Vitamin D-mediated upregulation of differentiation marker genes requires demethylating agents in neoplastic mast cells

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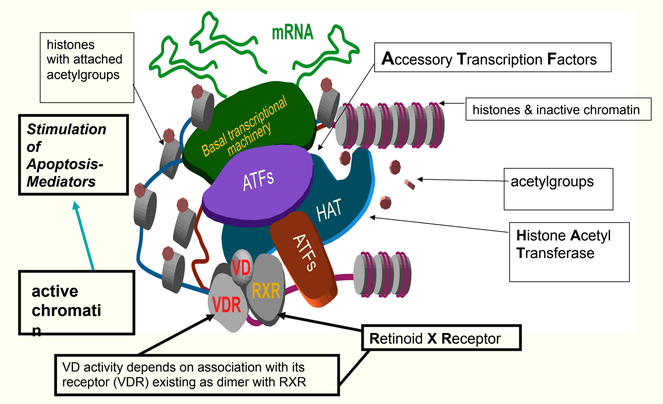
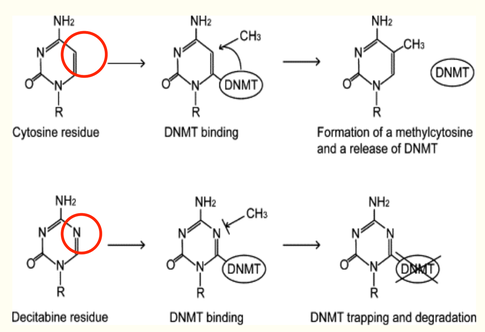
INTRODUCTION

Direct and indirect effects of vitamin D (VD) in prevention and treatment of malignancies are closely associated with its epigenetic effects. Its antineoplastic activity is mediated by induction of cellular differentiation and apoptosis. Considering the role of VD for disease prevention and promotion of therapy-success potentiation of treatment effects, the aim of this study was to evaluate possible epigenetic mechanisms, which are responsible for the supportive activity of demethylating agents such as (Decitabine, 5-aza-desoxycytidine DAC) or Simvastatin (Sim) on VD-mediated activation of differentiation-associated genes from mast cell leukemia-derived HMC-1.1 cells.

Epigenetics of Vitamin D3 action



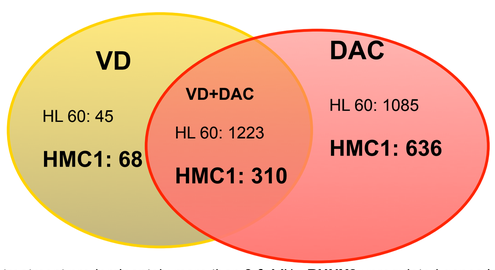
Mechanism of Decitabine action



Decitabine (5-aza-2-deoxycytosine) incorporates into DNA & prevents transfer of methyl groups by DNMT (= DNA (cytosine-5) - methyltransferase)

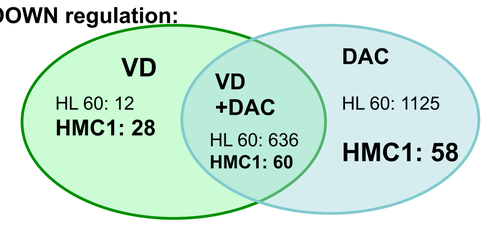
VENN Diagrams

VitaminD (VD)- and Decitabine (DAC) UP regulation:



Combined treatment predominantly more than 2-**fold)*** , **RUNX2** upregulated genes including
>The monocyte marker **CD14 (up to 200- (360-fold)***
>>>Osteocalcin (BGLAP) (up to +1000-fold)*
and **Fibroblast Growth factors (more than 2-fold)****

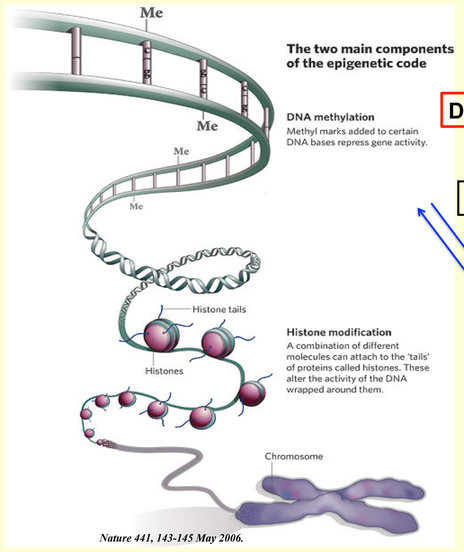
DOWN regulation:



Combined treatment downregulated genes from oncogenic signaling pathways

* Verified by Quantitative PCR
**mRNA expression analysis by Human ST1.0 Affymetrix Arrays from cell lines
Treated with 10nM Vitamin D3 and/or 2µM DAC for 3 days

Both Decitabine and Vitamin D act via Epigenetics:



Decitabine inhibits DNA-Methylation

Vitamin D acts on Chromatin

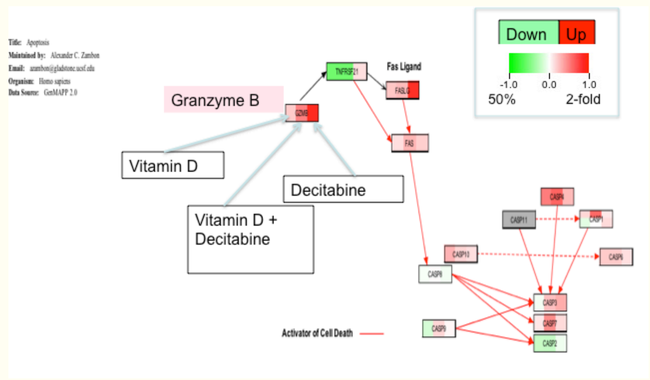
Chromatin remodeling

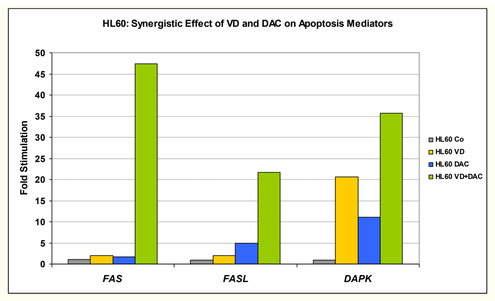
Histone code

Nuclear organization

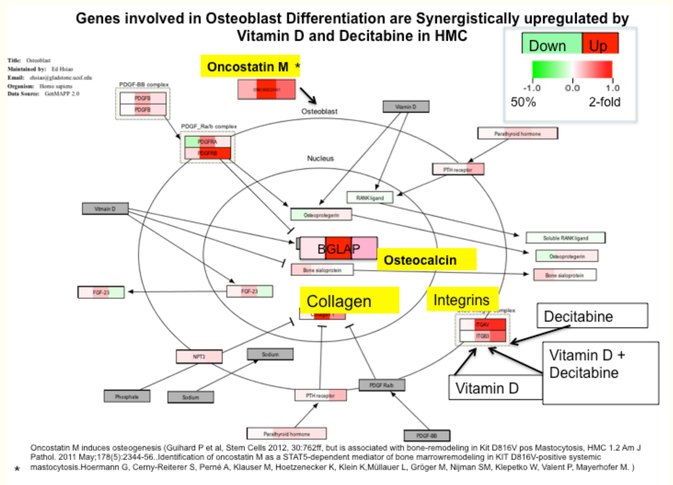
Untranslated RNA

Regulation of Apoptosis- Associated Genes



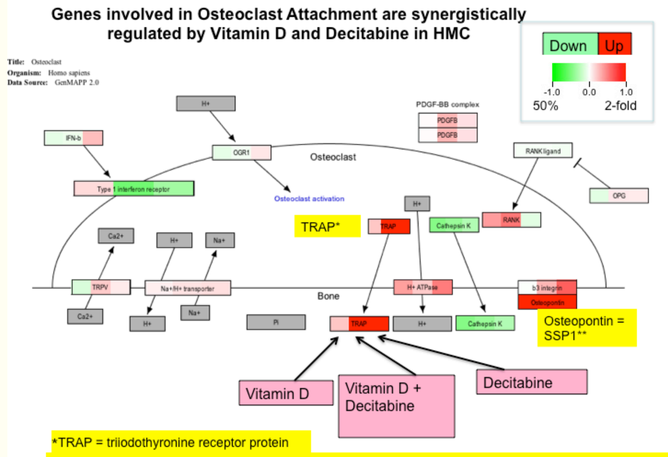


Genes involved in Osteoblast Differentiation are Synergistically upregulated by Vitamin D and Decitabine in HMC



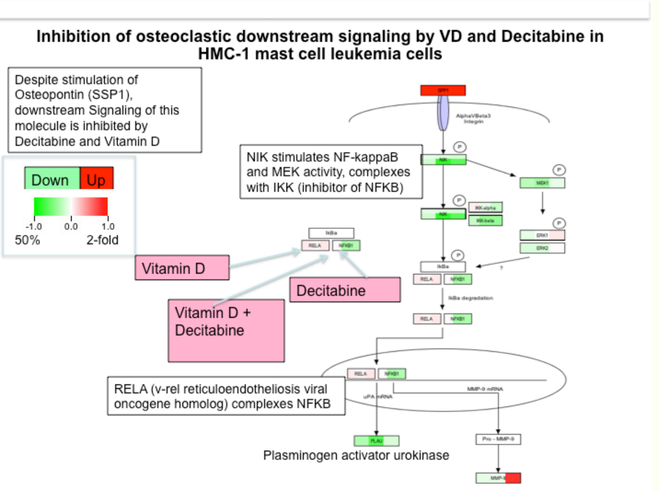
Oncostatin M induces osteogenesis (Guihard P et al, Stem Cells 2012, 30:762ff, but is associated with bone-remodeling in Kit D816V pos Mastocytosis, HMC 1.2 Am J Pathol. 2011 May;178(5):2344-56. Identification of oncostatin M as a STAT5-dependent mediator of bone marrowremodeling in KIT D816V-positive systemic mastocytosis.Hoermann G, Cerny-Reiterer S, Perné A, Klauser M, Hoetzenecker K, Klein K,Mullauer L, Gröger M, Nijman SM, Klepetko W, Valent P, Mayerhofer M.)

Genes involved in Osteoclast Attachment are synergistically regulated by Vitamin D and Decitabine in HMC

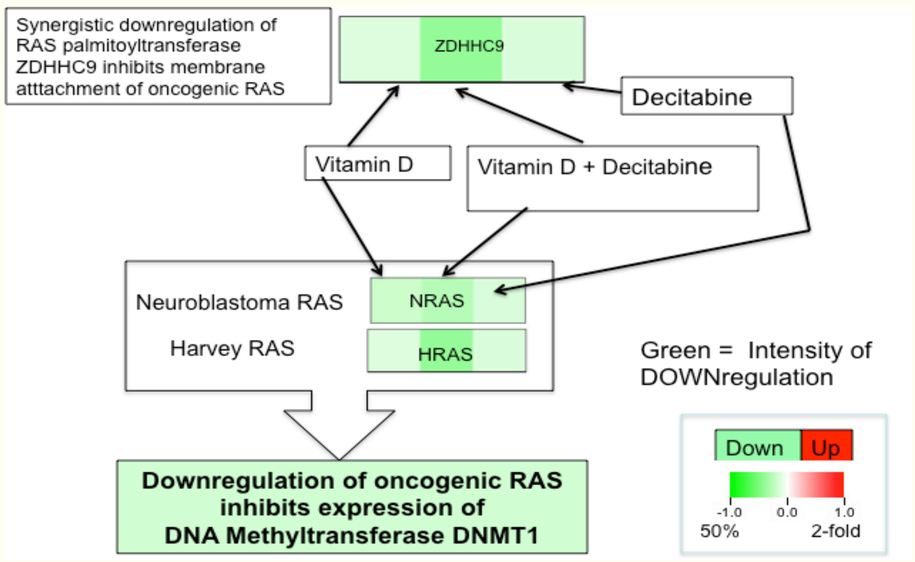


*TRAP = triiodothyronine receptor protein
**Osteopontin is responsible for attachment of osteoclasts to the mineralized bone matrix

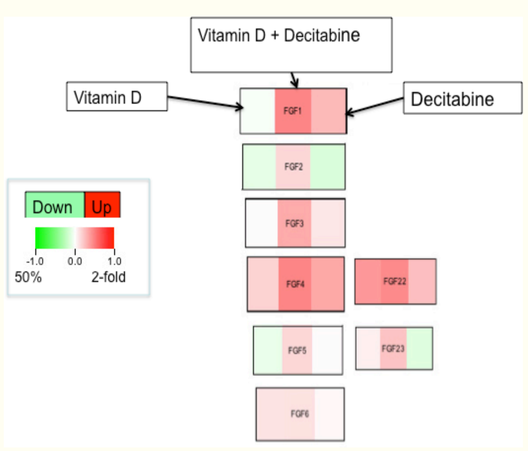
Inhibition of osteoclastic downstream signaling by VD and Decitabine in HMC-1 mast cell leukemia cells



Synergistic Downregulation of DNA Methyltransferase DNMT1 via RAS pathway



Induction of Differentiation is Closely Associated with Stimulation of Fibroblast Growth Factors (FGF)



Conclusion

1. Vitamin D3 and demethylating agents such as Decitabine regulate the 2 main epigenetic levels, namely
   - DNA-(de)methylation,
   - Regulation of HDACs
2. There is an impact on RAS signaling and associated downreguation of the DNA-methyltransferase1 (DNMT1)
3. **Stimulation of osteoblasts markers for (osteoblasts) differentiation and inhibition of osteoclastic downstream signaling by VD and Decitabine suggests a treatment option against osteolytic complications in mast cell leukemia.**

MATERIALS AND METHODS

Cell culture and treatment : Cells were cultured in cell culture flasks at 37°C and 5% CO_2 . The culture media were as recommended by ATCC for (MDA-MB-231 breast cancer DMEM containing 10% fetal calf serum (FCS), PC-3 prostate carcinoma DMEM-F12 with 10% FCS,
MG-63 and U2-OS osteosarcoma were cultured in AlphaMEM **medium** containing 10% FBS. For the HMC1.1 cell line, we used Iscove's modified Dulbecco's medium (IMDM) . thioglycerol (260 nM) with 20% of foetal bovine serum (FBS). All culture media contained 10 µg/mL gentamycin (Sigma). To guarantee optimal growth, cells were splitted two times a week and reseeded at a density of 2 – 5x10^5 cells/ml.
One day after splitting , 32 µM Simvastatin or 150 µM ibandronate were added to the culture medium for 72 hours.

Gene expression analysis: For comparative analysis of selected genes we synthesized cDNA using the First Strand cDNA Synthesis Kit as described by the supplier (Roche). The obtained cDNA was subjected to PCR amplification with a real-time cycler (Corbett Research). FAM-labeled TaqMan gene expression probes & primers-sets (all from Applied Biosystems) were used according to the conditions suggested by the suppliers. For normalization of expression we used VIC labelled GAPDH and 18S TaqMan primer & probe-sets in the same reaction vial (GAPDH 4310884E, 18S 4319413E, Applied Biosystems). Quantification of mRNA within the samples was examined using the comparative Ct method (Livak *et al.*, 2001, *Methods* **25**(4)**:** 402-408).

Transcriptomics and methylomics analysis: Analysis and data evaluation for the Affymetrix Arrays (Type Human Gene 1.0 ST Array) were commercially obtained from an internationally certified institution (Kompetenzzentrum für Biofluoreszenz, Regensburg). "Pathvisio" software (van Iersel et al., 2008, *BMC Bioinformatics* **9:** 399). was applied for specific analyses of defined pathways from Affymetrix Arrays (Type Human Gene 1.0 ST Array).

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Disclosure:

None of the authors has a relevant conflict of interest to declare.