Bridging nutrition, metabolism, the microbiome, and cancer susceptibility

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Diet & Microbiota Influence Cancer Risk and Progression

Dietary/digestive components + Gut microbiota = Metabolites that influence cancer

Oncometabolites: 2° bile acids (DCA), hydrogen sulfide

Tumor-suppressive metabolites: SCFAs (butyrate), equol, urolithins

Diet & Microbiota Influence Cancer Risk and Progression
Fiber-Microbiota-Butyrate Axis

- Fiber
- Bacterial fermentation
- SCFAs
- Butyrate
- Mitochondria: Energetics
- Nucleus: Epigenetics
- SCFAs
- FAO
- HDACi
- Ligand for GPCRs
- Nucleus: Epigenetics
Evidence for Butyrate & Cancer Prevention

Tumor-derived cell lines:
- Proliferation
- Apoptosis
- Differentiation

Microbiome studies:
- Butyrate producers: CRC cases < controls
A Limitation of Metagenomic Studies

Microbiota composition:
Cases vs controls
Association study: $\Delta$microbiome = cause or consequence?
Next Phase of Microbiome Research

Metagenomic Sequencing

Bacterial Culture

GEMMs

from cataloging to function

Gnotobiotic Facility
Does Dietary Fiber Protect Against Colorectal Cancer (CRC)?

Two proposed general mechanisms:

1. Maintains digestive movement (insoluble)

2. Bacterial fermentation (soluble)

But ...
...Lack of Universal Consensus: Conflicting Results from Human Epidemiology Studies

Ecologic and migration supportive; prospective cohort mixed:

<table>
<thead>
<tr>
<th>Not Protective</th>
<th>Protective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuchs et. al. 1999</td>
<td>Ferguson et. al. 2003</td>
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<tr>
<td>Lanza et. al. 2002</td>
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<td>Janike et. al. 2011</td>
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grain/fiber
Possible Reasons for Conflicting Results

Hypothesis: Dietary fiber protects against CRC in a microbiota- and butyrate-dependent manner.
Experimental Approach - Part 1

AOM

5 months

Sample tissue
AOM Tumors Resemble Human CRC

Similar mutation spectrum:

“Vogelgram”

Apc  Kras  Braf
β Catenin  p53

Similar transcriptome profiles:

Control    Control                      Control           Experimental
AOM
Low Fiber Diet
High Fiber Diet
Butyrate

Hypothesis: ↓ tumor burden
Combination of High Fiber & *B. fib* Confer Tumor Suppressive Effect

Experimental: Diminished incidence & multiplicity
Combination of High Fiber & \textit{B. fib} Also Inhibits Tumor Growth & Progression

Experimental: Diminished size
Combination of High Fiber & $B.\ fib$ Also Inhibits Tumor Growth & Progression
A Mutant Strain of *B. fibrisolvens* to Confirm Butyrate Mechanism

Butyryl-CoA synthesis operon:

*Asanuma et al. (2005) Current Microbiology*
Butyrate is a Causal Factor

High Dose (5 AOM)

Attenuated protective effect
What is Butyrate Doing Mechanistically?

#1 It accumulates in tumors…

LC-MS:
What is Butyrate Doing Mechanistically?

#2 …which increases histone acetylation due to HDAC inhibition and...

IHC:  

Western Blot:

\[
\begin{array}{c|c|c}
(-) B. fib & WT B. fib \\
\hline
HFD & LFD & TB & HFD & LFD \\
\end{array}
\]

H3ac  

H3
What is Butyrate Doing Mechanistically?

#3 … ↓ cell proliferation and ↑ apoptosis

Q: Why does butyrate accumulate in tumors in first place?

Accumulation
↓
HDAC inhibitor
↓
Epigenetic gene regulation
↓ ($\uparrow$p21)
↓ Cell proliferation (Ki-67)
↓ Fewer/smaller tumors

↓ Apoptosis (cleaved caspase 3)

($\uparrow$Fas)
A: Warburg Effect - Part 1 of 2

Tumors undergo Warburg effect ...

LDHA IHC:

LC-MS:

Relative Lactate Levels (AU)
A: Warburg Effect- Part 2 of 2

... butyrate dependent on Warburg effect in cell culture model

Butyrate \xrightarrow{+ \text{Warburg}} \text{Proliferation} \xrightarrow{- \text{Warburg}}

(siLDHA, glutamine>glucose)
Current Working Model

Treg cells & anti-inflammatory effects, barrier function, GPCR signaling

Caveats:
- Approach is reductionistic for diet and microbiome. One genetic bkg.
- Model is simplistic. Focused on cancer cell autonomous effects.

Nevertheless …
... Human Cancer Relevance

LC-MS:

H3ac WBA:

<table>
<thead>
<tr>
<th>Normal Colon (n = 11)</th>
<th>Adenocarcinoma (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyrate (pmols/µg of protein)</td>
<td></td>
</tr>
</tbody>
</table>

* Indicates significant difference

H3ac

H3
Advantages of Chemoprevention Strategy

Endogenous HDAC inhibitor:

- Butyrate
- Probiotics & prebiotics
- Minimal off-target/adverse effects

Synthetic HDAC inhibitors:

- HDACi
- HAT
- Anticancer chemotherapeutics
- Clinical trials & FDA approval

Tumor

- ↓ Proliferation
- ↑ Apoptosis

5 mM
0.5 mM

Future: Combinatorial Human Studies

Prospective-cohort fiber study

Cases

Controls

+ Gut microbiome

+ GWAS or exome sequencing

Hypothesis: Responders and non-responders should have microbiota and/or genetic differences. Resolve previous conflicting results.
Integrating Precision Medicine into Cancer Prevention

Population-based studies

Precision medicine

Genetic background/GWAS

Biomarkers: Dietary compliance or disease state

Mechanism: Etiology and disease progression

Translational potential

Microbiome

Metabolome
Emerging Evidence for Precision Medicine & Interactome

Nutrition (fiber)

Metabolism (butyrate)

Microbiome (Clostridium)

Genetic background

Immune/Inflammation (Treg cells)

Cancer susceptibility

Tumor

Normal

Tumor

Normal

Milk → SNPs → Bifidobacteria

Diet → SNPs → Choline production/1C metabolism
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[Logos of National Institutes of Health, USDA, American Institute for Cancer Research]
Questions?
Ongoing Experiments

Hypothesis: Butyrate mediates tumor suppression, in part, through a cancer cell non-autonomous manner via diminished inflammation.

Wild type
RAG2-deficient
IL10-deficient

Tumor burden
Inflammation

ChIP seq:
Colon, tumor, immune cells
H3K9ac, H3K27ac, HDACs

Other dietary factors?
Omega-3 fatty acids (PUFAs)
Synergize with butyrate in vitro
Beyond Butyrate: Gut Microbiota & Cancer

Dietary/digestive components + Commensal bacteria = Metabolites (oncometabolites, tumour-suppressive metabolites)

- Meat
- Bile acids
- Fiber
- Polyphenols

Intestinal Epithelium

Lamina Propria

- B cell
- T cell
- Immune cells
- Macrophage
- Dendritic cell

Cancer incidence

- 2° bile acids (DCA), hydrogen sulfide
- SCFAs (butyrate), equol, urolithins

Cancer treatment

- CpG oligo, platinum chemotherapy
- Anti-CTLA4, anti-PD-L1 immunotherapy
- β-glucuronidase & chemotherapy (CPT)

Systemic effects via circulation
Drugs Targeting Bacterial Enzymes to Prevent Adverse Effects of Chemotherapeutics in GI Tract

**Liver**

- Irinotecan (Prodrug)
  - Human Esterase → SN-38
  - Human UGT
    - SN-38-glucuronide
- SN-38-glucuronide
  - Bacterial GUS (Inactive)
  - SN-38 (Active)
  - Epithelial cell death
  - Dose-Limiting Diarrhea

**Large GI**

- SN-38-glucuronide
  - Human TOPO I (Efficacy)
Next Phase of Microbiome Research

Metagenomic Sequencing

Organoid co-culture system
- Higher throughput
- Less $
- Not Caco-2 cells
- Not existing miniguts

Bacterial Culture

GEMMs

Gnotobiotic Facility
3D Crypts-on-a-Chip

- Lgr5+ stem cells & each differentiated cell type on ECM scaffold in media with Wnt gradient
- Anatomically & physiologic normal crypt
- Customize genetic background & disease state
- Assay barrier function, transport, metabolism, etc.
- Amenable to microbial co-culture
- Can be interconverted to 2D culture system for high-throughput screening

Nancy Allbritton, UNC
Scott Magness, UNC
Intestinal Simulacra

Human crypts *in vivo*

3D crypts-on-a-chip
The Warburg Effect

Cancer cells shift from oxidative metabolism to aerobic glycolysis:

Glucose + O₂ → Glycolysis → Pyruvate → Oxidative Phosphorylation (OxPHOS)

2-4 ATP (Glycolysis) + 36 ATP (OxPHOS)

Otto Warburg
1931 Nobel Prize
Recently “rediscovered”
Cancer Cells Take Up Glucose at High Levels

Warburg effect is basis of tumor imaging in the clinic:
Warburg Effect: Conduit for Raw Materials to Double Cellular Biomass
Hypothesis: Butyrate can also increase histone acetylation by a mechanism other than HDAC inhibition.
Butyrate Regulates Histone Acetylation via ACL-Dependent Mechanism

Butyrate

ACL

TCA cycle

β-oxidation

mitochondria

Citrate

Acetyl CoA

nucleus

AcH3

HATs

H3 Acetylation

ACL

β-actin

siMock

siACL

Butyrate Dose (mM)

0 0.5 2.0 5.0

0 0.5 2.0 5.0

0% 20% 40% 60% 80% 100%

ACL-independent

ACL-dependent
TSA Control

![Diagram showing the metabolic pathways and regulatory roles of Butyrate, Glucose, Acetyl CoA, and H3ac with siACL and β actin expression under Vehicle and TSA conditions.](Image)
ACL-Dependent Mechanism Predominates in Normal Colonocytes

Butyrate → Butyrate → Butyrate → Acetyl CoA → Citrate → Citrate → Acetyl CoA

β oxidation

TCA cycle

mitochondria

Acetyl CoA → HATs → Ac → HDACs → nucleus

Glucose → AKT → Lipid biosynthesis

P-ACL (Ser454) → H3Ac

β-actin

Butyrate (10 mM) → Radiciol (100 μM)

+ + − − + +
A Cancerous Colonocyte

B Warburg Effect

C No Warburg Effect

D Acetyl-CoA Donor/HAT Mechanism

HDAC Inhibitor Mechanism

Regulation of Histone Acetylation and Transcription

Butyrate (mM)

Normal Colonocyte

Mitochondria

Nucleus

Histone

HATs

HDACs

CO₂

Acetyl-CoA

Glucose

Proliferation

Butyrate

Histone

Ac

Histone

Ac
Butyrate Increases Histone Acetylation by Stimulating HATs & Inhibiting HDACs

- **Normal cells; lower doses; proliferation genes**
- **Cancer cells; higher doses; pro-apoptotic genes (Fas)**

**Diagram:**
- Histone (Lys) + Acetyl-CoA → HATs → Histone (Ac)
- Histone (Lys) + Acetyl-CoA → HDACs → Histone (Lys)
Biological material (buccal swabs, stool samples, tissue biopsies, etc.):
- Can be obtained from disease cases and controls

Prepare genomic DNA

Next-generation DNA sequencing:
- 16S rRNA
- whole-genome shotgun (discard human sequence reads)

Computational assembly and analysis of microbial sequence reads to quantify abundance of microbiota

Principal Component Analysis to identify differences between samples such as disease cases and controls
 Identification of microbes significantly enriched in different body sites or at one site in cases vs controls
Bridging nutrition, metabolism, the microbiome, and cancer

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Microbiome

Cancer cell metabolism (Warburg effect)

Butyrate

Epigenetics (HDAC inhibitor)