Impact of the Total Western Diet for rodents on colon mucosal gene expression in a multi-generational murine model of colitis-associated colorectal cancer

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Colorectal cancer (CRC)

• CRC is the 2\textsuperscript{nd} leading cause of cancer-related death in the US.

• Majority of CRC incidence is attributed to diet.

Epigenetic gene expression signatures

Epigenetic gene expression signatures

central dogma

different cell types
- colon cell
- heart cell

Central dogma

Different cell types:
- Colon cell
- Heart cell

Normal vs. cancer:
- Normal cell
- Cancer cell

Epigenetic gene expression signatures

Heritable DNA methylation

RNA pol2

promoter

gene expression

exon 1

exon 2

rep

seq

TSG hypomethylation
active transcription
global hypermethylation
RepSeq inhibition

Heritable DNA methylation

RNA pol2

promoter

gene expression

exon 1

exon 2

normal cell

TSG hypomethylation
active transcription
global hypermethylation
RepSeq inhibition

tumor cell

TSG hypermethylation
silenced transcription
global hypomethylation
genome instability

Study design

- **Generation**: $F_0 \rightarrow F_1 \rightarrow F_2 \rightarrow F_3$
- **Control**: AIN AIN AIN AIN
- **Direct**: AIN AIN AIN TWD
- **Cumulative**: TWD TWD TWD TWD
- **Ancestral**: TWD AIN AIN AIN

Outcome: AAAA
Study design

<table>
<thead>
<tr>
<th>Generation</th>
<th>F₀</th>
<th>F₁</th>
<th>F₂</th>
<th>F₃</th>
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<tbody>
<tr>
<td>Control</td>
<td>AIN</td>
<td>AIN</td>
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<tr>
<td>Direct</td>
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<tr>
<td>Ancestral</td>
<td>TWD</td>
<td>AIN</td>
<td>AIN</td>
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</tr>
</tbody>
</table>

**CRC outcome**

Tumor burden (total mm³)

- aaaA
- aaaT
- tttT
- taaA

Legend:
- a
- b
- c
Knowledge gap

Objective:

evaluate differentially expressed genes (DEGs) of colonic mucosal cells from 3rd generation offspring.
Knowledge gap

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Hypothesis:
Total Western Diet (TWD) exposure will upregulate or downregulate genes that play a role in CRC.
Methods

1. Collect cells
Methods

1. Collect cells  
2. Extract RNA
Methods

1. Collect cells
2. Extract RNA
3. Sorting
Methods

1. Collect cells
2. Extract RNA
3. Sorting
4. Sequencing
Methods

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2. Extract RNA
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5. Quality control
Methods

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6. Trimming
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6. Trimming
7. Alignment
Methods

1. Collect cells
2. Extract RNA
3. Sorting
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7. Alignment
8. Quantification
Methods

1. Collect cells
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9. Filtering
Methods

1. Collect cells
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8. Quantification
9. Filtering
10. DEG analysis
Study design

- **Cancer vs. controls**
  - ~700-4500 DEGs

- **Cancer cohorts**
  - aaaA vs. aaaT
    - 36 DEGs
  - aaaA to tttT
    - >100 DEGs

- **Sham cohorts**
  - aaaT vs. tttT
    - 101 DEGs

Preliminary results

- **Cancer vs. controls**
  - ~700-4500 DEGs

- **Response types**
  - Defense response
  - Immune response
  - Response to interferon
Study design

Preliminary results

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Study design

Preliminary results

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  - aaaA to tttT
    - 119 DEGs
Study design

Preliminary results

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- **Cancer cohorts**
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    - 36 DEGs
  - \( \text{aaaA vs. tttT} \)
    - 119 DEGs

- **Sham cohorts**
  - \( \text{aaaT vs. tttT} \)
    - 101 DEGs
    - defense response
    - immune response
    - response to interferon
Summary & conclusions

• Multigenerational exposure to the Western dietary pattern may alter gene expression and health outcome in offspring.

Fehlker et al. 2014. BMC Cancer.
Sun et al. 2018. PeerJ.
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• Metanalysis of clinical CRC gene expression signature reveals DEGs related to immune function.
• Many DEGs in human CRC are associated with aberrant DNA methylation.
• Ongoing analysis will include methylation status.

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Acknowledgements

Benninghoff Laboratory
USTAR Applied Nutrition Research
USDA NIFA/AFRI Grant 2014-67017-21755
Utah Agricultural Experiment Station Project UTA-01178