Can Animal Models Extend our Understanding of the Role Vitamin D Plays in Cancer Prevention?

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Overview

• What are we trying to model?
  • Vitamin D biology
  • Cancer

• Is there in vivo proof of principle for vitamin D/cancer relationship?
  • Lack of vitamin D signaling (deficiency, knockouts)
  • 1,25(OH)2 D, Analogs

• How does dietary vitamin D influence cancer in animal models?
Challenge: Linking Population Science with Cell Biology

A. Inverse association between serum 25OHD and cancer risk


B. Cell culture: potential anticancer mechanisms for 1,25(OH)2 D

Fleet et al. 2011 Biochem J. In Press
Vitamin D is Metabolized and Acts Like a Hormone

Sunlight

25 hydroxyvitamin D

1,25 dihydroxyvitamin D

Active Hormone

Status Indicator
Vitamin D-Mediated Cancer Prevention May Require Local Activation

High Diet VD

Autocrine Production

1,25(OH)₂ D Action

Less Cancer

Calcium Regulating Endocrine System

Low 1α hydroxylase expression is in many tissues BUT... local production has not yet been demonstrated in vivo.
What are we trying to “model” in animals?

Part I: Human Cancers
Example: Clinical and Molecular Classification of Colon Cancer

Gene Mutations
- Loss of APC activity
- Activation of K-ras
- Loss of SMAD 2-4 activity
- Loss of p53 activity

Fearon and Vogelstein, 1990, Cell 61:759
When Can We Hope for Improved Vitamin D Status to Be Effective??

Early Stages of Carcinogenesis

Late Stages of Carcinogenesis

Vitamin D

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Potential Vitamin D-Mediated Chemoprevention

Normal Colon Cells

ACF

Polyp

Class I Adenoma

Class II Adenoma

Class III Adenoma

Carcinoma

Metastasis

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Fearon and Vogelstein, 1990, Cell 61:759
Proximal and Distal Colon Cancer is Different

Proximal

- Microsatellite Instability

Gene Defects
- DNA Mismatch Repair Enzymes
- $\beta$ catenin

Distal

- Adenoma to carcinoma
- Chromosome Instability

Gene Defects
- APC, K-ras, SMAD, p53

Vogelstein model

Colorectal Cancer

Bedenne et al., 1992, Cancer 69:833

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Pathway Based Classification of Breast Cancer Identifies 17 Tumor Subtypes

Microarray analysis of 1143 tumors
Pathway Signature Classification
Validation in 547 new tumor samples

Gatza et al., 2010 PNAS 107:6994

Histological subtypes

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Animal Models for Cancer Research

I. Chemically Induced (Rats, Mice)
- DNA damaging agents causing multiple mutations
- Relevance to human cancer?
- Multiple tissues affected

II. Spontaneous
- Companion Animals
- “Western Diet”-Induced

III. Genetic
- Knockout – elimination of genes that prevent cancer
- Transgenic – over-expression of specific cancer genes
- Natural Mutants (e.g. APC\textsuperscript{min}, FAP model)

IV. Implanted Tumors/Cells (late stage model)
- Xenograph – across species (requires immune deficient mouse)
- Orthograph – within species

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Example; Choosing an Animal Model for Prostate Cancer Research

The Human Prostate Cancer Cascade

Timeline:
* Initiation (mutations)
* Promotion
* Invasion and Metastasis
* Progression: Androgen Dependent Metastases
  Castrate Resistant Metastases

Correlation with Experimental Models:

- **Chemical**
  - NMU-Androgen: 1 year

- **Transgenic**
  - APT121 Tg: 6 months
  - TRAMP: 4 months
  - PTEN +/-: 1 year

- **Implanted**
  - LNCaP xenografts: 1 month

- **Pre-initiation**
  - Castration/Repletion: 2 weeks

* Fleet 2011*
What are we trying to “model” in animals?

Part II: Vitamin D Status
Challenge: There are two Hypotheses to Be Tested

**Hypothesis 1:** Benefit comes from preventing deficiency

**Hypothesis 2:** Additional benefit from optimizing serum 25OHD

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**Graphs:**

- **Risk of Cancer** vs. **Serum 25OHD (nmol/L)**
  - **Y-axis:** Risk of Cancer
  - **X-axis:** Serum 25OHD (nmol/L)
  - **Legend:** IOM Level
  - **Graph 1:** Decreasing risk with increasing serum 25OHD levels
  - **Graph 2:** Decreasing risk with increasing serum 25OHD levels

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Assessing VD Status with Serum 25OH D

- Deficient: < 25 nmol/L
- Insufficient: IOM: < 50 nmol/L
- Proposed optimal: > 80 nmol/L

Modeling Human Health Relevant Ranges of Vitamin D Status in Mice

Mouse: 11 week feeding Trial

No UV light

“optimal”

deficient

Diet VD₃ (IU/kg)

Serum 25OH D (nmol/L)

Data from 10 published human trials

Total Vitamin D Intake (IU/d)

Fleet 2008 J. Nutr. 138:1114
What can Treatment with $1,25(\text{OH})_2\text{D}$ or Analogs tell us?

- 100’s of analogs have been developed
- Most cause hypercalcemia
- Some have been developed for treatment of cancer
- None have been approved for clinical use

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Is cancer development accelerated in mice lacking the vitamin D signal?

Vitamin D receptor (VDR) knockout mice
(CYP27B1 KO mice)
**Caution: VDR Knockout Mice have Disrupted Calcium Metabolism**

**Phenotype**

- Hypocalcemia*
- Osteomalacia*
- Alopecia
- Low Body Fat.....

* Corrected by High Calcium Rescue Diets

**VDR KO =**

Type II Genetic Rickets

No evidence that VDR KO mice spontaneously develop internal organ cancers

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VDR Deletion Alters Normal Mammary Growth

Mice fed high Ca “rescue” diet to prevent hypocalcemia

Zinser et al. 2002, Development 129:3067
VDR KO Increases Breast Cancer Development

MMTV-neu Transgenic mice

<table>
<thead>
<tr>
<th></th>
<th>VDR&lt;sup&gt;+&lt;/sup&gt;/+</th>
<th>VDR&lt;sup&gt;+&lt;/sup&gt;/−</th>
<th>VDR−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>% thick/dilated ducts</td>
<td>17</td>
<td>67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Duration to first tumor (months)</td>
<td>8.5</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Time to 50% tumor incidence (months)</td>
<td>18.5</td>
<td>14.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Zinser and Welsh 2004, Carcinogenesis 25:2361
VDR Deletion Alters Normal Colonic Epithelium

Distal colon, 12-15 wks old

Fed on standard diet with extra Calcium in drinking water

Kallay et al., 2001, Carcinogenesis 22:1429
VDR Deletion Alters Tumor Size in APC^{min} Mice

Fed standard chow diet 5 months

Larriba et al., 2011, PLoS One 6:e23524

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Dietary Vitamin D Studies

Severe Vitamin D deficiency
“Western” Diet
Supplementation Trials
Fleet/Clinton collaboration

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Vitamin D Deficiency Increases Growth of Implanted Tumor Cells

Model of Late Stage Colon Cancer
MC-26 Mouse Colon Tumor Cells (Orthologous model)

Vitamin D Status
Deficient
25OHD < 12.5 nmol/L

Sufficient
25OHD < 66±15 nmol/L

Vitamin D Deficiency Enhances Bone Lesions Caused by Prostate Cancer Cells

Plasma 25OHD
- 98±10 nmol/L
- 5±2 nmol/L

Model for Bone Damage Caused by Cancer Metastasis
- Nude Mouse +/- VD diet
- Inject PC-3 into bone
- uCT (14 wks)

Similar studies done with breast cancer cell lines (Ooi et al. 2010 Cancer Res. 70:1835)

Zheng et al., 2011, Prostate 71:1012

Fleet 2011
Does a “Western Diet” Cause Cancer?

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NWD₁</th>
<th>NWD₂</th>
</tr>
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<tbody>
<tr>
<td>Ca (%)</td>
<td>0.5</td>
<td>0.05</td>
<td>0.7</td>
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<tr>
<td>Vitamin D IU/kg</td>
<td>1000</td>
<td>110</td>
<td>2300</td>
</tr>
<tr>
<td>Kcal/g</td>
<td>3.6</td>
<td>4.5</td>
<td>4.5</td>
</tr>
</tbody>
</table>

High fat, low folate, choline, methionine too

> 46 papers published using this diet

Hyperplasia and hyperproliferation seen in......
epithelial cells of the Breast, Colon, Exocrine Pancreas, Prostate, and Bladder

Colon effect inhibited by dietary Calcium Only

Richter et al., 1995, Carcinogenesis 16:2685
**Goal:** Model Human Health Relevant Ranges of Nutrient Intake in Mice

**Vitamin D Status**

- **Diet VD₃ (IU/kg)**
  - 0
  - 200
  - 400
  - 600
  - 800
  - 1000

- **Serum 25OH D (nmol/L)**
  - a
  - b
  - c
  - d

- **“optimal”**
- **deficient**

**Low Ca Diets Reduce Bone**

- **AIN93G**

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Fleet 2008 J. Nutr. 138:1114
“Western” Diet Increases Spontaneous Colon Cancer in Mice

Yang K et al. 2008, Cancer Res 68:7803

<table>
<thead>
<tr>
<th></th>
<th>AIN</th>
<th>NWD</th>
<th>NWD +</th>
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<tbody>
<tr>
<td>Ca (%)</td>
<td>0.5</td>
<td>0.05</td>
<td>0.7</td>
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<td>Kcal/g</td>
<td>3.6</td>
<td>4.5</td>
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“Western” Diet Increases Cancer in \( \text{APC}^{\text{min}} \) Mice

Human FAP model; most tumors in small intestine

<table>
<thead>
<tr>
<th></th>
<th># Tumors/mouse</th>
<th>Tumor Volume (mm(^3))</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>AIN76A</td>
<td>Western</td>
</tr>
<tr>
<td>p21 +/-</td>
<td>1.7 ± 1.9</td>
<td>4.3 ± 1.9(^d)</td>
</tr>
<tr>
<td></td>
<td>9.6 ± 8.9</td>
<td>17.5 ± 13.0(^e)</td>
</tr>
<tr>
<td>p21 +/-</td>
<td>2.1 ± 1.5</td>
<td>6.6 ± 3.9(^{b,d})</td>
</tr>
<tr>
<td></td>
<td>12.1 ± 10.7</td>
<td>23.0 ± 15.2(^{b,d})</td>
</tr>
<tr>
<td>p21 +/-</td>
<td>3.7 ± 1.4(^c)</td>
<td>7.5 ± 4.9(^{b,f})</td>
</tr>
<tr>
<td></td>
<td>16.7 ± 13.3(^c)</td>
<td>27.3 ± 20.2(^{c,d})</td>
</tr>
</tbody>
</table>

*Mice surviving to 36 wks only*

*Effect of p21 deletion and Western diet are independent*

Yang et al., 2001, Cancer Res. 61:565
Many Dietary Vitamin D Studies Supplement at High Levels

<table>
<thead>
<tr>
<th>Study</th>
<th>Vitamin D levels (IU/kg diet)</th>
<th>Effect</th>
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</thead>
<tbody>
<tr>
<td>DMBA/TPA-induced Skin Cancer Pence, 1991 Nutr. Cancer</td>
<td>200 to 4000</td>
<td>None</td>
</tr>
<tr>
<td>DMH induced colon cancer Comer 1993 Nutr. Cancer</td>
<td>250 to 10,000</td>
<td>None</td>
</tr>
<tr>
<td>DMH induced colon cancer Beaty 1993 J. Nutr.</td>
<td>1000 to 4000 (0.5, 1.0, or 1.5% Ca)</td>
<td>45% lower; 4000 IU + 1.5% Ca (p=0.12);</td>
</tr>
<tr>
<td>PTEN+/− endometrial lesions Yu 2010 Canc. Prev. Res.</td>
<td>1,800 vs 25,000</td>
<td>25% reduction in lesions</td>
</tr>
<tr>
<td>APC&lt;sup&gt;−/−&lt;/sup&gt; +DSS, intestinal cancer Irving 2011 Arch Biochem Biophys</td>
<td>Serum 25OHD 65 vs 725 nmol/L</td>
<td>None;</td>
</tr>
</tbody>
</table>

• 25OH D levels > 65 nmol/L (All hypothesis II)
• 25OH D >400 nmol/L = VDR activation = pharmacologic

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Studies on Dietary Vitamin D and Early Prostate Cancer in Mice

Collaboration between Fleet lab (Purdue) and Clinton Lab (Ohio State U)
Examination of Normal Prostate Biology using the Castration-Repletion Model

“recapitulates the high risk phases of mutagen sensitivity during human adolescence, when prostate gland size increases 5-fold and when PIN first appears”

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Examination of Normal Prostate Biology using the Castration-Repletion Model

**Normal Prostate**

Intact -> Castration at 9 wk -> Prostate Regression (Castrated) -> Testosterone infusion for 5d at 10 wk -> Prostate Growth (Castrated + T)

**PEC Apoptosis**

TUNEL Staining

**PEC Proliferation**

Ki-67 Staining

**Dietary Vitamin D₃ from Weaning**

<table>
<thead>
<tr>
<th>Diet VD (IU/kg)</th>
<th>Serum 25OHD (nmol/L)</th>
<th>Condition Modeled</th>
</tr>
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<tbody>
<tr>
<td>25</td>
<td>25±1</td>
<td>Deficient</td>
</tr>
<tr>
<td>200</td>
<td>83±5</td>
<td>“Optimal”</td>
</tr>
<tr>
<td>10,000</td>
<td>230±6</td>
<td>Supraphysiologic</td>
</tr>
</tbody>
</table>

Fleet 2011
Dietary Vitamin D Deficiency Increases PEC Proliferation and Apoptosis: Wild Type Mice

Kovalenko et al. 2011, Cancer Prev Res 4:1617

* p < 0.05
n = 6 per group
Anterior Prostate Lobe

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Example: Choosing an Animal Model for Prostate Cancer Research

- Slow growing
- Doesn’t progress

**The Human Prostate Cancer Cascade**

* Initiation (mutations)*
* Promotion*
* Invasion and Metastasis*
* Progression: Androgen Dependent Metastases
* Castrate Resistant Metastases

**Correlation with Experimental Models**

- **NMU-Androgen**: 1 year
- **APT121 Tg**: 6 months
- **TRAMP**: 4 months
- **PTEN +/-**: 1 year
- **LNCaP xenografts**: 1 month
- **Castration/Repletion**: 2 weeks

_Fleet 2011_
Dietary Vitamin D Deficiency Increases PEC Proliferation and Apoptosis: APT121 Mice

* p < 0.05

n = 6 per group
Anterior Prostate Lobe

Kovalenko et al. 2011, Cancer Prev Res 4:1617  
Fleet 2011
Creation of Mice with Prostate Epithelial Cell Specific VDR Deletion

This avoids whole body effects of VDR deletion on Calcium metabolism.....

VDR mice with a floxed Exon 2 allele

Crossed to ......

Probasin-Cre transgenic mice (PEC-specific expression upon maturation)

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Prostate Epithelial Cell Specific VDR Deletion Increases Proliferation

AIN76A diet with 200 IU vitamin D₃/kg

Proliferation

Apoptosis

n = 8 per group
Anterior Prostate Lobe

Kovalenko et al. 2011, Cancer Prev Res 4:1617
What Effect Does Diet Have on Prostate Cancer Promotion?

- APT121 Mice
- Feed diets from Weaning to 7 months
- Assessments:
  - Serum Vitamin D metabolites
  - H&E Stained Sections
  - 2001 Bar Harbor Pathology Workshop

<table>
<thead>
<tr>
<th>% Diet Ca</th>
<th>25</th>
<th>150</th>
<th>1000</th>
</tr>
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<tbody>
<tr>
<td>0.2</td>
<td>n = 30</td>
<td>n = 30</td>
<td>n = 30</td>
</tr>
<tr>
<td>0.5</td>
<td>n = 30</td>
<td>n = 30</td>
<td>n = 30</td>
</tr>
<tr>
<td>1.5</td>
<td>n = 30</td>
<td>n = 30</td>
<td>n = 30</td>
</tr>
</tbody>
</table>

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Does High Diet Ca Increase Prostate Cancer Risk?

**Chan et al. 1998**
Cancer Causes Control 9:559

Sweden: Ca > 1189 mg/d
- \( RR_{total} = 1.91 \)
- \( RR_{metastatic} = 2.64 \)

At least 10 other studies show a similar increase in risk!!!

Potential Mechanism:
Suppression of \( 1,25(OH)_2 D \)

High Diet Ca = Low PTH = Low serum \( 1,25 \) D = reduced VDR signaling
Diets Caused Expected Changes in Serum VD Metabolites

![Graph showing changes in 25OH D (nmol/L) and 1,25(OH)₂ D (pg/ml) with different diets and percent diet calcium.](image-url)
Development of Prostate Cancer in the Anterior Lobe of APT121 Mice

Anterior Prostate Lobe

- normal
- mPIN
- microinvasive carcinoma

E-H: Variants of adenocarcinoma

H&E 400X

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High VD Reduces the Progression to Advanced Phenotypes

mPIN (early lesion)  Micro-invasions (late lesion)

n = 90/group

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Are there Different Requirements for Prostate vs Bone Health?

Prostate Cancer

Bone Mineral Density

Diet VD3 (IU/kg)
Diet Calcium (%)

Driven by Diet Vitamin D
Driven by Diet Calcium

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Principle Proven: Lifelong Improvements in Vitamin D Status Protect against Prostate Cancer

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Summary

• Model selection is important

• Vitamin D deficiency, loss of VDR, 1,25(OH)₂ D, or analogs:
  • proof of principle
  • limited applicability for prevention

• Human-relevant ranges of vitamin D status and cancer?
  • Few studies do this
  • Few models have been evaluated
  • Our current studies in prostate cancer suggest a benefit to serum 25OHD that are higher than those that protect bone health

• Missing link: Demonstrating local production of 1,25(OH)₂ D
Questions?

CLOSE TO HOME

“I know there’s no sunshine down here, but I bet we still get good vitamin D from the flames!”

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