Understanding Colorectal Cancer Initiation

via co-evolutionary study of disease tissue and gut microbiome

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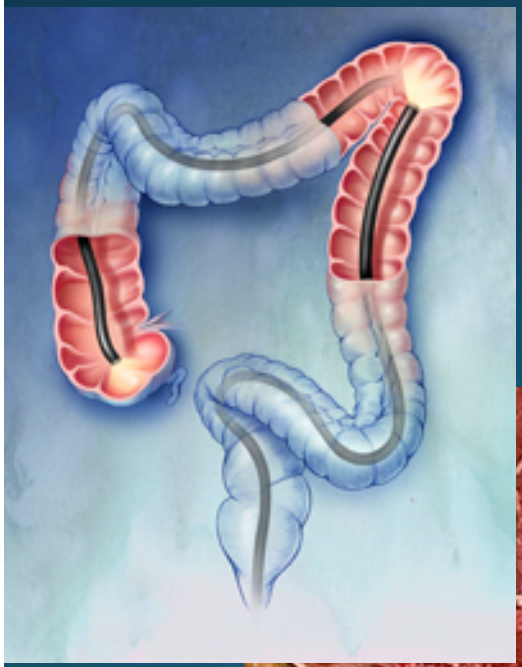
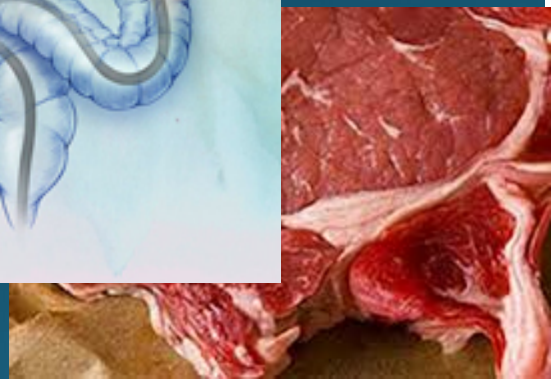
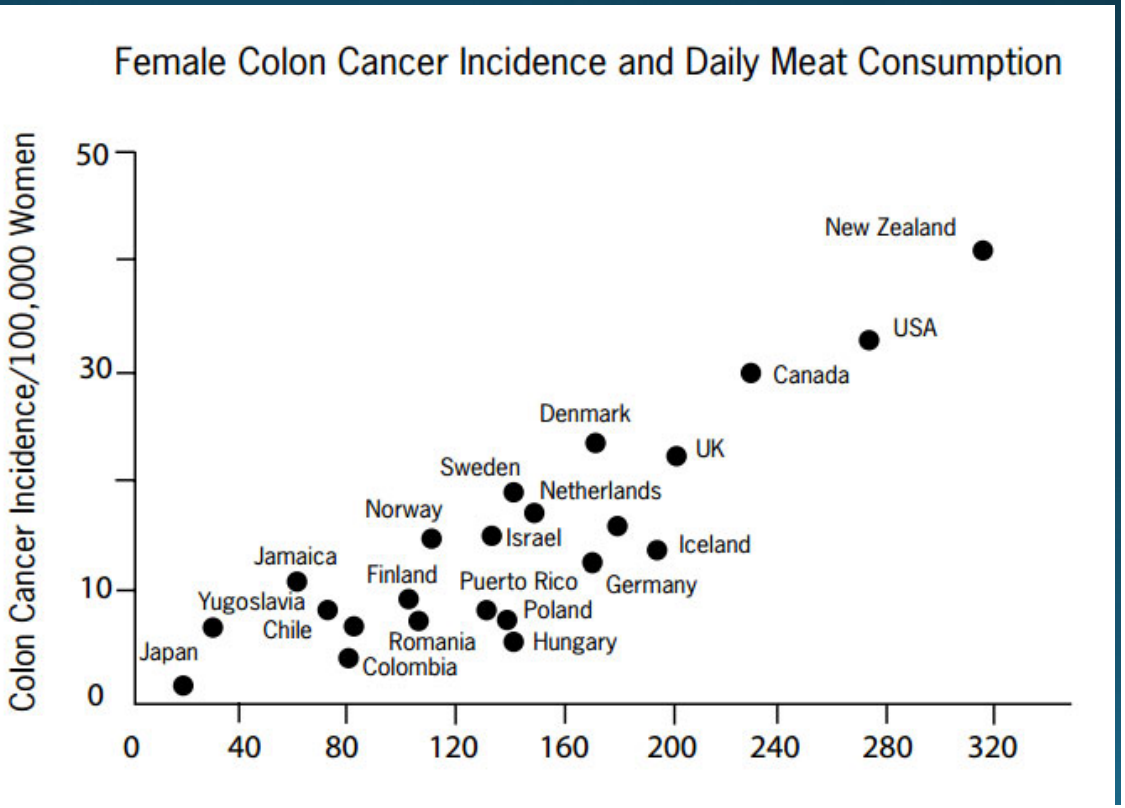
Colorectal Cancer Statistics

- One in every 20 adults in the US will develop colon cancer during his or her lifetime with female having slightly lower chance than male.
- Epidemiology studies revealed a strong correlation between red meat consumption and colorectal cancer rate







Main Scientific Questions

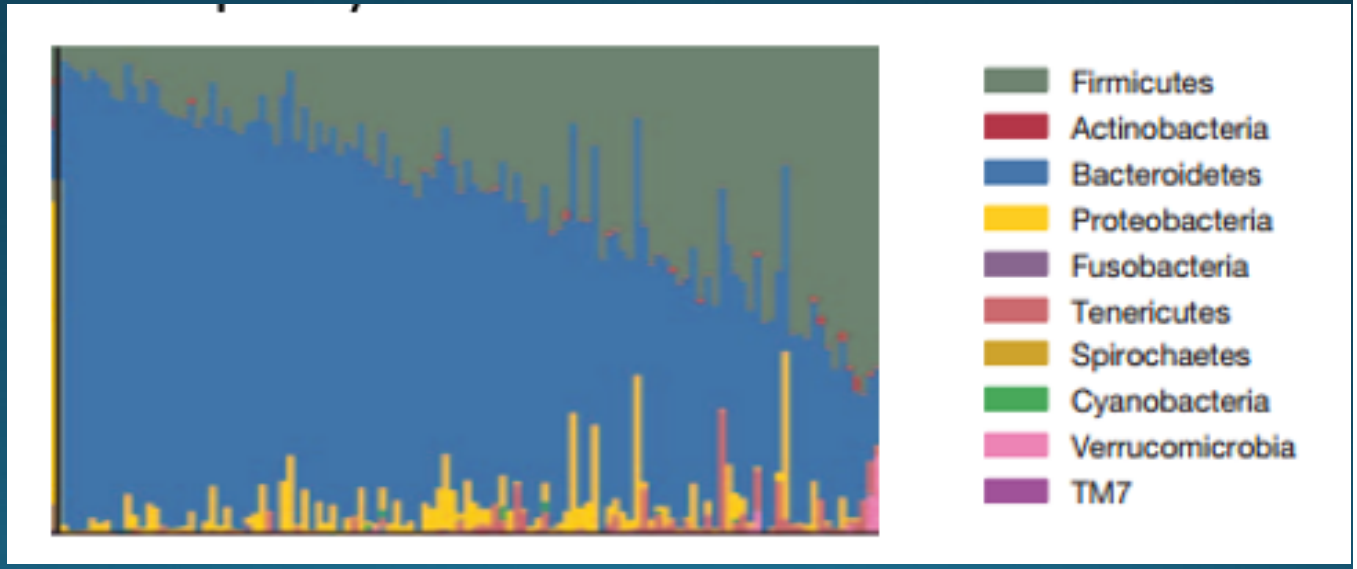
- Multiple studies suggest that microbiome of human guts may play important roles in the development of colon cancer but the mechanism remains largely unknown

- Main questions to address:
  - How human gut microbiome evolves as a colorectal disease evolves towards the formation of cancer?
  - What roles the human gut microbiome may play leading to the development of colon cancer?
  - What are the key drivers of a colon cancer?

What Are Known About the Disease

- For a healthy person, the subpopulations of different microbes in a human gut changes as the person ages

- The five most dominating species are
  - Bacteroidetes
  - Firmicutes
  - Proteobacteria
  - Actinobacteria
  - Fusobacteria



Nature 486, 207–214 ,2012

What Are Known About the Disease

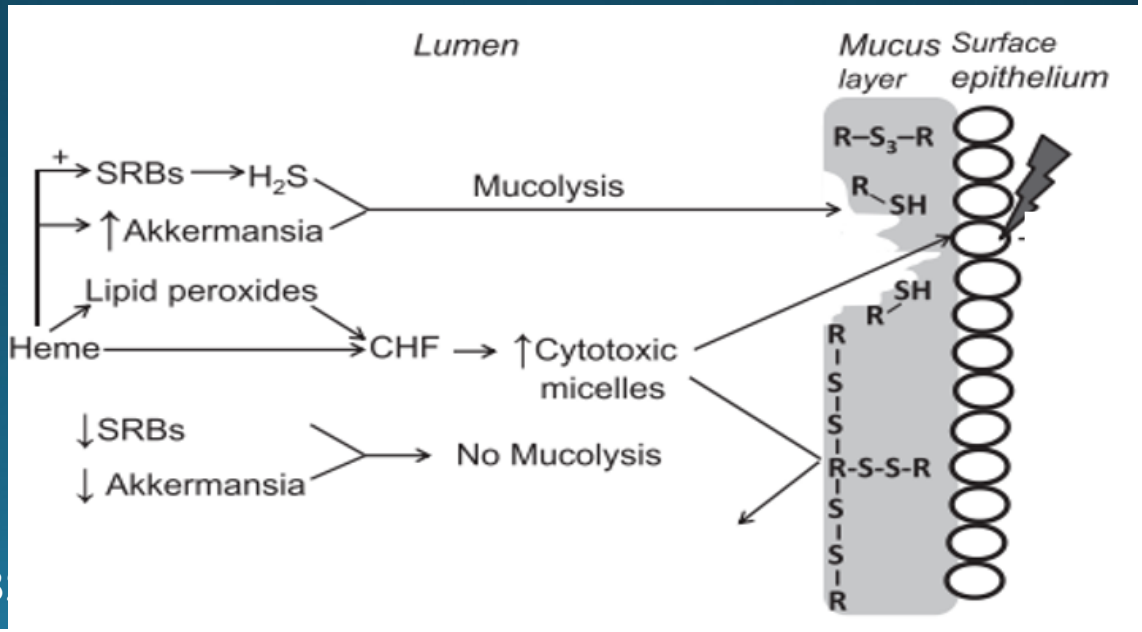
- In patients with inflammatory bowel disease (IBD), e.g., Crohn's disease and ulcerative colitis, leading towards colon cancer, *Fusobacteria* tend to have the largest population increase

	Inflammatory Bowel Disease (UC/CD)	Carcinoma
Fusobacteria		
Fusobacterium	↑	↑
F.nucleatum	↑	↑
Bacteroidetes		
Bacteroides	↑/↓	↑
Firmicutes		
Clostridia	↑/↓	↑/↓
Actinobacteria		
Bifidobacterium	↓	↓
Proteobacteria		
Enterobacter	↑	↑
Campylobacter	↑	↑
Euryarchaeota		
Methanobacter	↑	↑

Rev Esp Enferm Dig. 2015 Sep 8;107

What Are Known About the Disease

- Iron-containing heme (from red meat) has been found to increase populations of certain species of Bacteroidetes and Proteobacteria (Gram-negative) and decrease populations of Firmicutes, leading to
  - increased production of some metabolites harmful to mucus of the colon epithelial layer, damaging the mucus layer and exposing the colonocytes to more challenging environments
  - decreased production of the beneficial short-chain fatty acids such as butyrate



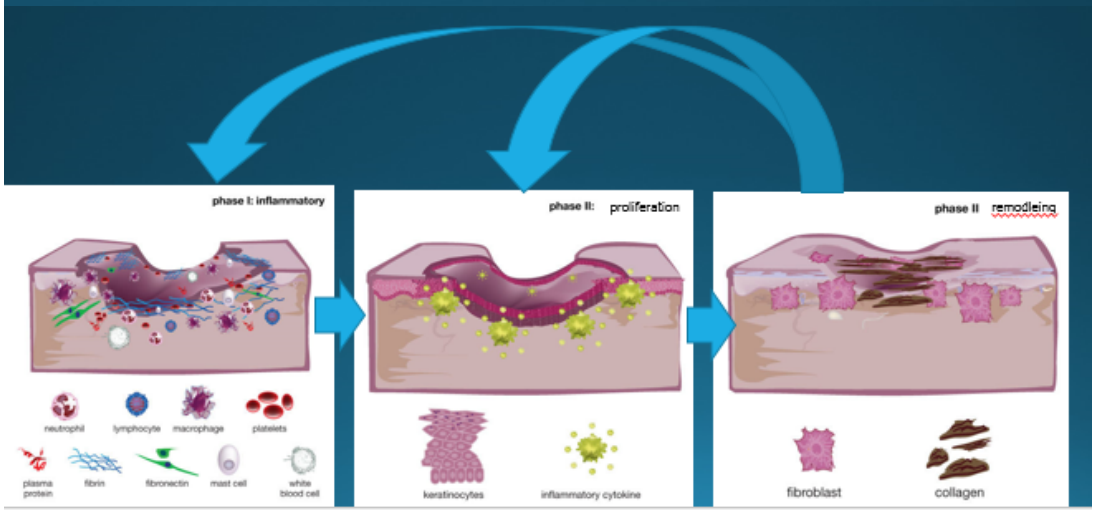
Proc Natl Acad Sci U S A. 2015;112(3

What Are Known About the Disease

- The increased populations of the Gram-negative bacteria will give rise to increased concentration of lipopolysaccharides (LPS), key bacterial antigens, leading to increased immune attacks, including releasing of reactive oxygen species (ROS), and tissue damages

- Combination of the above in a persistent manner will lead to chronic inflammation, such as IBD

Phase	Cellular and Bio-physiologic Events
Hemostasis	1. vascular constriction 2. platelet aggregation, degranulation, and fibrin formation (thrombus)
Inflammation	1. neutrophil infiltration 2. monocyte infiltration and differentiation to macrophage 3. lymphocyte infiltration
Proliferation	1. re-epithelialization 2. angiogenesis 3. collagen synthesis 4. ECM formation
Remodeling	1. collagen remodeling 2. vascular maturation and regression



Chronic Inflammation and Cancer

- Recent studies suggest that chronic inflammation is a critical component in initiation and progress of all (sporadic) cancers
- To understand the relationship between chronic inflammation and cancer, we have recently studied 18 types of chronic inflammation
- Nine of them are considered as cancer prone such as Crohn's disease & ulcerative colitis and nine considered as cancer independent such as pulmonary sarcoidosis & asthma
- Question: do cancer-prone types of inflammation share common characteristics, which are not shared by cancer-independent ones? If yes, what are they?

Data Used

- We have analyzed the transcriptomic data of the 18 types of chronic inflammation vs controls from the GEO database, plus some additional data

Cancer Prone inflammation	Moderately cancer prone	Largely cancer independent
Cirrhosis	Chronic obstructive pulmonary disease	Alcoholic hepatitis
Barrett's esophagus	Idiopathic pulmonary fibrosis	Non-alcoholic steatohepatitis
Atrophic gastritis	Chronic rhinosinusitis	Cystitis
Ulcerative colitis	Atopic dermatitis	Pulmonary sarcoidosis
Crohn's disease		Asthma
Chronic viral hepatitis C		Psoriasis
		Irritable bowel syndrome

- Each disease type has at least 10 pairs of samples
- Their relevance to cancer is based on statistics in the public domain

Differences between CPIs vs CIIs

- At the tissue level: CPIs have higher levels of the following than CIIs
  - Increased CD_4 populations and decreased CD8 population while CIIs have the opposite
  - Macrophage populations
  - ROS production by immune cells and oxidative stress
  - Angiogenesis and lymph-angiogenesis
  - Hypoxia
  - Total cytokines
  - Syntheses of all extracellular matrix (ECM) component types, suggesting ECM damage and repair

Zhang, Yao et al, in preparation, 2015.

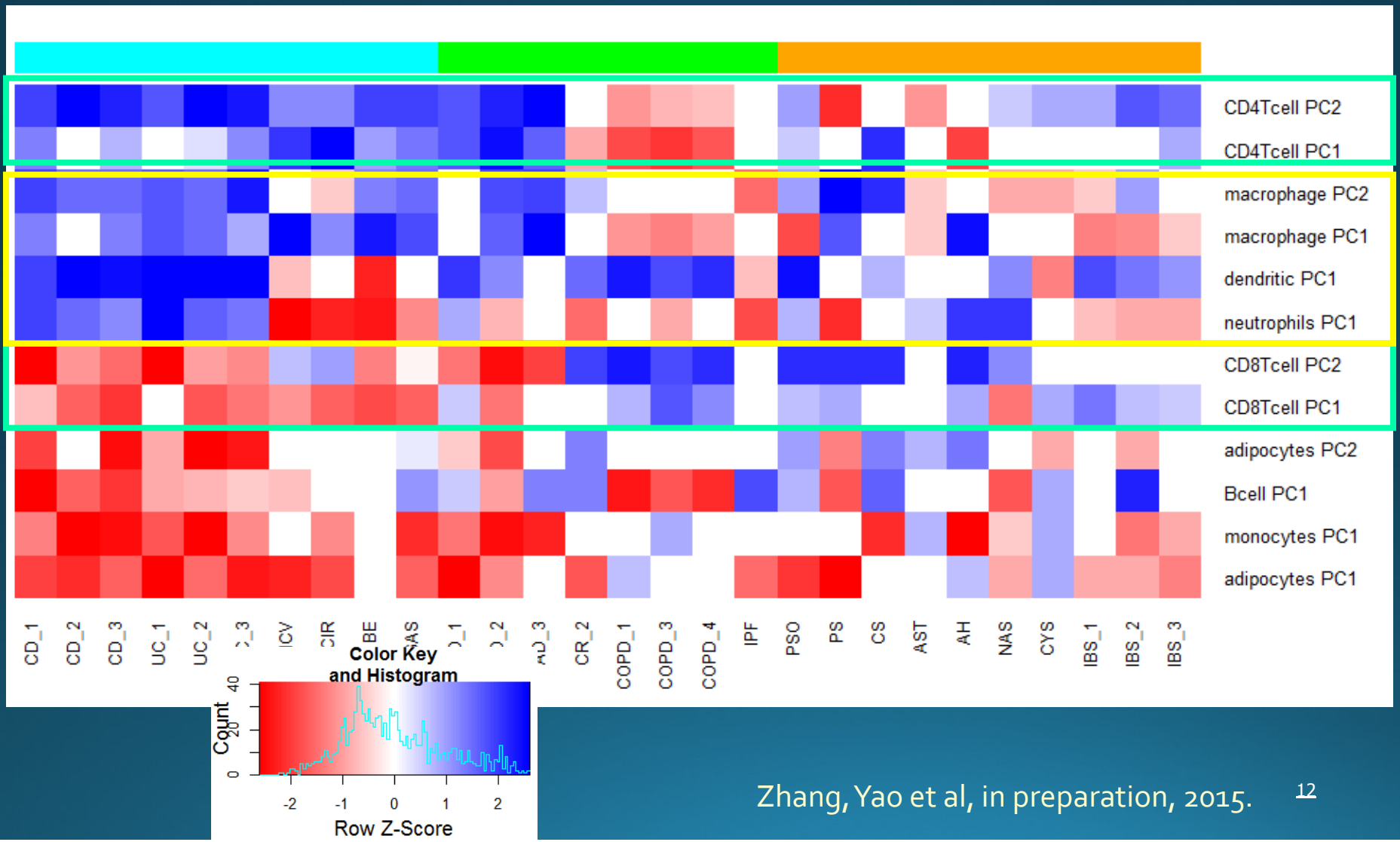
Differences between CPIs vs CIIs

- At the cellular level, CPIs have higher levels of the following than CIIs
  - Epithelial cell proliferation
  - Glycosylation, particularly O-linked
  - Synthesis of glucosaminoglycans, e.g., heparan, chondroitin & keratan sulfates, and hyaluronic acids for some CPIs
  - Expression of sialic acid-containing mucins
  - Iron accumulation
  - Hypoxia
  - Oxidative stress
  - Phospholipid synthesis, suggesting damages to phospholipids, leading to production of pro-inflammatory signals such as prostaglandins

Zhang, Yao et al, in preparation, 2015.

CPIs have increased CD4 and decreased CD8 populations while CIIs have the opposite

Cancer prone Moderately cancer prone Cancer independent



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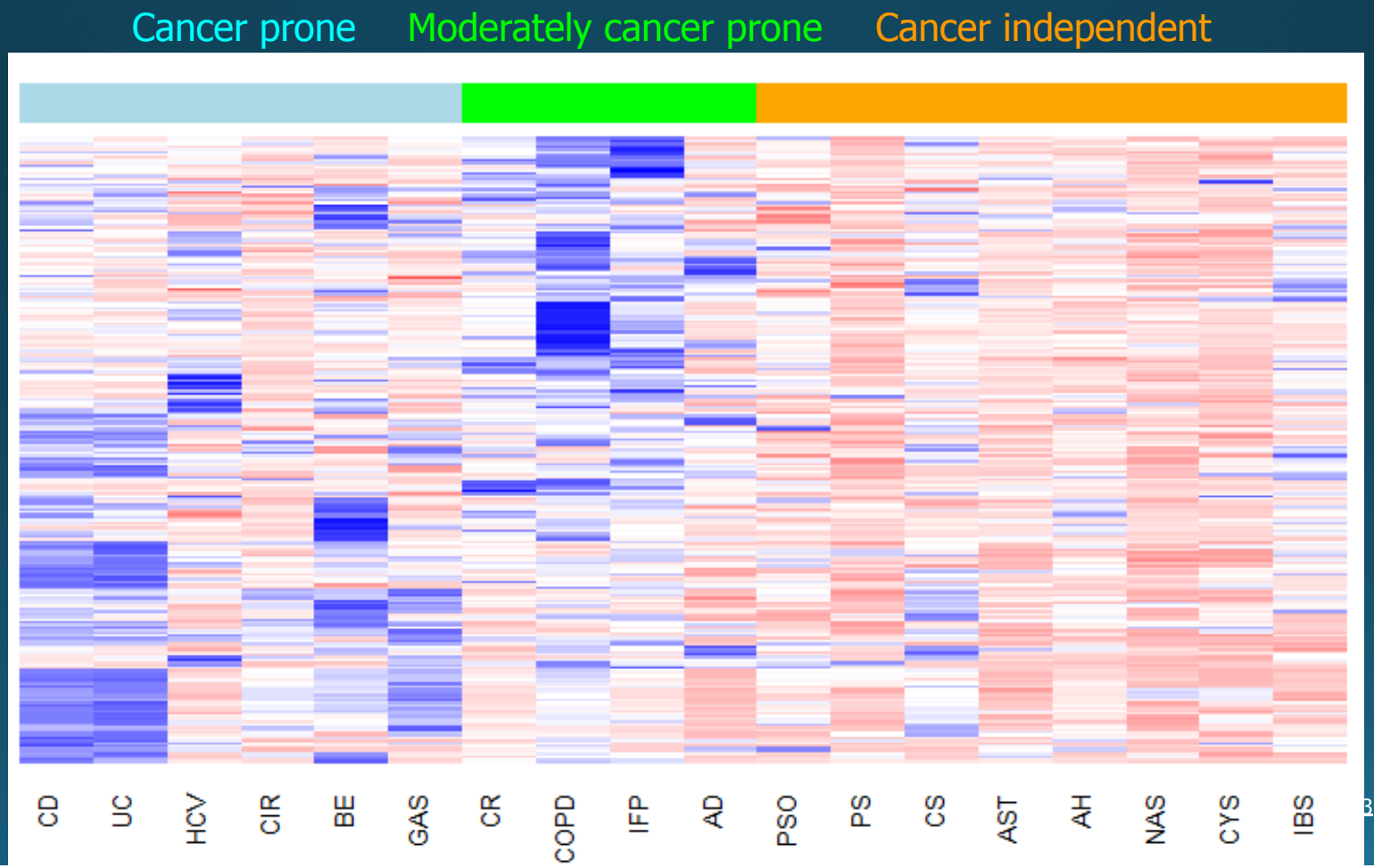
ECM Syntheses

- Substantially increased syntheses of multiple components of ECM such as collagens and glucosaminoglycan are observed in CPIs vs CIIs



Iron Accumulation

cytoplasm

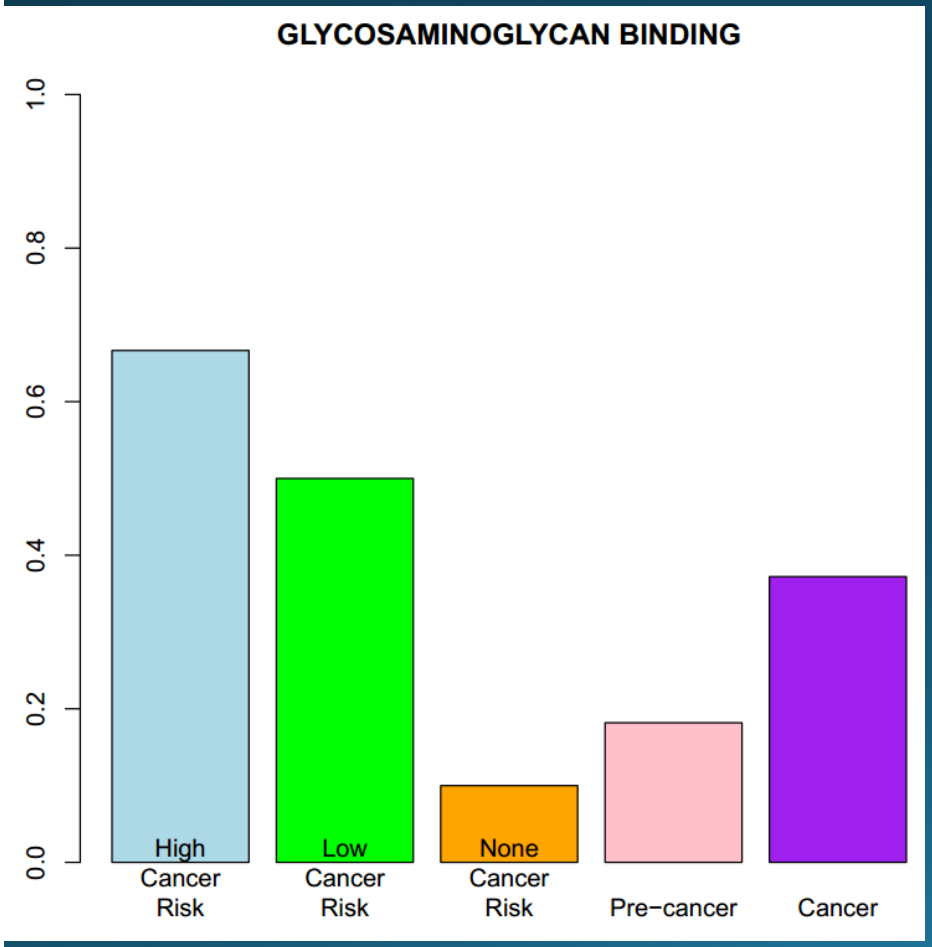
Gene name	Full name	Function
FTH1	Ferritin, heavy polypeptide	Iron storage
FTL	Ferritin, light polypeptide	Iron storage
SLC11A2(D		
TFRC		
SLC40A1(F		
CD163		
CD91		
HMOX1		
CP		
HEPH		
FLVCR1		

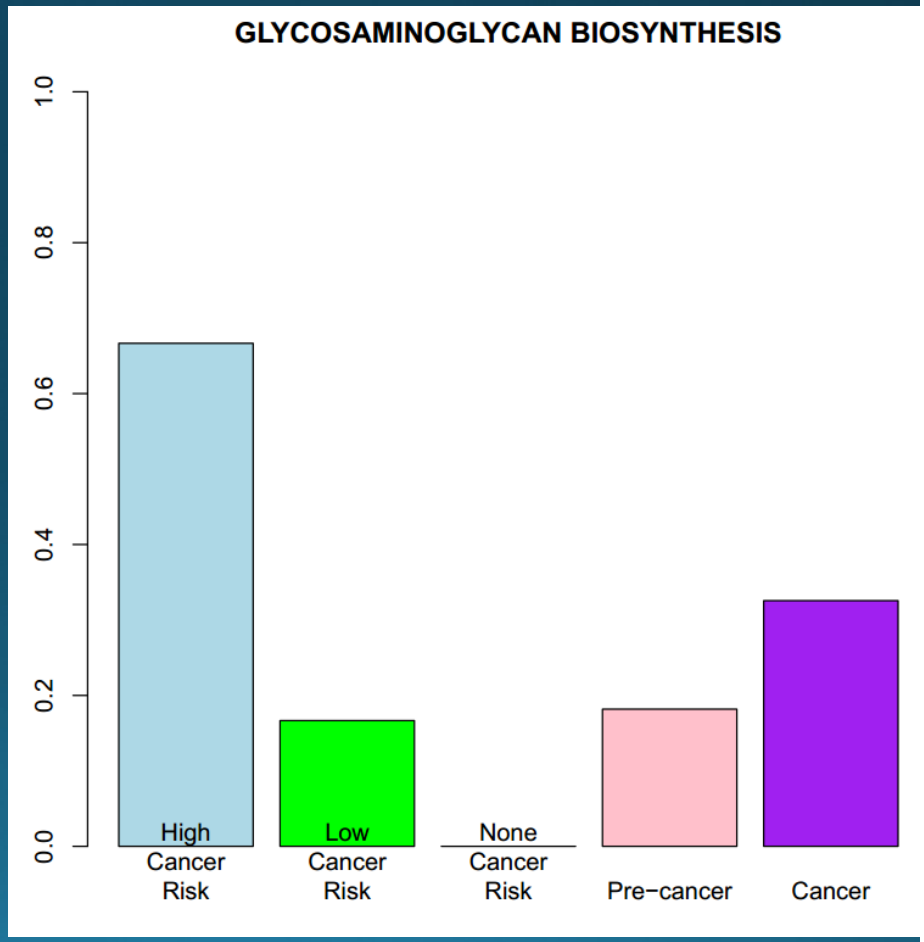
mitochondria

Gene name	Full name	function
Transporters		
SLC25A37	Solute Carrier Family 25 (Mitochondrial Iron Transporter), Member 37	Iron importer for the synthesis of mitochondrial heme and iron-sulfur clusters
SLC25A28	Solute Carrier Family 25 (Mitochondrial Iron Transporter), Member 28	
ISC assembly machinery		
NFS1	Cysteine Desulfurase, Mitochondrial	Sulfur donor
LYRM4	Mitochondrial Matrix Nfs1 Interacting Protein	In complex with NFS1
FDXR	Ferredoxin Reductase	Ferredoxin-NADP+ reductase
FDX1	ferredoxin	Electron transport
FDX1L	Ferredoxin-1-Like Protein	Electron transport
GLRX5	Glutaredoxin 5	Cluster transfer
HSCB	HscB Mitochondrial Iron-Sulfur Cluster Co-Chaperone	Cluster transfer
ISCA1	Iron-Sulfur Assembly Protein 1	Maturation of radical SAM-dependent proteins and aconitase
ISCA2	Iron-Sulfur Assembly Protein 2	
ISCU	Iron-sulfur cluster scaffold homolog	scaffold
NFU1	Scaffold protein	Alternative scaffold protein
HSPA9	Heat Shock 70kDa Protein 9 (Mortalin)	Cluster transfer
Heme synthesis		
ALAS1	5'-Aminolevulinate Synthase 1	Catalyzes the rate-limiting step in heme (iron-protoporphyrin) biosynthesis.
ALAS2	5'-Aminolevulinate Synthase 2	catalyzes the first step in the heme biosynthetic pathway
FECH	Ferrochelatase	catalyzes the insertion of the ferrous form of iron into protoporphyrin IX in the heme synthesis pathway.
Others		
FTMT	Ferritin Mitochondrial	Stores iron

Glycosylation and Glycan Synthesis

- Higher level of glycosylation and glycan synthesis in CPIs vs CIIs

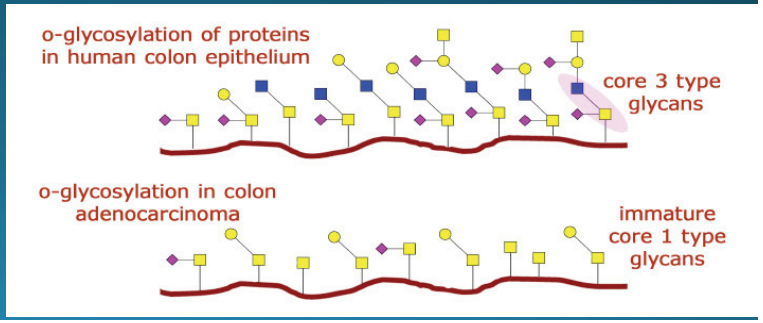




Zhang, Yao et al, in preparation, 2015.

- Observation 1: There is an increased influx of Ferrous ions , which strongly correlates with the level of H_2O_2 production by innate immune cells and ECM damages
- Observation 2: Strong indication by multiple evidence of continuous Fenton reaction in mitochondria: $H_2O_2 + Fe^{2+} \rightarrow {\cdot}OH + OH^- + Fe^{3+}$, giving rise to continuous production of ·OH, the most reactive ROS in human cells, which does not have a natural scavenger
- Observation 3: There is a strong correlation between the estimated ·OH production and the total production of glycans
- Observation 4: When ·OH react with glysidic bonds in glycans. It produces aldehyde-containing glycan fragments; and a recent study (PNAS, 2014) found that such "incomplete" glycans are highly oncogenic

Zhang, Yao et al, in preparation, 2015.



Our Prediction

- Our data analyses strongly suggest that there is a common pathway for a chronic inflammation to become cancerous
  - damaged ECM with high ROS level -> increased iron accumulation -> persistent production of hydroxyl radicals ▪OH -> syntheses of glycan as their scavengers -> some oxidized glycans are highly oncogenic
- Genomic mutations in pre-cancerous colorectal adenoma genomes are predominantly involved in
  - ECM components
  - ECM-cell interaction and cell-cell adhesion
  - Cell-cycle control
  - Growth factor signaling
  - Glycoprotein synthesis

Alignment Between Disease Progression and Microbiome Evolution

- Heme intake for an extended period of time may play a key role in the development and maintaining of chronic inflammation in colon, which also altered the proportions of different species in gut microbiome

- Increased oxidative state and hypoxia in the colorectal environment further drive the alteration of the microbial populations, increasing anaerobic microbial populations and decreasing aerobic microbes, leading to increased immune responses and further damages to colon tissue and decreased protection against cytotoxin (data not shown)

Tang, et al, in preparation, 2015.

Alignment Between Disease Progression and Microbiome Evolution

- Co-expression analyses of colon cancer tissues and matching microbiome, using public data, revealed a number of important connections between metabolisms of colon cancer and gut microbiomes

- Example : The increased glycans on cancer cell surfaces correlate with the increased bacterial production of lipopolysaccharides through well understood metabolism, hence providing a mechanistic explanation of
  - How cancer cells feed fusobacteria, giving rise to its increased population, and
  - How fusobacteria offer cancer microenvironment with antigens to keep innate immune attacks and chronic inflammation, needed for cancer development

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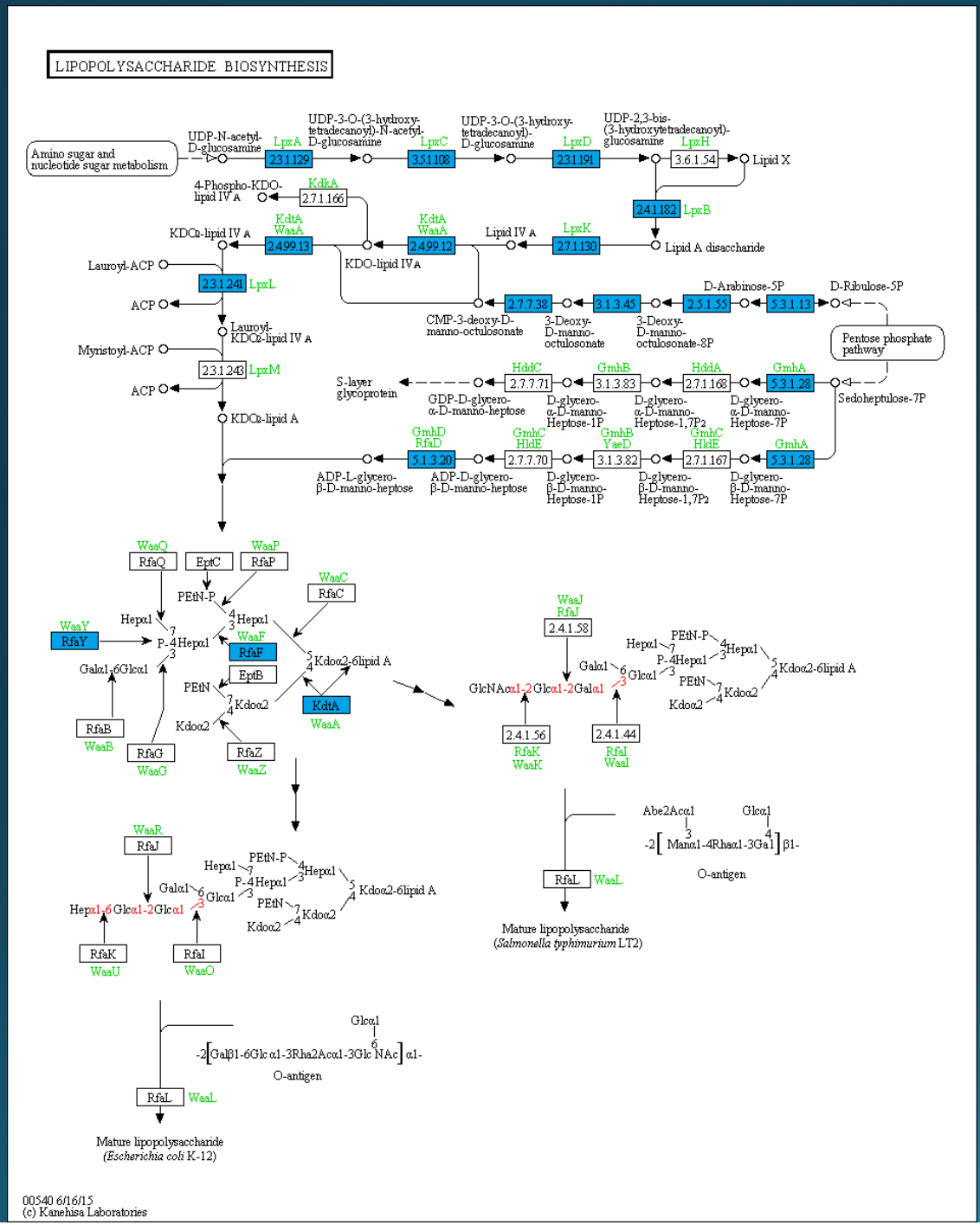
Tang, et al, in preparation, 2015.



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Alignment Between Disease Progression and Microbiome Evolution

- Example: increased cholesterol uptake/synthesis and metabolism in colon cancer gives rise to increased production of bile acids, which can lead to the production of secondary bile acids by multiple bacteria types, which may serve as additional growth signals to cancer

- Example: Some increased bacteria population such as Roseburia may be the result of increased glycan production by cancer cells but may not contribute to cancer development

Summary

- The project is still on going but substantial amount of new insights has been gained regarding possible pathways from chronic inflammation to colon cancer initiation
- There is clear co-evolution between the colon disease and gut microbiome, which leads to chronic inflammation characterized by
  - Deeply damaged ECM
  - Continuous generation of hydroxyl radicals due to iron accumulation and Fenton reaction
  - Glycans seem to be a key element, which has been widely observed on cancer cell surfaces but not studied on one hand as scavenger of hydroxyl radicals and on the other hand as an oncogene.
- The observed statistical relations provide a set of highly informed hypotheses, which can be possibly validated using experiments

Tang, et al, in preparation, 2015.

Acknowledgement

- Professor Tongyu Tang, visiting scholar
- Wei Du, postdoctoral fellow
- Chi Zhang, Ph.D. student
- Ning Dong, Ph.D. student
- Tao Sheng, Ph.D. Student
- Fang Yao, Ph.D. student

- Georgia Research Alliance