Mechanisms Associated with Dietary Energy Balance Effects on Tumor Development: Alterations in Growth Factor and Energy Sensing Pathways

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Presentation Outline

• Part I

- Dietary energy balance modulates skin tumor promotion via altered growth factor signaling (e.g., Akt/mTOR) and proliferation during tumor promotion.

- Possible role of altered IGF-1R/EGFR crosstalk in dietary energy balance effects on tumor promotion.

- Targeting mTORC1 as a strategy for inhibition of tumor promotion mimicking the effects of CR.

• Part II

- Dietary energy balance modulates prostate cancer progression in Hi-Myc mouse model

- Dietary energy balance effects growth factor signaling pathways in prostate

- Possible role of inflammation and inflammatory signaling in prostate cancer progression in Hi-Myc Mice
Potential Mechanisms Underlying the Link Between Energy Balance and Cancer

Moore et al, ANYAS, 2011
Dietary Energy Balance Modulation Alters Steady State Growth Factor Signaling Pathways in Epithelial Tissues of Normal Mice

Caloric Density of Diets Used to Examine the Effect of Dietary Energy Balance on Growth Factor Signaling in Epithelial Tissues

<table>
<thead>
<tr>
<th>Experimental diet</th>
<th>Experimental Phenotype</th>
<th>Protein (kcal%)</th>
<th>Carbohydrate (kcal%)</th>
<th>Fat (kcal%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30% CR\textsuperscript{1}</td>
<td>Lean</td>
<td>29</td>
<td>57</td>
<td>14</td>
</tr>
<tr>
<td>15% CR\textsuperscript{2}</td>
<td>Normal</td>
<td>24</td>
<td>65</td>
<td>12</td>
</tr>
<tr>
<td>AIN76A\textsuperscript{3}</td>
<td>Overweight</td>
<td>20</td>
<td>70</td>
<td>10</td>
</tr>
<tr>
<td>DIO</td>
<td>Obese</td>
<td>20</td>
<td>20</td>
<td>60</td>
</tr>
</tbody>
</table>

Effect of Dietary Energy Balance Modulation on Weight Distribution and Serum Hormone Levels in Male Mice

**FVB Mice**

![Graph showing weight distribution in FVB mice with different dietary energy balance modulations.]

**C57BL/6 Mice**

![Graph showing weight distribution in C57BL/6 mice with different dietary energy balance modulations.]

Effect of Dietary Energy Balance Modulation on Growth Factor Signaling in Epithelial Tissues of Untreated Mice

Dietary Energy Balance Modulation and Two-Stage Skin Carcinogenesis

• Calorie restriction (CR) significantly reduces tumor incidence, multiplicity, and papilloma size during two-stage skin carcinogenesis (Boutwell, 1964; Birt et al, 1991; Birt et al, 1993).

• Inhibitory effects of CR are observed primarily during tumor promotion (Birt et al, 1991).

• Potential mechanisms of inhibition:
  - 40% CR inhibits ERK, leading to decreased proliferation (Liu et al, 2001);
  - Levels of corticosterone may mediate inhibitory effects of 40%CR (Stewart et al, 2005);
  - Reduced PI3K and Ras signaling following treatment with TPA (Xie et al, 2007);
  - Altered growth factor signaling via reduced circulating IGF-1 (Moore et al, CPR 2008; Cancer Res., 2008)

• Effect of positive energy balance is less clear.
Dietary Energy Balance Modulation of Skin Tumor Promotion by TPA in ICR Mice

Initiation 25 nmol DMBA

Start Experimental Diet Week 4

Begin Promotion 3.4 nmol TPA Week 8

Continue promotion until multiplicity plateaus

Moore et al, unpublished data
Dietary Energy Balance Does Not Affect the Rate of Malignant Conversion of Papillomas to SCCs

<table>
<thead>
<tr>
<th>Experimental Diet</th>
<th>Total Number of Mice</th>
<th>Average Papillomas Per Mouse&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Carcinoma Incidence (%)</th>
<th>Carcinomas per Mouse</th>
<th>Conversion Ratio&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>60kcal% fat</td>
<td>25</td>
<td>8.86</td>
<td>96.0</td>
<td>2.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26</td>
</tr>
<tr>
<td>10kcal% fat</td>
<td>27</td>
<td>8.20</td>
<td>92.3</td>
<td>1.58</td>
<td>0.20</td>
</tr>
<tr>
<td>15% CR</td>
<td>29</td>
<td>6.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.0</td>
<td>1.59</td>
<td>0.26</td>
</tr>
<tr>
<td>30% CR</td>
<td>26</td>
<td>4.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.7</td>
<td>0.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.23</td>
</tr>
</tbody>
</table>

<sup>1</sup>Data taken at 27 weeks of promotion with 3.4 nmol TPA after which the papilloma response had reached a plateau

<sup>2</sup>Ratio of the average number of SCCs at 50 weeks to average number of papillomas at 27 weeks

<sup>a</sup>Significantly different from all groups, Wilcoxon Rank Sum (p<0.05)

Moore et al, unpublished data
Dietary Energy Balance Modulation Alters Epidermal Proliferation Following TPA Treatment

Moore et al, unpublished data

* Significantly different from other groups Wilcoxon Rank Sum p < 0.05
Weight Gain and Selected Serum Protein Profiles of Female ICR Mice in the Two-Stage Skin Carcinogenesis Study

![Graph showing weight gain and serum protein profiles](image)

- **IGF-1 (ng/mL)**
  - 30% CR: 100, 300
  - 15% CR: 200, 400
  - 10Kcal%: 300, 500
  - 60Kcal%: 400, 600

- **Insulin (pg/mL)**
  - 30% CR: 1000
  - 15% CR: 1200
  - 10Kcal%: 1500
  - 60Kcal%: 1800

- **Leptin (pg/mL)**
  - 30% CR: 5000
  - 15% CR: 7000
  - 10Kcal%: 9000
  - 60Kcal%: 11000

*a, b* Significantly different from values with the same lettering Wilcoxon Rank Sum (*p < 0.05*)

Moore et al, unpublished data
Summary of Dietary Energy Balance Effects on Growth Factor Signaling and Cell Cycle Related Proteins in Epidermis During Tumor Promotion

Moore et al, CAPR, 2008 and unpublished data
Reduced Circulating IGF-1 Inhibits Two-Stage Skin Carcinogenesis

**LID Mice**

- Circulating IGF-1 is produced primarily by the liver
- Cre/loxP system was utilized to create a targeted deletion of liver specific IGF-1 production
- Results in a 75% reduction in circulating IGF-1
- Targeted deletion had no effect on post-natal growth

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**Graph A**

- TPA 13.6, Wt
- TPA 13.6, LID

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**Graph B**

- Papillomas per mouse
- Percent of mice with papillomas

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Reduced Circulating IGF-1 Decreases Epidermal Proliferation Both in the Absence and Presence of TPA Treatment

*Significantly different (p<.05) from values obtained from wild-type mice"
Reduced Circulating IGF-1 Decreases Activation of EGFR, IGF-1R and Akt/mTOR Signaling Induced by TPA

Mechanisms for IGF-1R/EGFR Cross-Talk

Moore et al, unpublished data
Working model for EGFR/IGF-1R crosstalk

Cell proliferation, cell growth, cell survival
Rapamycin is a Highly Potent Inhibitor of Skin Tumor Promotion by TPA

Initiation
25 nmol DMBA

Week 2: Start Rapamycin treatments followed by promotion with 6.8nmol TPA

Continue promotion until multiplicity plateaus

Rapamycin is a Highly Potent Inhibitor of Skin Tumor Promotion by TPA

* denotes significance. Differences in the average number of papillomas per mouse at 25 weeks between the TPA control group and corresponding Rapamycin treated groups for each dose were statistically significant (P < 0.05, Mann-Whitney U Test).

** Significantly different from 6.8nmol TPA (P < 0.05, Chi-square test).

Checkley et al, CAPR, 2011
Rapamycin Treatment Inhibits TPA-Induced Epidermal Hyperproliferation

* P < 0.05

Checkley et al, CAPR, 2011
Topical Rapamycin Inhibits TPA-Induced Epidermal mTORC1 Signaling Primarily Through the p70S6K Downstream Signaling Pathway

Checkley et al, CAPR, 2011
Rapamycin Potently Inhibits Skin Tumor Promotion by TPA in Overweight and Obese Mice

Initiation
25 nmol DMBA

Start 10 KCAL %
or 60 KCAL% diet
Week 2

Begin Promotion:
6.8 nmol TPA +
rapamycin
Week 8

Continue promotion
until multiplicity plateaus

Overweight

Obese

* Indicates significantly different from
6.8 nmol TPA (p <.05)

Checkley et al, unpublished
Metformin Given in the Drinking Water Inhibits Skin Tumor Promotion by TPA in Overweight Mice

- **Initiation**: 25 nmol DMBA
- **Start**: 10 KCAL % diet Week 2
- **Begin Promotion**: 6.8 nmol TPA + metformin Week 8
- **Continue promotion until multiplicity plateaus**

**Graphs**:
- **Papillomas Per Mouse**
  - 6.8 nmol TPA
  - 250 mg/kg met + TPA
  - 50 mg/kg met + TPA

- **Percent of Mice with Papillomas**
  - T + M 50
  - T + M 250

**Table**:
- **Checkley et al, unpublished**
  - Ace
  - MET 250
  - TPA
  - TPA + MET 250 50

**Images**:
- **pAMPKα**
- **AMPK α**
mTORC1 Signaling Pathway as a Potential Target for Prevention of Obesity Related Cancer Promotion

Obesity → GFR → PI3K → p-T308 → Akt → mTORC1 → p-S473 → mTORC2 → Tumor Promoters

AMPK → mTORC1

- p70S6K → S6 Ribosomal Protein Translation
- 4E-BP1 → eIF4E → Protein Translation → Tumor Promotion

Metformin, Phytochemicals, CR

Rapamycin, Rapalogs, Phytochemicals, CR
Hi-Myc Mouse Model of Prostate Cancer

• Overexpression of c-myc in ARR2PB-myc-PAI transgenic mice resulted in complete penetrance of PIN as early as 2 to 4 weeks of age.

• Progression to locally invasive adenocarcinomas occurs within ages 3 to 6 months for Hi-myc mice and within 10 to 12 months in mice expressing low c-myc levels.

• Ventral Prostate, Dorsolateral Prostate, and Anterior Prostate are the target of c-Myc overexpression.

• Metastases are not present in these transgenic mice.

• This mouse model was the first nonSV40 model to develop advanced adenocarcinoma without NE features.

Elwood-Yen et al, Cancer Cell 4:223-228, 2003

<table>
<thead>
<tr>
<th></th>
<th>30% CR</th>
<th>Overweight control</th>
<th>DIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial mass (g)</td>
<td>25.9 ± 0.9</td>
<td>24.1 ± 0.96</td>
<td>22.3 ± 0.88</td>
</tr>
<tr>
<td>Final mass (g)</td>
<td>23.9 ± 1.2</td>
<td>40.1 ± 1.2</td>
<td>44.9 ± 1.6</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>21.1 ± 0.84</td>
<td>38.0 ± 0.87</td>
<td>41.2 ± 1.2</td>
</tr>
<tr>
<td>Feed consumption (g/wk)</td>
<td>21.2 ± 0.04</td>
<td>29.8 ± 0.43</td>
<td>28.1 ± 0.8</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>383.7 ± 47.1</td>
<td>550 ± 56.8</td>
<td>893. ± 90.9</td>
</tr>
<tr>
<td>Insulin (pg/mL)</td>
<td>2624.6 ± 235.9</td>
<td>3934.2 ± 557.9</td>
<td>5682.9 ± 557.5</td>
</tr>
<tr>
<td>Leptin (pg/mL)</td>
<td>801.6 ± 293.9</td>
<td>10385.9 ± 960.3</td>
<td>10392.1 ± 608.9</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>12205.7 ± 898.5</td>
<td>9417.3 ± 666.9</td>
<td>9654.7 ± 568.2</td>
</tr>
<tr>
<td>Resistin (pg/mL)</td>
<td>1516.1 ± 84.7</td>
<td>2003.7 ± 213.4</td>
<td>2538.5 ± 167.3</td>
</tr>
</tbody>
</table>

\( ^{a} \text{N}=12\text{ mice/group; values are means ± SEM. Serum analyses were performed at study's end.} \\
^{b} \text{Value for each diet group differed significantly (p<0.05, Wilcoxon rank sum) from that in the other groups for this parameter.} \\
^{c} \text{Value differed significantly (p<0.05, Wilcoxon rank sum) in the 30\% CR group for this parameter relative to the other two groups.} \)
Characterization of Representative Lesions in Ventral Prostate HI-Myc Mice on the Different Diets at 6 Months

30% CR   Overweight Control   Obese

Blando et al, CAPR, 2011
Dietary Energy Balance Effects Progression of Prostate Cancer in Hi-Myc Mice on At Both 3 and 6 Months

3 Months

6 Months

Blando et al, CAPR, 2011
Dietary Energy Balance Manipulation Modulates Growth Factor Signaling Pathways in Prostate of Male C57BL/6 and FVB/N Mice

Dietary Energy Balance Effects Growth Factor Signaling in Ventral Prostate of Hi-Myc Mice

Blando et al, CAPR, 2011
IHC Staining for KI67, Cyclin D1, CD31 and p-NFkB IN Ventral Prostate of Hi-Myc Mice
Dramatic Increases are Seen in Stromal Inflammatory Cell Infiltration in Ventral Prostate of Hi-Myc Mice on an Obesity Inducing Diet
Perilipin Staining of Adipocytes in Ventral Prostate of Hi-Myc Mice on Different Diets at 6 Months

30% CR  Overweight Control  Obese
Dietary Energy Balance Dramatically Effects Expression of Inflammatory Cytokines and VEGF Family Members

IL-23 levels are dramatically upregulated.
The IL-23 Receptor is Highly Upregulated in Ventral Prostate of Hi-Myc Mice on an Obesity Inducing Diet at 6 Months

30% CR

Overweight Control

Obese
Obesity Increases Prostate Cancer Progression Through Multiple Mechanisms

From Kruijsdijk et al, CEBP 2009
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