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

Sumira Phatak
Aaron Thomas
Rakesh Kaundal
Rousselene Jones
Korry Hintze
Abby Benninghoff
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

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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 → exon 1 → exon 2

gene expression

normal cell
- TSG hypomethylation
- active transcription
- global hypermethylation
- RepSeq inhibition

Heritable DNA methylation

RNA pol2

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

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

CRC outcome

- **Control**: AIN > AIN > AIN > AIN
- **Direct**: AIN > AIN > AIN > TWD
- **Cumulative**: TWD > TWD > TWD > TWD
- **Ancestral**: TWD > AIN > AIN > AIN

**Tumor burden (total mm³)**

- **aaaA**
- **aaaT**
- **tttT**
- **taaA**
Knowledge gap

Objective:

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

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
4. Sequencing
5. Quality control
Methods

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

1. Collect cells
2. Extract RNA
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5. Quality control
6. Trimming
7. Alignment
Methods

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

1. Collect cells
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9. Filtering
Methods

1. Collect cells
2. Extract RNA
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4. Sequencing
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7. Alignment
8. Quantification
9. Filtering
10. DEG analysis
Study design

Preliminary results

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

Preliminary results

- Cancer vs. controls
  - ~700-4500 DEGs
- Cancer cohorts
  - aaaA vs aaaT
  - 36 DEGs
Study design

Control
- F0: AIN
- F1: AIN
- F2: AIN
- F3: AIN

Direct
- F0: AIN
- F1: AIN
- F2: AIN
- F3: TWD

Cumulative
- F0: TWD
- F1: TWD
- F2: TWD
- F3: TWD

Ancestral
- F0: TWD
- F1: AIN
- F2: AIN
- F3: AIN

Preliminary results

- Cancer vs. controls
  - ~700-4500 DEGs

- Cancer cohorts
  - aaaA vs aaaT
    - 36 DEGs
  - aaaA to tttT
    - 119 DEGs
Study design

Preliminary results

• Cancer vs. controls
  • ~700-4500 DEGs

• Cancer cohorts
  • aaaA vs. aaaaT
    36 DEGs
  • aaaA to tttT
    119 DEGs

• Sham cohorts
  • aaaaT 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.
Summary & conclusions

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  • defense/immune response: down regulation promotes CRC metastasis
  • response to interferon: down regulation promotes CRC development

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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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Sun et al. 2018. PeerJ.
Acknowledgements

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