Role of Vitamin D receptor (VDR) in HIV induced tubular injury

Mohammad Husain

Department of Biotechnology, Jamia Millia Islamia

New Delhi

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INTRODUCTION

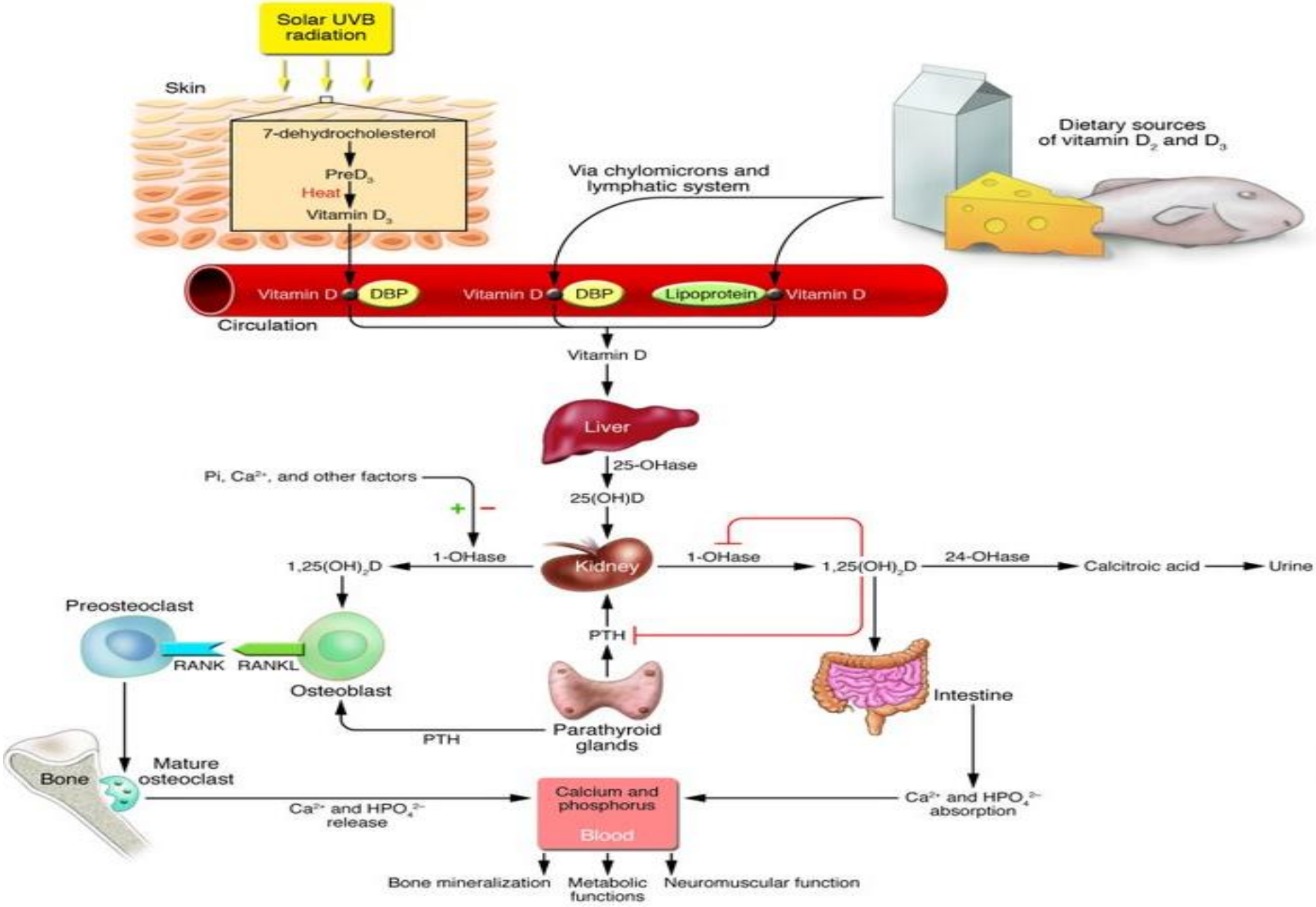
- Vitamin D is technically not a Vitamin; rather, a prohormone produced photo chemically in the skin from 7- dehydrocholestrol.
- Expression and nuclear activation of Vitamin D Receptor (VDR) is necessary for the effects of VIT D.
- VDR tissue distribution and cellular localization has been reported in almost every organ.
- VDR regulates the expression of approximately >500 of the 20488 genes in human genome.

Biological actions of 1α 25 (OH)2 D3 through VDR

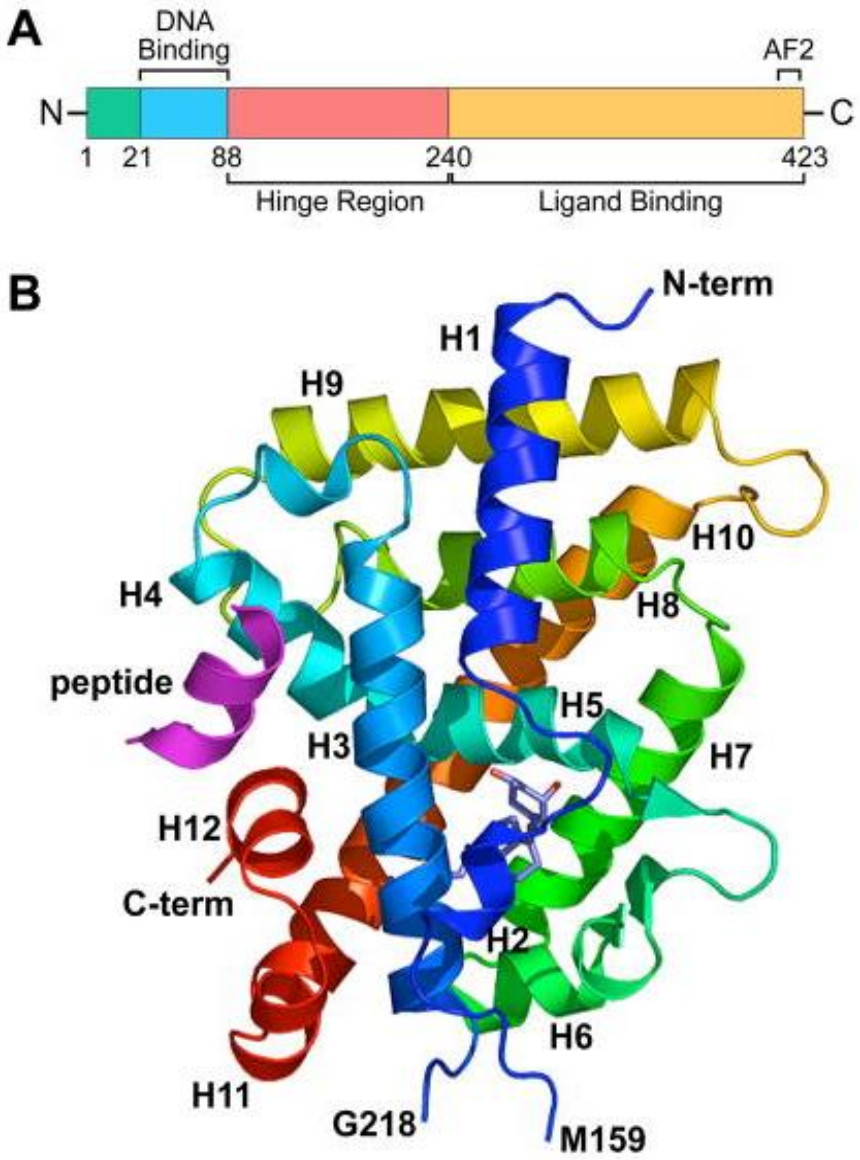
Functions as a pluripotent hormone in different arenas are:

- Adaptive Immune system
- Innate Immune system
- Insulin secretion by pancreatic beta cells
- Multifactorial heart functioning and Blood pressure regulation
- Reduction of Proteinuria
- Control of systemic Inflammation..

Vitamin D synthesis



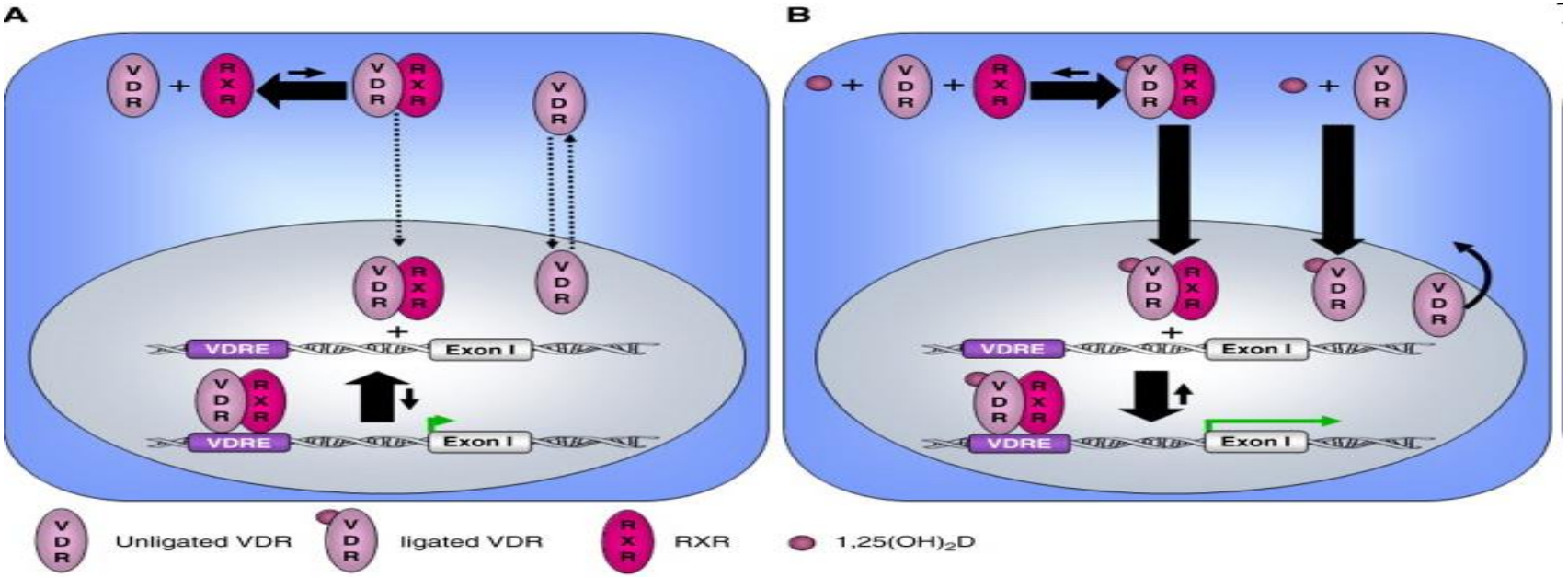
Vitamin D receptor (VDR)



A .Schematic of the VDR protein comprised of a DNA binding domain, a large ligand binding domain and a hinge region that links the two functional domain of the protein together. N, amino terminal end; C, carboxy terminal end; AF2, activation function 2. Amino acid numbers are shown.

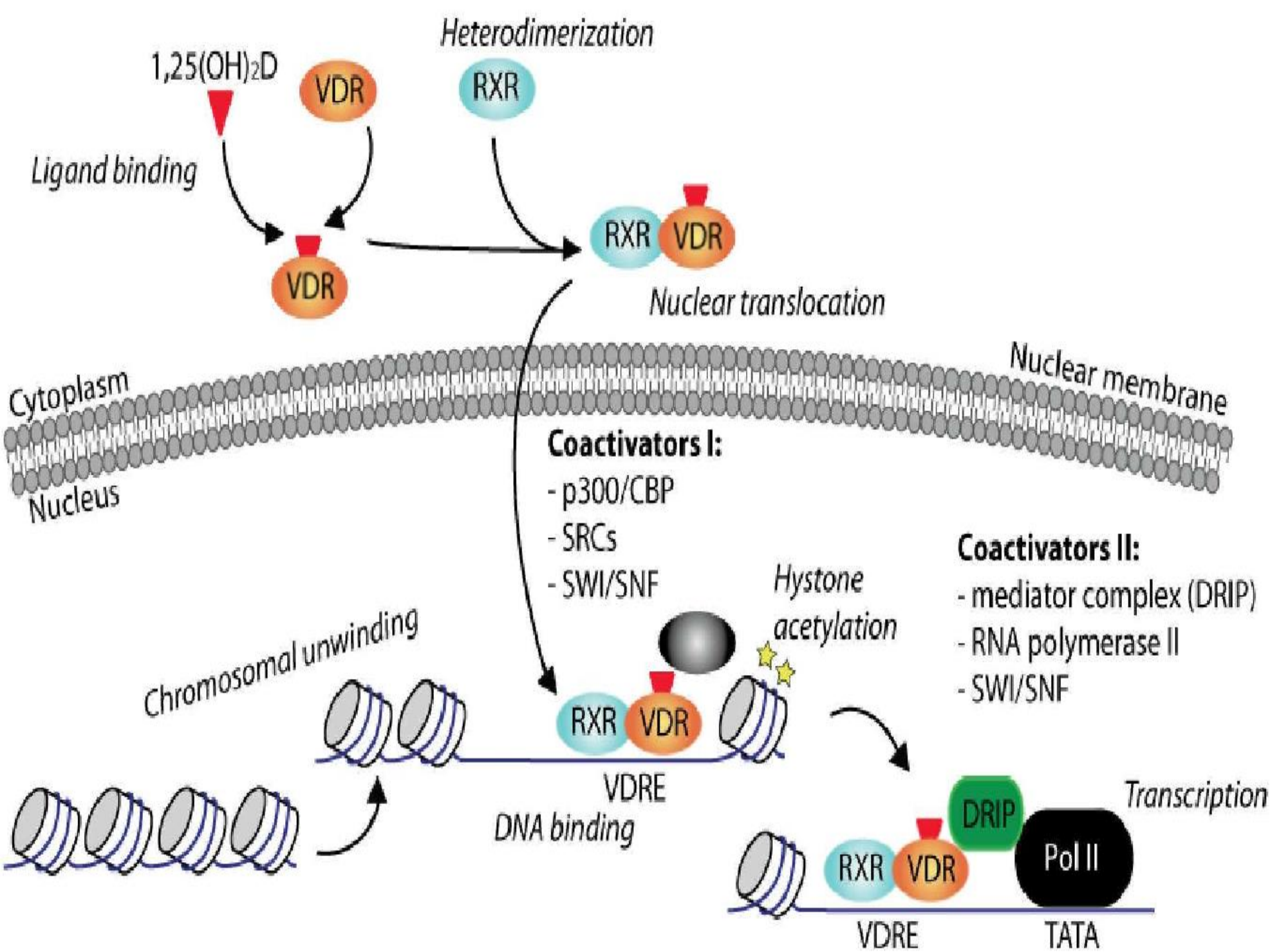
B. Crystal structure of the VDR ligand binding domain comprised of 12 α-helices (H1–H12). The N-terminal and C-terminal portions of the molecule are shown.

VDR - Member of a family of Nuclear steroid hormone receptors



- Intracellular organization of the VDR. In the absence of 1,25(OH)2D,
- VDR shuttles between cytoplasm & nucleus.
- The VDR can dimerize with RXR, but the formed heterodimer is not stable and has a low affinity for the VDRE.
- This results in basal regulation of target gene transcription

- Upon binding 1,25(OH)2D, VDR is activated and translocated to the nucleus
- The VDR/RXR heterodimer is stabilized, giving it a high affinity for the VDRE, which results in an increased transactivation or transrepression of the genes


Heterodimerization
1,25(OH)2D
VDR
RXR
Ligand binding
VDR
RXR VDR
Nuclear translocation
Cytoplasm
Nuclear membrane
Nucleus
Coactivators I:
- p300/CBP
- SRCs
- SWI/SNF
Coactivators II:
- mediator complex (DRIP)
- RNA polymerase II
- SWI/SNF
Hystone acetylation
Chromosomal unwinding
RXR VDR
VDRE
DNA binding
Transcription
DRIP
Pol II
RXR VDR
VDRE
TATA

HIV infection and VITAMIN D deficiency

- **Observational studies have revealed very high low 25(OH)D levels in HIV-infected patients Could be due to** :
  - **Traditional risk factors: Lack of UVB exposure, darker skin pigmentation.**
  - **Antiretroviral therapy (ART)–As VIT D and PI(protease inhibitors) and NNRTI (Non Nucleoside reverse transcriptase inhibitor) are metabolized by Cytochrome P 450 system.**
- **The mechanism of HIV on VDR remains to be investigated?**

VDR polymorphism and HIV infection susceptibility and progression

- The risk of HIV disease infection and progression has been examined in relation to the presence of a number of polymorphism in the VDR gene.

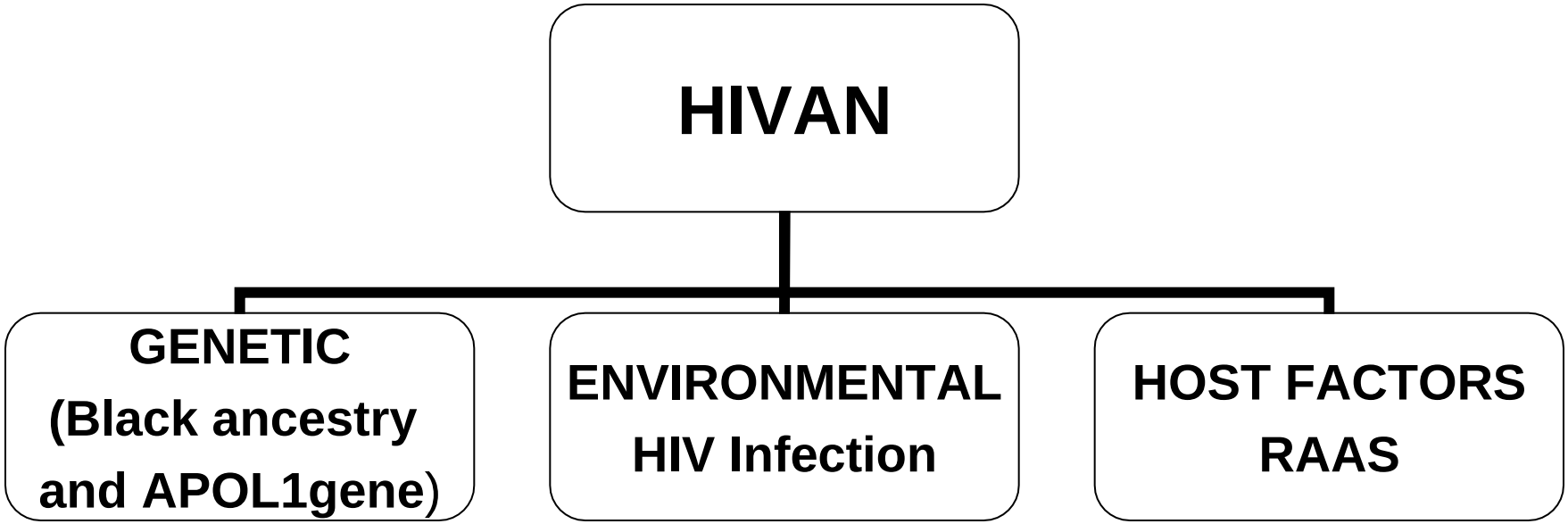
- The study by Nieto et al. and Barber et al. have examined the prevalence of mutations in a region of the VDR gene corresponding to *Bsm* I restriction enzyme and *Fok* I in relation to HIV status.

- The heterozygous genotype Bb and Ff was significantly associated to progression of AIDS and CD4 count drop to under 200/mm3

HIVAN

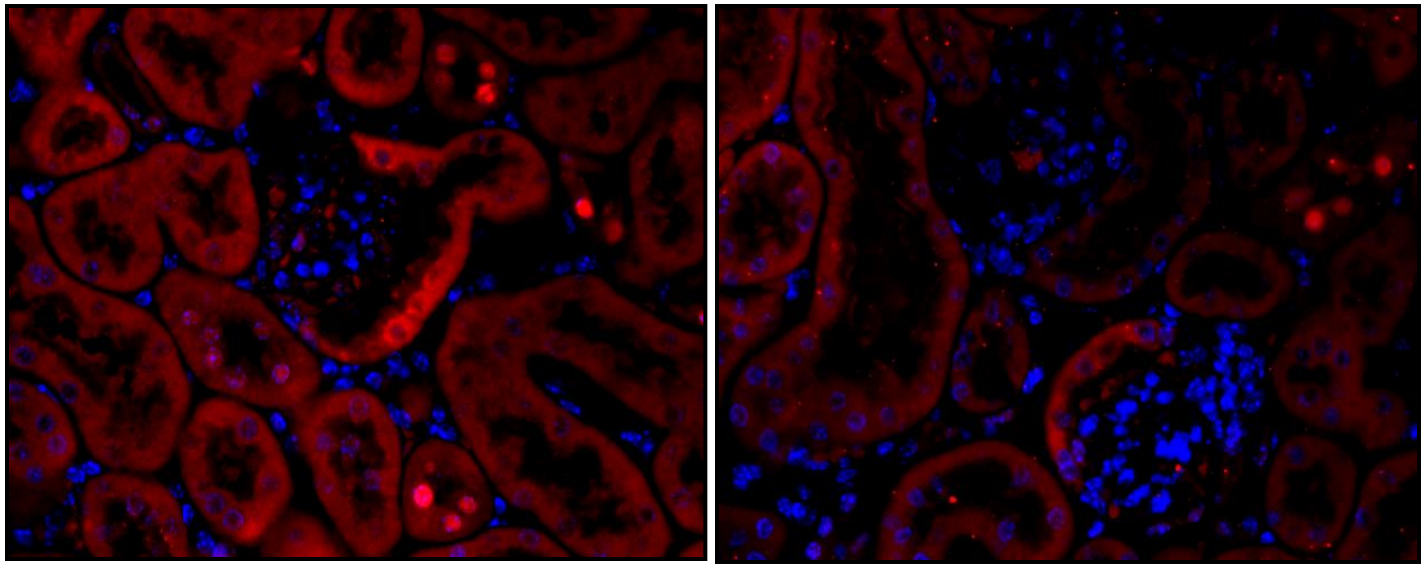
- HIV-associated nephropathy (HIVAN) is a combined glomerular and tubular injury characterized by:

  - **Collapsing glomerulopathy (CG) with collapse of glomerular capillary structures and a striking hyperplasia of podocytes.**
  - **Microcystic dilatation of renal tubules.**
  - **Interstitial inflammation and fibrosis consequent to the glomerular and tubular injuries.**



VDR expression in murine model of HIVAN(Tg26)

VDR/DAPI

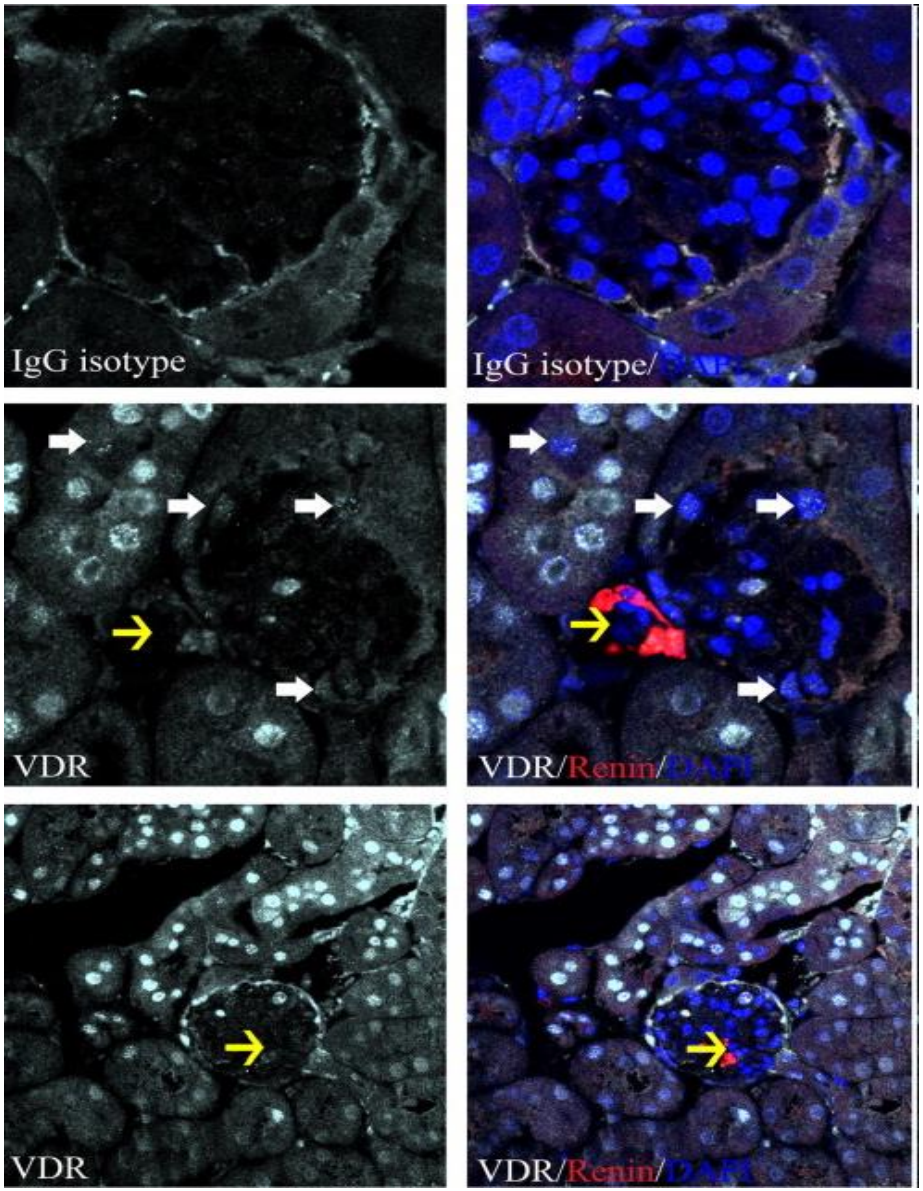


Control **Tg26**

Tg26 mouse model was developed using gag/pol deleted clone of HIV-1 to render the virus non-replicative

VDR localization in Kidney

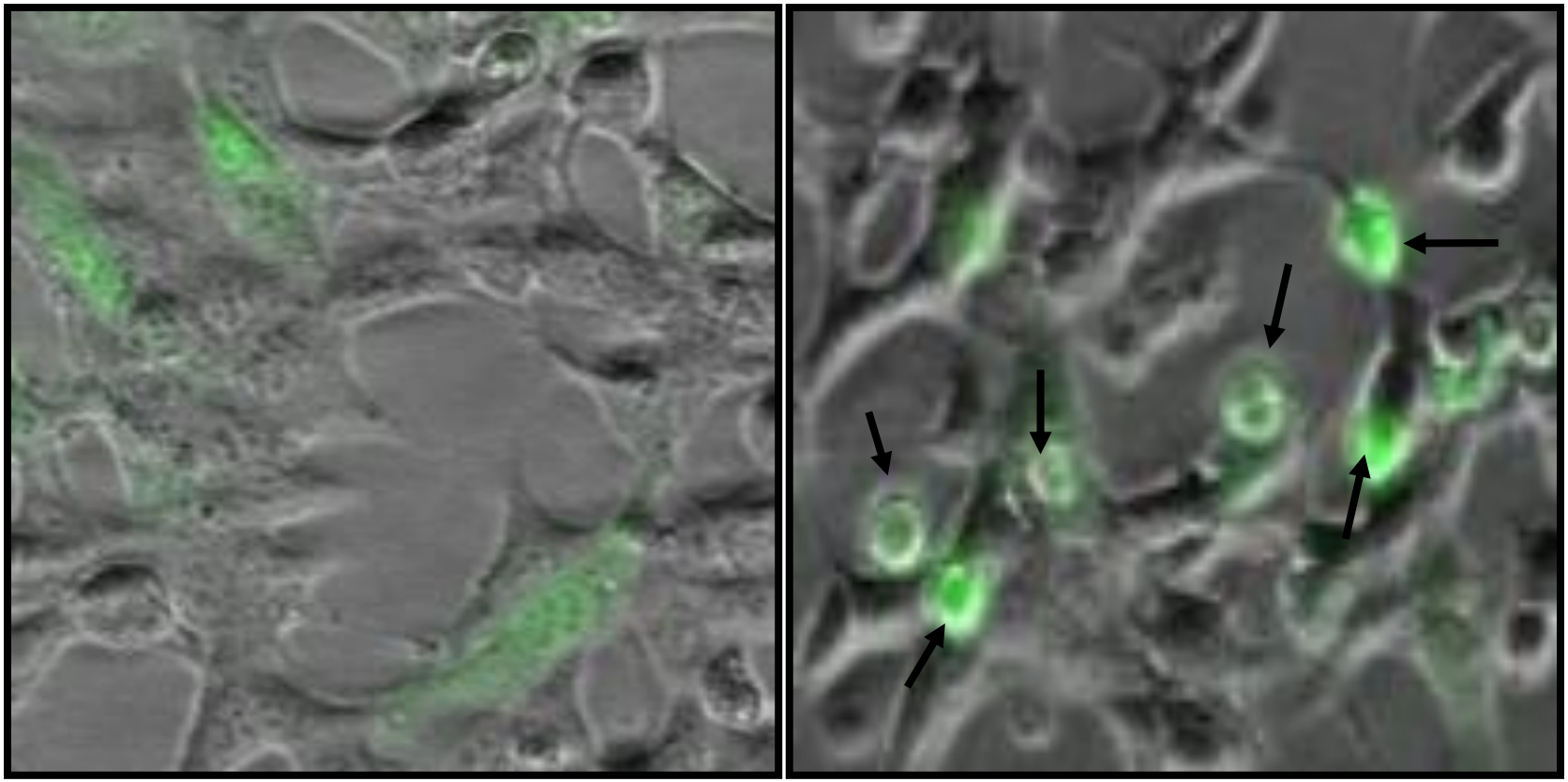
VDR in mouse proximal renal Tubule, Distal tubule, Glomerulus.



HIV induces apoptosis

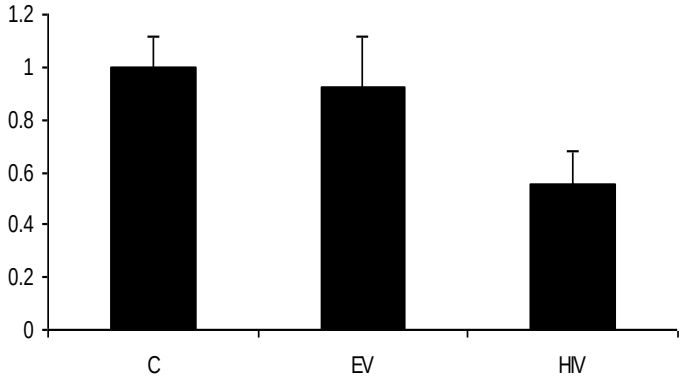
- HIV has been reported to induce apoptotic phenotype in tubular cells.
- Oxidative stress is involved in the induction of HIV-induced kidney cell apoptosis. It can contribute to HIV disease pathogenesis by
  - **Enhancing viral replication**
  - **Increasing the inflammatory immune response**
  - **Accelerating the loss of immune function** .
- HIV-1-induced overproduction of ROS, has been proposed to account for the activation of apoptosis in several cell types including tubular cells.
- Role of VDR in HIV induced ROS generation in tubular cells?

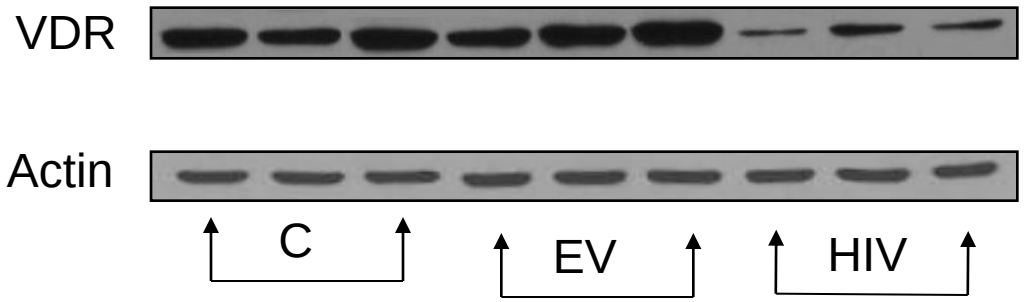
Morphological effect of HIV-1 expression on tubular cells



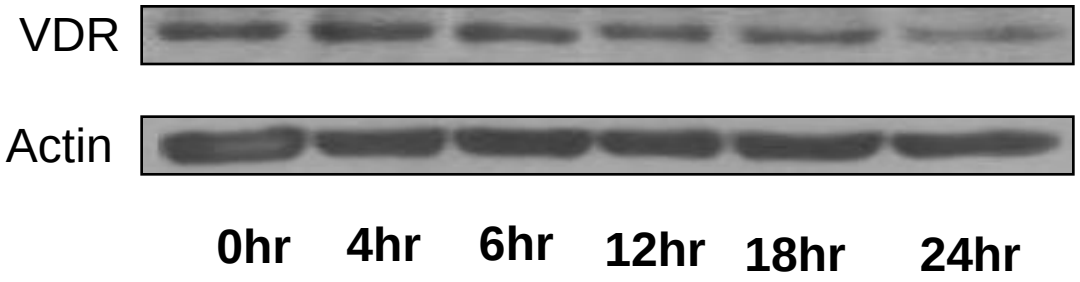
EV HIV

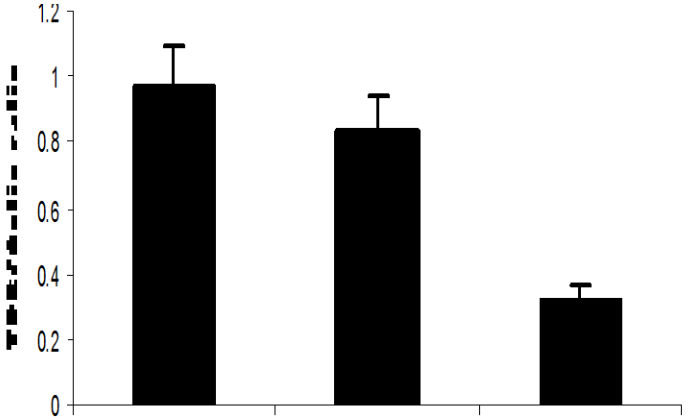
HIV down regulates VDR in tubular cells





mRNA expression of VDR

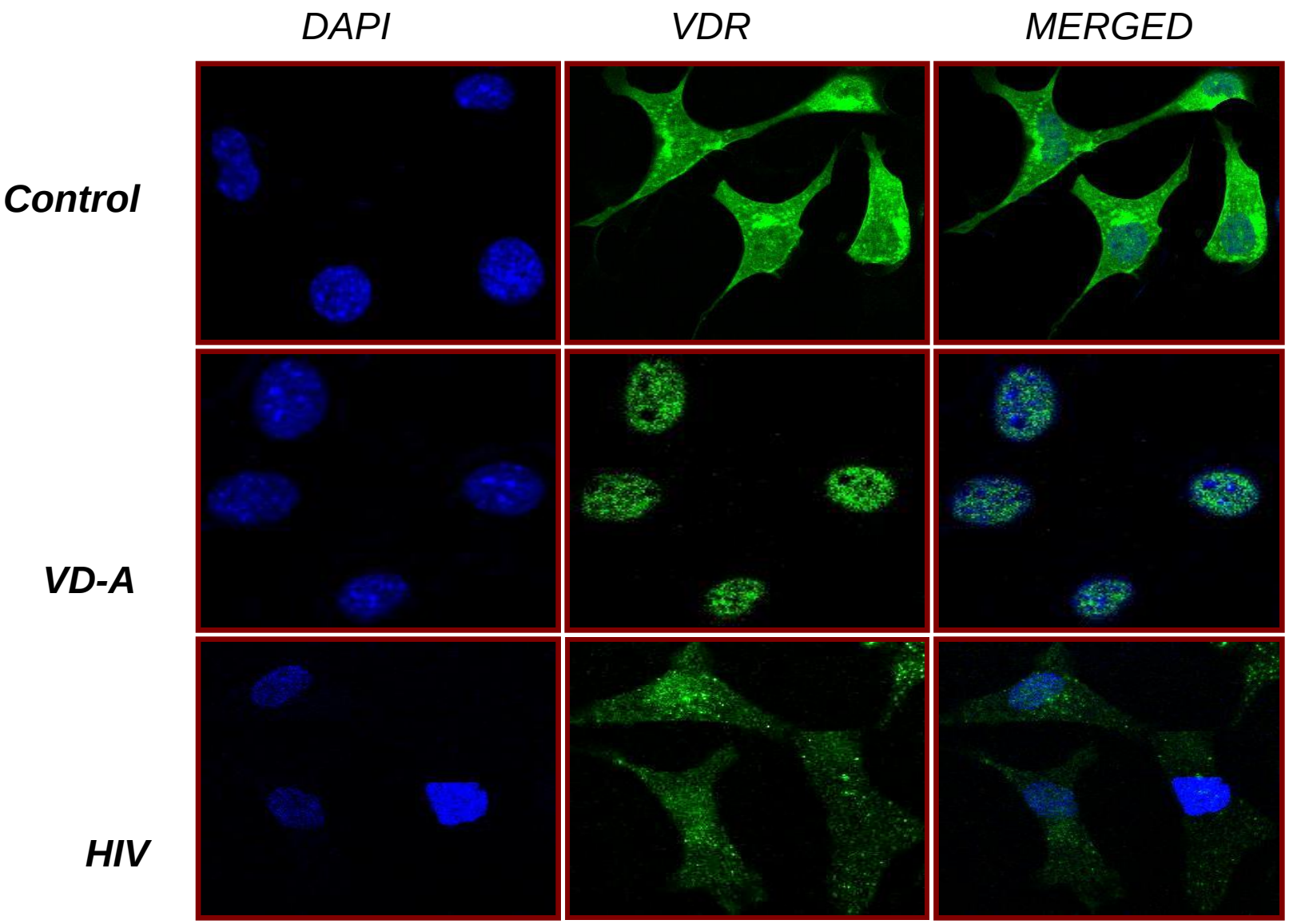




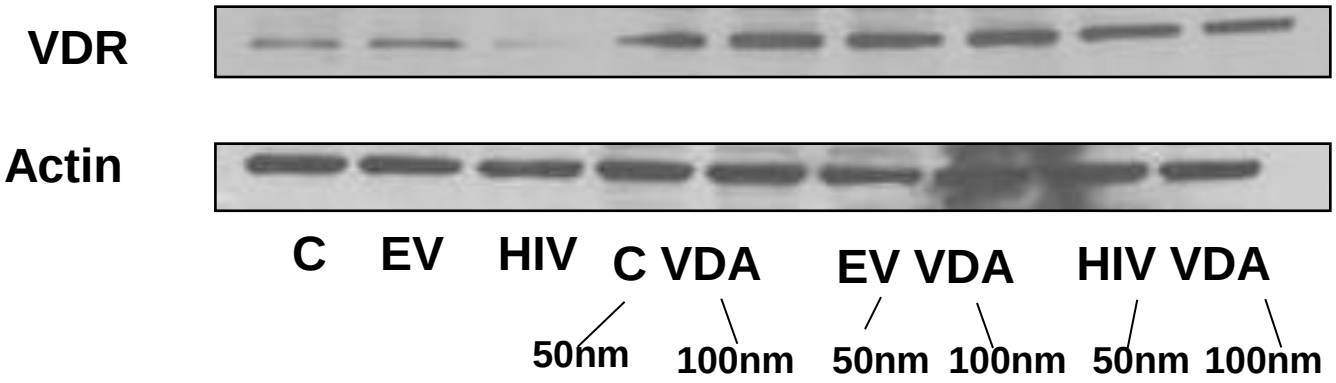
TIME course of VDR down regulation with HIV

Protein expression

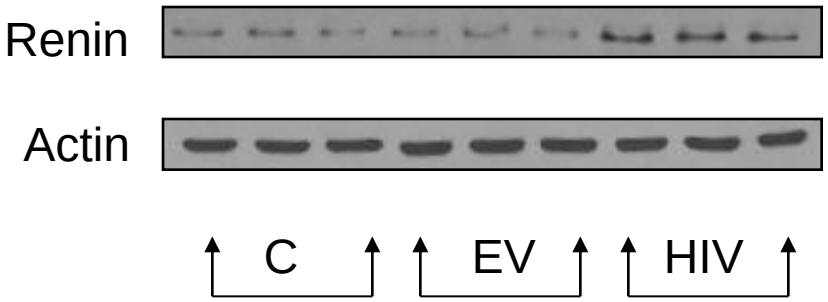
Immunofluoroscence study of VDR expression in tubular cells

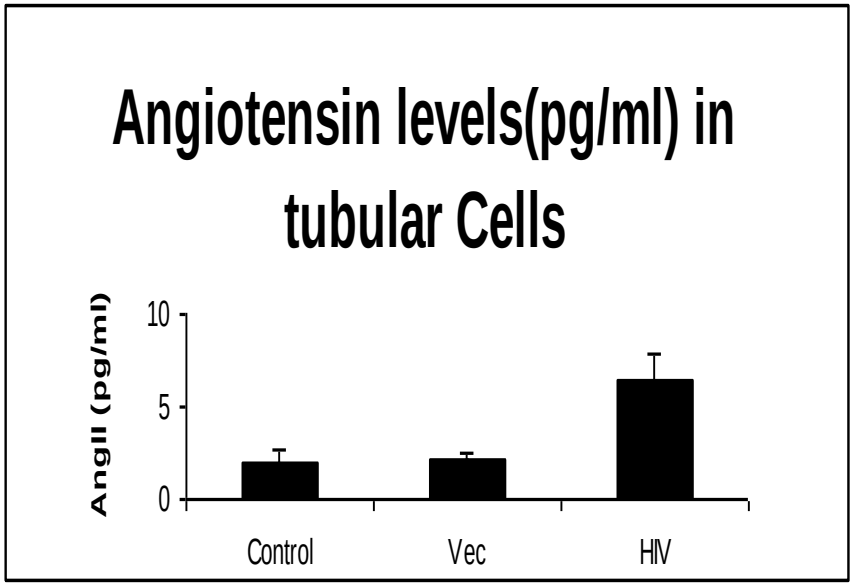


Effect of VDA agonist (EB1089)

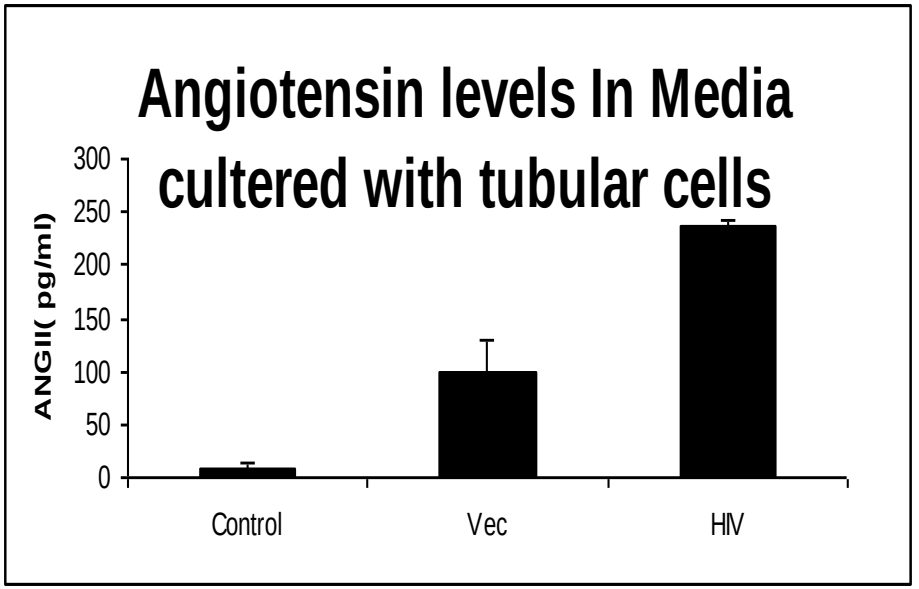


HIV activates tubular cell Renin Angiotensin system



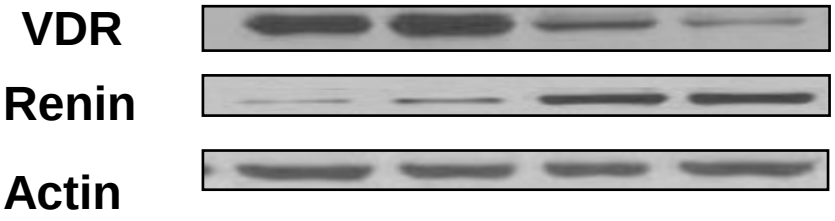


p < 0.0131



p< 0.001

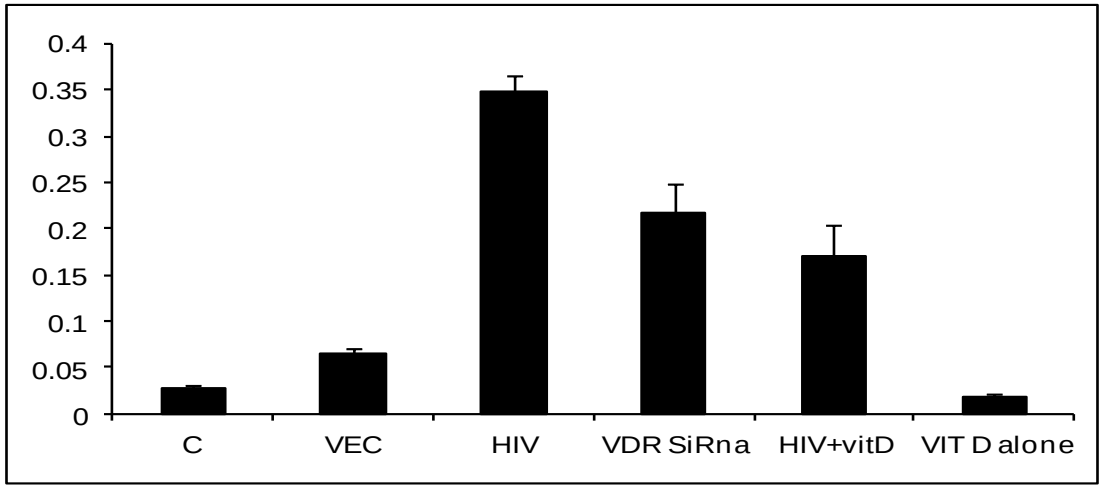
VDR as a negative regulator of RENIN



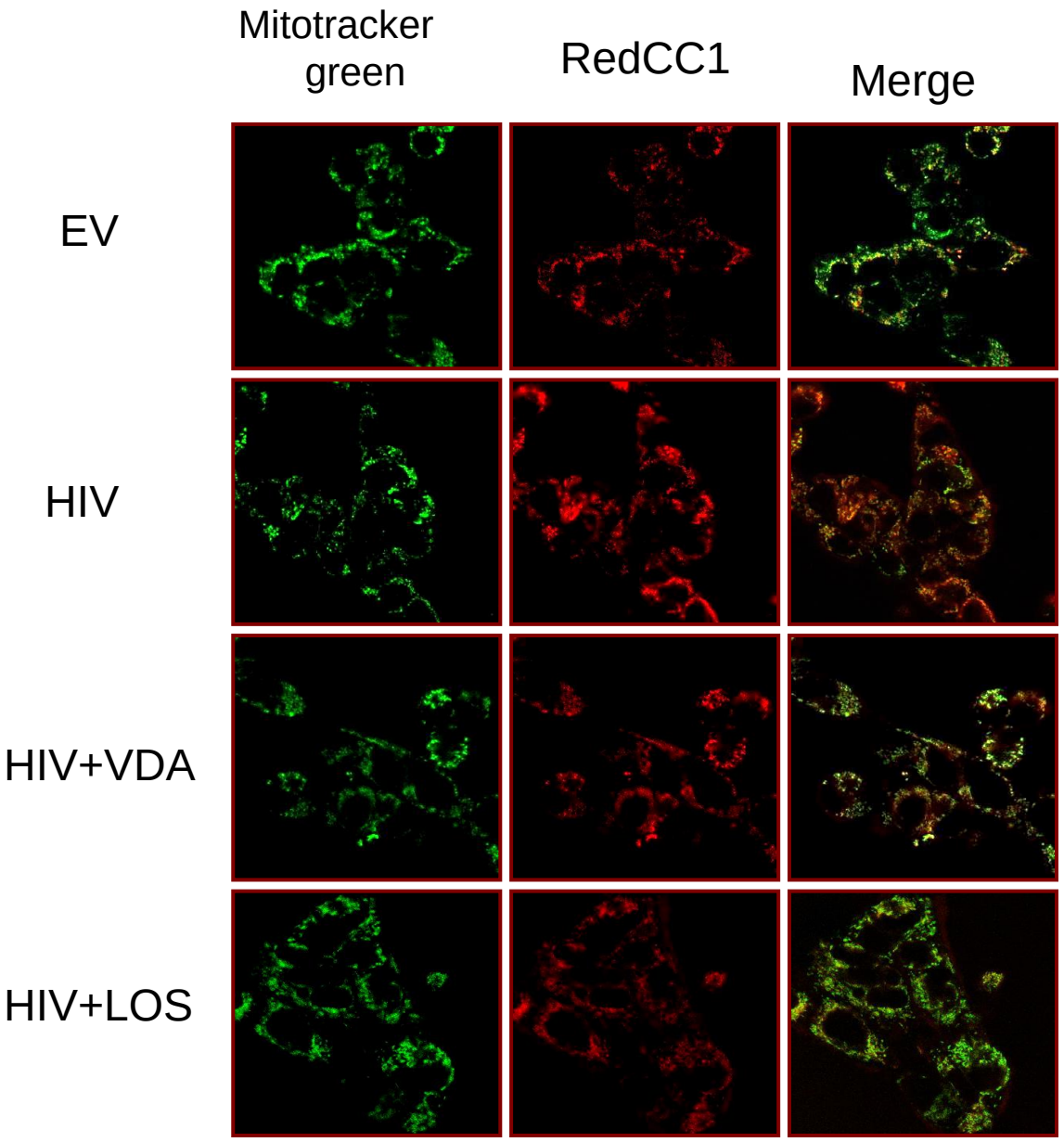
Cont **Cont siRNA** **VDR siRNA** **HIV**

- **SiRNA- small interfering RNA**

ANGIOTENSIN II ELISA



ROS generation in Mitochondria

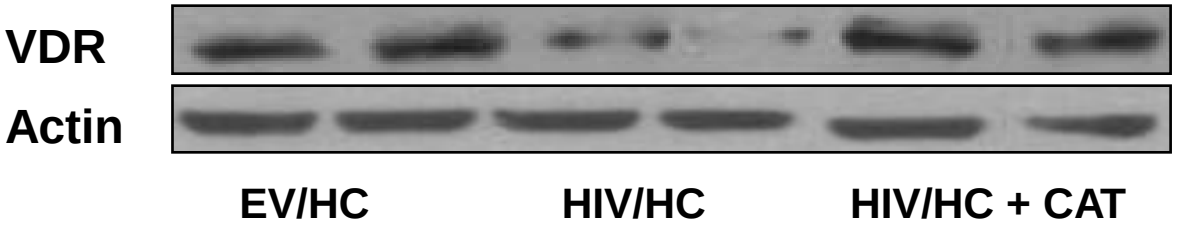


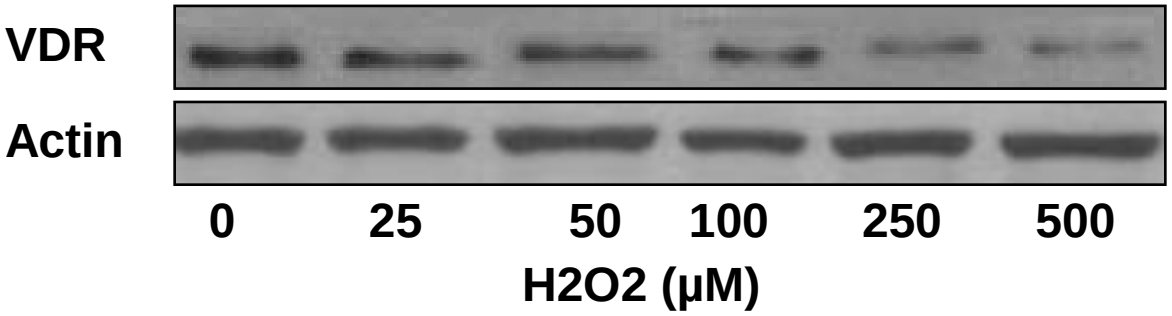
To determine the site of ROS in HIV milieu and its modulation by VDA and Ang II blockade, Both EV/MC and HIV/MC were treated with either buffer, VDA (50 nM), or losartan (10-7M) and then labeled with Red CC1 and MitoTracker green followed by fluorescence microscopy. HIV stimulated mitochondrial ROS generation; whereas, both VD and losartan partially inhibited HIV-induced ROS generation.



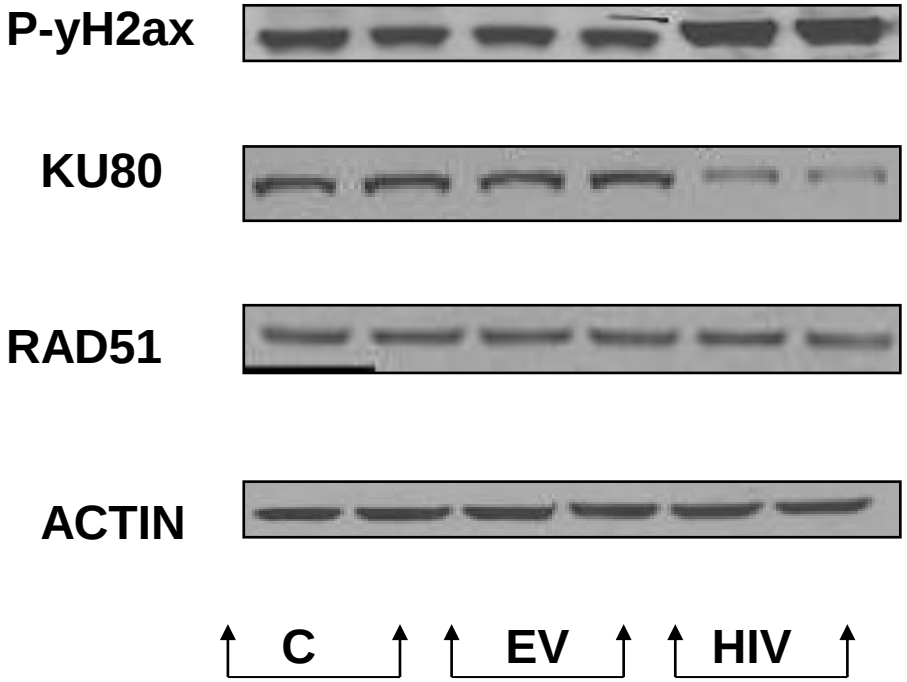
HOW HIV Down regulates VDR????

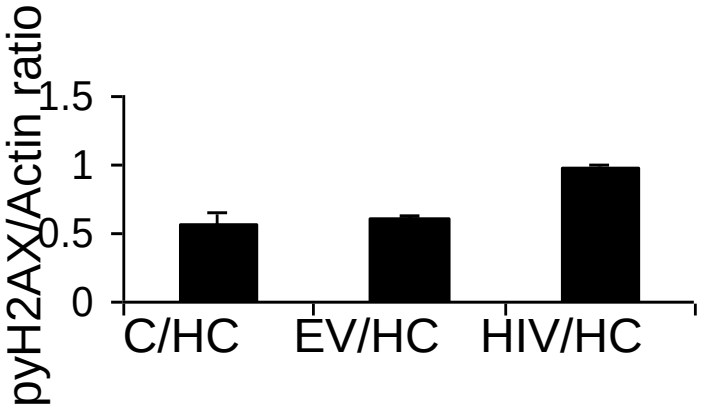
Oxidative stress / Reactive oxygen species

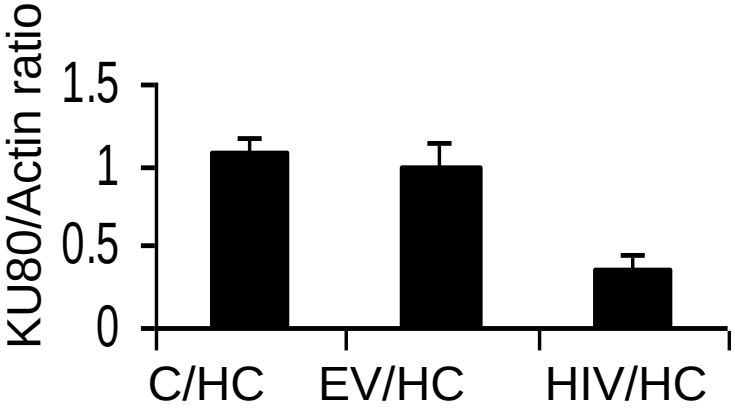




1. DNA Damage response



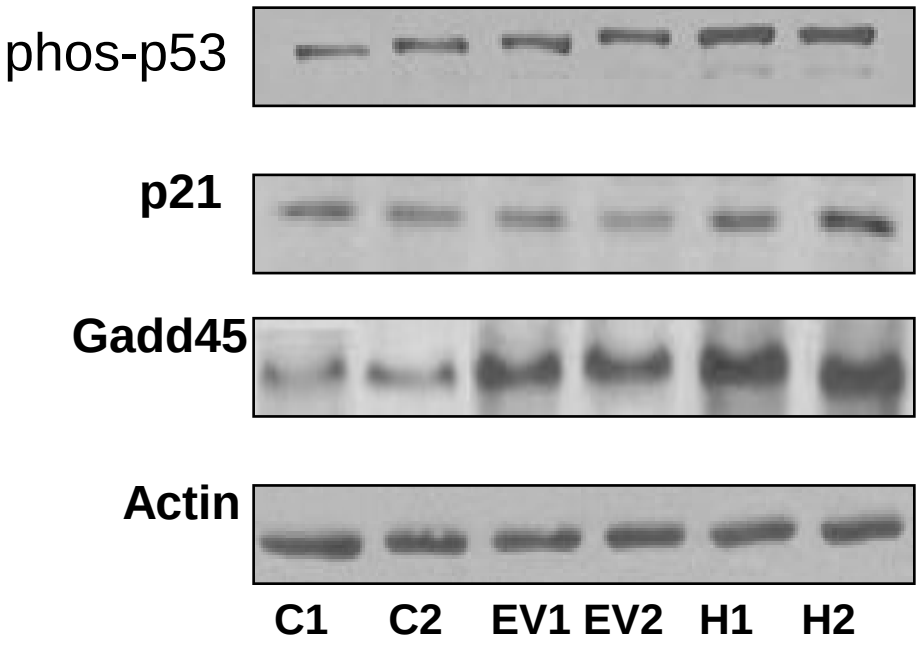


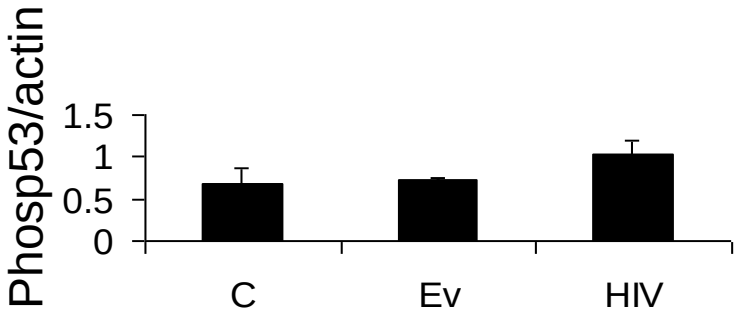


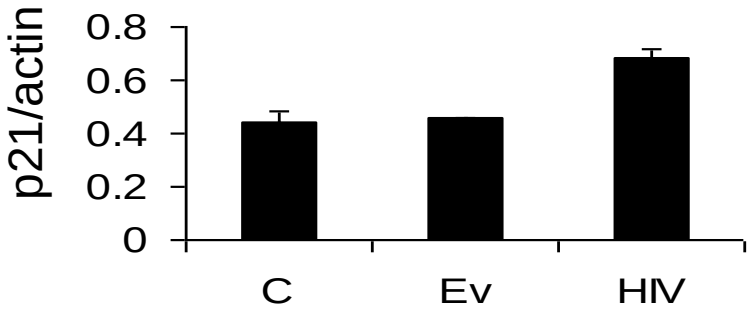
KU80- Non homologous end joining protein In G2 phase of cell cycle.

RAD51: Homologous recombination protein In S phase of cell cycle

Cell Cycle arrest genes



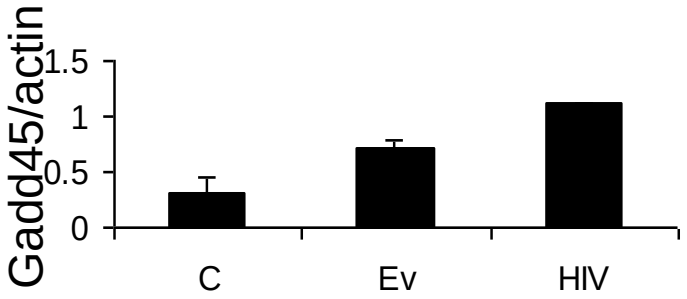




p53-Tumour Suppressor gene

p21- Cyclin dependent kinase inhibitor -1

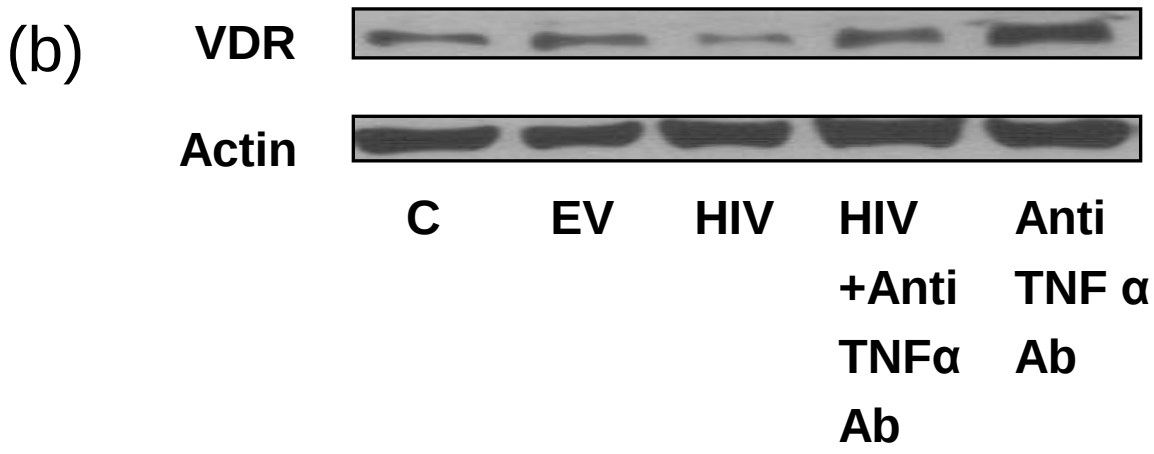
Gadd45- Growth arrest and DNA damage inducible 45 alpha



2. Role of Inflammation

- HIV infection induces high **TNF-α** state (Tumour necrosis factor)
- TNF-α is associated with decreased 125(OH)2D3 levels.



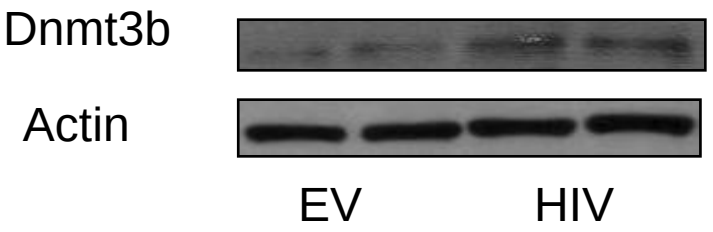


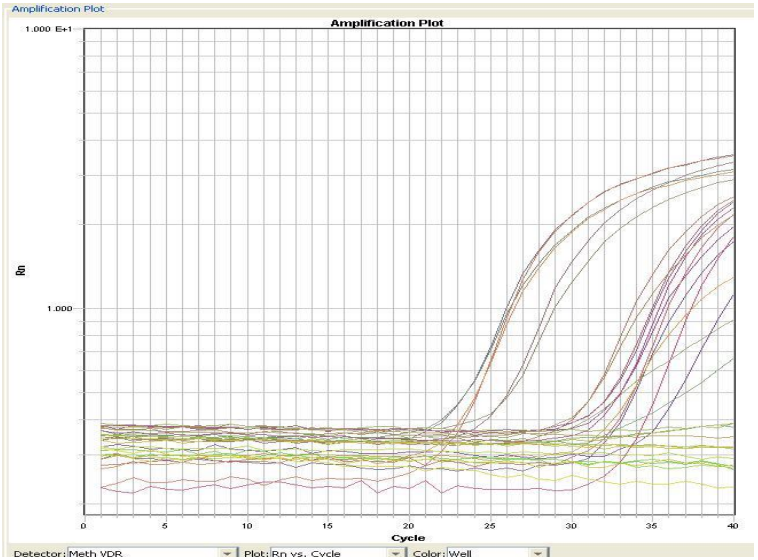
3. EPIGENETIC Modifications

- Two changes are **DNA methylation** and **Histone modification** both of which serve to regulate gene expression without altering the underlying DNA sequence.

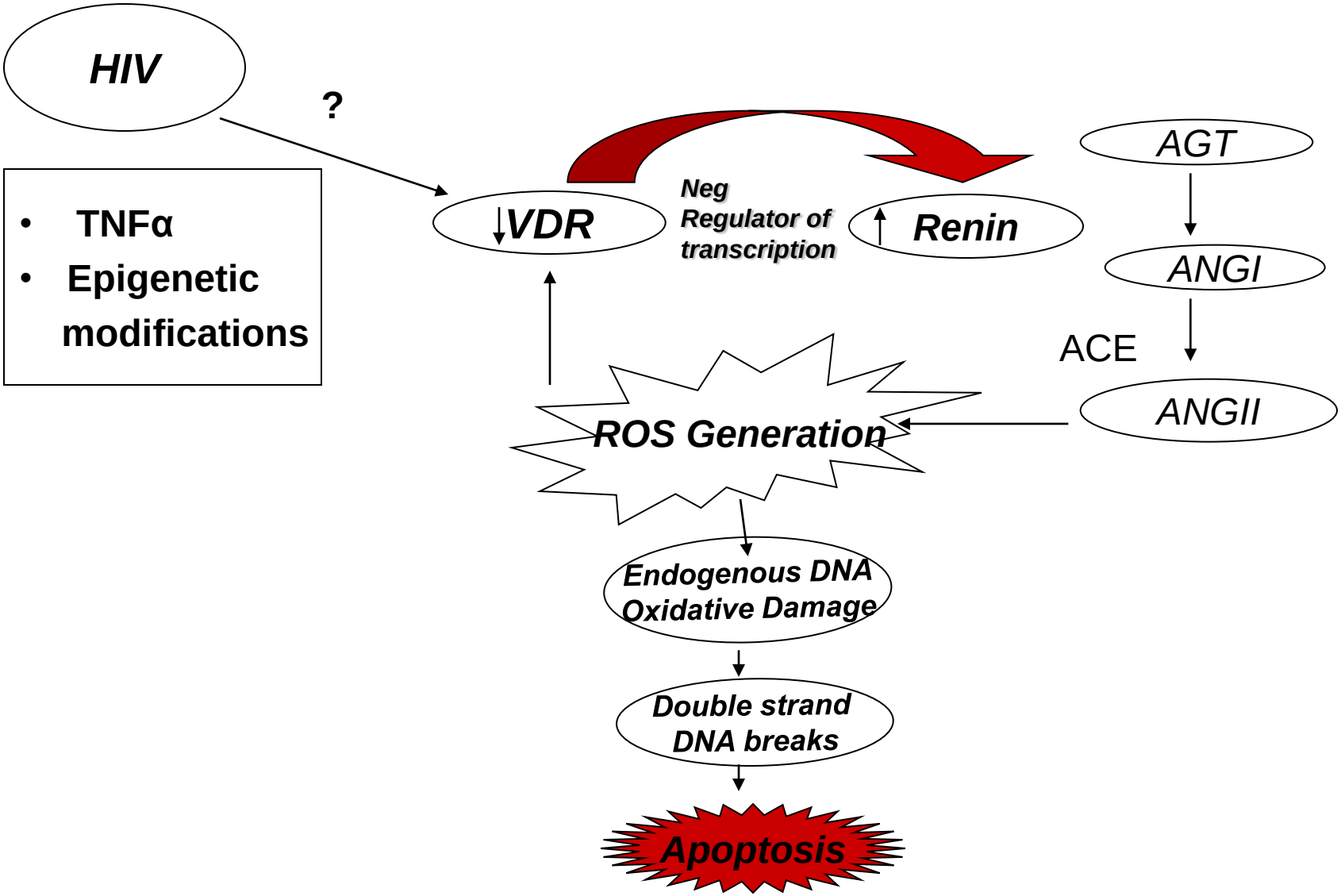
- **DNA methylation** in vertebrates typically occurs at **CpG sites** (cytosine-phosphate-guanine sites, that is, where a cytosine is directly followed by a guanine in the DNA sequence by **DNA methyltransferase** (DNMT) enzymes.

Methylation Studies





Summary


HIV
?
• TNFα
• Epigenetic modifications
↓VDR
Neg Regulator of transcription
↑ Renin
AGT
ANGI
ACE
ANGII
ROS Generation
Endogenous DNA Oxidative Damage
Double strand DNA breaks
Apoptosis

THANK YOU!!