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University at Albany Cancer Research Center
Rensselaer, NY
Vitamin D Biology

Endogenous vitamin D₃ (skin)

Dietary Vitamin D₂ and D₃ (intestine)

Vitamin D → 25(OH)D → 1,25(OH)₂D → Vitamin D Receptor

Catabolism

(Cyp2r1) (Cyp24a1) (Cyp27b1)

Circulating form (nM) Active form (pM)
Vitamin D and Cancer: Evidence and Mechanisms

- Epidemiologic
  - Serum 25D & risk
  - VDR polymorphisms
- Animal Models
  - Dietary vitamin D reduces tumor development
- Laboratory
  - Control of cell cycle, apoptosis, differentiation

Possible Link between Vitamin D, Immune Responses and Breast Cancer?
Effects of 1,25D on Breast Cancer Cells

MCF-7 cells

Control

1,25D

1,25D

↑ p21, p27
↓ D cyclins/cdks
↑ G_0/G_1
↓ S phase

Growth Arrest, Differentiation

bcl2/bax

cyt c release

DNA fragmentation

Apoptosis, Autophagy

Effects of 1,25D require VDR

Narvaez and Welsh
Mice lacking VDR have increased rate of mammary carcinogenesis

In situ cancer

Breast Tumor Incidence

WT VDR HET

MMTV-neu transgenic model

Suggests that presence of VDR in normal tissue prevents cancer development

Zinser and Welsh
What is the Role of the Vitamin D Pathway in Non-transformed Mammary Cells?

• Human mammary epithelial (HME) cells
  • Isolated from surgical resections
  • Immortalized with hTERT
• Normal morphology and features

Kemmis and Welsh
HME cells Internalize 25D and Synthesize 1,25D

25D-DBP Uptake

1,25D Synthesis

Growth Inhibition

Growth inhibition in response to physiologic concentrations of circulating 25D
“Autocrine” Vitamin D Action

Blood vessel

25D → 25D

Growth arrest, differentiation, genomic stability

Changes in Gene Expression

VDR

1,25D

Cyp27b1

25D → 1,25D

Allows for cellular control of VDR activation
Systemic vs Local VDR Activation

What specific pathways are regulated by 1,25D/VDR in HME cells?
<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Fold Increase</th>
</tr>
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<tbody>
<tr>
<td>Cyp24A1</td>
<td>CYP24</td>
</tr>
<tr>
<td>Cluster of Differentiation 14</td>
<td>CD14</td>
</tr>
<tr>
<td>Bone Morphogenic Protein 6</td>
<td>BMP6</td>
</tr>
<tr>
<td>Triggering receptor expressed on myeloid cells 1</td>
<td>TREM-1</td>
</tr>
<tr>
<td>Interleukin 1 receptor-like 1</td>
<td>Ilrl1</td>
</tr>
<tr>
<td>Mal, T-cell differentiation protein-like</td>
<td>MALL</td>
</tr>
<tr>
<td>Cathelicidin</td>
<td>hCAP18</td>
</tr>
<tr>
<td>Macrophage Colony Stimulating Factor</td>
<td>MCSF</td>
</tr>
<tr>
<td>Toll-like Receptor 4</td>
<td>TLR4</td>
</tr>
<tr>
<td>Interleukin 8</td>
<td>Il-8</td>
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<tr>
<td>TNFα-induced protein 6</td>
<td>TNFAI6</td>
</tr>
<tr>
<td>Beta1-defensin</td>
<td>Defb1</td>
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</table>

Enrichment of Immune Regulatory Genes

Simmons and Welsh
Possible Functions of Immune Response in Mammary Gland

- Protect the exposed tissue from infection
- Respond to injury
- Regulate tissue remodeling/apoptosis
- Modulate cancer risk

Evidence for control of immune response by vitamin D in vivo?
Altered Lymphoid Tissues in VDR Knockout mice

Mammary Lymph Nodes

Lymph Node Size

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>VDRKO</th>
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<tbody>
<tr>
<td>Size</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>±10</td>
<td>±10</td>
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</table>

Lymphoma Development

Tumor Incidence (%)

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>VDRKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>50</td>
<td>75</td>
</tr>
</tbody>
</table>

Matthews, Zinser and Welsh
Chronic Inflammation in Mammary Gland of VDR Knockout Mice

VDR suppresses inflammation in normal tissue

Zinser and Welsh
Confirmation of Immune Response Gene Regulation in HME cells by 1,25D

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Real Time PCR Fold Change</th>
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<tbody>
<tr>
<td>CYP24</td>
<td>248</td>
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<tr>
<td>CD14</td>
<td>77</td>
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<tr>
<td>BMP6</td>
<td>20</td>
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<tr>
<td>TREM-1</td>
<td>In Progress</td>
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<tr>
<td>Ilrl1</td>
<td>10</td>
</tr>
<tr>
<td>MALL</td>
<td>In Progress</td>
</tr>
<tr>
<td>hCAP18</td>
<td>3</td>
</tr>
<tr>
<td>MCSF</td>
<td>In Progress</td>
</tr>
<tr>
<td>TLR4</td>
<td>3</td>
</tr>
<tr>
<td>Il-8</td>
<td>In Progress</td>
</tr>
<tr>
<td>TNFAI6</td>
<td>In Progress</td>
</tr>
<tr>
<td>Defb1</td>
<td>2</td>
</tr>
</tbody>
</table>
CD14: Multi-functional Cytokine
Up-Regulated by 1,25D

- Pattern recognition receptor
  - Binds LPS on bacteria
  - Recognizes apoptotic cells
- Induced during mammary gland remodeling
- Activates Toll-like receptor 4
- Expressed on many immune cells and epithelial cells
Subcellular Localization of CD14

- Function of soluble CD14?

[Image: Comparison of Con and 1,25D treated HME cells with images of blue and green staining. Diagram showing ELISA results with bars for different concentrations of 1,25D (nM).]
### 1,25D Regulated Immune Genes in HME cells code for Secreted/Soluble Proteins

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Fold Change</th>
<th>Secreted or Soluble Form?</th>
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<tr>
<td>CYP24</td>
<td>203</td>
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<td>63</td>
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<td>15</td>
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<td>TREM-1</td>
<td>11</td>
<td>Potential</td>
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<tr>
<td>Ilrl1</td>
<td>10</td>
<td>Potential</td>
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<tr>
<td>MALL</td>
<td>8</td>
<td>Yes</td>
</tr>
<tr>
<td>hCAP18</td>
<td>4</td>
<td>Yes</td>
</tr>
<tr>
<td>MCSF</td>
<td>4</td>
<td>Yes</td>
</tr>
<tr>
<td>TLR4</td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td>Il-8</td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td>TNFAI6</td>
<td>3</td>
<td>Yes</td>
</tr>
</tbody>
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Identification of 1,25D Regulated Secreted Proteins by Cytokine Arrays

1,25D ± LPS
24h

HME cells

Collect Media

60 cytokine antibody array

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytokines Up-Regulated in Conditioned Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25D</td>
<td>FGF-7, IGFBP-1, IGFBP-2, IGFBP-4, IL-6, IL-15, IL-16</td>
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<tr>
<td>LPS</td>
<td>EGF, GM-CSF, RANTES</td>
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<tr>
<td>1,25D + LPS</td>
<td>BDNF, BMP-6, CKB-8-1, Eotaxin-2, FGF-6, Fractalkine, I-309, IGF-1, IL-1β, IL-1α, IL-1Ra, IL-7, IL-13, LIGHT, MCP-1, MCP-2, MCP-3, MCP-4, MIP-3α, PDGF-BB, TARC</td>
</tr>
</tbody>
</table>
Immune Surveillance in Cancer Prevention

- Tumor Antigens
- Secreted Signals
- Normal Epithelium
- In situ Cancer
- Tumor Surveillance & Elimination
- Carcinogens
- Chronic inflammation
- Inherited genetic mutations
- Radiation
- Viral infection
- Repair, senescence, and/or apoptosis: intrinsic tumor suppression
- Loss of polarity
- Loss of ECM contact
- Innate and adaptive immunity
- IFN-γ
- Perforin
- TRAIL
- IFN-α/β
- NKG2D
- Protection (i.e., extrinsic tumor suppression)
### Transformation Alters Immune Gene Regulation by 1,25D

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>HME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyp24</td>
<td>600</td>
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<tr>
<td>CD14</td>
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<td>Il1lr1</td>
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<td>Bmp6</td>
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<tr>
<td>Il33</td>
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Working Model: Vitamin D & Immune Responses in Mammary Cells

- **Vitamin D**
- **Immune Genes**
- **Growth Regulators**
- **Cytokines**
- **Anti-Microbial Peptides**
- **Injury, inflammation**

**Key Elements**:
- **sCD14**
- **TLR4**
- **IL1RL-1**
- **TREM-1**
- **Immune Genes**
- **Mammary epithelial cell**
- **VDR**
- **1,25D**
- **IGF-BPs, BMPs, FGFs**
- **Cathelicidin Beta Defensin 1**

**Processes**:
- Shedding?
- Growth Regulators
- Injury, inflammation
In Mice, deletion of VDR is associated with:
• Enlarged lymph nodes
• Chronic tissue inflammation
• Increased cancer risk

In Human Mammary Epithelial Cells:
• Vitamin D metabolites induce growth arrest and differentiation
• Enrichment of immune response genes detected on microarray profiles after treatment with 1,25D
• 1,25D increases expression and secretion of multiple growth factors, cytokines and anti-microbial peptides
• Vitamin D regulation of immune responses becomes corrupted with cancer progression
Significance/Take Home Message

• Vitamin D signaling enhances the synthesis and secretion of immune regulatory proteins in mammary cells

• Possible link between the tumor suppressive actions of the vitamin D pathway and regulation of immune responses in mammary gland
Acknowledgements

NIH CA69700, CA11114
DOD Breast Cancer Research Program
American Institute for Cancer Research
Komen Foundation
Vitamin D, Inflammation & Colon Cancer

• Inflammatory bowel disease
• Autoimmune disorder
• Mediated by auto-reactive T cells
• Increased risk for colon cancer

• Mouse models
• DSS - inflammation
• IL10 KO mouse - IBD
• Azoxymethane (AOM) - preneoplasia
VDRKO Mice are Hypersensitive to DSS induced colitis

Survival

Inflammatory Cytokines

Tissue Damage

Froicu and Cantorna, BMC Immunology, 2007
Vitamin D/VDR alters severity of IBD in IL10 KO mice

Dietary Vitamin D Deficiency

VDR Ablation

Froicu et al, Mol Endo 2003
Colon Carcinogenesis in WT & VDRKO mice

Welsh lab, unpublished