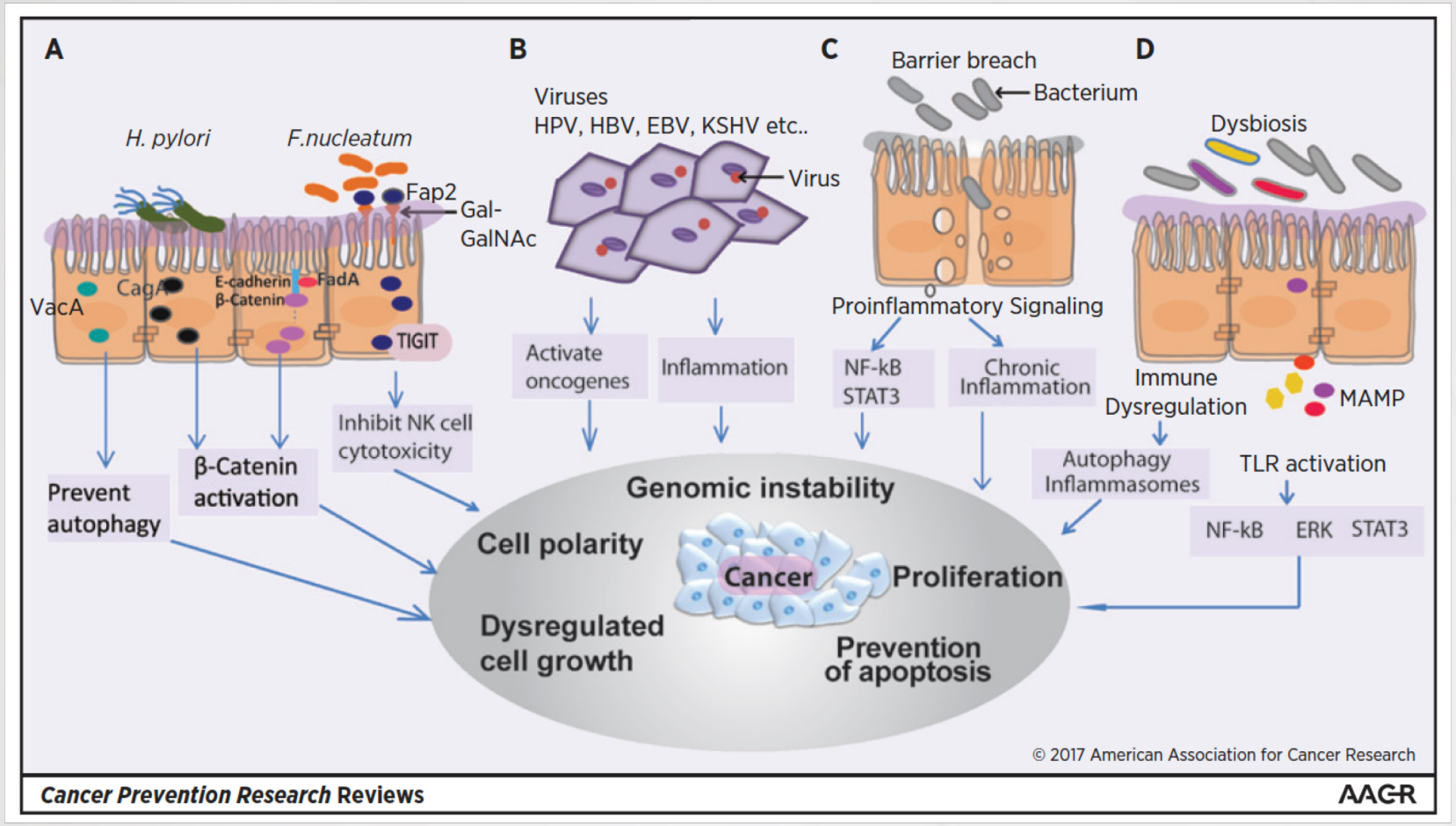
Cancer Contributor



Microbes	Induced cancer
Epstein-Barr virus	Lymphoma Nasopharynx cancer
HBV, HCV	Liver cancer
Helicobacter pylori	Gastric adenocarcinoma
Papilloma virus	Cervical cancer
Herpes virus	Kaposi sarcoma

Brennan, C. A. and W. S. Garrett (2016). "Gut Microbiota, Inflammation, and Colorectal Cancer." Annu Rev Microbiol **70**: 395-411.

Mechanisms of microbiome carcinogenesis



- Microbes inject **effectors** into the host cells.
- **Oncoproteins** of tumor virus transform cells types.
- **Barrier breach** results in proinflammatory signaling for carcinogenesis.
- **Dysbiosis** and altered microbiota-host interaction can induce carcinogenesis.

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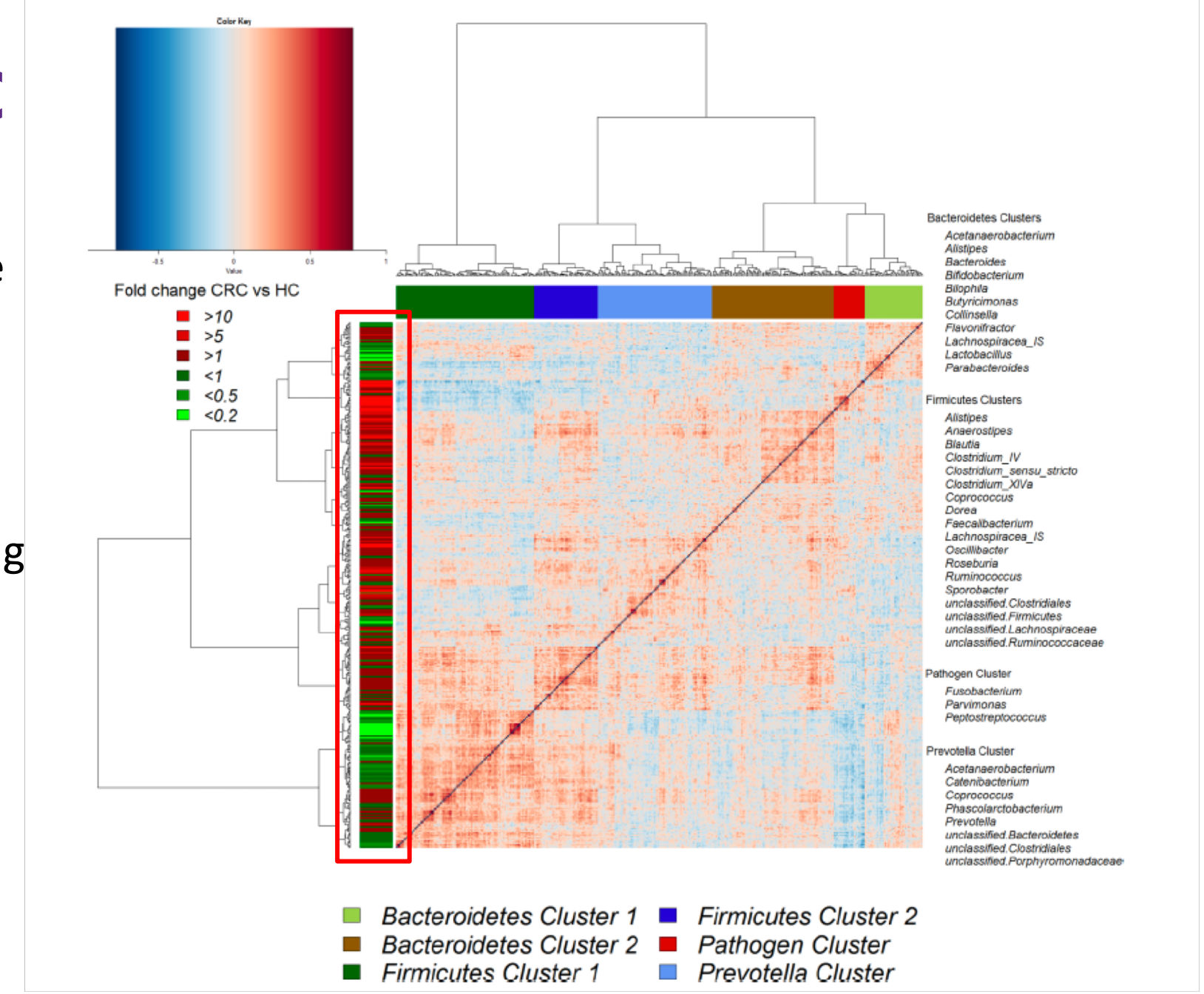
Rajagopala, S. V., et al. (2017). "The Human Microbiome and Cancer." Cancer Prev Res (Phila) 10(4): 226-234.

- Brief on human microbiota and cancer
- **Fusobacterium and Colorectal Cancer (CRC)**

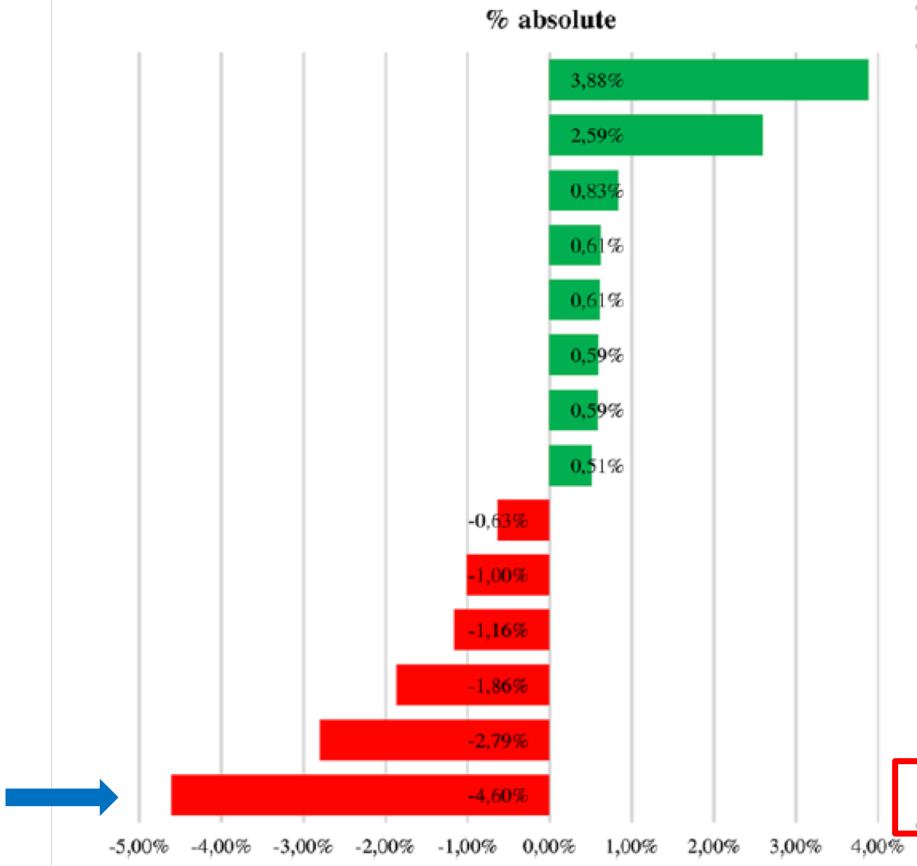
Microbiota in CRC

- The abundance of diverse microbes is different in CRC tissue and normal tissue.
- There is correlation among microbes.
- The dysbiosis cause the CRC.

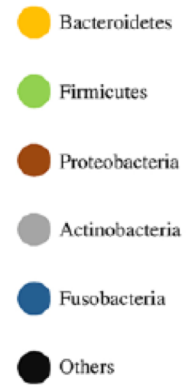


Flemer, B., et al. (2017). "Tumour-associated and non-tumour-associated microbiota in colorectal cancer." Gut **66**(4): 633-643.

Microbiota in CRC



Taxonomy	CRC	CTRL	p-value
Bacteroides	16.04%	19.93%	0.00
Faecalibacterium	3.33%	5.92%	0.00
Lachnoclostridium	2.41%	3.24%	0.04
Alistipes	1.19%	1.80%	0.01
U. m. of *Lachnospiraceae* family	1.49%	2.09%	0.03
Parabacteroides	1.05%	1.65%	0.00
Subdoligranulum	0.70%	1.29%	0.00
Sutterella	0.37%	0.88%	0.00
Others	1.41%	0.78%	0.05
Streptococcus	2.49%	1.49%	0.05
Leptotrichia	1.28%	0.11%	0.00
U. m. of Bacteria kingdom	2.48%	0.62%	0.00
U. m. of *Proteobacteria* phylum	6.18%	3.39%	0.02
Fusobacterium	6.73%	2.13%	0.00

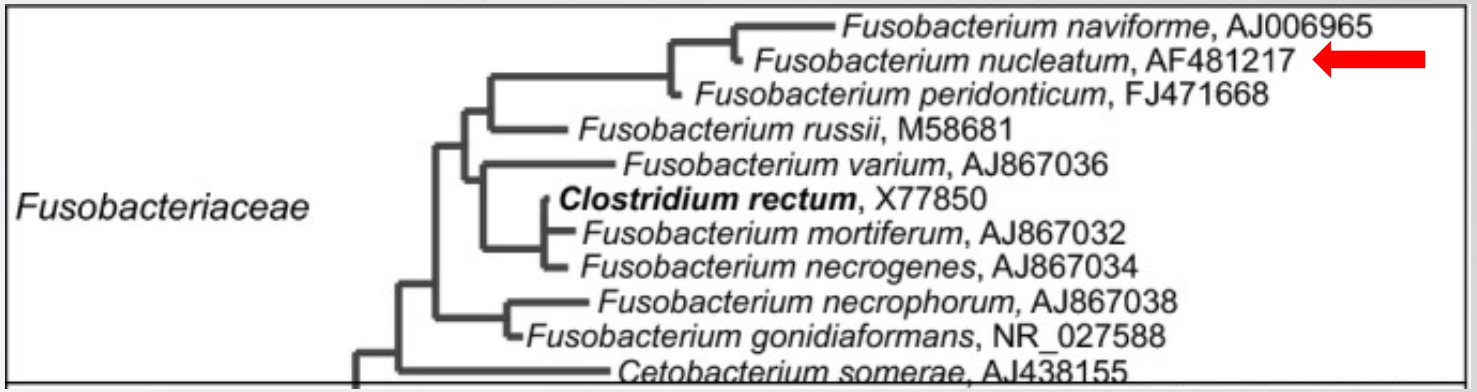


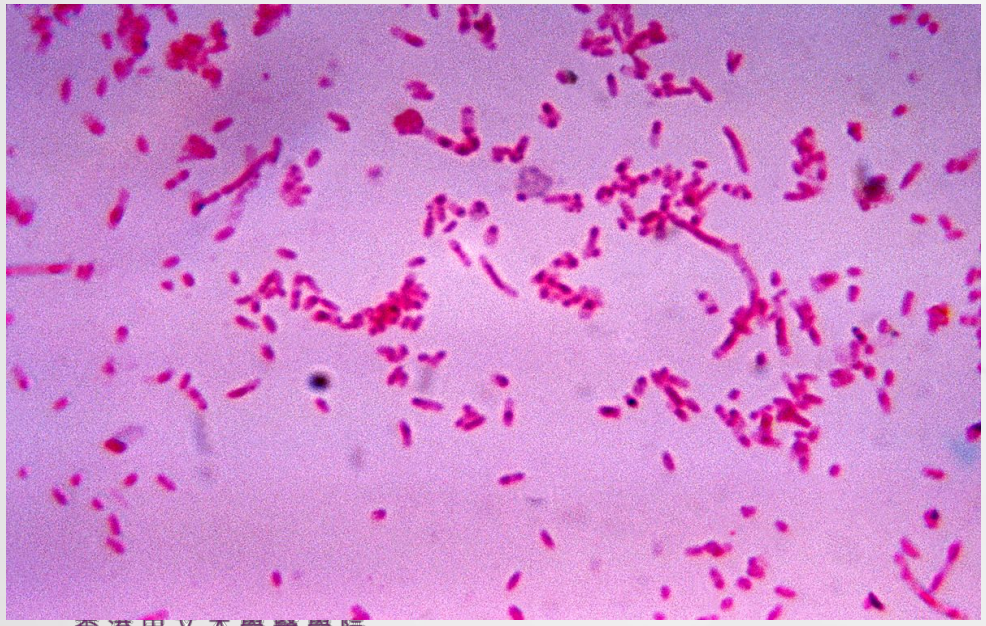
- Fusobacterium is the key phylotypes that contribute to the dysbiosis in CRC patients.
- Compared to health tissue, *Fusobacterium* is more abundant in CRC tissue.

Mancabelli, L., et al. (2017). "Identification of universal gut microbial biomarkers of common human intestinal diseases by meta-analysis." FEMS Microbiol Ecol **93**(12).

Introduction of Fusobacteria

- Gram-negative anaerobic bacterium.
- Pleomorphic, but usually spindle-shaped.





- Over 30 species within *Fusobacterium.*
- *F. nucleatum* is mostly linked to oral diseases.

Fusobacterium as the biomarker in CRC

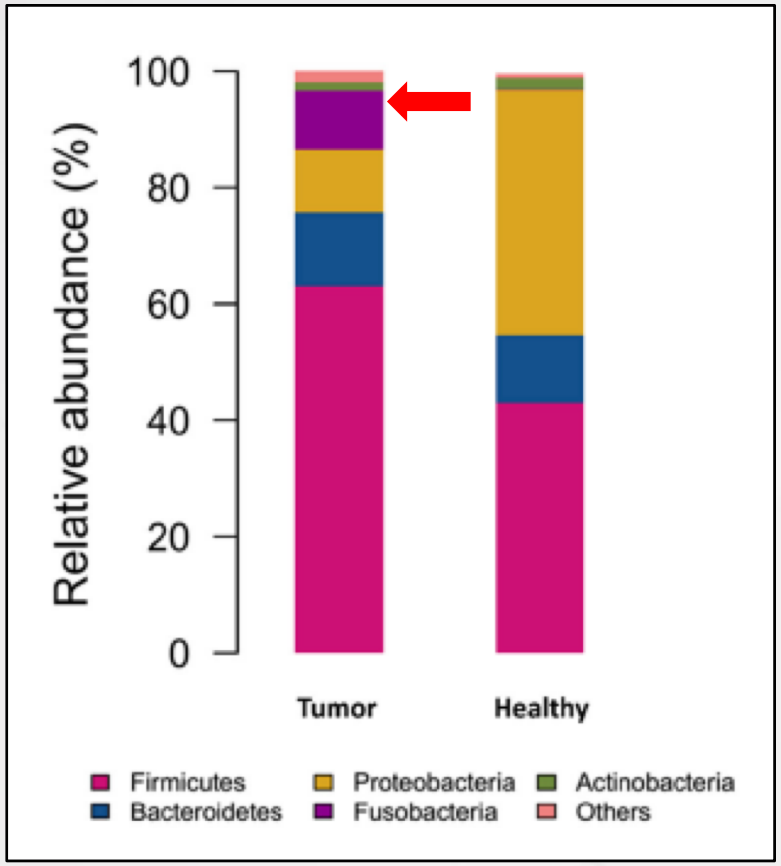


Table 2. Association between *Fusobacterium* abundance and colorectal adenomas.

Categories*		Case (n = 48)	Control (n = 67)	OR (95% CI)**
Tertile 1	low	8	23	Reference
Tertile 2		12	22	1.57 (0.54–4.57)
Tertile 3	high	28	22	3.66 (1.37–9.74)
P trend				

*The abundance of *Fusobacterium* among control subjects were used to generate tertile cut-off. The lowest tertile of *Fusobacterium* abundance was considered as the reference.
**Odds ratio and 95% confidence interval.
Compared to subjects with a low copy number, subjects with high abundance of *Fusobacterium* are more likely to be adenoma cases than controls.

- *Fusobacterium* is significantly more abundant in CRC tissue than normal tissue.

McCoy, A. N., et al. (2013). "Fusobacterium is associated with colorectal adenomas." PLoS One **8**(1): e53653

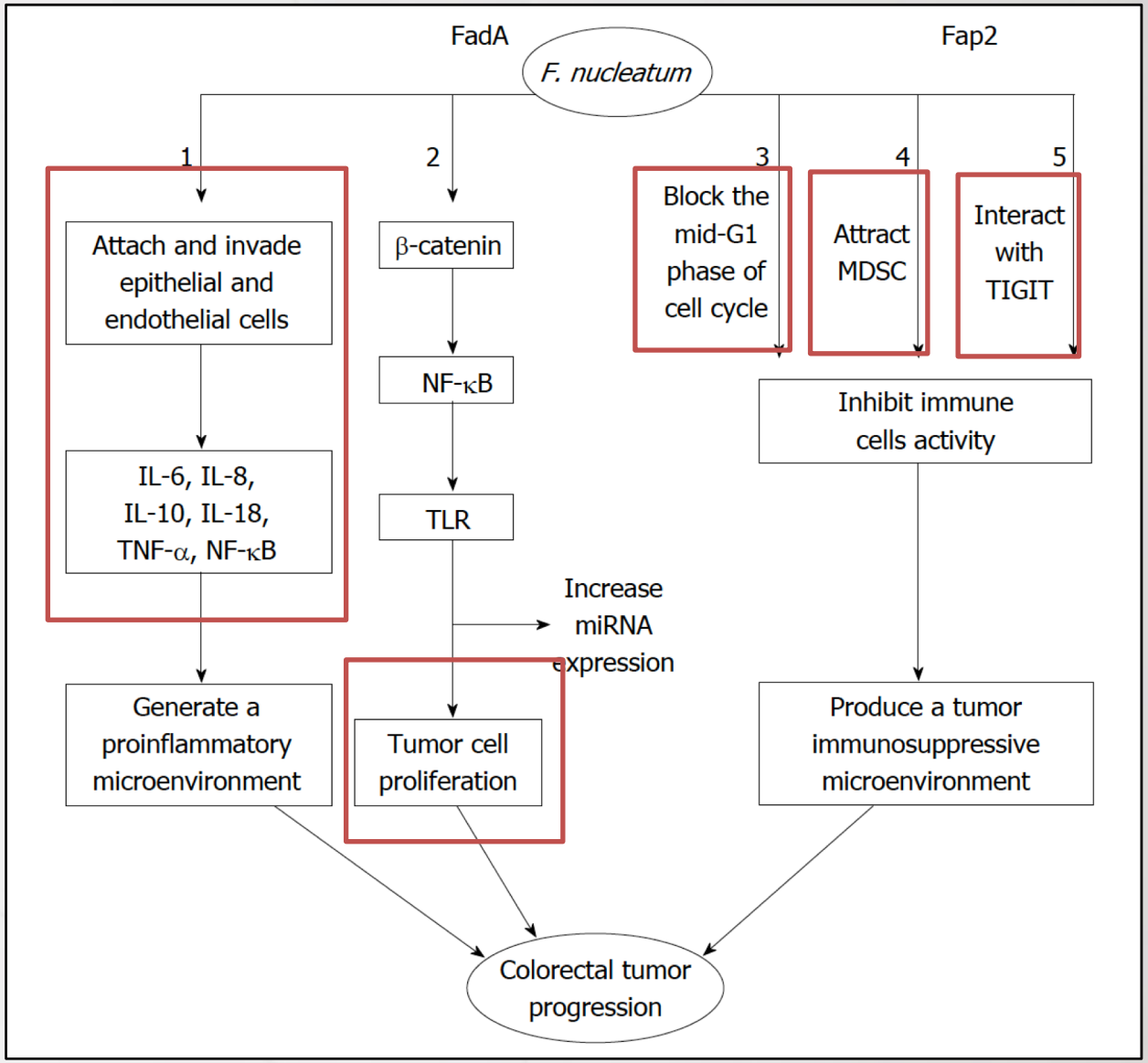
Mechanism of *F. nucleatum* carcinogenesis

FadA (*Fusobacterium* adhesion A)

- Induce cytokines to generate a pro-inflammatory microenvironment.
- Activate the β-catenin signaling pathway to promote tumor cell proliferation.

Fap2 (fibroblast activation protein 2)

- Block the G1 phase of cell cycle.
- Attract MDSC to promote tumor progression.
- Interaction with TIGIT to protect tumor cell from immune cell attack.



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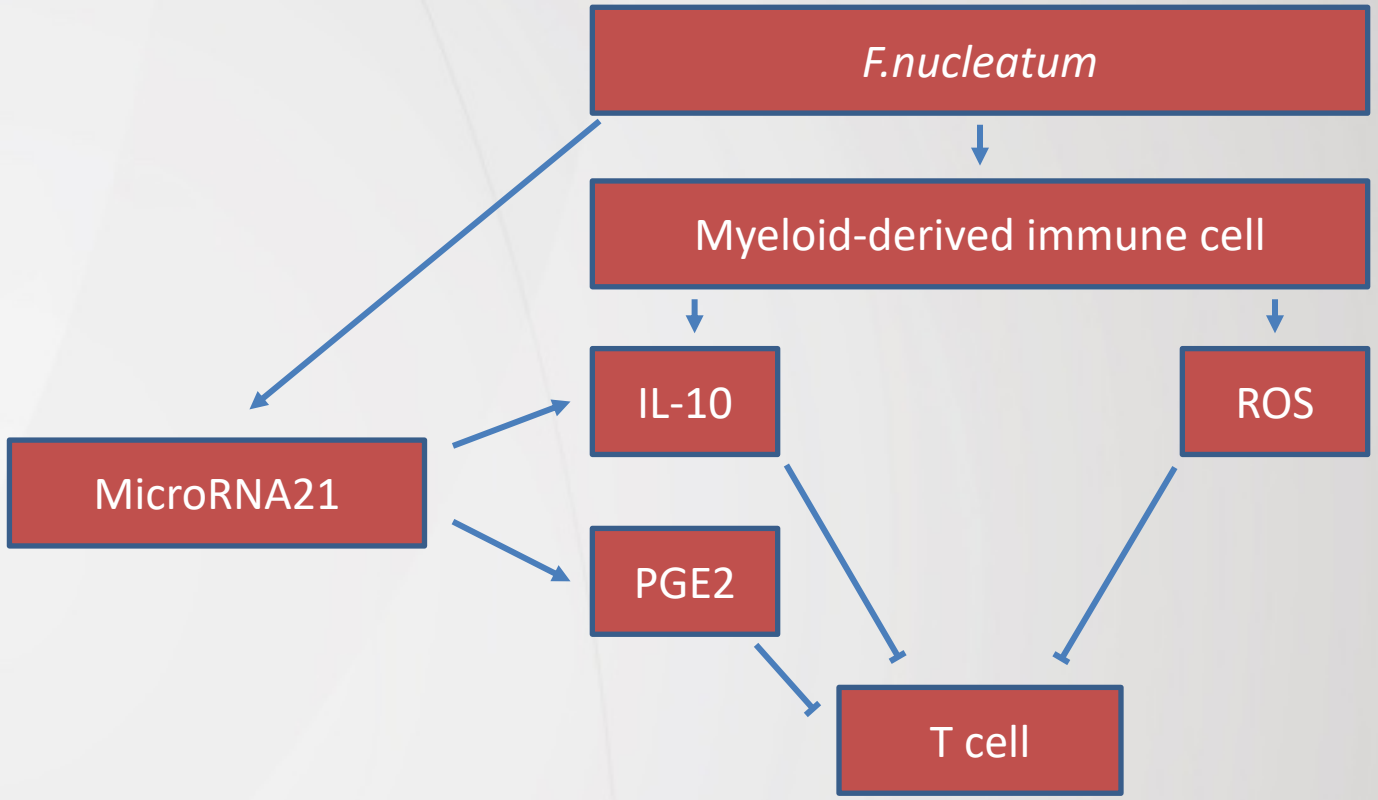
Immunity in *F. nucleatum* infection

- Myeloid-derived immune cells were enriched in *F. nucleatum* infected host.
- IL-10 and ROS were accumulated by myeloid-derived immune cell.
- Increased MicroRNA21 promote the expression of PGE2
- IL-10,ROS and PGE2 suppressively modulated T-cell-mediated adaptive immunity



Nosho, K., et al. (2016). "Association of Fusobacterium nucleatum with immunity and molecular alterations in colorectal cancer." World J Gastroenterol **22**(2): 557-566.

Fusobacteria associated diseases

Anatomic sites	Disease
Oral infection	Aggressive periodontitis
	Chronic periodontitis
	Endodontic infections
Adverse pregnancy outcomes	Chorioamnionitis
	Neonatal sepsis
GI disorders	Colorectal cancer
	Appendicitis
Other infections	Cerebral aneurysm
	Alzheimer's disease
	Lemierre's syndrome

Han, Y. W. (2015). "Fusobacterium nucleatum: a commensal-turned pathogen." Curr Opin Microbiol **23**: 141-147.

Detection of *F. nucleatum* in CRC

Total cases (*n*)	Positive cases (*n*)	Positive percentage	Detection method	Detection samples
101	88	87.13%	FISH and FQ-PCR	Frozen tissue and FFPE tissue
598	76	13%	qPCR	FFPE tissue
511	44	8.6%	qPCR	FFPE tissue
149	111	74%	qPCR	Genomic DNA
511	286	56%	qPCR	FFPE tissue
158	85	54%	ddPCR	Feces

- **METHOD** --- FISH (Fluorescence *in situ* hybridization), FQ-PCR, qPCR and ddPCR are the usual method in clinical detection.
- **SAMPLE** --- Feces are difficult to detect *F. nucleatum,* FFPE (Formalin-fixed paraffin embedded) tissues and frozen tissue are limited by surgery or colonoscopy.
- **CONPARISON** --- qPCR is most usual in detection, ddPCR in higher detection rate of low concentration of sample, FQ-PCR displays higher sensitivity and specificity.

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Therapeutic planning

Chemoprevention

- Use of **Aspirin**, COX-2 inhibitor, EO2 antagonist.
- Induces neutrophils apoptosis and lipoxin-driven **immune –regulatory effect**
- Aspirin may support the immune system and prevent the development of *F. nucleatum*-associated CRC.

Immunotherapy

- Antibody treatment, immune-checkpoint blockade therapy, and adoptive cell transfer.
- Eg. 1) **Anti-Fap2 antibody** may favor antitumor immune response; 2) The **blockade of CTLA-4 and PD-1** may shape the antitumor immune response.

ARTICLE

www.nature.com/ismej

ORIGINAL ARTICLE

Review

Fusobacterium and Enterobacteriaceae: Important players for CRC?

Fusobacterium and *Escherichia*: models of

Tumor Targeting by *Fusobacterium nucleatum*: A Pilot Study and Future

Cell

Fusobacterium nucleatum Promotes Chemoresistance to Colorectal Cancer by Modulating Autophagy

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Summary

- Gut microbiota has been extensively associated with diverse cancers and diseases.
- *F. nucleatum* may contribute to CRC *via* multiple mechanisms.
- Chemoprevention and immunotherapy strategies could be most potential approaches to cure microbiota-related cancers.

Thanks for attention

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Wang, T., et al. (2012). "Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers." ISME J **6**(2): 320-329

McCoy, A. N., et al. (2013). "Fusobacterium is associated with colorectal adenomas." PLoS One **8**(1): e53653

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