Dissecting the Obesity-Cancer Link: Mechanistic Insights from Animal Models

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and
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Today’s Presentation

• Links between obesity, energy balance and cancer in animal models
• Lessons from studies in mice regarding molecular targets and strategies for breaking the obesity-cancer link
  - Calorie Restriction
  - Diet- and Genetically-Induced Obesity
  - Physical Activity
  - Calorie Restriction Mimetics (mTOR/IGF-1R inhibitors; bioactive food components)
Disclosure Information
Stephen D. Hursting

I have no financial relationships to disclose.
I will discuss an experiment using Rad001 (Everolimus) in my presentation.
The Shape of Things to Come. The Economist 12/11/03

(http://www.economist.com/displaystory.cfm?story_id=2282754)
Metabolic Syndrome

Describes a state of metabolic dysregulation characterized by:

- Insulin resistance
- Elevated bioavailable IGF-1
- Pro-inflammatory state (elevated CRP, cytokines)
- Altered adipokines (elevated leptin)
- Pro-coagulant changes
- Dyslipidemia (hypertriglyceridemia)

Associated with many types of cancer
(Calle, et al., NEJM 2003: 14% of cancer deaths in men; 20% in women due to overweight/obesity)
Energy Balance and Cancer Prevention

Energy Balance:
\[ \text{kcal in} = \text{kcal out} \]

- Amount
- Type
- Pattern

- Physical Activity
- Routine Metabolism
- Thermoregulation
- Growth
- Storage
Calorie Restriction (~20%) Extends Lifespan in Multiple Species


% Increase in Longevity (versus *ad libitum*-fed controls)
**Control**

<table>
<thead>
<tr>
<th>8.5 yrs.</th>
<th>22.0 yrs.</th>
</tr>
</thead>
</table>

| 9.0 yrs. | 22.4 yrs. |

**CR**

<table>
<thead>
<tr>
<th>8.5 yrs.</th>
<th>22.0 yrs.</th>
</tr>
</thead>
</table>

| 9.0 yrs. | 22.4 yrs. |

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**Weindruch: CR Study in Rhesus Monkeys**  
(Colman, et al., *Science* 2009)
Calorie Restriction Inhibits Spontaneous Tumorigenesis in Multiple Model Systems

<table>
<thead>
<tr>
<th>Experimental System</th>
<th>Animals</th>
<th>Degree of Calorie Restriction</th>
<th>Ratio of incidence in AL/CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary</td>
<td>DBA mice</td>
<td>33%</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>MMTV-neu TG</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MMTV-Wnt-1 TG</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wistar rats</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mammary</td>
<td>C3H mice</td>
<td>33%</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Swiss mice</td>
<td>20%</td>
<td>7</td>
</tr>
<tr>
<td>Liver</td>
<td>AK mice</td>
<td>25%</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>F344 rats</td>
<td>25%</td>
<td>9.3</td>
</tr>
<tr>
<td>Leukemia</td>
<td>CD1</td>
<td>40%</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>Sencar mice</td>
<td>40%</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Wistar rats</td>
<td>20%</td>
<td>6</td>
</tr>
<tr>
<td>Skin</td>
<td>Wistar rats</td>
<td>20%</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>Swiss mice</td>
<td>20%</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>H:NMRI mice</td>
<td>20%</td>
<td>4.1</td>
</tr>
<tr>
<td>Pituitary</td>
<td>COBS rats</td>
<td>30%</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>K-ras x Ink4A mice</td>
<td>30%</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>K5-COX-2 TG mice</td>
<td>30%</td>
<td>5.5</td>
</tr>
<tr>
<td>Pancreas</td>
<td>F344 rats</td>
<td>40%</td>
<td>1.7</td>
</tr>
<tr>
<td>Testes</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Cancer: A Complex Foe

The essential aberrations of cancer

- Dysregulated growth signals and cellular energetics
- Inflammation
- Genomic instability
- Tissue invasion and metastasis
- Sustained angiogenesis
- Limitless replicative potential
- Evading growth suppression, apoptosis, and immune surveillance

Adapted from: Hanahan & Weinberg, Cell (2000) and Cell (2011)
Modeling Energy Balance and Human Cancer in Mice by Altering Key Genes and Pathways

Hursting, et al., *Mutation Res*, 2005
### Growth Factor Levels and MMTV-Wnt-1 TG Mammary Tumor Growth in CR, Overweight and Diet-Induced Obese (DIO) Mice

<table>
<thead>
<tr>
<th></th>
<th>IGF-1 (ng/ml)</th>
<th>Insulin (pg/ml)</th>
<th>Leptin (ng/ml)</th>
<th>Adiponectin (ng/ml)</th>
<th>L/A</th>
<th>Tumor Vol (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CR (30%)</strong></td>
<td>390</td>
<td>380</td>
<td>1.9</td>
<td>9.4</td>
<td>0.2</td>
<td>120</td>
</tr>
<tr>
<td>(29% body fat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Overweight</strong></td>
<td>526</td>
<td>398</td>
<td>5.3</td>
<td>9.2</td>
<td>0.6</td>
<td>510</td>
</tr>
<tr>
<td>(35% body fat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DIO</strong></td>
<td>718</td>
<td>596</td>
<td>16</td>
<td>9.1</td>
<td>1.8</td>
<td>1485</td>
</tr>
<tr>
<td>(47% body fat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n=12 mice/group

Adapted from: Nunez, et al., *Nutrition and Cancer*, 2007

Dr. Nomeli Nunez
Genetic Reduction of Systemic IGF-1

~75% of IGF-1 in serum -- liver

Ecuadorians with Laron Syndrome Have Very Low IGF-1, Increased Longevity, and Virtually No Cancer or Diabetes. NY Times 2/16/11.

S. Yakar and D. LeRoith, PNAS, 1999
Mammary Tumor Growth in Control and Liver IGF-1 Deficient (LID) Mice

Dr. Nikki Ford

Serum IGF-1 Levels

Control

LID

LID/CR

LID/DIO

Tumor vol (mm³)

Days after tumor cell injection

Dr. Nikki Ford
Diabetic A-Zip/F-1 Mice Lack WAT But Display Increased Susceptibility to Mammary and Skin Carcinogenesis


**Serum:**
- Insulin, IGF-1
- Cytokines
- Adipokines

**Tissue:**
- pAkt
- pmTOR
Dietary Energy Balance Modulation of Akt/mTOR Signaling (normal and tumor tissue)

Skin
Liver
Prostate
Colon
Pancreas
Mammary

Hursting, et al., Cancer Res, 2007
Moore, et al., Cancer Prev Res, 2008;
Olivo-Marston, et al., Mol Carcinogenesis 2009
deAngel, et al., Mol Carcinogenesis, in press
The Effect of IGF-1 Infusion on Growth of Orthotopically Transplanted MMTV-Wnt-1 Mammary Tumors in Calorie Restricted Mice

Consistent with Kalaany and Sabatini (*Nature* 2009)—PIK3CA ablates CR effects

Phenotypes of Genetically Obese \textit{db/db} and \textit{ob/ob} Mice

- Morbid obesity - early onset
- Hyperphagic –2X the intake of controls
- Hyperglycemic
- Hyperinsulinemic, but low IGF-1 and HMW adiponectin
- \textit{db/db}: Hyperleptinemic/leptin resistant - mutant leptin receptor
- \textit{ob/ob}: Hypoleptinemic; no circulating WT leptin
Wnt-1 Mammary Tumor Growth Is Increased in \( db/db \) Mice but Suppressed in \( ob/ob \) Mice

( Zheng, et al., Endocr Relat Cancer, 2011 )

<table>
<thead>
<tr>
<th></th>
<th>( ob/ob )</th>
<th>( WT )</th>
<th>( db/db )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin:</td>
<td>Ê Ê Ê</td>
<td>Ê</td>
<td>Ê Ê Ê</td>
</tr>
<tr>
<td>IGF-1:</td>
<td>í</td>
<td>Ê</td>
<td>í</td>
</tr>
<tr>
<td>L/A:</td>
<td>í</td>
<td>Ê</td>
<td>Ê Ê Ê</td>
</tr>
<tr>
<td>Tumor Wt (g)</td>
<td>0.21 ± 0.07</td>
<td>0.62 ± 0.13</td>
<td>1.68 ± 0.33</td>
</tr>
</tbody>
</table>
Converging Signaling Pathways

Obesity/Insulin Resistance

Insulin, IGF-1

IRS

PI3K

Akt

JAK2

STAT3

AdipoR1

AmphoR1

AMPK

TSC1

TSC2

mTOR

Rapamycin
Survival plots for male and female mice, comparing control mice to those fed rapamycin (2.4 mg/kg/day) in the diet starting at 600 days of age, pooling across the three test sites.
CR and Rapamycin (but not Treadmill Exercise) Reverse the Effects of DIO on Transplanted Wnt-1 Tumor Growth

Tumor Weight (mg)

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>61 ± 19</td>
</tr>
<tr>
<td>Treadmill Exercise</td>
<td>347 ± 211</td>
</tr>
<tr>
<td>DIO</td>
<td>394 ± 243</td>
</tr>
<tr>
<td>+ Rapamycin</td>
<td>Not done</td>
</tr>
<tr>
<td>- Rapamycin</td>
<td>92 ± 23</td>
</tr>
</tbody>
</table>

n=15 mice/group

Nogueria, et al., *Endocr Relat Cancer*, in press
# Mouse Models of Physical Activity and Carcinogenesis

## CR, Voluntary Wheel Running Decreases Polyp Formation in \( APC^{\text{min}} \) Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Polyps (SE)</th>
<th>Polyps &gt; 2mm (SE)</th>
<th>Survival 100 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=20)</td>
<td>26.2 (2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.3 (1.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wheel (n=21)</td>
<td>21.2 (2.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.2 (2)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CR (n=20)</td>
<td>3.8 (0.6)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.1 (0.4)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The mTOR Inhibitor RAD001 (Everolimus) Inhibits Wnt-1 Mammary Tumor Growth in Lean, Control and Obese Mice

DeAngel, et al. Mol Carcinogenesis, in press
Cancer: A Complex Foe

Energy balance impacts the essential aberrations of cancer

- Dysregulated growth signals and cellular energetics
- Inflammation
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Adapted from: Hanahan & Weinberg, Cell (2000) and Cell (2011)
Dynamics of epithelial tissues: epithelial-mesenchymal transition (EMT)

Specific epithelial cells receive signals to differentiate.

Cells protrude out of epithelium, disassemble cell-cell junctions.

Cells become ‘mesenchymal’ (invasive and migratory) and then differentiate to a new cell type in another location.

Does Obesity Promote EMT? Enrich Breast Cancer Stem Cells?
Effects of Energy Balance on M-Wnt (Claudin-low) and E-Wnt (Basal-like) Mammary Tumors and EMT Markers (Dunlap, et al., Cancer Res, submitted)
Energy Balance Modulates Adipocyte/Tumor Cell Interactions in M-Wnt (Claudin-low) Mammary Tumor Cells

Sarah Dunlap, et al., Cancer Res, submitted
Targets and Pathways for Intervention

Inflammation

Cell Signaling

Epigenetics

Breaking the Obesity Cancer Link
Acknowledgements

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National Cancer Institute
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Cleveland Clinic/Case Western
  Ofer Reizes

University of Kansas Medical Center
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