Mechanisms of Calorie Restriction-Mediated Inhibition of Epithelial Carcinogenesis: Identifying Targets for Cancer Prevention

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Dietary energy balance (CR vs DIO) modulates steady state growth factor signaling in epithelial tissues in untreated mice.

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.
  – Rapamycin
  – Metformin
Energy Balance: Concept Defined

- **Normal**: $18.5 < \text{BMI} < 25$
- **Positive**: $\text{BMI} > 25$
- **Negative**: $\text{BMI} < 18.5$

**Energy Balance**

- **Energy Consumption**
- **Energy Expenditure**
Dietary Energy Balance and Cancer

• Many human cancers are associated with obesity.

*In Women*
- Breast (postmenopausal)
- Endometrial
- Cervical
- Uterine
- Ovarian
- Colorectal
- Kidney
- Liver/Gall Bladder
- Pancreatic
- Esophageal
- Hematopoietic

*In Men*
- Prostate
- Stomach
- Colorectal
- Kidney
- Liver/Gall Bladder
- Pancreatic
- Esophageal
- Hematopoietic

• Animal studies confirm an association between obesity and increased risk of cancer development in many tissues.

• Calorie restriction (CR) is a potent inhibitor of carcinogenesis in many animal models (i.e., chemical- or radiation-induced carcinogenesis; tumor transplant models; spontaneous tumorigenesis in knockout and transgenic models).

Renenhan et al., *Lancet*, 2008
Calle et al., *NEJM*, 2003
Potential Mechanisms Underlying the Link Between Energy Balance and Cancer

Moore et al, ANYAS, 2011
Dietary Energy Balance Modulates Steady State Growth Factor Signaling in Epithelial Tissues of Untreated Mice

Effect of Dietary Energy Balance Manipulation on Skin Tumor Promotion in ICR Mice
Multistage Epithelial Carcinogenesis in Mouse Skin: An Excellent Model for Identifying Targets and Mechanisms


**INITIATION**
- Apply subcarcinogenic dose of initiating agent
- Time: 2 wks

1. Metabolic activation of procarcinogens and covalent binding to DNA
2. DNA repair/cell replication and fixation of mutation
3. Mutation induction in critical target genes (e.g., Ha-ras) of “stem” cells in bulge region of hair follicle or basal compartment of interfollicular epidermis

**PROMOTION**
- Commence continual delivery of promoting agent
- Time: 10 - 40 wks

1. Increased DNA synthesis, inflammation
2. Altered gene expression/enzyme activities
3. Expansion of initiated stem cell population

**PROGRESSION**
- Time: 20 - 50+ wks

1. Production and maintenance of chronic cell proliferation
2. Development of clonal outgrowths called papillomas
3. Diploid lesions
4. Additional genetic events occur stochastically
5. Aneuploidy, LOH
6. Dysplasia
7. Conversion of papilloma to squamous cell carcinoma
8. Invasion
9. Metastasis

Normal skin → Hyperplastic epidermis → Papilloma → SCC

Elevated Akt Activity Contributes Significantly to Skin Tumor Promotion Through Multiple Downstream Signaling Pathways

Segrelles et al, Cancer Res., 2007
Lu et al, MCR, 2007
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.
Caloric Density of Diets Used to Examine the Effect of Dietary Energy Balance on Skin Tumor Promotion

<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&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Lean</td>
<td>29</td>
<td>57</td>
<td>14</td>
</tr>
<tr>
<td>15% CR&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Normal</td>
<td>24</td>
<td>65</td>
<td>12</td>
</tr>
<tr>
<td>AIN76A&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Overweight</td>
<td>20</td>
<td>70</td>
<td>10</td>
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<tr>
<td>DIO</td>
<td>Obese</td>
<td>20</td>
<td>20</td>
<td>60</td>
</tr>
</tbody>
</table>

Moore et al, CAPR, 2008; Moore et al, unpublished
Weight Gain and Selected Serum Protein Profiles of Female ICR Mice in the Two-Stage Skin Carcinogenesis Study

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

Moore et al, unpublished data
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

**Tricia 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(^1)</th>
<th>Carcinoma Incidence (%)</th>
<th>Carcinomas per Mouse</th>
<th>Conversion Ratio(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60kcal% fat</td>
<td>25</td>
<td>8.86</td>
<td>96.0</td>
<td>2.28(^a)</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(^a)</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(^a)</td>
<td>57.7</td>
<td>0.96(^a)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

\(^1\)Data taken at 27 weeks of promotion with 3.4 nmol TPA after which the papilloma response had reached a plateau

\(^2\)Ratio of the average number of SCCs at 50 weeks to average number of papillomas at 27 weeks

\(^a\)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

Tricia Moore et al, unpublished data

* Significantly different from other groups Wilcoxon Rank Sum p < 0.05
Summary of Dietary Energy Balance Effects on Growth Factor Signaling and Cell Cycle Related Proteins During Tumor Promotion

Moore et al, CPR, 2008 and unpublished data
Overexpression of IGF-1 in Epidermis of Transgenic Mice (BK5.IGF-1) Leads to Elevated Levels of Cell-Cycle Regulatory Proteins

Liver IGF-1 Deficient (LID) Mice as a Model for Reduced Circulating IGF-1

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.


Yakar et. al. *PNAS*, 1999
Reduced Circulating IGF-1 Inhibits Two-Stage Skin Carcinogenesis


TPA 13.6, Wt
TPA 6.8, Wt
TPA 13.6, LID
TPA 6.8, LID
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

Treatment of Primary Mouse Keratinocytes with IGF-1 Leads to Activation of EGFR and ErbB2

Moore et al, unpublished
Dietary Energy Balance Alters Heterodimer Formation of the IGF-1R and EGFR

Moore et al, unpublished
HK1.IGF-1 Transgenic Mice: A Model of Inducible IGF-1 Expression in Mouse Epidermis

Bol et al., 1997; Wilker et al., 1999
Inducible Expression of IGF-1 Leads to Enhanced Activation of EGFr & IGF-1R in Epidermis of TPA Treated HK1.IGF-1 Mice

Wilker et al., Mol Carcinogenesis 1999
Summary and Conclusions I

- Dietary energy balance (CR vs DIO) modulates steady state growth factor signaling in multiple epithelial tissues of normal, untreated mice.

- CR inhibited while DIO enhanced epidermal proliferation and skin tumor promotion by TPA.

- CR decreased while DIO increased activation of the IGF-1R and EGFR, as well as signaling downstream to multiple effectors, including Akt and mTOR during tumor promotion. Levels of cyclin D1, cyclin E and c-myc directly correlated with caloric density, while levels of p27 and p21 inversely correlated with caloric density.

- Reduced circulating IGF-1 levels (i.e., LID mice) inhibited TPA skin tumor promotion, TPA-induced epidermal hyperproliferation and signaling downstream of both the IGF-1R and EGFR. Alterations in circulating IGF-1 levels modulated cross talk between the IGF-1R and EGFR in keratinocytes both in vitro and in vivo.

- Dietary energy balance affects susceptibility to skin tumor promotion, at least in part, through alterations in IGF-1R/EGFR signaling pathways (including Akt/mTOR). These changes subsequently modulate levels and/or activity (phosphorylation) of cell cycle related proteins, thus altering epidermal proliferation.
Can We Identify Calorie Restriction Mimetic Compounds that Can Block Tumor Promotion?

- Rapamycin
- Metformin
- Pentacyclic Triterpines (P. frutescens)
Targeting mTORC1 Signaling

Growth factors → PI3K → Akt → mTORC1 → S6K1, 4E-BP1 → mRNA translation → Cell growth↑, Autophagy↑

mTORC2 → PRAS40 → mTORC1

mTORC1 → Raptor, GβL → mTORC2

PTEN → PI3K

MEK → MAPK → RSK

TSC2, TSC1 → PI3K, Akt

GSK-3 → REDD1, AMPK

Hypoxia → REDD1

Energy stress → AMPK

LKB1 → AMPK

Wnt signaling → GSK-3, REDD1

Rapamycin, Metformin
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

acetone
TPA 6.8 nmol
Rapa 200nmol only
Rapa 200nmol + TPA
Rapa 100nmol + TPA
Rapa 50nmol + TPA
Rapa 20nmol + TPA
Rapa 5nmol + 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

Checkley et al, CAPR, 2011

* P < 0.05
Rapamycin Treatment Inhibits TPA-Induced Skin Inflammation

Checkley et al, CAPR, 2011
Treatment with Rapamycin and a Phytochemical Combination Suppresses Proliferation/Migration of Keratinocyte Stem Cells During Tumor Promotion

From Alonso & Fuchs, PNAS, 2003
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

TPA

Papillomas Per Mouse

Weeks of Promotion

Percent of Mice with Papillomas

Weeks of Promotion

250 mg/kg met + TPA
50 mg/kg met + TPA

Checkley et al, unpublished
Metformin Given in the Drinking Water Attenuates TPA Induced Epidermal Hyperproliferation

![Graphs showing labeling index and epidermal thickness across different treatment groups.](image)

* , p< 0.05  

Checkley et al, unpublished data
Metformin Activates Epidermal AMPK and Attenuates TPA Induced Signaling Through mTORC1

Multiple (4X) Treatment Protocol

<table>
<thead>
<tr>
<th></th>
<th>Ace</th>
<th>MET 250</th>
<th>TPA 250</th>
<th>TPA + MET 50</th>
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<tbody>
<tr>
<td>actin</td>
<td></td>
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</tr>
<tr>
<td>pS6r\textsuperscript{235/236}</td>
<td></td>
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<tr>
<td>pS6r\textsuperscript{240/244}</td>
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<tr>
<td>PDCD4</td>
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</tr>
<tr>
<td>p-4E-BP\textsuperscript{37/4}</td>
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</tr>
</tbody>
</table>

\* p < 0.05

Checkley et al, unpublished
Multiple Downstream Pathways are Regulated by AMPK

From van Veelen et al, Oncogene 30:2289, 2011
Summary and Conclusions II

• Rapamycin was an extremely potent inhibitor of skin tumor promotion by TPA. Topical doses as low as 5 nmol per mouse produced significant inhibition.

• Rapamycin effectively inhibited TPA induced epidermal hyperproliferation at doses that inhibited skin tumor promotion.

• Rapamycin also blocked TPA-induced skin inflammation as shown by a reduced dermal infiltration of multiple inflammatory cells.

• Rapamycin effectively inhibited mTORC1 activity primarily through the p70S6K downstream pathway.

• Preliminary experiments show that Metformin given in the drinking water also blocked TPA skin tumor promotion and downstream mTORC1 signaling.

• CR mimetic compounds that block mTORC1 signaling are effective inhibitors of skin tumor promotion by TPA.

• Targeting mTORC1 signaling pathways may be an effective strategy either alone or in combination with other pytochemicals for mimicking CR and for chemoprevention of epithelial cancer.
mTORC1 Signaling Pathway as a Potential Target for Mimicking the Effects of CR on Cancer Promotion

GFR → PI3K → Akt → mTORC1

p-T308 → p-S473

mTORC2

S6 Ribosomal Protein Translation → Tumor Promotion

AMPK

Metformin, Phytochemicals, CR

Rapamycin, Rapalogs, Phytochemicals, CR
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