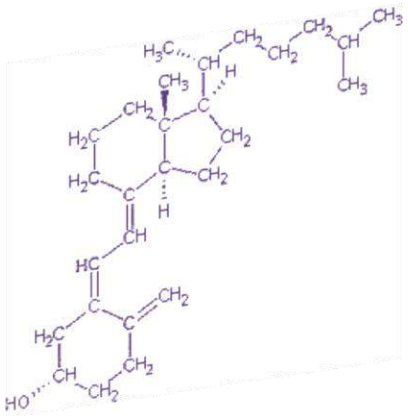
The Molecular Mechanisms of Vitamin D Action

The 1st Abu Dhabi International Conference on Vitamin D Deficiency
Abu Dhabi 2012



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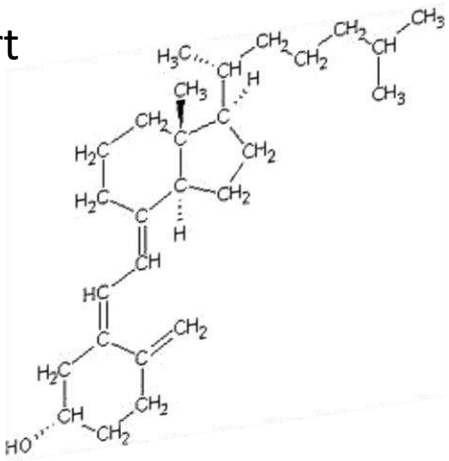
American University in Dubai

Vitamin D

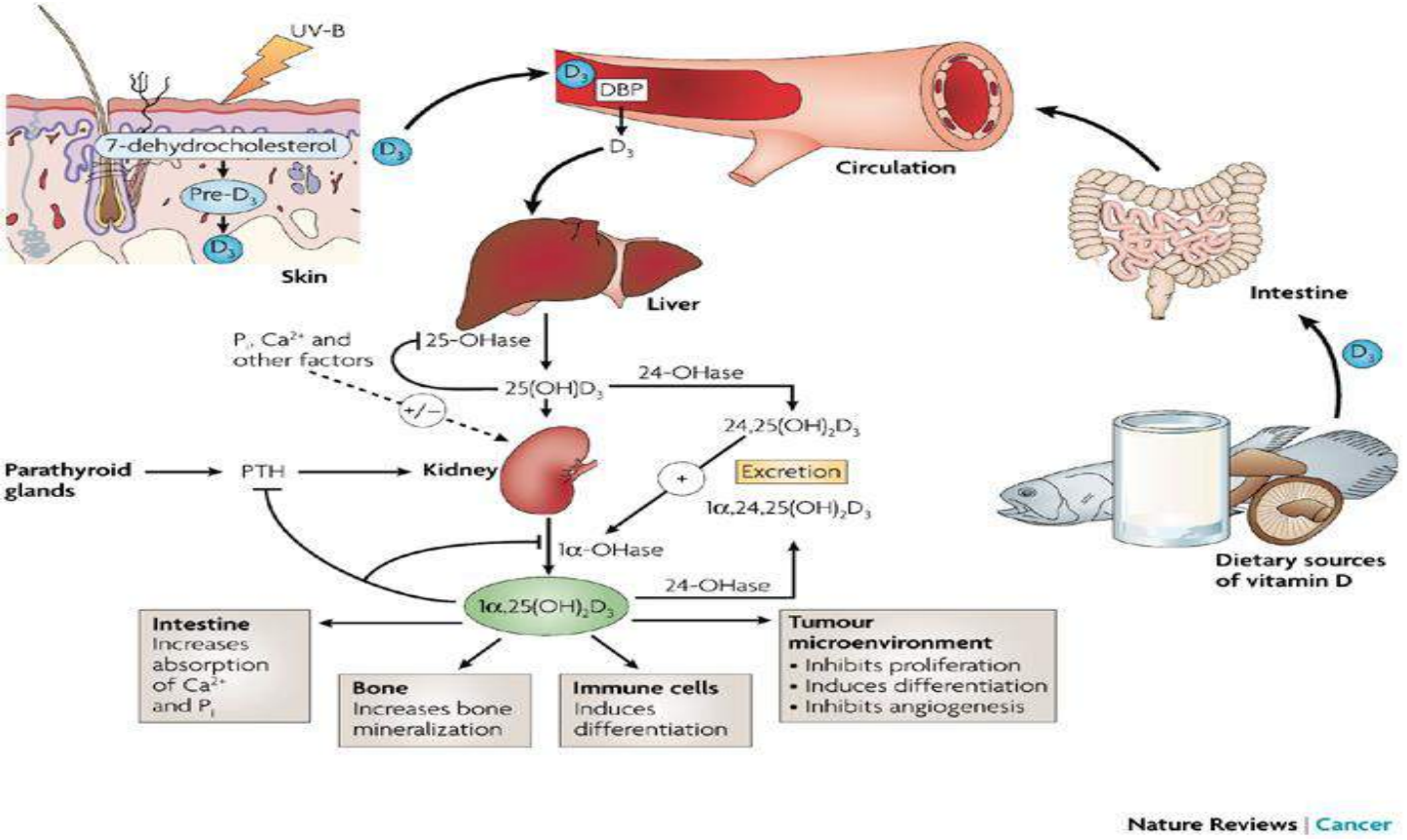
- Vitamin D_2 is made naturally by plants, and vitamin D_3 is made naturally by the body when the skin is exposed to ultraviolet radiation in sunlight.

- It can also be obtained through the diet in foods (e.g. fatty fish, fish liver oil, and eggs, food supplements).

- **Vitamin D** can exert **pleiotropic** effects:
  - Regulate Calcium/Phosphorous uptake and transport
  - Modulate Immune function (reduces inflammation)
  - Arrest proliferation and growth
  - Affect cellular differentiation
  - Modulate metabolic responses e.g. insulin secretion
  - Activate Apoptosis

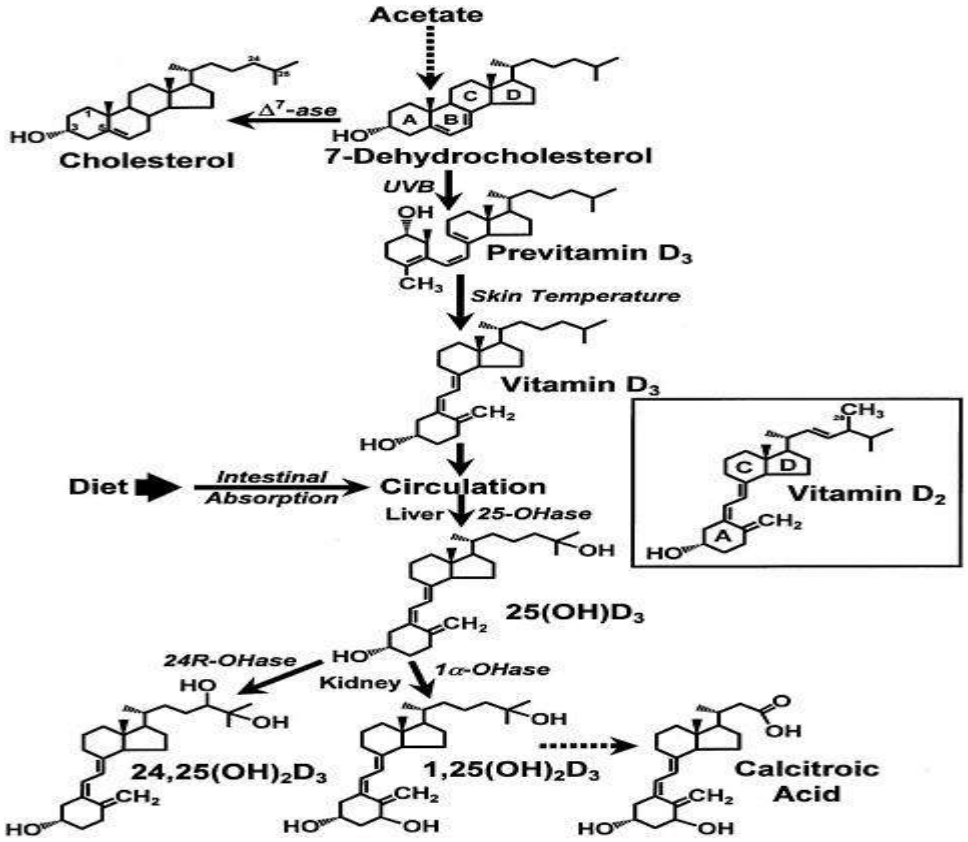


Biochemical Pathways of Vitamin D_3 Metabolism in the Human Body



Deeb K et al 2007

Hydroxylation in Human Tissues



The photochemical, thermal, and metabolic pathways for vitamin D_3 activation to biologically active molecule

Holick MF. 1996

Vitamin D_3 Action on Target Tissues in the Human Body

Classification	Target tissue or cell*	Specific effects† Moukayed 2012
Intestine	Duodenum (1)	↑ Intestinal calcium absorption (TRPV6 intestinal calcium transporters) ↑ Calbindin D28k
	Jejunum (2) (brush border and basolateral membranes)	↑ Intestinal phosphate transport (TRPV6 intestinal calcium transporters)
Bone	Osteoblasts (and in turn osteoclasts) and chondrocytes	↑ Bone formation: bone mineralization and matrix formation ; ↑ Osteocalcin; ↑Osteopontin/SPP1; ↑ RANKL for osteoblasts to activate osteoclasts
Parathyroid hormone	Chief cells	↓ PTH
Kidney	Distal tubules (Ca) Proximal Tubules (Phosphate)	↑ reabsorption of Calcium (↑ TRPV5, Calbindin) ↑ Reabsorption of phosphate (↑NPT1 and NPT2) ↑ Detoxification of 1α25 VitD_3 (CYP24A1 OHase) ↑ Calbindin D9k
Immune system	Monocytes/macrophages and T-lymphocytes (helper type 1)	Suppression of γ-interferon and IL-1 through IL-6
Central nervous	Dorsal root ganglia glial cells, and hippocampus	↑ Production of Nerve Growth factor (NGF), Neurotrophin-3 and Leukemia inhibitory factor

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Vitamin D_3 Action on Target Tissues in the Human Body

Classification	Target tissue or cell*	Specific effects†
Epithelium	Epidermal skin/kertinocyte	↑ Differentiation
	Hair follicle	↑ Differentiation
	Female reproductive tract	Uterine development
	Mammary	↓ Cell growth
	Prostate	↓ Cell growth
	Colon	↓ Cell growth
Endocrine Target tissues	Thyroid gland	↓ TSH
	Pancreatic β- cells	↑ Insulin secretion (Calbindin 28K)
Many systems	Diverse cells and cancer cell lines	↓ Cell growth (↓c-*fos*, ↓c- myc) ↑ Differentiation (↑p21, ↑p27) ↑ Apoptosis (↓ *Bcl-2*) ↓ Angiogenesis

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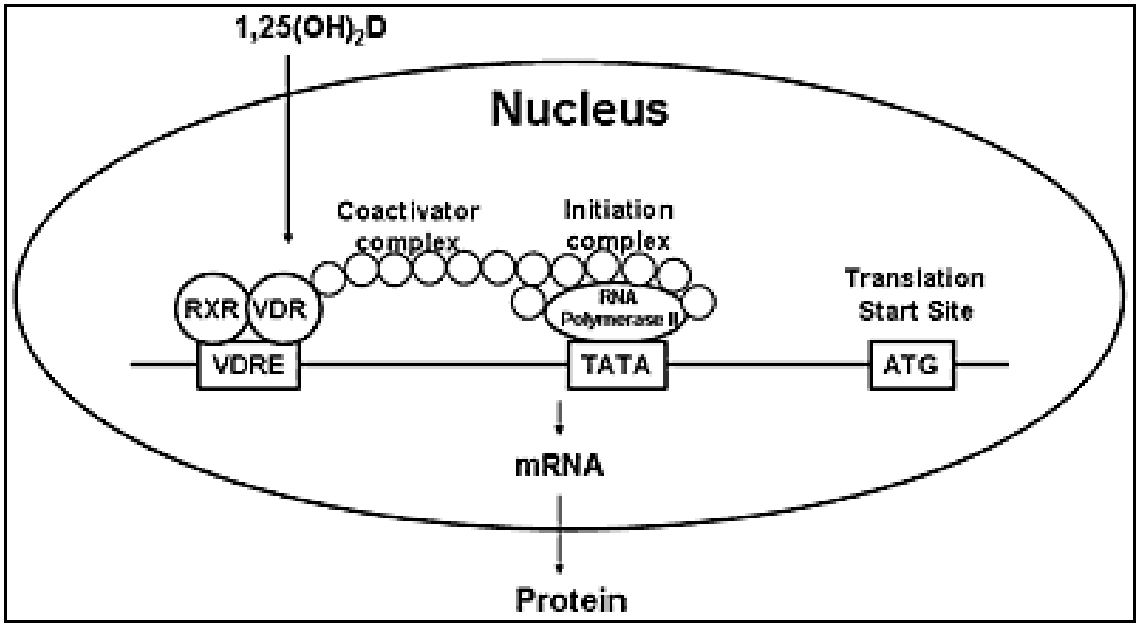
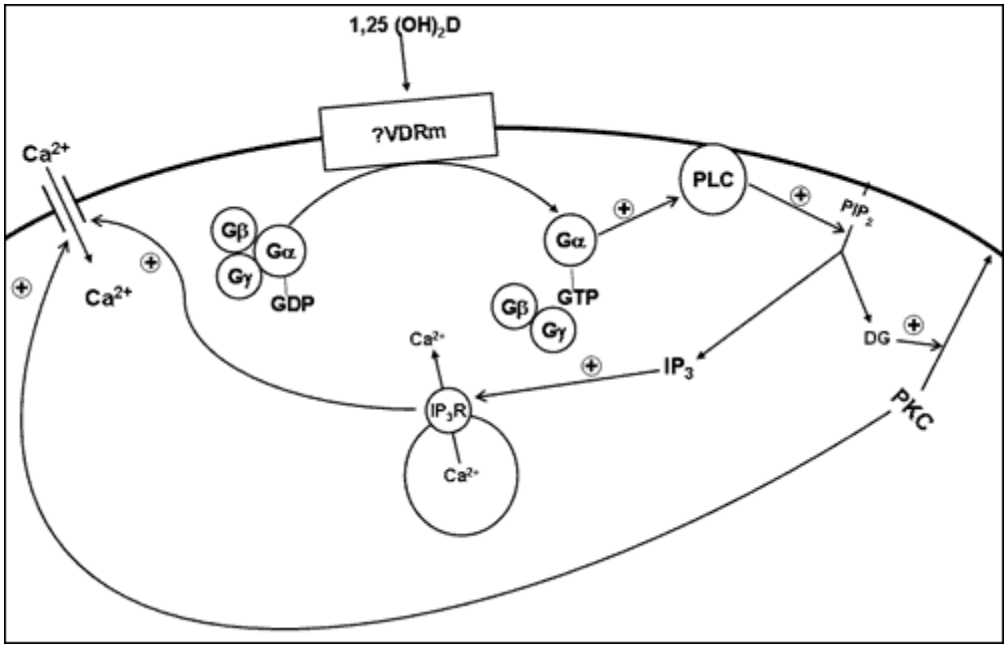
Genomic Action of Vitamin D_3: $1,25(OH)_2D_3$- regulated gene transcription



$1,25(OH)_2D_3$ enters the target cell and binds to its receptor, VDR. The VDR heterodimerizes with the retinoid X receptor (RXR). The dimer VDR/RXR binds onto the vitamin D response element (VDRE)

Bickle et al. 2009

Non Genomic Actions of Vitamin D_3



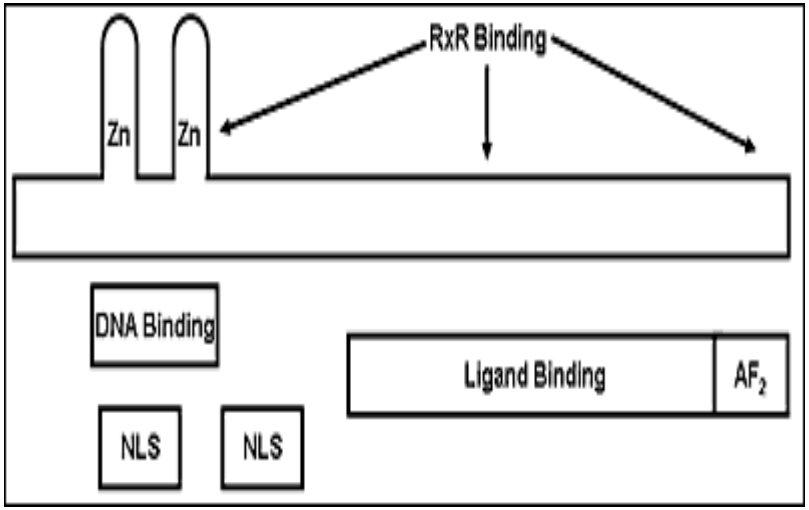
This response is thought to exist to facilitate a **quick response** to the effect of 1,α25 (OH) Vitamin D_3. This has been observed in a number of cell types including osteoblasts, liver, muscle and intestine. This facilitates the rapid flux of Ca^{2+} release by activation of PLC and PKC pathways through a G-protein coupled process.

The Vitamin D Receptor (VDR)

- Protein produced by gene 12q13-q14
- The Vitamin D Receptor is a 427 residue long protein composed of two domains:
  - DNA binding domain (DBD)
  - ligand binding domain (LBD)

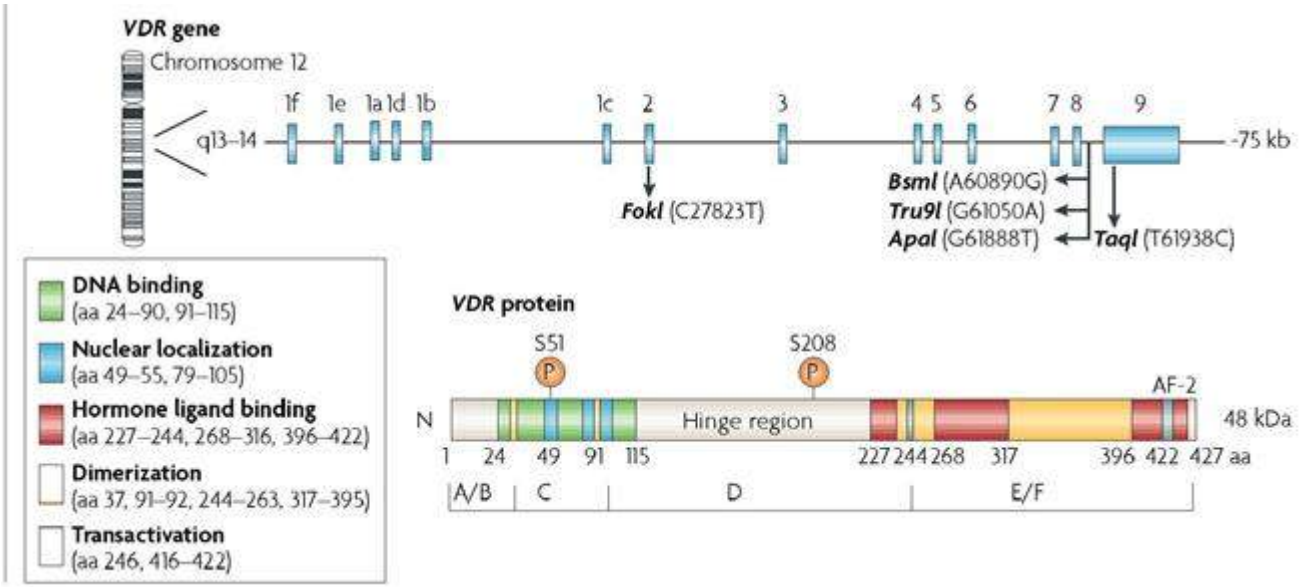
- Three human transcripts available.
- The VDR belongs to the nuclear receptor (NR) superfamily including receptors for the steroid, retinoid and thyroid hormones.

- DNA binding domains (DBD) of these receptors are generally highly conserved however ligand binding domains (LBD) exhibit limited similarity.



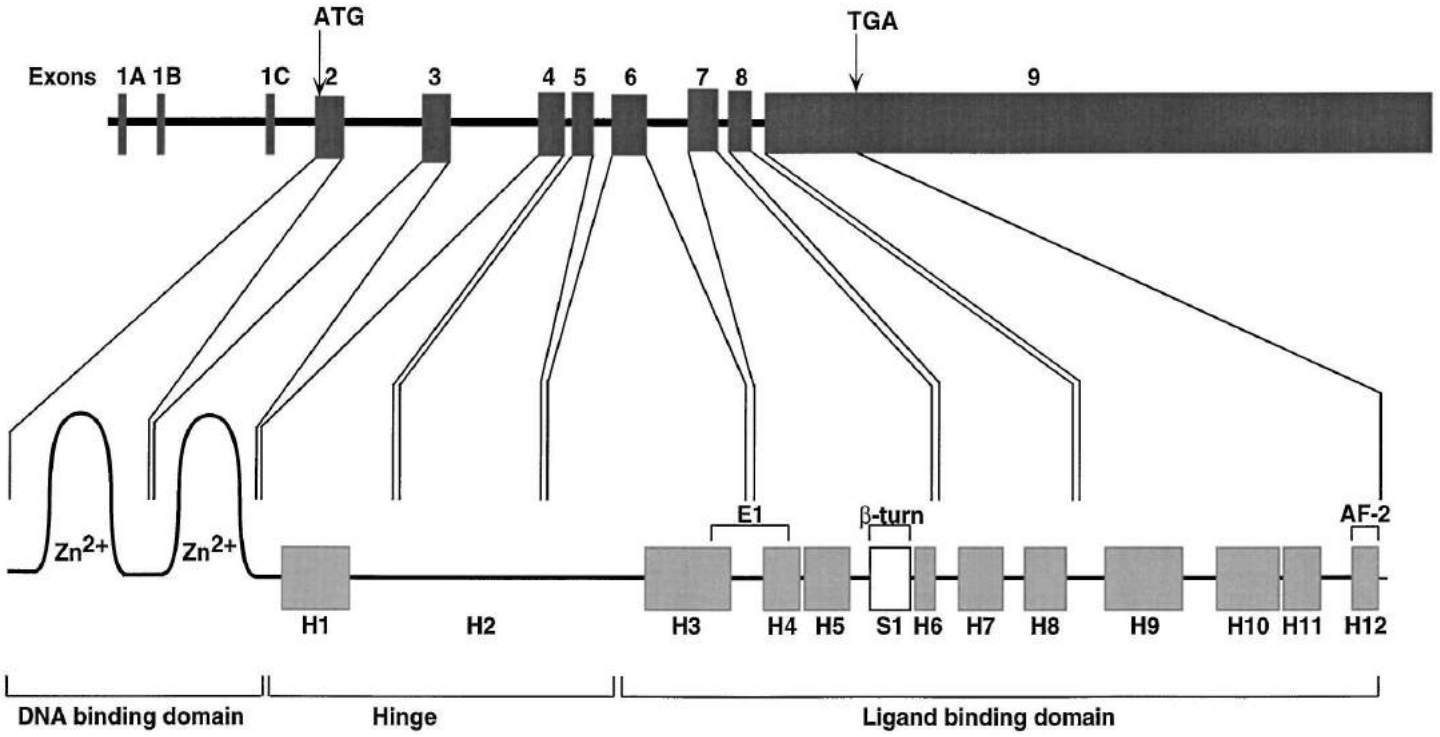
Binding Domains for VDR

Human VDR mRNA and protein

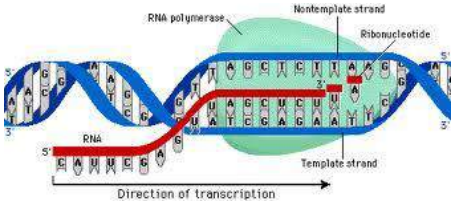


Deeb K et al 2007

The Vitamin D Receptor (VDR)



Binding Domains for VDR
Three mRNA isoforms exist for VDR.



VDR Element

- Consensus sequence is

5’ GGTCCA NNN GGTCCA 3’

- DR3 in the promoter region of target genes; DR4 and DR6 exist.
- 5’ end bound by RXR and 3’ end bound by the VDR
- Regulations at the VDRE could be:
    - **Activation** via one VDRE in the gene.
    - Some genes may have more than one VDRE
    - **Gene repression**.
    - Repression at VDRE may occur via binding orientation reversal or VDRE interference with TATA sequences.
    - **Modulation** of transcription:
        - via interaction with other overlapping *cis*-elements flanking the DR3
        - or interaction with a second gene transcription factors.
- RXR ligand binding (by 9-*cis* retinoic acid) further augments gene regulation at the RXR/VDR response element sequences. The effects could be antagonistic or synergistic.

VDRE Regulated Genes

Tissues	Target tissue or cell*	Gene
Intestine	Duodenum and Jujenum	↑ Calbindin D28k ↑ NPT2 Intestinal phosphate transport ↑ TRPV6 intestinal calcium transporters
Heart	Myocytes	↓ ANP (atrial natriuretics peptide) acts as vasodialator
Bone	Osteoblasts (and in turn osteoclasts) and chondrocytes	↑ Osteocalcin; ↑Osteopontin/SPP1; ↑ RANKL for osteoblasts to activate osteoclasts
Parathyroid hormone	Chief cells	↓ PTH
Kidney	Distal tubules (Ca) Proximal Tubules (Phosphate)	↑ reabsorption of Calcium (↑ TRPV5, Calbindin D28k and D9k)) ↑ Reabsorption of phosphate (↑NPT1 and NPT2) ↑ Detoxification of 1α25 VitD_3 (CYP24A1 OHase)
Cellular adhesion		↑β-3 integrins promoting cellular adhesion
Many systems	Diverse cells and cancer cell lines	↓ Cell growth (↓c-*fos*, ↓c- myc) ; ↑ Differentiation (↑p21, ↑p27) ↑ Apoptosis (↓ *Bcl-2*)

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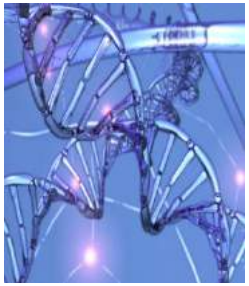
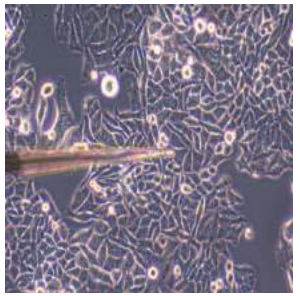
Human Genome Wide Association Scan and *In Vitro* studies

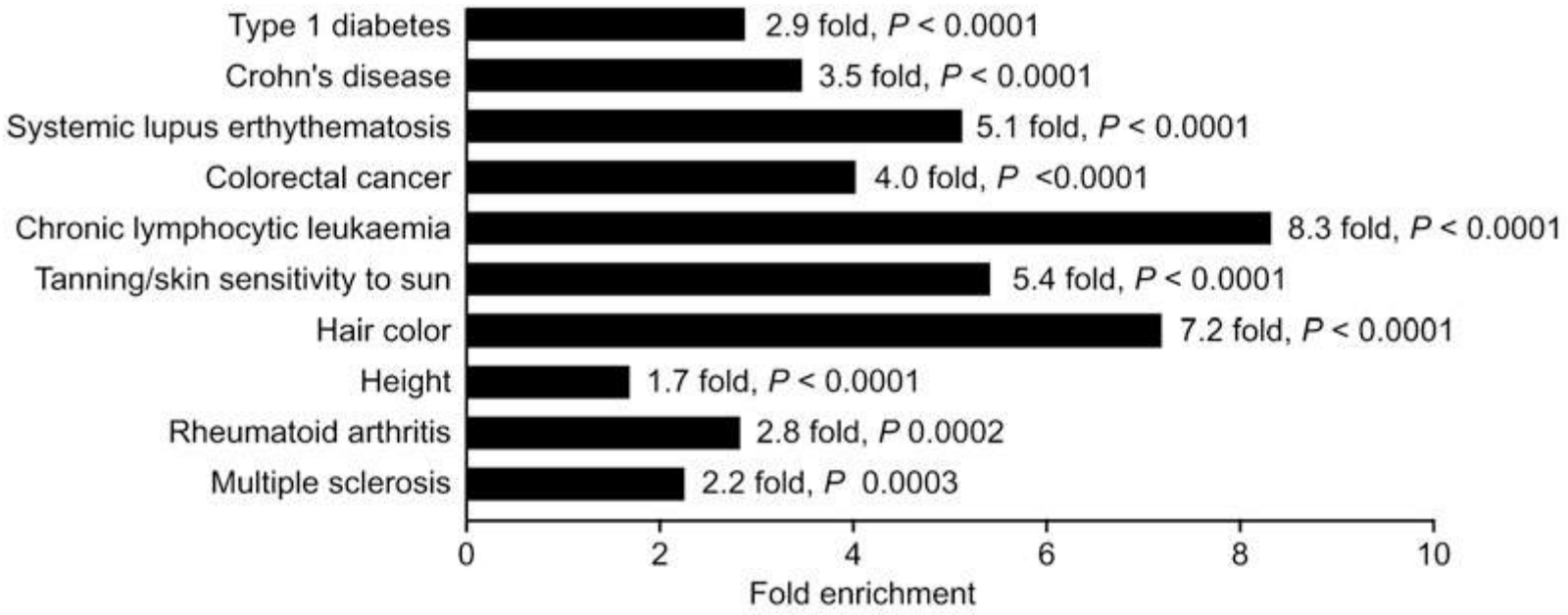




- 2776 genomic positions occupied by the VDR
- 229 genes with significant changes upon *in vitro* stimulation with Vitamin D_3
- Motifs used between 5 and 30 bp long.
- Regions identified include: intergenic, intronic, upstream, downstream and UTR regions.

Ramagopalan S. et al. Genome Res. 2010

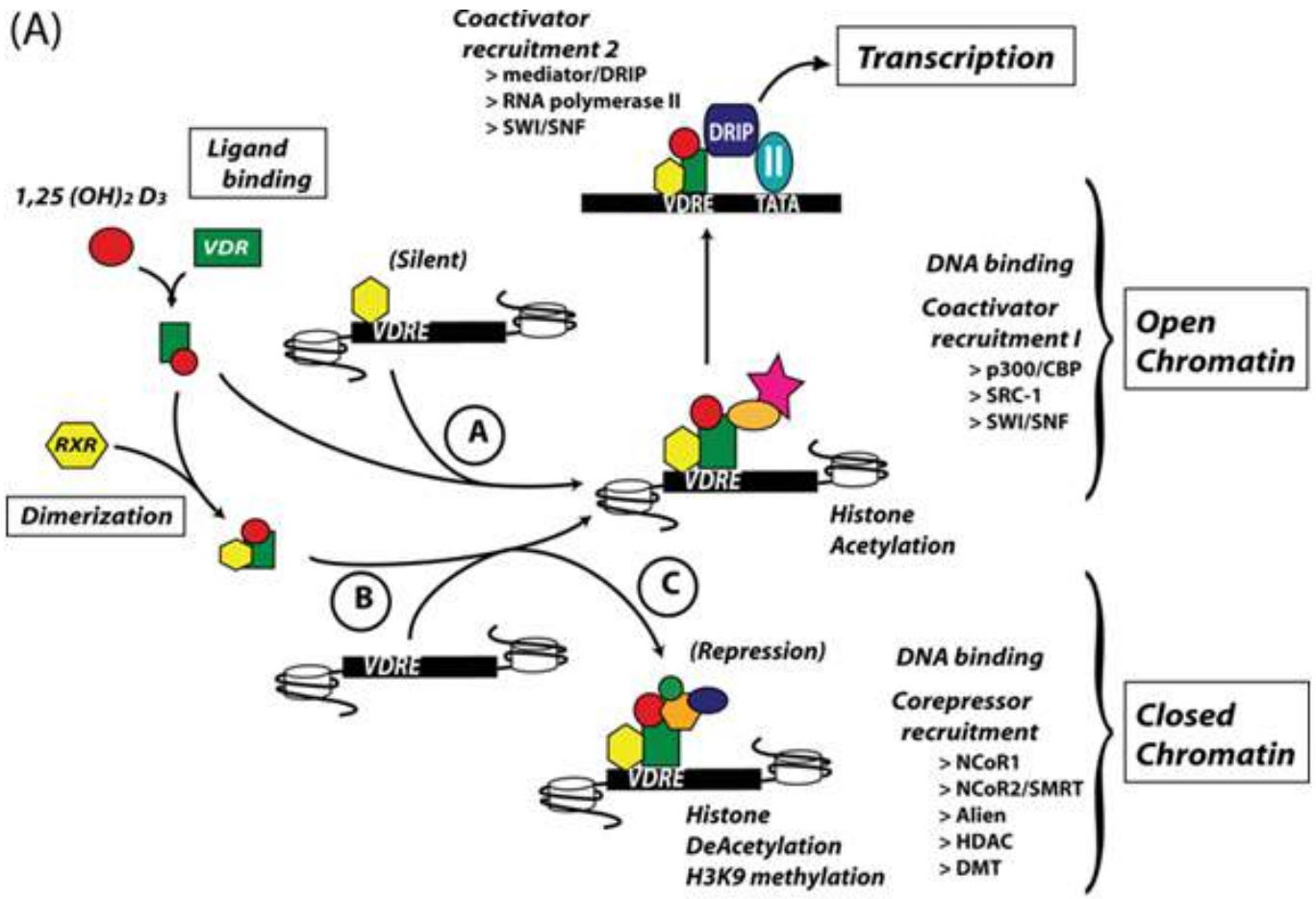
VDR binding and Human traits



Common traits showing enrichment of VDR binding identified by ChIP-sequencing after calcitriol stimulation.

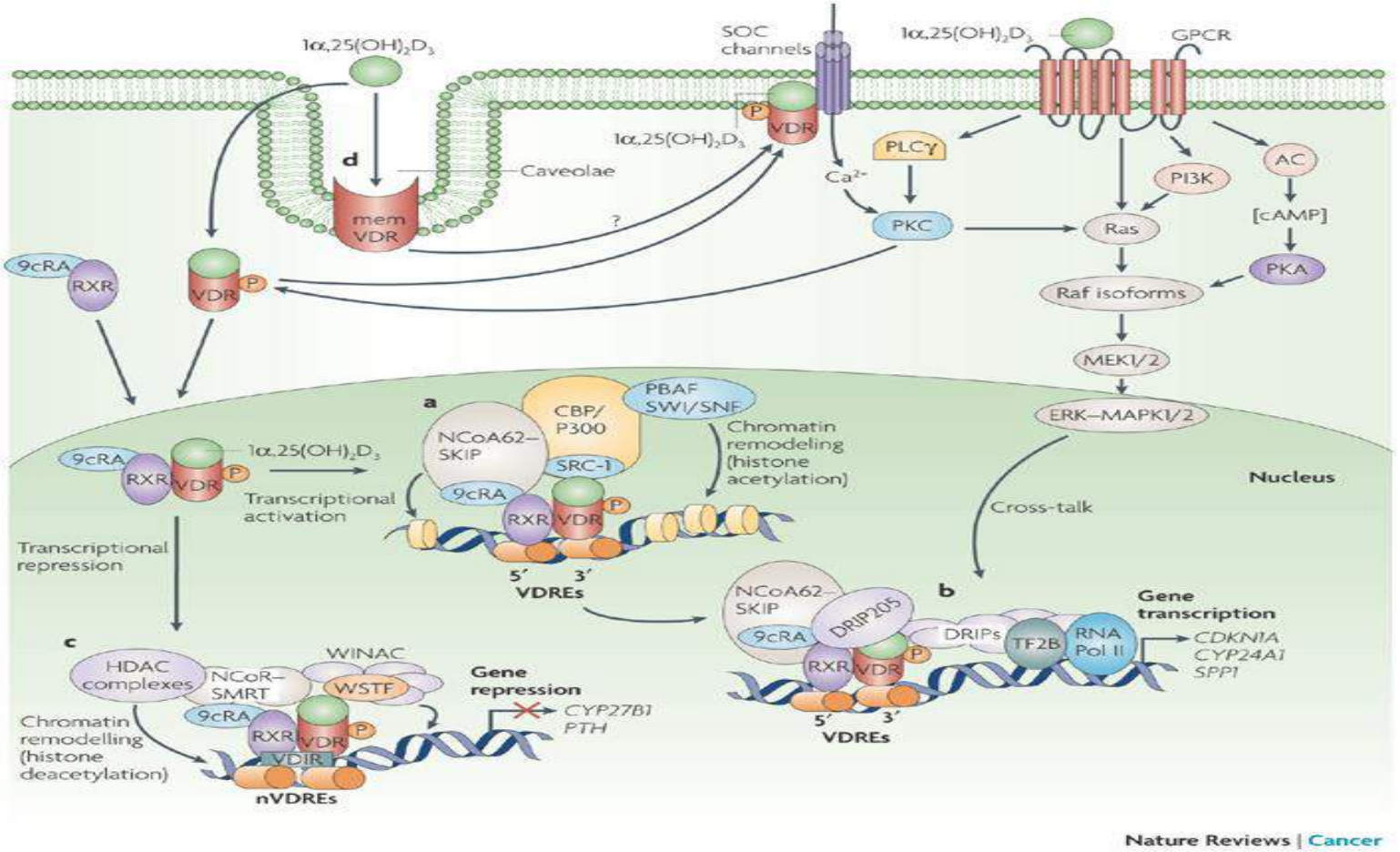
Ramagopalan S. et al. Genome Res. 2010

Vitamin D-mediated Gene Transcription through VDR



Fleet et. al

Signalling Pathways in Vitamin D Action



Deeb K et al 2007

VDR Knock Out (KO) Mice

- Two models generated initially:
  - Labs of Marie Demay MB 1997 and Shigeaki Kato, 1997
- **Phenotype**
  - Growth retardation
  - Vitamin D deficiency rickets type II (VDDR II) and osteomalacia
  - Alopecia
  - Hypocalcemia
  - Hyperparathyroidism
  - Impaired bone formation
  - female infertility, uterine hypoplasia, impaired folliculogenesis
  - Cancer phenotype: Hyperproliferation of descending colon (increased proliferating cell nuclear antigen (PCNA)and cyclin D1 expression); colorectal cancer
  - Decreased survival; KO die at an age of 10.6 months v.s. 20.5 months in WT

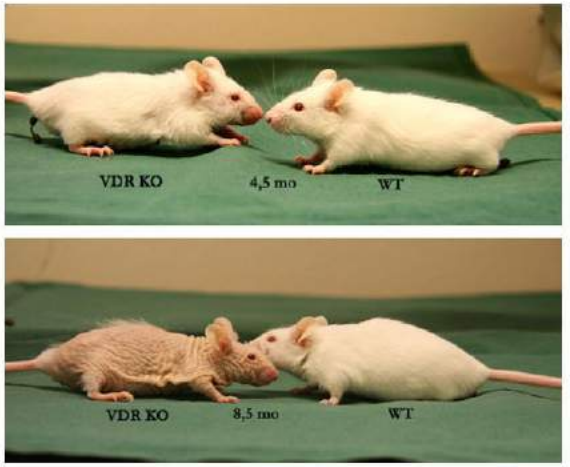
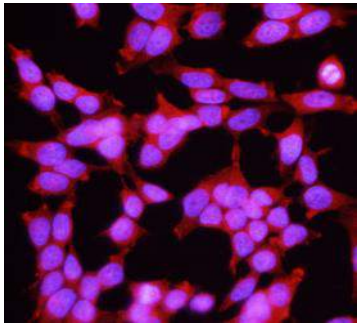


Fig. 2. Phenotype of VDR knockout mouse (KO) compared to wildtype littermate (WT; NMRI background strain) at the age of 4.5 (top) and 8.5 (bottom) months.

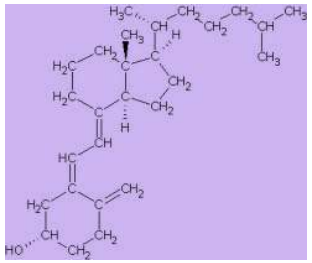


Vitamin D and Cancer

- ***In vitro* and animal studies:**
  - indicated that vitamin D may have anti-cancer benefits, including against progression and metastasis, against a wide spectrum of cancers.
  - Data from cell lines or cancers of the lung, bone, colon, kidney, melanocyte , retina , breast, prostate.

- **Geographical correlation studies (USA):**
  - indicated higher rates of total cancer mortality in regions with less UV-B radiation, among African-Americans and overweight and obese people, each associated with lower circulating vitamin D.

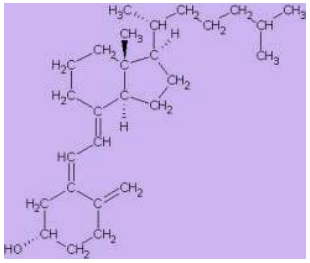
- A large number of scientific studies have investigated a **possible** role for vitamin D in cancer prevention



Vitamin D and Protective Role from Tumour Development

- **KO mouse model studies** (Zeisner and Welsh; 2002 and 2005) and **carcinogens** does indicate the importance of **VDR** in cancer and that optimal VDR signalling may be required to **suppress tumourigenesis.**
  - Mice were fed a high calcium diet to prevent disturbances in calcium homeostasis
  - Mice were gavaged with dimethylbenzanthracence **(DMBA)** using a protocol designed to induce tumors.

- **In KO mice compared to WT littermates:**
  - **Skin:** higher number of 40% sebaceous, 25% squamous and 15% follicular papillomas; other infrequent lesions include basal cell carcinoma
  - **Mammary tissues:** higher incidence of alveolar and ductal hyperplasias
  - **Lymph nodes and thymus**: Lymphoblastic and thymic lymphoma higher in Vdr–/– (27%) compared with WT mice (11%)
  - NO effect on tumor development in **ovary, uterus, lung or liver.**
  - Decreased survival



Vitamin D and Cancer

- **Randomized clinical trials** designed to look at Vitamin D effect on **other** outcomes e.g. bone mineralization, **also** suggest that postmenopausal women who take Vitamin D supplements have reduced cancer incidence.

  (Evidence from re-analysis 2011: 14-17% reduction in breast cancer; 20% in colorectal cancers).

- The **biological evidence** for an anti-cancer role of 25(OH)D is also strong for **prostate cancer**, but the **epidemiologic data have not been supportive.** Although not entirely consistent, some studies suggest that higher circulating 1,25(OH)2D may be more important than 25(OH)D for protection against aggressive, poorly-differentiated prostate cancer.

- **Observational studies:** Epidemiologic studies of the association between vitamin D and the risk of colorectal cancer have provided some indications that higher levels of intake are associated with a reduced risk. However, the data are inconsistent.

Vitamin D and Cancer

- **Observational studies:**

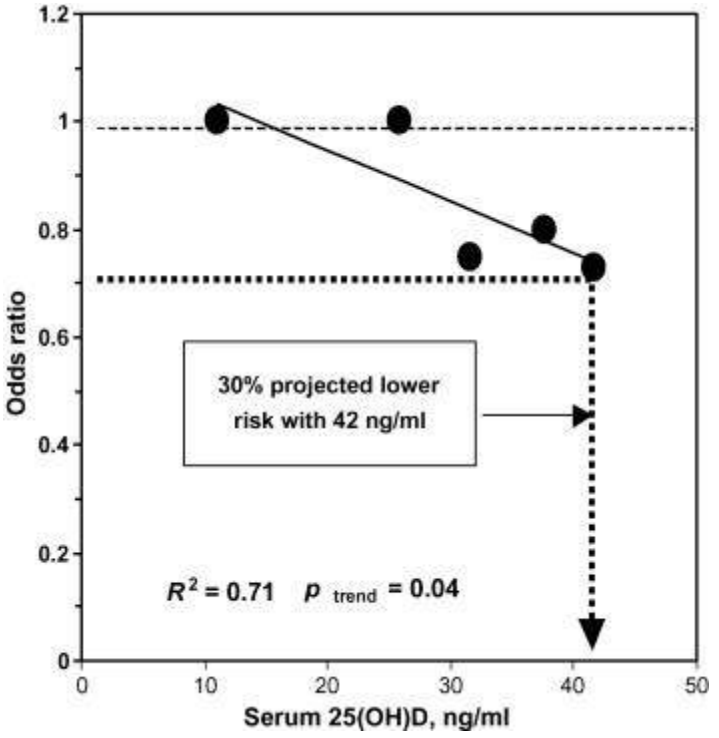
  Vitamin D and **prevention** of breast cancer: pooled analysis

  (Garland CF et al. J Steroid Biochem. Mol. Biol. , 2007 Mar; 103 (3-5):708-11)

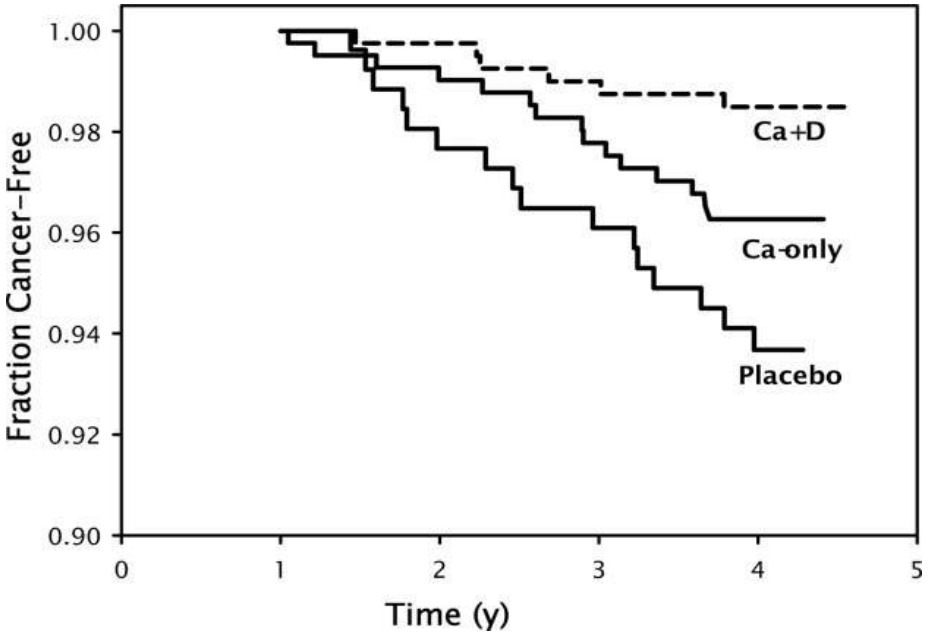
  – Data was pooled from two studies

  – Indicate a dose–response association between serum 25(OH)D and risk of breast cancer.

  – Intake of **2000 IU/day** of VitaminD_3, and very moderate exposure to sunlight (12 min/day), could raise serum 25(OH)D to **52 ng/ml**, a level associated with **reduction by 50%** in incidence of breast cancer, according to **observational studies**

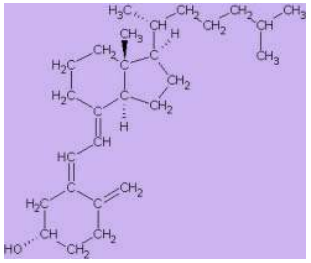


Survival with Cancer-free Outcome Improved with Vitamin D Administration



This was a 4-y, population-based, double-blind, randomized placebo-controlled trial. The primary outcome was fracture incidence, and the principal secondary outcome was cancer incidence. Improving calcium and vitamin D nutritional status substantially reduces all-cancer risk in postmenopausal women.

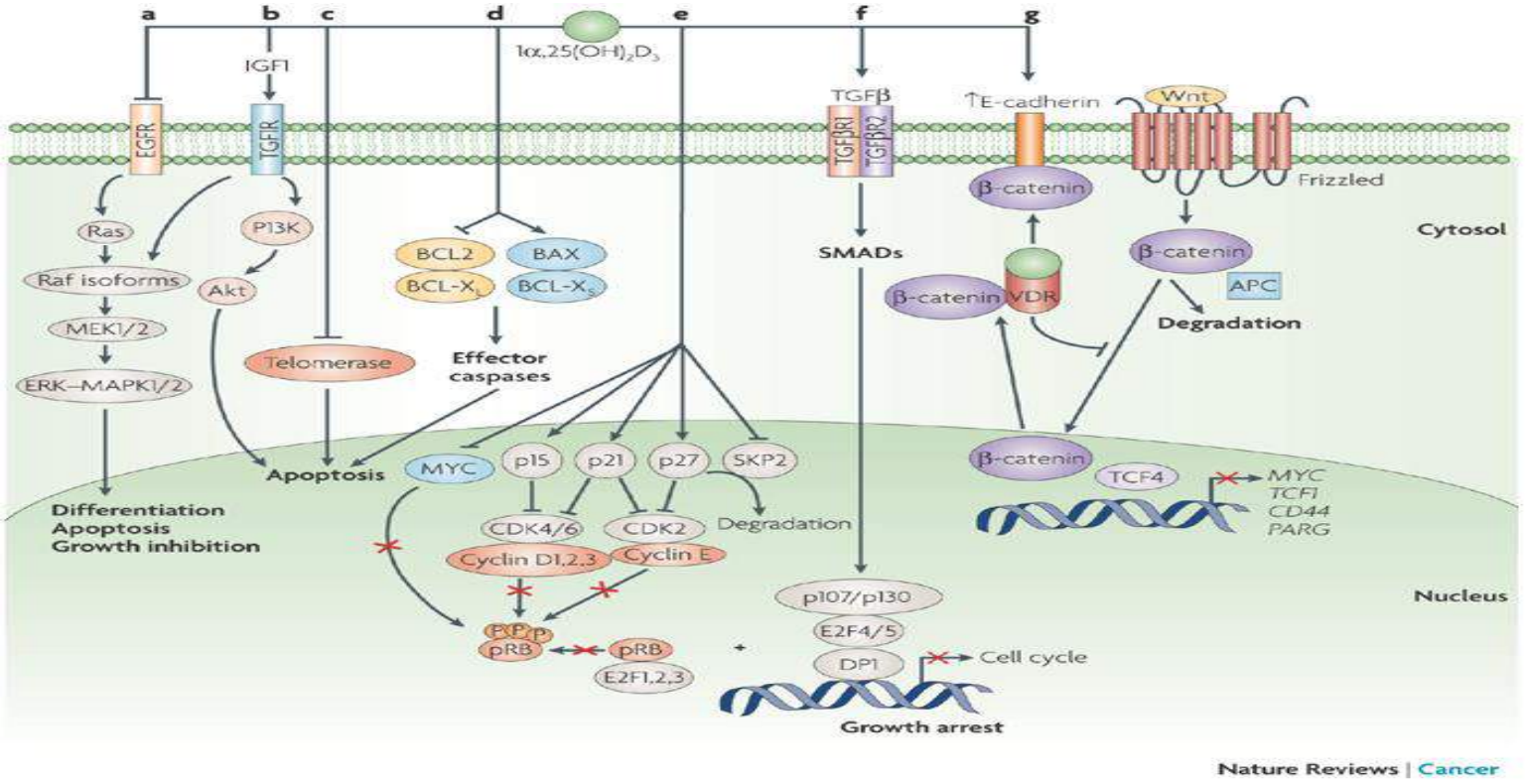
*Lappe J et al. (*2007) *Am J Clin Nutr* 2007;85:1586 –91.



Vitamin D and Cancer

- National Cancer Institute at the National Institute of Health “Although some evidence suggests that vitamin D may provide some protection against colorectal and possibly other cancers, the evidence of potential benefit is **limited** and inconsistent.
- This leaves the need for further research into the direct effects of dose dependent treatment of 1a,25 Vitamin D3 and analogues on different cancers.

Cross-talk of $1,25(OH)_2D_3$ with Other Growth Factors



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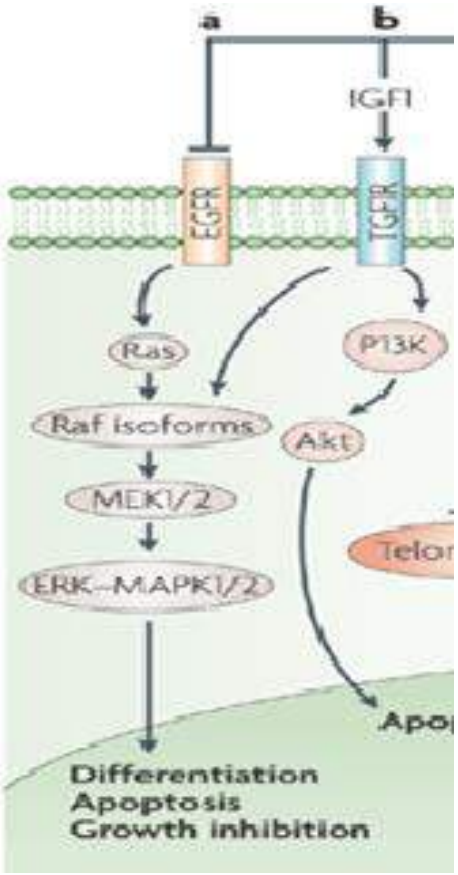
Vitamin D3 is may modulate differentiation, growth and apoptosis cellular signals through cross-talk with other growth factors and cytokines thus affecting cell cycle progression and cell survival.

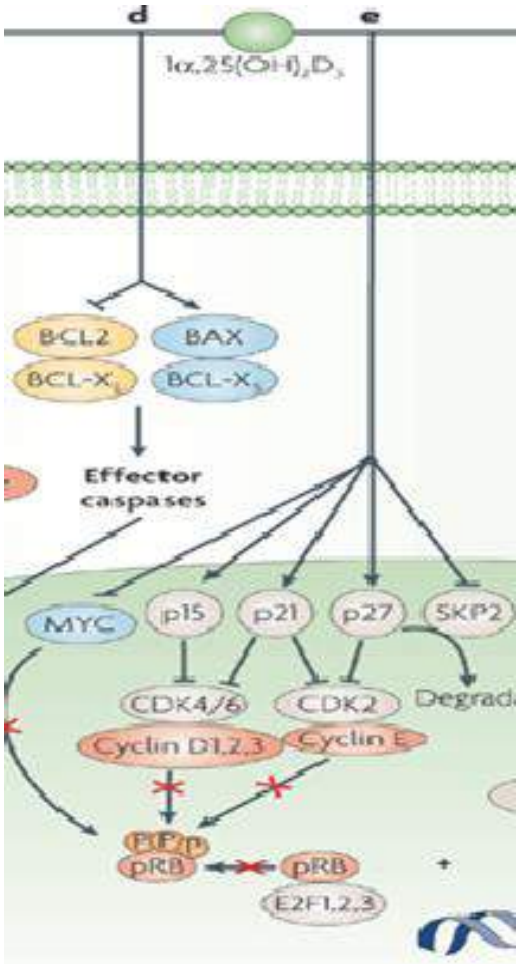
Cross talk with Receptor Tyrosine Kinases



- Must do something about the error with IGFI re
- Cepto
- **Vitamin D derivatives inhibit the mitogenic effects of IGF-I on MCF-7 human breast cancer cells; NFKB depressed therefore if Vitamin D acting.**
- **Vitamin D analogues suppress IGF-I signalling and promote apoptosis in breast cancer cells**

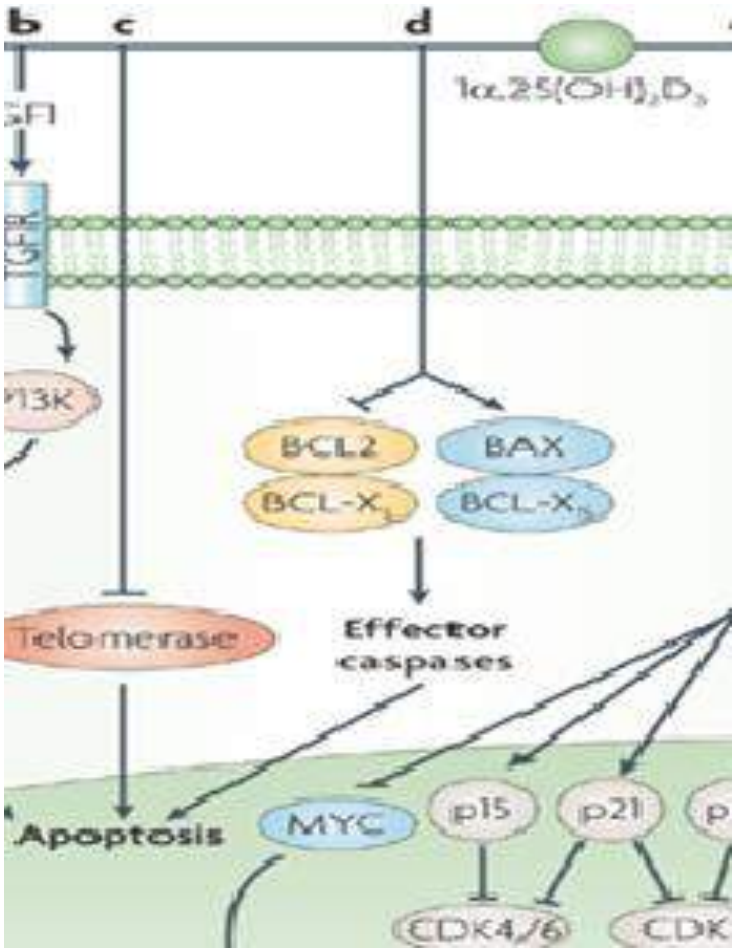
Vitamin D_3 Blocks Cell Cycle Progression

- P21: VDRE mediated effect on gene transcription.
  - P21 arrests the cell cycle and progression G1->S phase
- P27: No VDRE but Vitamin D3:
  - VDR interacts with the transcription factors activating p27 promoter namely with Sp1 and NF-Y
  - VDR activates the PTEN gene which stabilizes p27 by dephosphorylation and therefore prevents p27 degredation
  - VD reduces CDK2 activity (S phase arrest) and Skip2 abundance.



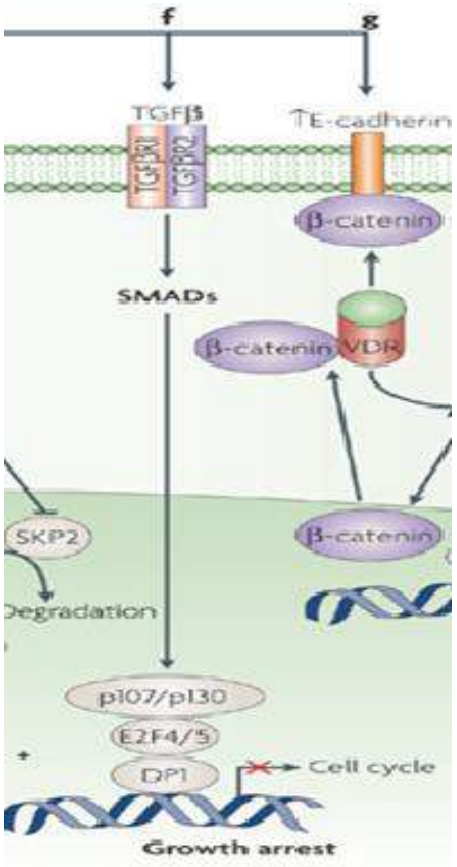
Vitamin D_3 and Apoptosis Pathways

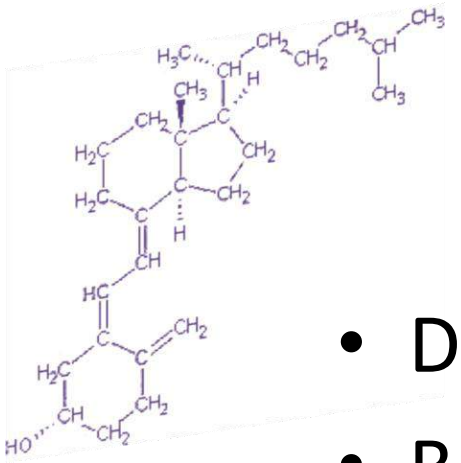
- Telomerase data is contradictory and varies:
  - Some studies show that Vit D3 in epithelial cells destabilizes Telomerase Reverse Transcriptase (TERT) mRNA therefore inducing Apoptosis.
  - Other studies show that in PMBC telomerase activated . Mononuclear cells ie monocytes macrophages.



Vitamin D_3 and TGFβ

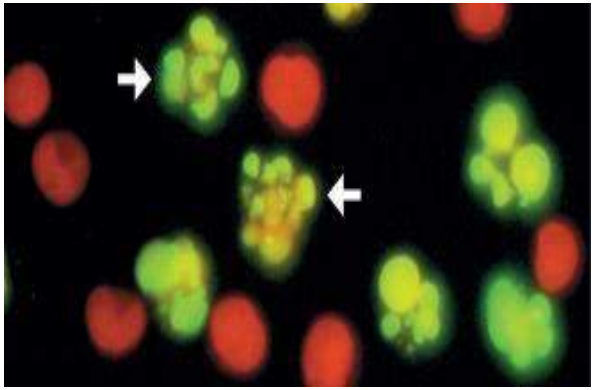
- Vitamin D_3 potentiates TGFβ pro-apoptotic signals
- Cell cycle transitions to Mitosis are blocked (S/G2/M)
- Upregulate genes transcription of proapoptotic proteins





Vitamin D and Apoptosis

- Direct effects on calcium fluxes
- Blocking Telomerase activity
- Blocking RTK activity e.g. AKT/mTOR pathways



$1,25(OH)_2D_3$ Regulates Genes Involved in Regulating Replication and Apoptosis

Gene	Effect of 1α 25 $(OH)_2$ Vitamin D_3	Cellular process	Mode of regulation
Thymidine synthetase (TYMS)	+ (VDRE)	DNA replication	S phase arrest
Thymidine Kinase (TK1)	+ (VDRE)	DNA replication	S phase arrest
Telomerase (TERT)	+ (ID)	DNAreplication	VDRE destabilizes telomerase's reverse transcriptase portion TERT's mRNA; Telomere attrition
P70 S6 kinase	+ (ID)	Protein translation	S transition affected
GADD45	+	Cell cycle and Apoptosis	G2 growth arrest and DNA damage repair blocked; G2/M transition blocked
Bax α	**+ (ID)**	Apoptosis	Apoptosis induced (upregulated)
Bax γ	+ (ID)	Apoptosis	Apoptosis induced (upregulated)
Bax δ	+ (ID)	Apoptosis	Apoptosis induced (upregulated)
FADD (Fas-associated death domain)	+ (ID)	Apoptosis	Apoptosis induced (upregulated)
CASP8	+ (ID)	Apoptosis	Caspase 8 activated
DAP 3 (Death Associated Protein 3)	+ (ID)	Apoptosis	Apoptosis induced (upregulated)

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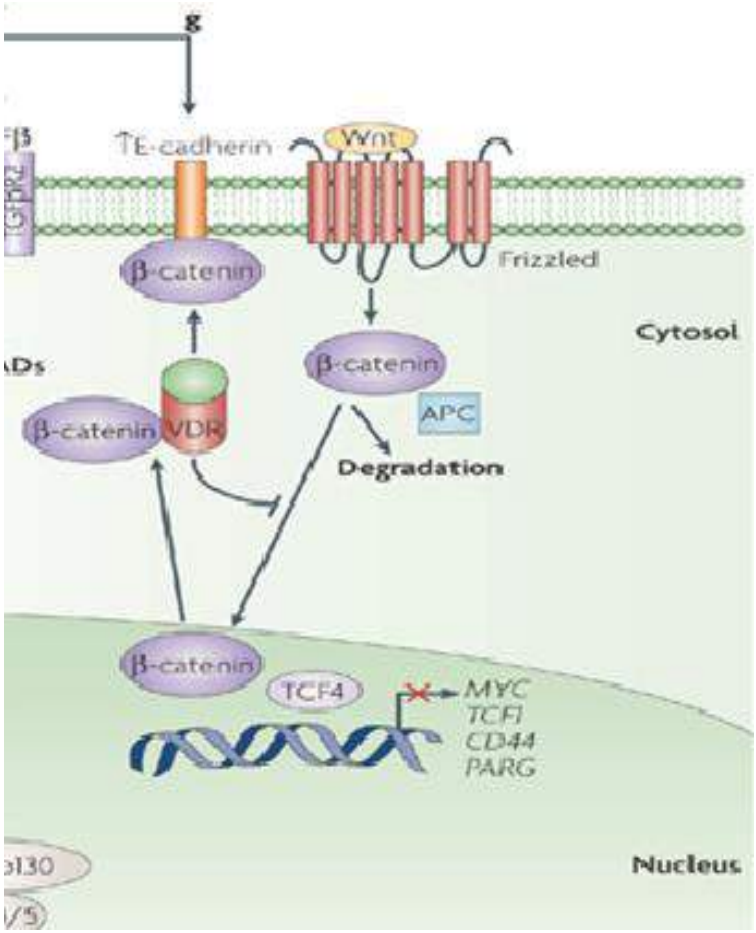
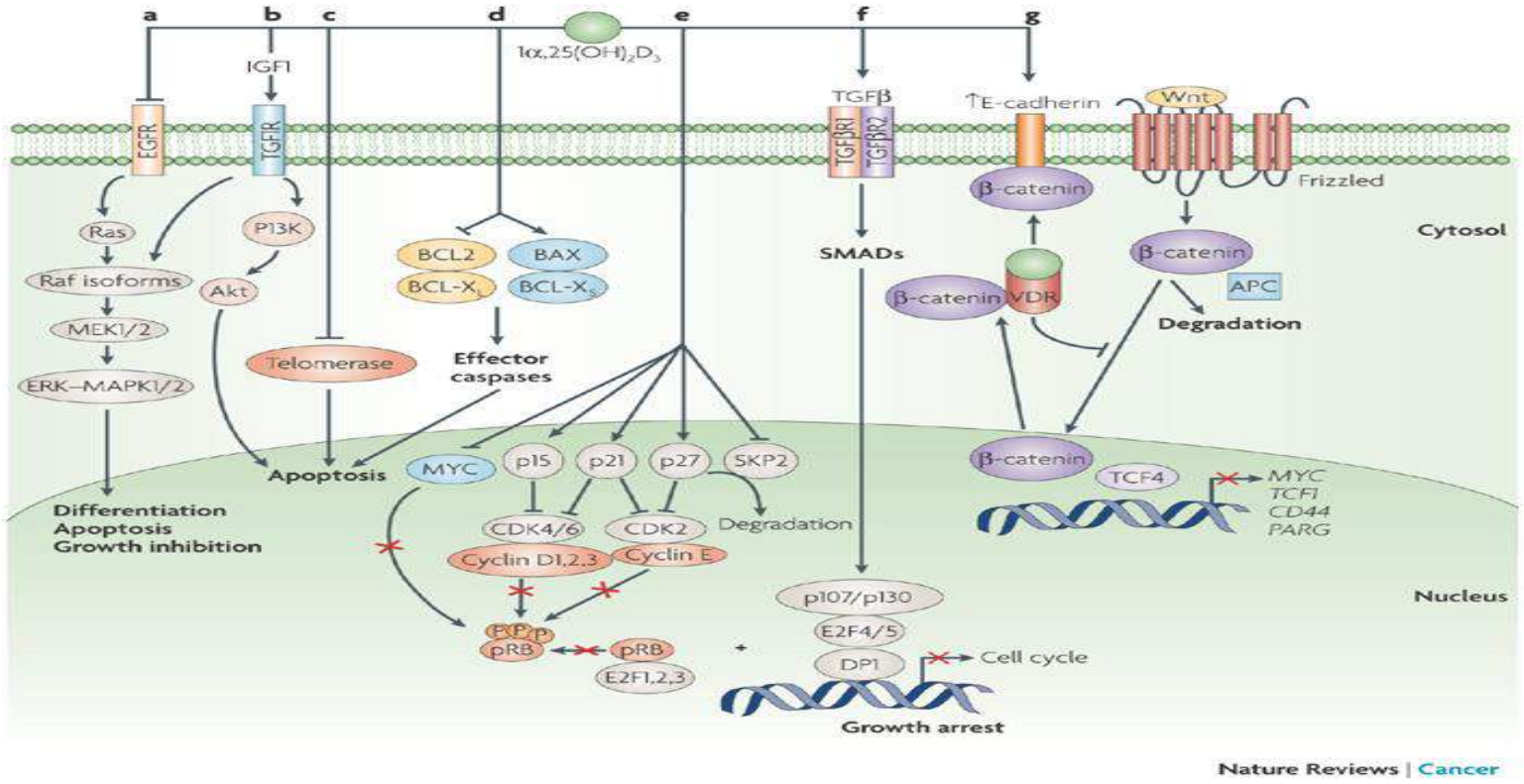
Vitamin D_3 and Wnt Signalling

- Wnt proteins have mitogenic and morphogenic effects
- Important in embryogenesis, proliferation and cancer
- Vitamin D_3 blocks translocation of β-catenin to the nucleus where it promotes cell proliferation
- Very relevant in **colon cancer.**
- *In vitro* evidence in Caco-2 and HT-29 cell lines
- Vitamin D_3 may also E-cadherin which is able to bind β-catenin and accumulate in the cytosol
- Vitamin D_3 can also upregulate inhibitor of Wnt



$1,25(OH)_2D_3$ Regulates Cell Survival

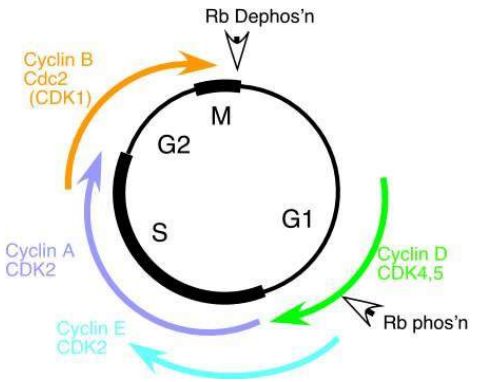


Deeb K et al 2007

Vitamin D3 is may modulate differentiation, growth and apoptosis cellular signals through cross-talk with other growth factors and cytokines like EGFR, IGF-I, TGFβ and Wnt. Vitamin D3 can modulating MAPK- ERK siginalling and PI3 Kinase- Akt signalling thus affecting cell survival.

Vitamin D and Cell Growth

- Antiproliferative effects of 1α,25(OH)2D3 have been demonstrated in various cell lines and tumour types.
- The anti-tumour effects of 1α,25(OH)2D3 involve mechanisms that are associated with
  - Arrest at all stages of the cell cycle
  - promoting differentiation
  - induction of apoptosis
  - Modulating cellular DNA repair
  - inhibiting tumour angiogenesis.



$1,25(OH)_2D_3$ Regulates Genes Involved in Regulating Cell Growth and Replication

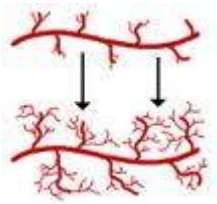
Gene	Effect of 1α 25 $(OH)_2$ Vitamin D_3	Cellular process	Mode of regulation
P21 (CDKN1)	+ (VDRE)	Cell growth	VDRE mediated effect on gene transcription. P21 arrests the cell cycle and progression G1->S phase
P27	+ (ID)	Cell growth	a) VDR interacts with the transcription factors activating p27 promoter namely with Sp1 and NF-Y b) VDR activates the PTEN gene which stabilizes p27 by de-phosphorylation and therefore prevents p27 degradation c) VD reduces CDK2 activity (S phase arrest) and Skip2 abundance.
HOXA10	+ (VDRE)	Cell cycle	G1 arrest
MN1	+	Cell cycle	G1 transition slow down
Cyclin D1	-(VDRE)	Cell cycle	Levels reduced and activity impaired; Through cross talk with EGFR (i.e. EGFR and RTK activation of cycle D1 is blocked)
FOXO	+ (VDRE)	Cell cycle	Down regulates Cyclin D i.e. G1 arrest
INK4 (CDK inhibitor)	+ (VDRE)	Cell cycle	G1 arrest

Moukayed 2012

Vitamin D3, Angiogenesis and Tumour Growth

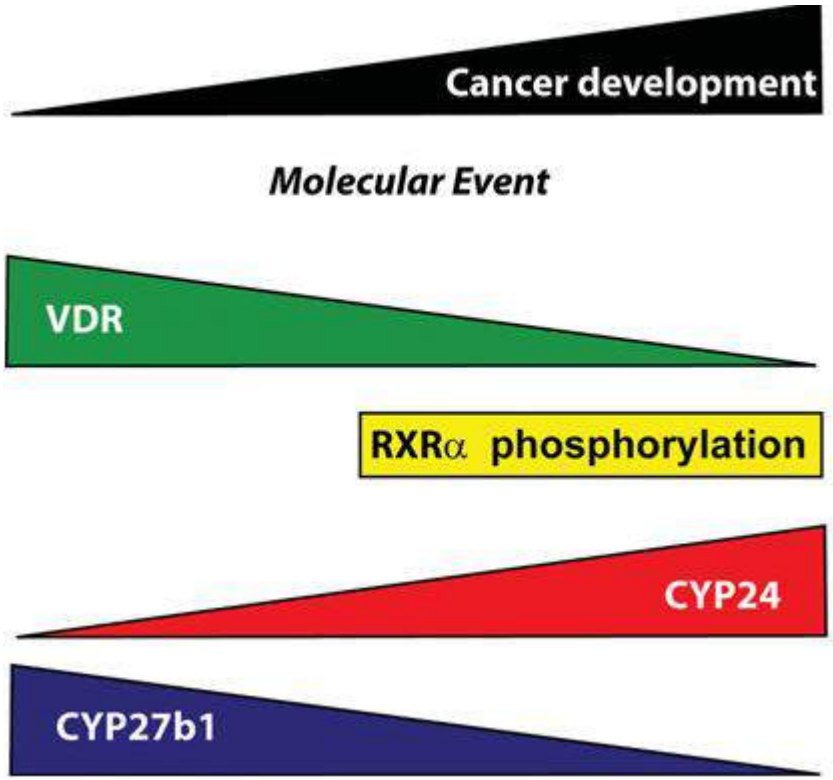
- Hypoxia induced HIF-1α in tumour cell lines blocked (mRNA and protein level)
- Hypoxic action blocked

Gene	Effect of 1,a, 25 $(OH)_2$ Vitamin D3	Cellular process	Mode of regulation
VEGF	**+/-**	**Angiogenesis**	**Inhibit RTK signaling AKT and Ras pathways**
Thrombospondin (THBS1)	+	Angiogenesis	mRNA levels increased to block angiogenesis



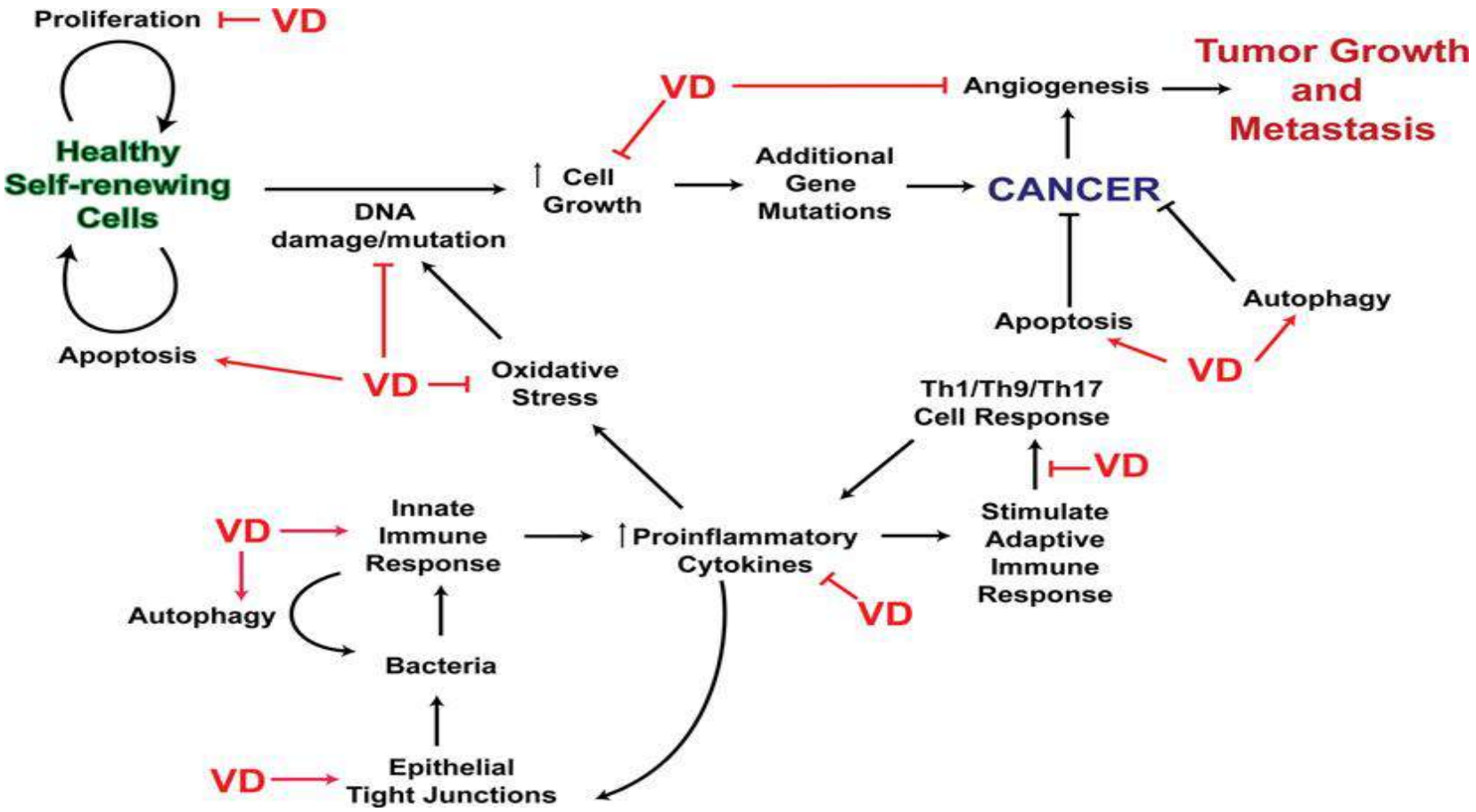
Moukayed 2012

The Cellular and Molecular Mechanisms Propose a Role for Vitamin D_3 in Tumourigenesis

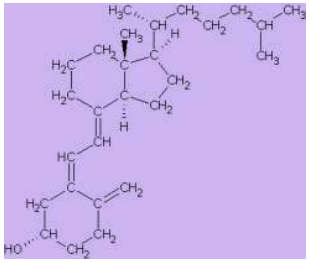


Fleet et al. 2012 with modification

Understanding the Molecular Events Regulated by $1,25(OH)_2\ D_3$ (VD) Helps Modulate Therapies



Fleet et al. 2012



Vitamin D Analogues as Treatments?

- Molecules that mimic Vitamin D and mediate Vitamin D molecular signalling mechanisms
- Still more experimentation and studies to be done but potentially may be used to improve health outcomes
- Additive to complement classical therapies
- **Promising potential**

ERGO

Let’s go out in the Sun and get some Vitamin D