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Identifying Sex Differences in Colon Cancer Metabolism

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Abstract: Colon cancer is the second most common cancer to affect women worldwide. While women have a 30-40% lower incidence of colon cancer than men, they have a higher likelihood of cancer presentation on the right-side of the colon. This is of high concern because patients with right-sided colon cancer have poorer clinical outcomes than those with left-sided colon cancers. The reasons for this difference in outcome are not known, however, it has been proposed that female hormones influence colonic metabolism and affect tumor growth in this region of the colon. We have examined the metabolic differences between 207 colon tumor tissues from men and women with left and right-sided colon cancer using untargeted liquid chromatography mass spectrometry-based metabolomics. Using pathway analysis tools, we show region-specific and sex-specific differences in metabolites that may influence tumor growth in women with colon cancer.

Introduction

❑ Sex chromosomes and steroid sex hormones heavily influence metabolic processes.

Energy balance
Glucose metabolism
Lipid metabolism

→
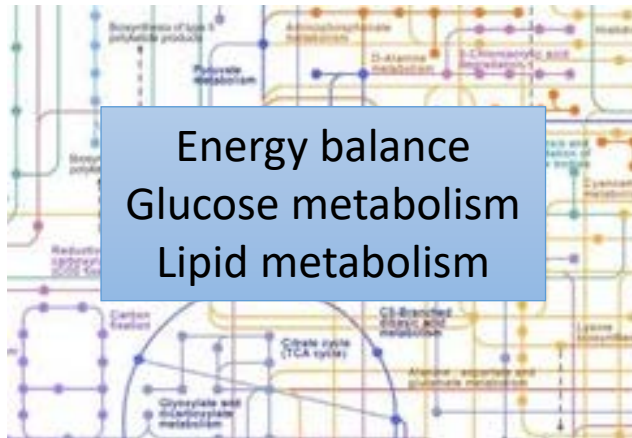
Obesity
Diabetes
Cancer
Cardiovascular Disease

Clegg, D.J., et al. Mol. Metab 2018 15:1-2

Current biases in research

❑ Basic and preclinical research predominantly uses male animal models.

❑ Research on chronic diseases are heavily biased towards men.

❑ Sex-based treatments may improve clinical outcomes for both men and women.

Clegg, D.J., et al. Mol. Metab 2018 15:1-2

NOT-OD-15-102: Consideration of Sex as a Biological Variable in NIH-funded Research

Sex-specific differences in colon cancer presentation

SEER (U.S)

55 % women

45 % men

ONS (U.K.)

56 % women

44 % men

RIGHT-SIDED CRC
Inferior outcomes
with cetuximab
Poorer prognosis

Sessile serrated polyps
CMS1 and CMS3
CIMP-high
Midgut
BRAF mutant
MSI-high
Bile acid exposure
Invasive bacteria biofilms
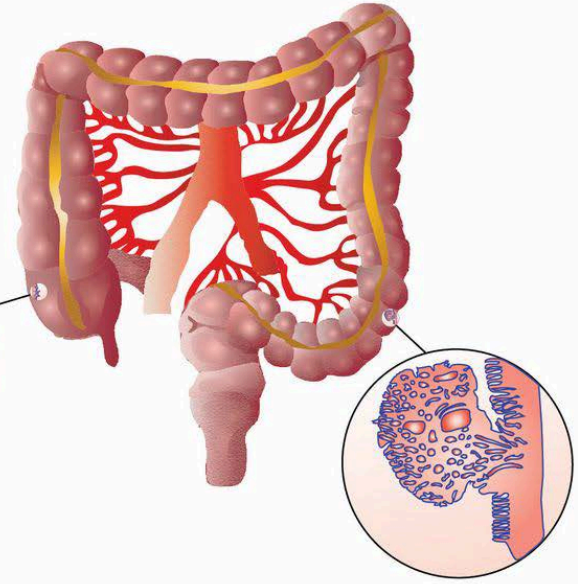
LEFT-SIDED CRC
Superior outcomes
with cetuximab
Better prognosis

Tubular adenoma
CMS2 and CMS4
Higher EREG/AREG
expression
Hindgut

SEER

48 % women

52 % men

ONS

50 % women

50 % men

Lee, M.S, et al. J Natl Compr Canc Netw. 2017, 15 (3); 411-419

Patient outcomes and tumor location in the colon

Figure 2. 5-Year observed specific colon cancer survival according to tumor location and incidence period.

Gervaz, P, et al. EJSO, 2016 42(9):1343-9
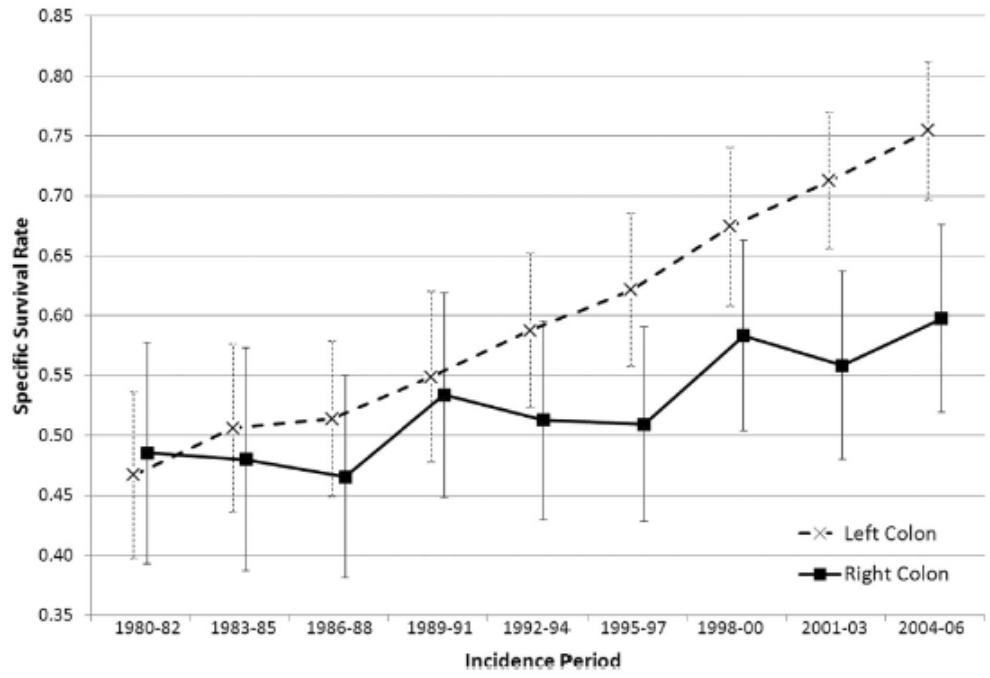
20% increased risk of death from cancer arising on right compared to left

Prognostic Survival Associated With Left-Sided vs Right-Sided Colon Cancer
A Systematic Review and Meta-analysis

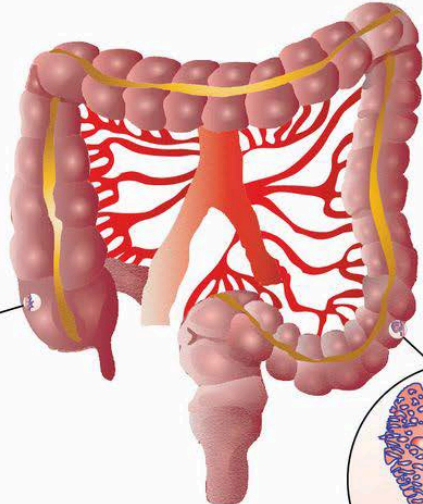**Petrelli, F., et al. Jama Oncology, 2017, 3(2):211-219**

Drivers of right-sided colon cancer

RIGHT-SIDED CRC
Inferior outcomes
 with cetuximab
Poorer prognosis

Sessile serrated polyps
CMS1 and CMS3
CIMP-high
Midgut
BRAF mutant
MSI-high
Bile acid exposure
Invasive bacteria biofilms

**Metabolites &
Microbiome?**

LEFT-SIDED CRC
Superior outcomes
 with cetuximab
Better prognosis

Tubular adenoma
CMS2 and CMS4
Higher *EREG/AREG*
 expression
Hindgut

Lee, M.S, et al. J Natl Compr Canc Netw. 2017, 15 (3); 411-419

Hypothesis

Women with RCC have a metabolic subtype driven by hormonal regulation that differs from women with LCC and men with RCC.

❑ **Main Goal:** Identify metabolites that discriminate tumors in women with RCC from LCCs, and from men.

Study Design

❑ **Analysis:** Discovery-based metabolomics approach to examine metabolites present **in patient tumor tissues** using HILIC and RPLC-ESI-QTOFMS.

    ❑ Stage I-III (some stage IV patients have chemotherapy before surgery)

    ❑ No oral antibiotics, PPIs, or any other drug treatment before surgery

    ❑ Postmenopausal women and age-matched men

    ❑ No transverse or rectal tumors

Sample Cohort

Philip B. Paty MD,
Memorial Sloan Kettering Cancer Center (MSKCC)

Co-PI Sajid A. Khan MD, FACS
Surgical Oncology, YSM

1990-2001 samples were collected from 760 colorectal cancer patients undergoing surgery at MSKCC. All samples flash frozen in liquid nitrogen and stored at -80ºC. Additional samples included metastasis, mucosa, muscularis.

Women RCC = 52
Women LCC = 49
Men RCC = 50
Men LCC = 56

Sample Collection

Biocision ThermalTray
Placed in a rectangular ice pan in dry ice.
Covered with foil, changed every 5 samples.

Tissue placed on chilled thermal tray, held with tweezers, and cut under weighing paper

❑ Prevents tissue projectiles
❑ Allows viewing on tissue through paper
❑ Cleaner cutting
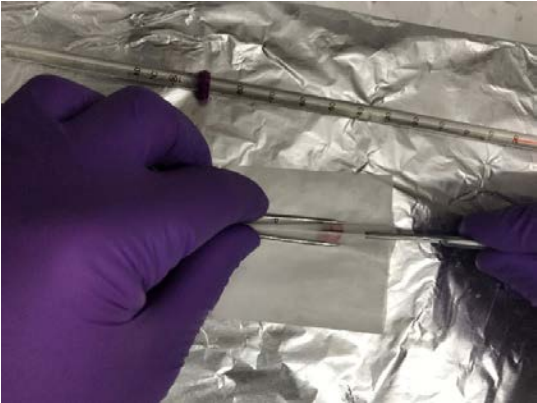
~2500 samples cut by NR and CJ in Justin Cross lab at MSKCC, and shipped o/n on dry ice

= 48 hours on MTA Metro-North Railroad

Sample Extraction
Methods to minimize batch effects and sample degradation

Sample homogenization
Precellys with Cryolys attachment

Larger-scale sample drying
All samples dried in one day
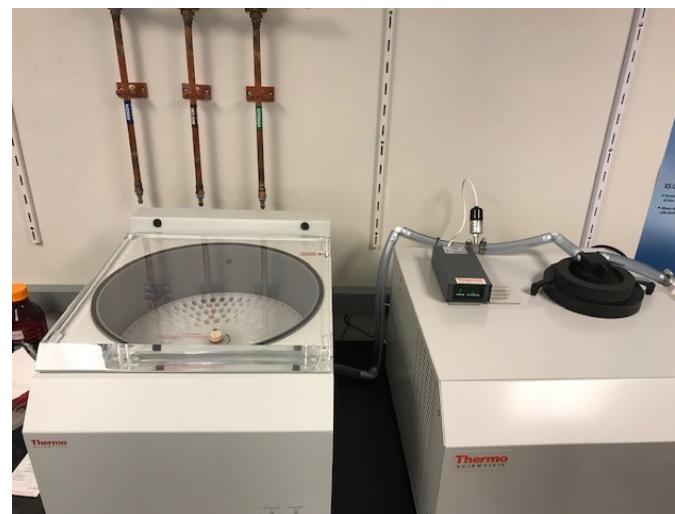
LC-MS-based metabolomics

UPLC I-Class-Xevo®G2-XS QTof

HILIC ESI +ve and –ve
Acquity UPLC BEH Amide
12 min run basic pH 9.6

RPLC ESI +ve and –ve
Acquity UPLC BEH C18
14 min run acidic

3 batches on 96 well plates

Each plate had a global QC

QCs…10 samples…blk…QC….10 samples…blk…QC

Data preprocessing
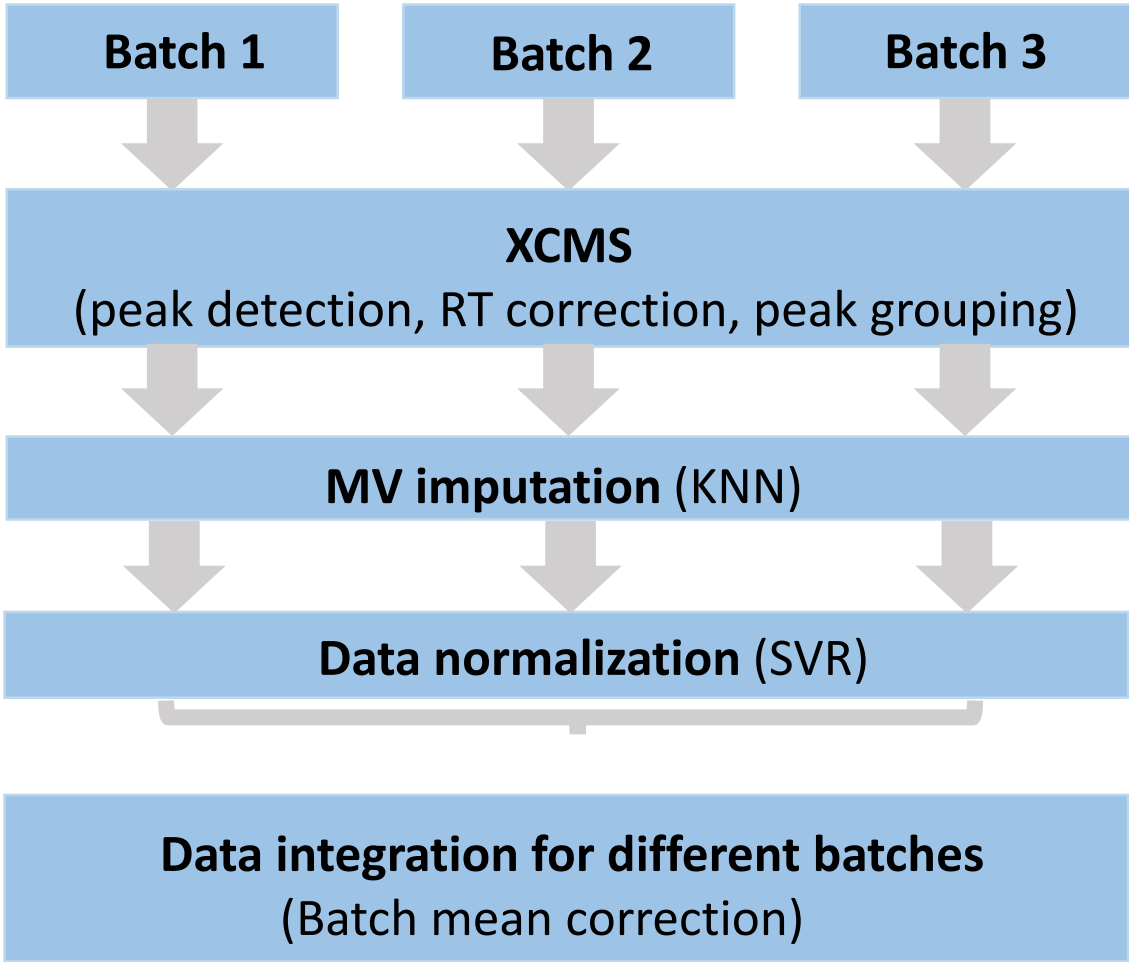
Data preprocessing

raw   normalized
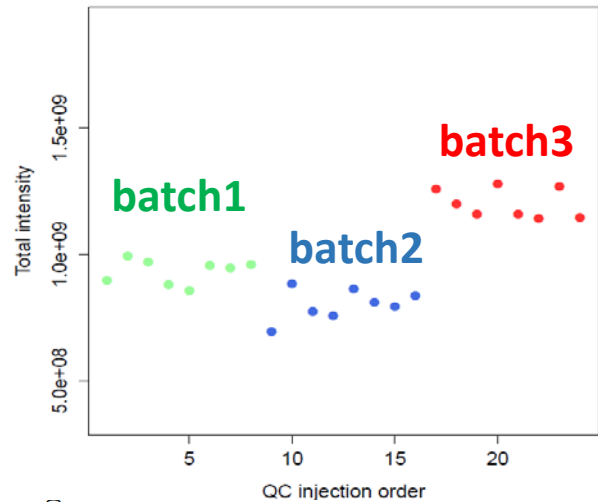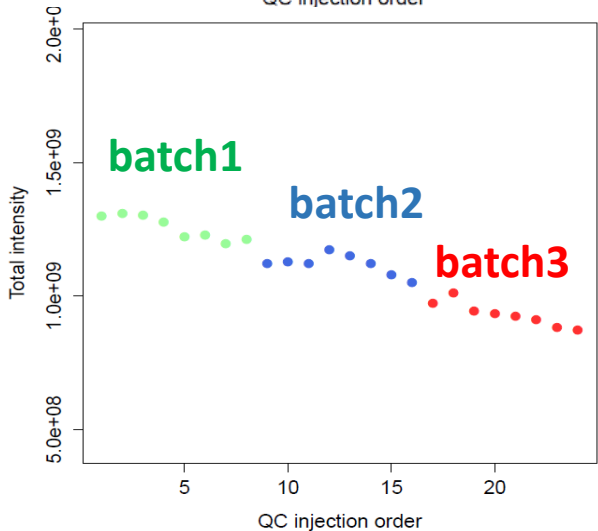
HILIC-MS (+)

HILIC-MS (-)
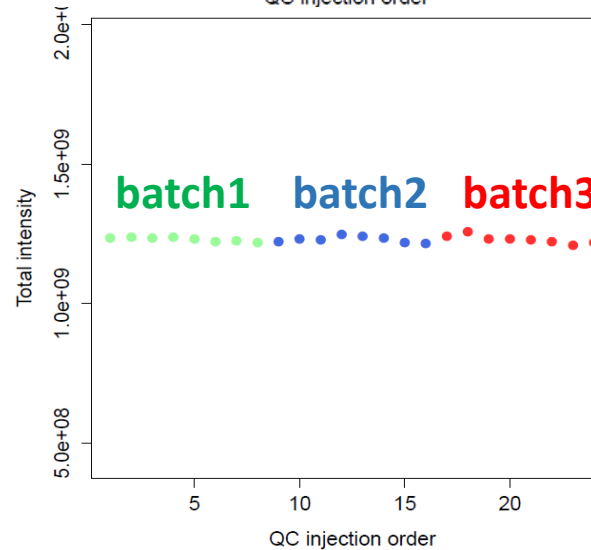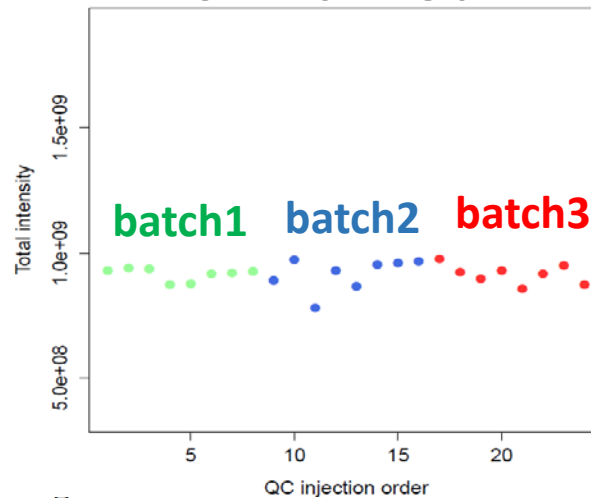
Statistical Analysis

Multivariate Analysis

PCA

Data quality check and assessment of unbiased variation between samples

OPLS-DA

2-group models, determine variables that contribute to classification VIP >1

Univariate Analysis

ROC AUC >0.7

Mann-Whitney *U* test p<0.01, q<0.1

To identify false positives and focus on most important variables
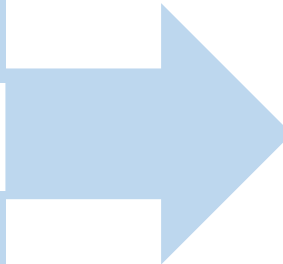
Pathway Analysis

Mummichog, MetDNA

Metabolite Identification

Multivariate Analysis
PCA
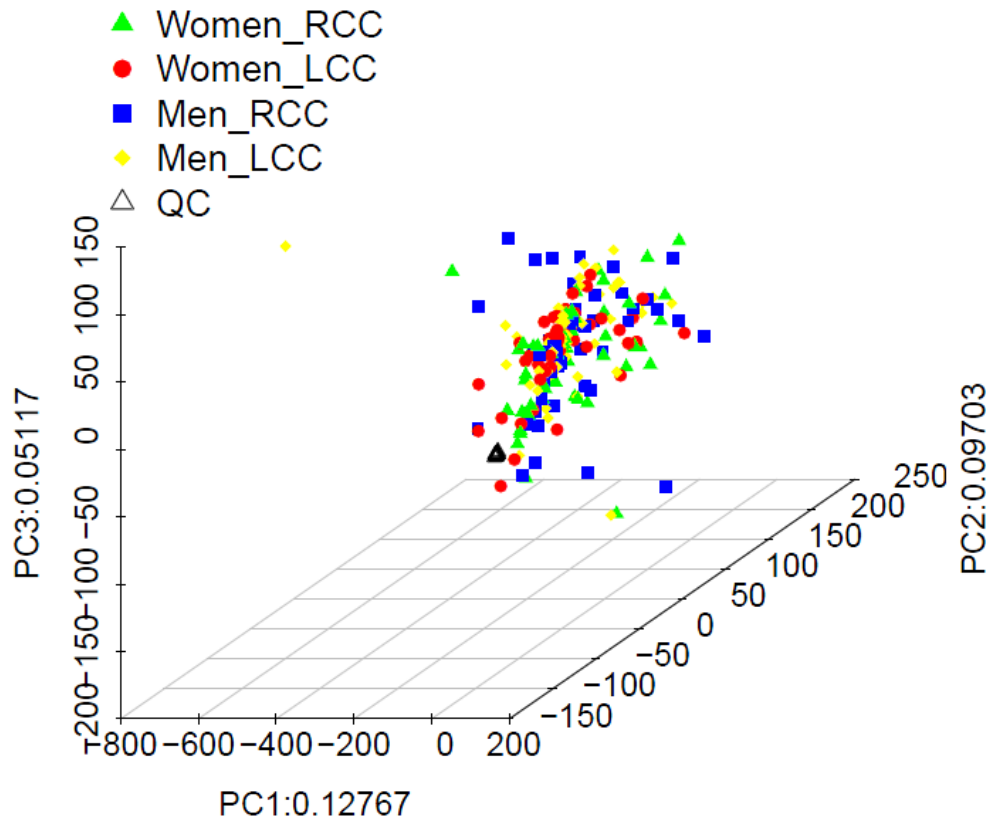
QCs are well aligned

Variation within groups is large

No obvious grouping of samples by sex or primary tumor location

Multivariate Analysis
PCA
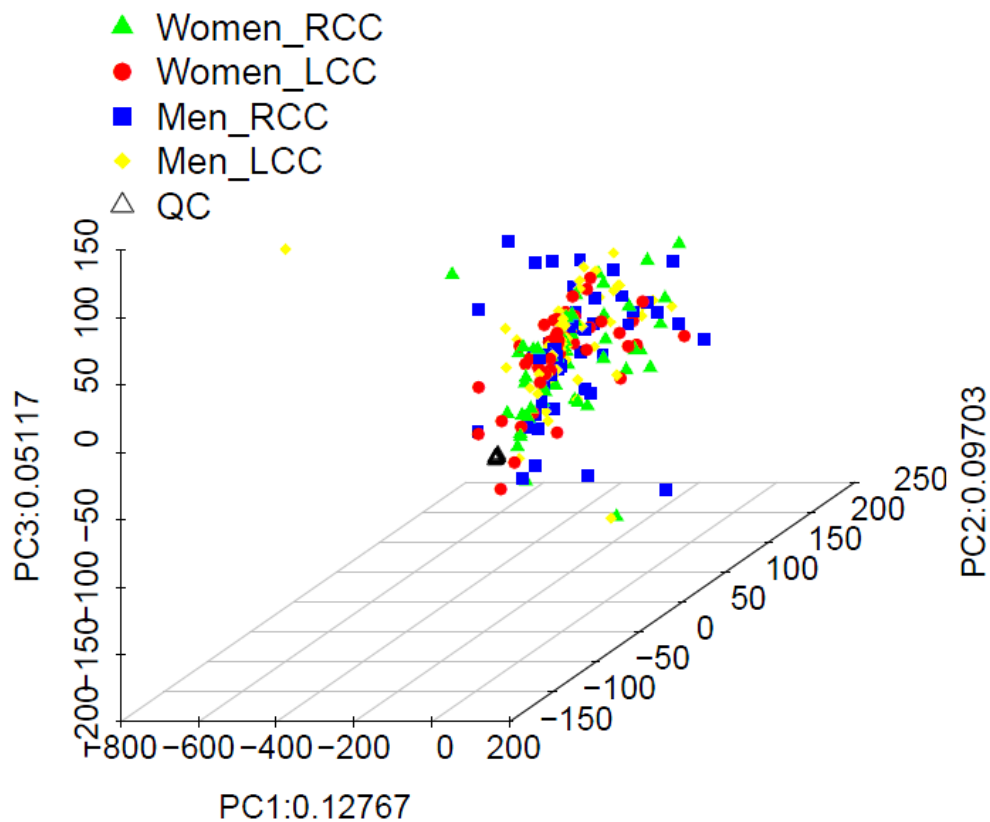
QCs are well aligned

Variation within groups is large

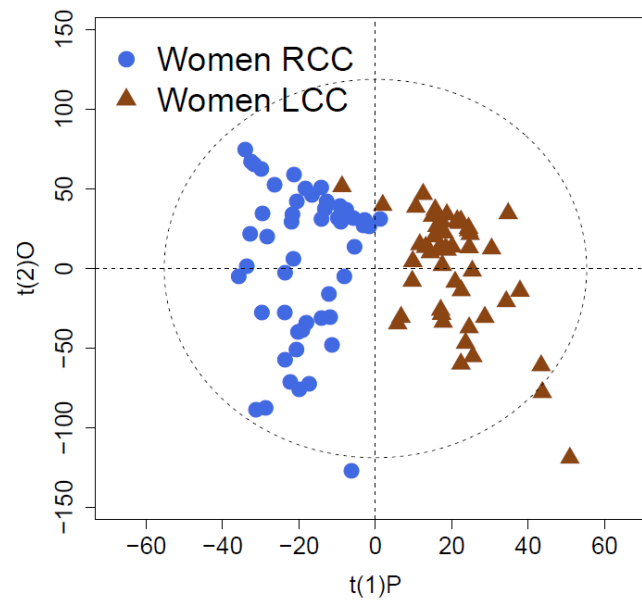No obvious grouping of samples by sex or primary tumor location
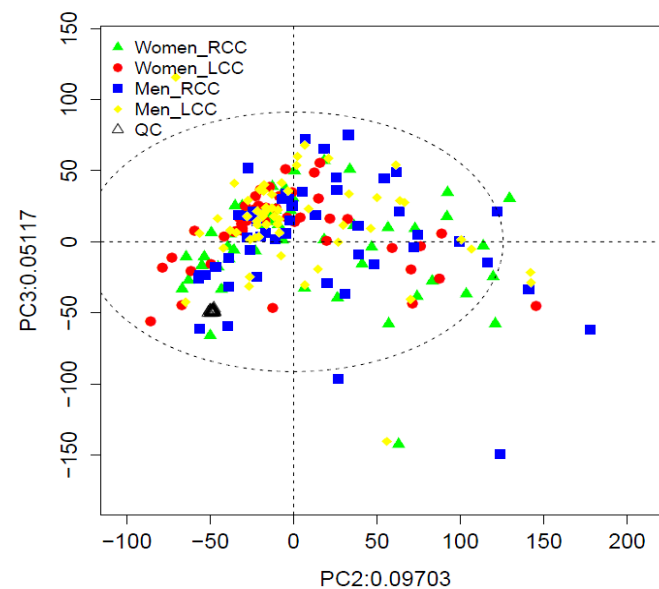
Multivariate Analysis
OPLS-DA
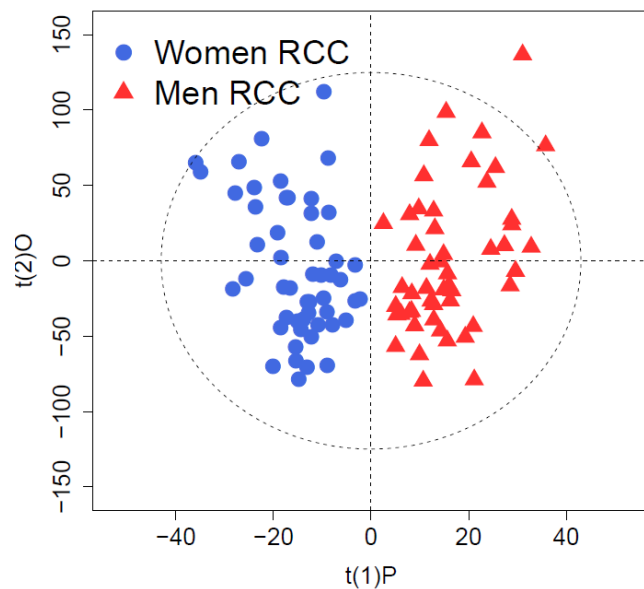
Sex-specific differences

$R^2X(cum) = 0.20$

$R^2Y(cum) = 0.80$

$R^2Q(cum) = 0.012$

Location-specific differences

$R^2X(cum) = 0.21$

$R^2Y(cum) = 0.77$

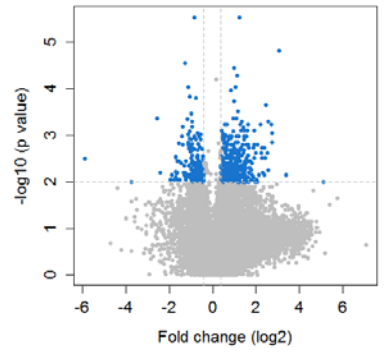$R^2Q(cum) = 0.07$

Multivariate Analysis
OPLS-DA <u>Stage III</u>
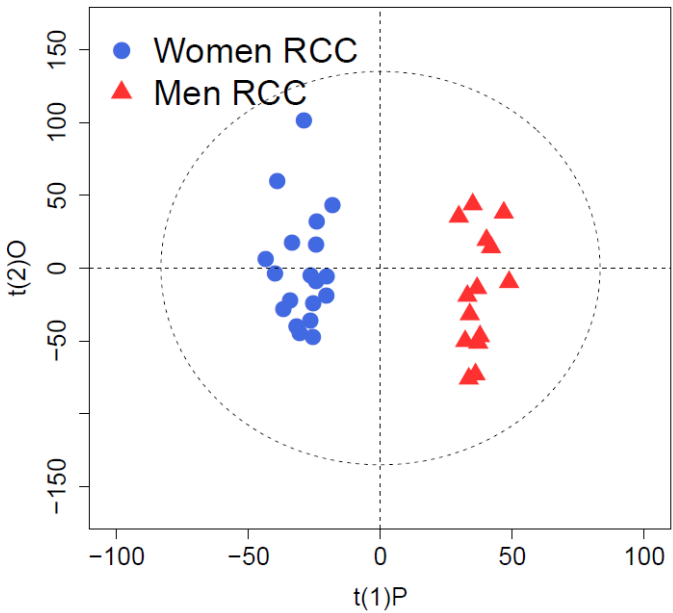
R²X(cum) = 0.28

R²Y(cum) = 0.97

R²Q(cum) = 0.25

27,176 features
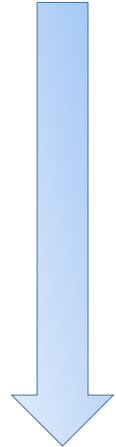
VIP >1

AUC >0.7
P-value <0.01
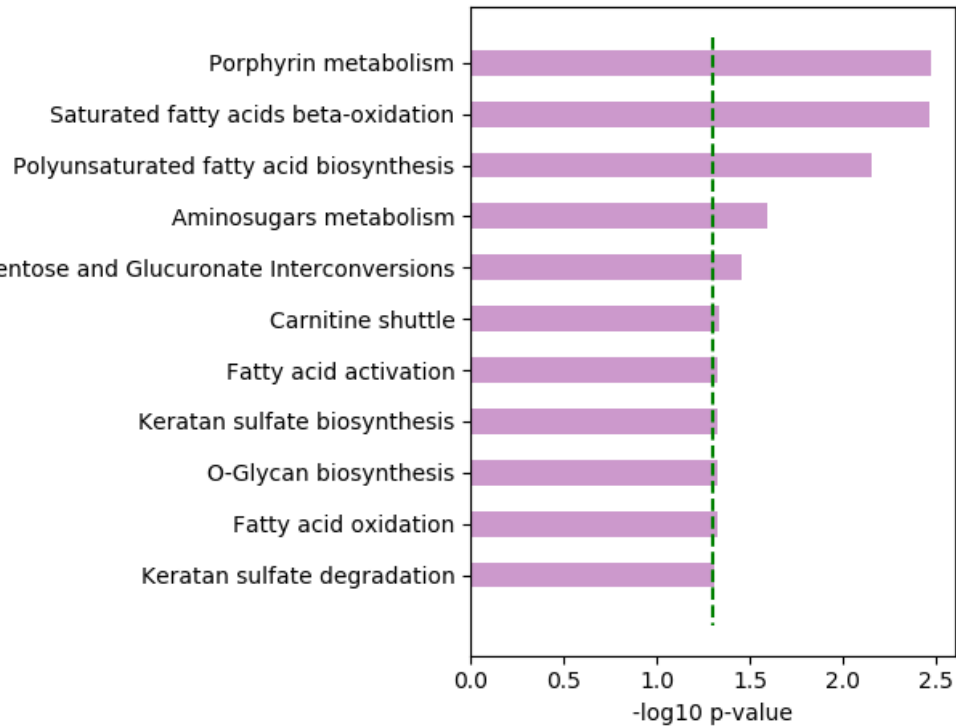*Fold change 1.3*

242 features

Pathway Analysis
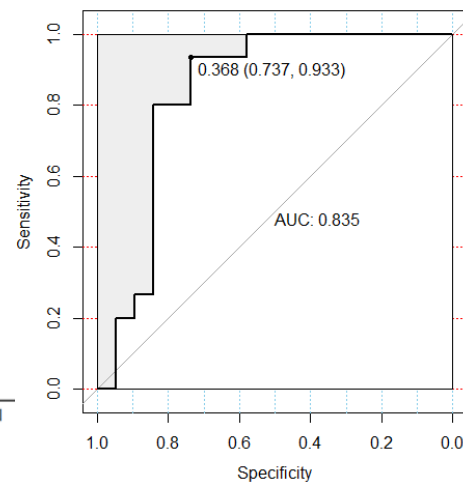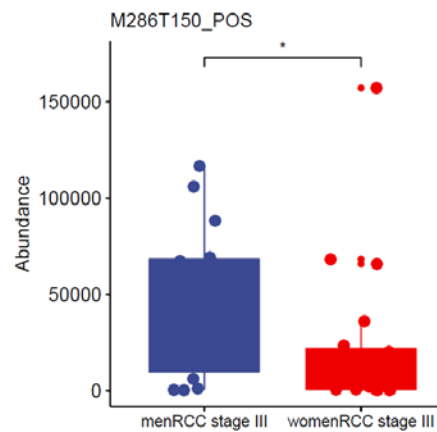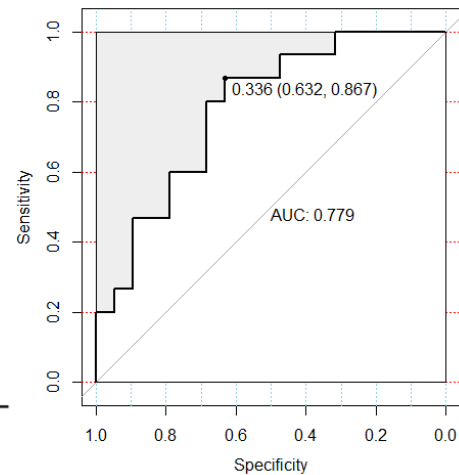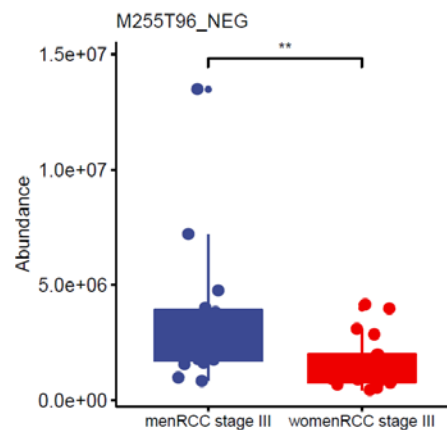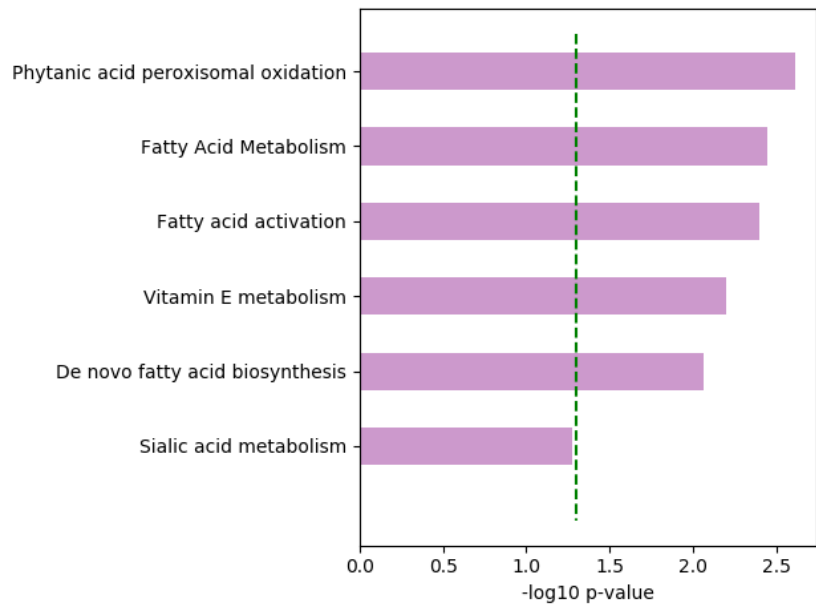
Mummichog

Pathway Analysis

Mummichog

RPLC-MS

Fatty acid pathway changes confirmed by RPLC-MS

Multivariate Analysis
OPLS-DA <u>Stage II</u>

women RCC vs. women LCC

- Women RCC
- Women LCC
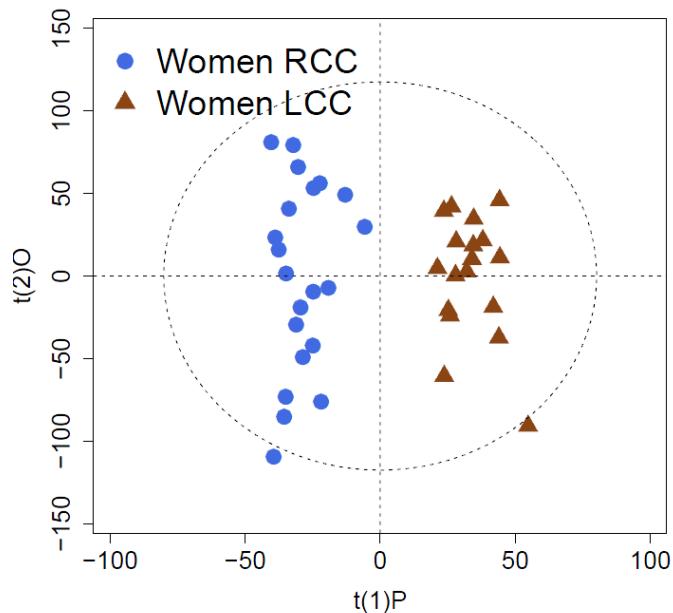
$R^2X(cum) = 0.24$

$R^2Y(cum) = 0.92$

$R^2Q(cum) = 0.18$

27,176 features

VIP >1

AUC >0.7
P-value <0.01
Fold change 1.3

297 features

Strategy

CRC stage I   CRC stage II   CRC stage III

❑ What is the metabolite trajectory by stage in men and women with RCC?

❑ How do other factors play in? Genetic mutations, microbiome?

Conclusions

❑ Non-targeted metabolomics reveals high metabolic inter-tumor heterogeneity.

❑ Sex- and tumor location-specific differences in colon cancer metabolism can be better examined when stratified by tumor stage.

❑ These differences could be related to hormonal regulation of fatty acid metabolism.

Acknowledgements

- Drs. Philip Paty, Kuo-Shun Hsu, Justin Cross
  Memorial Sloan Kettering Cancer Center.

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