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

central dogma

Epigenetic gene expression signatures

- Central dogma
- Different cell types
  - Colon cell
  - Heart cell

Epigenetic gene expression signatures

central dogma

different cell types
- colon cell
- heart cell

normal vs. cancer
- normal cell
- cancer cell

Heritable DNA methylation

RNA pol2

promoter

gene expression

RNA

pol2

gene expression

exon 1

RNA pol2

gene expression

exon 2

rep

seq

normal cell

TSG hypomethylation

active transcription

global hypermethylation

RepSeq inhibition

Heritable DNA methylation

RNA pol2

gene expression

promoter

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

- **Control**
  - F_0: AIN
  - F_1: AIN
  - F_2: AIN
  - F_3: AIN

- **Direct**
  - F_0: AIN
  - F_1: AIN
  - F_2: AIN
  - F_3: TWD

- **Cumulative**
  - F_0: TWD
  - F_1: TWD
  - F_2: TWD
  - F_3: TWD

- **Ancestral**
  - F_0: TWD
  - F_1: AIN
  - F_2: AIN
  - F_3: AIN
Study design

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

Tumor burden (total mm³)

CRC outcome

- aaaA
- aaaT
- tttT
- taaA
Objective:
evaluate differentially expressed genes (DEGs) of colonic mucosal cells from 3rd generation offspring.
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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

1. Collect cells
2. Extract RNA
3. Sorting
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5. Quality control
Methods

1. Collect cells
2. Extract RNA
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6. Trimming
Methods

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2. Extract RNA
3. Sorting
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6. Trimming
7. Alignment
Methods

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8. Quantification
Methods

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7. Alignment
8. Quantification
9. Filtering
Methods

1. Collect cells
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5. Quality control
6. Trimming
7. Alignment
8. Quantification
9. Filtering
10. DEG analysis
Study design

Preliminary results

- Cancer vs. controls
  - ~700-4500 DEGs
Study design

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- Cancer cohorts
  - aaaA vs aaaT
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Study design

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- Cancer cohorts
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    - 119 DEGs
- Sham cohorts
  - 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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• Ongoing analysis will include methylation status.

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