POTENTIAL CONFLICTS OF INTEREST

• CO-FOUNDER & SAB MEMBER OF CYTOCHROMA INC DEVELOPING VITAMIN D ANALOG FOR USE IN CHRONIC KIDNEY DISEASE

• SAB MEMBER OF RECEPTOR THERAPEUTICS DEVELOPING ANTI-CANCER VITAMIN D ANALOGS FOR USE IN CANCER THERAPY
Cellular & Molecular Actions of Vitamin D in Cancer

Objectives:

• Review current knowledge of Vitamin D Metabolism
  - New information about the enzymes/cytochrome P450s involved
    - Activating CYPs: 25-Hydroxylase & 1α-Hydroxylase (CYP27B1)
    - Concept of extra-renal 1α-hydroxylase
    - Inactivating CYPs: 24-Hydroxylase (CYP24A1)

• Molecular Actions of 1,25-(OH)$_2$D$_3$ in Normal & Cancer Cells
  - General Transcriptional Mode of Action
  - Effects of 1,25-(OH)$_2$D$_3$ & its Analogs on Cancer Cells
  - Dysfunctional vitamin D signaling in cancer cells
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Activation of Vitamin D$_3$

Vitamin D$_3$ → CYP2R1 CYP27A1 Liver → 25-OH-D$_3$ → CYP27B1 Kidney & Extra-renal Tissues → 1α,25-(OH)$_2$D$_3$ Calcitriol

Similar pathway exists for vitamin D$_2$
Activation of Vitamin D₃

Vitamin D₃ → 25-OH-D₃

CYP2R1, CYP27A1, CYP3A4
CYP2R1

• LIVER MICROSOMAL VITAMIN D 25-HYDROXYLASE

• SGC (TORONTO) CRYSTALLISED A FUNCTIONAL HUMAN CYP2R1 WITH VITAMIN D₃ IN ACTIVE SITE

• SUBSTRATES INCLUDE VITAMINS D₃ & D₂ & PRODRUGS (eg 1α-OH-D₂ & 1α-OH-D₃)

• WORKS AT NANOMOLAR SUBSTRATE & PROBABLY THE PHYSIOLOGICALLY-RELEVANT VIT.D-25-HYDROXYLASE

• GENOME-WIDE STUDY OF THE DETERMINANTS OF SERUM 25-OH-D LEVEL IDENTIFIED CYP2R1 & CYP24A1

• MUTATION IN hCYP2R1 AT L99P CAUSES RICKETS

- Serum 25-OH-D concentrations in 33,996 individuals of European descent from 15 cohorts

- 4 Main Determinants of plasma 25-OH-D:
  a) 7-dehydrocholesterol reductase -----skin synthesis of D precursor
  b) vitamin D binding protein (DBP or Gc) --transport of vitamin D
  c) CYP2R1 (chromosome 11p15)---25-hydroxylase of vitamin D
  d) CYP24A1 (chromosome 20q13)--24-hydroxylase of 25-OH-D

See also: Ahn J, et al Human Molecular Genetics 2010; 19:2739-2745
POLYMORPHISMS & MUTATIONS REPORTED IN CYP2R1 (25-HYDROXYLASE)

POLYMORPHISMS

MUTATION CAUSING RICKETS
Activation of Vitamin D$_3$

Vitamin D$_3$ → 25-OH-D$_3$ → 1α,25-(OH)$_2$D$_3$

CYP2R1 = 25-HYDROXYLASE
CYP27A1 = Liver
CYP27B1 = 1α-HYDROXYLASE
Calcitriol
CYP27B1

- KIDNEY MITOCHONDRIAL 1α-HYDROXYLASE
- MUTATIONS IN hCYP27B1 CAUSE VDDR-RICKETS TYPE 1
- REGULATION: KIDNEY CYP27B1—PTH & FGF-23; Ca & PO$_4$
- PROBES & ANTIBODIES REVEAL PRESENCE OF CYP27B1 IN EXTRA-RENAL TISSUES eg PROSTATE, BREAST etc
- REGULATION: EXTRA-RENAL CYP27B1 -- CYTOKINES
POLYMORPHISMS & MUTATIONS OF HUMAN CYP27B1

- CYP27B1 SNP
- ERR Triad
- Heme binding
- Beta Helix
- VDDR-I
- Mutagenesis

POLYMORPHISMS

MUTATIONS = RICKETS
Roles of FGF23
1) Increase PO_4^3- excretion
2) Decrease CYP27B1
3) Increase CYP24A1 & NET INCREASE IN D CATABOLISM
CYP27B1

- KIDNEY MITOCHONDRIAL 1α-HYDROXYLASE
- MUTATIONS IN hCYP27B1 CAUSE VDDR-RICKETS TYPE 1
- REGULATION: KIDNEY CYP27B1 – PTH & FGF-23; Ca & PO₄
- PROBES & ANTIBODIES REVEAL PRESENCE OF CYP27B1 IN EXTRA-RENAL TISSUES eg PROSTATE, BREAST etc
- REGULATION: EXTRA-RENAL CYP27B1 -- CYTOKINES
Vitamin D

Liver 25-hydroxylase

Serum 25-OHD

FGF_{23}

Renal 1α-hydroxylase

Serum 1,25-(OH)_{2}D

Bone turnover & mineralization

Intestinal Ca absorption

Calcemic effects
EXTRA-RENAL 1α-HYDROXYLASE

Calcemic effects

- Bone turnover & mineralization
- Intestinal Ca absorption

Extra- calcemic effects

- Insulin secretion
- Renin synthesis
- Skeletal muscle strength

Non-renal tissue cell

25-OHD

Extra-renal 1α-hydroxylase

1,25-(OH)₂D

Gene transcription

- Tumor cell proliferation & Angiogenesis
- Macrophage anti-microbial peptides
- Dendritic-Tcell interaction & CD4 cell activation

Vitamin D

Liver 25-hydroxylase

Diet

UV-B

Serum 25-OHD

Renal 1α-hydroxylase

Serum 1,25-(OH)₂D

FGF₂₃

PTH
METABOLISM OF VITAMIN D₃

Inactivation of 25-Hydroxyvitamin D₃ (25-OH-D₃) through the 24-hydroxylase (CYP24A1) pathway.

CYP27A1: Conversion of vitamin D₃ to 25-OH-D₃.
CYP27B1: Conversion of 25-OH-D₃ to 1α,25-(OH)₂D₃.
CYP24A1: Conversion of 1α,25-(OH)₂D₃ to 1α,24R,25-(OH)₃D₃, a lactone, and calcitriol.

CYP24A1

- Mitochondrial 25-OH-D 24-hydroxylase in kidney and all target cells

- Substrates include 25-OH-D₃ & 1α,25-(OH)₂D₃ & CYP24A1 is a multicatalytic enzyme

- Major role is catabolic and exists to attenuate action of calcitriol inside target cells

- CYP24A1 knockout mouse shows 50% lethality

- CYP24A1 mutations in humans cause idiopathic infantile hypercalcaemia
CYP24A1

- MITOCHONDRIAL 25-OH-D 24-HYDROXYLASE IN KIDNEY AND ALL TARGET CELLS

- SUBSTRATES INCLUDE 25-OH-D$_3$ & 1α,25-(OH)$_2$D$_3$
  & CYP24A1 IS A MULTICATALYTIC ENZYME

- MAJOR ROLE IS CATABOLIC AND EXISTS TO ATTENUATE ACTION OF CALCITRIOL INSIDE TARGET CELLS

- CYP24A1 KNOCKOUT MOUSE SHOWS 50% LETHALITY

- CYP24A1 MUTATIONS IN HUMANS CAUSE IDIOPATHIC INFANTILE HYPERCALCEMIA
Mutations in CYP24A1 and Idiopathic Infantile Hypercalcemia

Karl P. Schlingmann, M.D., Martin Kaufmann, Ph.D., Stefanie Weber, M.D., Andrew Irwin, B.Sc., Caroline Goos, Ulrike John, M.D., Joachim Misselwitz, M.D., Günter Klaus, M.D., Eberhard Kuwertz-Bröking, M.D., Henry Fehrenbach, M.D., Anne M. Wingen, M.D., Tülay Güran, M.D., Joost G. Hoenderop, Ph.D., René J. Bindels, Ph.D., David E. Prosser, Ph.D., Glenville Jones, Ph.D., and Martin Konrad, M.D.

RESULTS

Sequence analysis of CYP24A1, which encodes 25-hydroxyvitamin D 24-hydroxylase, the key enzyme of 1,25-dihydroxyvitamin D₃ degradation, revealed recessive mutations in six affected children. In addition, CYP24A1 mutations were identified in a second cohort of infants in whom severe hypercalcemia had developed after bolus prophylaxis with vitamin D. Functional characterization revealed a complete loss of function in all CYP24A1 mutations.

CONCLUSIONS

The presence of CYP24A1 mutations explains the increased sensitivity to vitamin D in patients with idiopathic infantile hypercalcemia and is a genetic risk factor for the development of symptomatic hypercalcemia that may be triggered by vitamin D prophylaxis in otherwise apparently healthy infants.
POLYMORPHISMS & MUTATIONS IN HUMAN CYP24A1

CYP24A1

POLYMORPHISMS

MUTATIONS CAUSING HYPERCALCEMIA

JONES, PROSSER, KAUFMANN. Arch Biochem Biophys, in press
CYP24A1 and kidney disease
Martin Petkovich and Glenville Jones

Purpose of review
Patients with chronic renal disease have elevated serum phosphate levels, elevated fibroblast-like growth factor 23 (FGF-23), and declining vitamin D status. These changes are related and may be responsible for elevated 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) and dysfunctional vitamin D metabolism. This review focuses on the biochemistry and pathophysiology of CYP24A1 and the utility of blocking this enzyme with CYP24A1 inhibitors in chronic kidney disease (CKD) patients.

Recent findings
CYP24A1 is the cytochrome P450 enzyme that catalyzes the conversion of 25-hydroxyvitamin D$_3$ (25-OHD$_3$) and its hormonal form, 1,25-dihydroxyvitamin D$_3$ [1,25-(OH)$_2$D$_3$], into 24-hydroxylated products targeted for excretion. The CYP24A1-null phenotype is consistent with the catabolic role of CYP24A1. A number of polymorphisms of CYP24A1 have recently been identified. New data from the uremic rat and humans suggest that dysfunctional vitamin D metabolism is due to changes in CYP24A1 expression caused by phosphate and FGF-23 elevations.

Summary
Changes in serum phosphate and FGF-23 levels in the CKD patient increase CYP24A1 expression resulting in decreased vitamin D status. Vitamin D deficiency may exacerbate defective calcium and phosphate homeostasis causing renal osteodystrophy and contribute to the other complications of renal disease. These findings argue for increased focus on correcting vitamin D deficiency in CKD patients by blocking CYP24A1 activity.

Keywords
25-hydroxyvitamin D-24-hydroxylase, chronic kidney disease, cytochrome P450, fibroblast-like growth factor 23, phosphate, vitamin D metabolism

VITAMIN D: ENDOCRINE, AUTOCRINE, PARACRINE SYSTEM

Liver
- CYP27A1
- CYP2R1

Kidney
- CYP27B1
- Megalin/cubulin

Extra-renal 1α-hydroxylating target cells
- CYP24A1
- RXR/VDR
- VDRE
- mRNA
- p21
- Cytokines
- Cathelicidin

Normal target cells
- RXR/VDR
- VDRE
- mRNA
- Calbindins
- RANK-L
- Osteopontin

Vitamin D-dependent genes
- Calcitroic acid
- DBP
- Meigalin/cubulin

Jones G (2008)
Pharmacokinetics of Vitamin D Toxicity.
Amer J Clin Nutr 88: 582S-586S.
Vitamin D: Endocrine, Autocrine, Paracrine System

Serum 25-OH-D₃ is a surrogate for cellular 1,25-(OH)₂D₃.

- **liver**
  - Nucleus
  - CYP27A1
  - CYP2R1

- **kidney**
  - Nucleus
  - CYP27B1
  - Megalin/cubulin

- **extra-renal 1α-hydroxylating target cells**
  - CYP27B1
  - CYP24A1
  - RXR/VDR
  - mRNA
  - cytokines
  - cathelicidin

- **normal target cells**
  - RXR/VDR
  - VDRE
  - mRNA
  - osteopontin
  - calbindins

- **VITAMIN D: ENDOCRINE, AUTOCCRINE, PARACRINE SYSTEM**
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- 1α,25-(OH)₂D₃
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ANTI-PROLIFERATIVE EFFECT OF 1,25-(OH)$_2$D$_3$ IS VDR-DEPENDENT IN MURINE MAMMARY TUMOUR MODEL.

CELL GROWTH

1,25-(OH)$_2$D$_3$ REDUCES GROWTH

25-OH-D$_3$ Activated by CYP27B1 MIMICS 1,25-(OH)$_2$D$_3$

MATTHEWS D, LAPORTA E, ZINSER GM, NARVAEZ C, WELSH J-E

Mechanism of Action of Vitamin D

Microarray Analysis of Calcitriol Treatment Breast Cancer Cells

MCF-7 ERa(+)  
- 6 Cell Adhesion
- 19 Cell Cycle/Apoptosis
- 3 DNA Repair
- 7 Growth/Immune Mod.
- 2 Steroid Receptors
- 2 Oncogenes
- 8 Others

MB231 ERa(-)  
- 2 Cell Adhesion
- 5 Cell Cycle/Apoptosis
- 2 Growth/Immune Mod.
- 1 Steroid Receptors
- 1 Oncogene
- 3 Trans Factors/Kinases

2000 Gene Probes

Figure 3 | Key cancer-related signalling pathways targeted by $\alpha,\beta(\text{OH})_2D_3$. 
MOLECULAR PATHWAYS MEDIATING THE ANTI-INFLAMMATORY ACTIONS OF 1,25-(OH)₂D₃

EVIDENCE OF DYSFUNCTIONAL VITAMIN D METABOLISM IN CANCER CELLS

• Progressive **decline in CYP27B1** (1α-hydroxylase) expression in prostate, colon & breast tumorigenic cell lines (eg. Chen TC et al J Cell Biochem 2003)


• Suggestion that **CYP24A1 is an independent prognostic marker of survival** in patients with lung adenocarcinoma (Chen G et al. Clin Cancer Res 2011)


**BOTTOM LINE:** BALANCE BETWEEN CYP27B1 & CYP24A1 IS UPSET
CYP27B1 in Prostatic Cell Lines

CYP27B1 in Primary Prostate Cancer cells

Chen G (2011) Clin Cancer Res
CYP24A1-Survival Marker
in Lung Adenocarcinoma

CYP24A1 in Prostate Cancer

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DYSFUNCTIONAL CELLULAR METABOLISM OF 1,25-(OH)$_2$D$_3$ REDUCES ANTI-PROLIFERATIVE EFFECT IN CANCER CELLS
EVIDENCE OF DYSFUNCTIONAL VDR-MEDIATED SIGNALING

• PRESENCE OF **VDR** IN CANCER CELL IS **ESSENTIAL** TO MEDIATE ANTI-PROLIFERATIVE EFFECTS OF 1,25-(OH)_{2}D_{3}

• IF **VDR** IS PRESENT THEN **HYPERCALCEMIC SIDE-EFFECTS** RESULTING FROM EXCESSIVE VDR-MEDIATED SIGNALLING CAN LIMIT VITAMIN D THERAPY

• **TRANSCRIPTION FACTOR SNAIL** REPRESSES VDR EXPRESSION IN HUMAN **COLON CANCER CELLS** (Palmer HG et al Nature Med, 2004); & IN **OSTEOSARCOMA** (Yang G H et al Euro J Pharmaocology 2011)
EVIDENCE OF DYSFUNCTIONAL VDR-MEDIATED SIGNALING

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SW-480-ADH differentiates into epithelial cell when treated with 1,25-(OH)$_2$D$_3$
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