Published in final edited form as:

J Steroid Biochem Mol Biol. 2014 October ; 144PA: 19–21. doi:10.1016/j.jsbmb.2013.09.005.

1,25-Dihydroxyvitamin D₃ Induced Histone Profiles Guide Discovery of VDR Action Sites

Mark B. Meyer*, **Nancy A. Benkusky**, and **J. Wesley Pike**

University of Wisconsin at Madison, Madison, WI 53706 USA

Abstract

The chromatin environment dictates activity throughout the genome. Post-translational modification of the N-terminal tails of histone proteins allow nucleosomes to shift, the chromatin to relax and genes to become activated. Histone modification events and markers will change in response to environmental stimuli; therefore they present a method for identification of sites of transcription factor activity. 1,25-Dihydroxyvitamin D₃ induces the vitamin D receptor (VDR) to bind to DNA and activate transcription. These actions alter the chromatin environment and can be detected by increases or decreases in the histone modifications. In fact, in genomic loci with multiple enhancers, selective modulation of those enhancers after vitamin D₃ stimulation can be readily detected by histone modifications. We provide an example of these actions on the *Mmp13* gene locus where VDR binds selectively to an enhancer 10 kilobases upstream of the transcriptional start site. This binding event is accompanied by an enhancer-selective increase in histone 3 lysine 9 acetylation (H3K9Ac). ChIP-seq analysis of histone modifications requires less genomic material than transcription factor ChIP-seq, thus proving advantageous to *in vivo* assays with limited cellular material. Therefore, histone modification activity alone may be used as a guide for discovering sites of VDR action for further biochemical analysis.

Keywords

Histone Modification; ChIP-seq; VDR; Mmp13; H3K9Ac

1. Introduction

Several decades ago, the Chromatin Immunoprecipitation (ChIP) assay was first developed by Solomon and Varshavsky to link histone proteins to DNA within intact cells [1, 2]. Histones give the genome the ability to wrap immense amounts of DNA in a very small space; however they leave their N-terminal tails exposed. These N-terminal tails undergo post-translational modification by enzyme complexes adding modifications such as

© 2013 The Authors. Published by Elsevier Ltd. All rights reserved.

*Corresponding Author: Mark B. Meyer, Ph.D. Department of Biochemistry, University of Wisconsin-Madison, 433 Babcock Drive, Madison, WI 53706. Tel: (608) 262-8230; Fax: (608) 263-9609; mmeyer@biochem.wisc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

acetylation, methylation, phosphorylation and deamination that alter the structure of DNA wrapping and allow for transcription to occur [3]. These modifications can occur in numerous combinations on the same histone tail or on different histone tails concurrently revealing a higher level of chromosomal control or histone code [4]. Through enormous efforts such as the Encyclopedia of DNA Elements (ENCODE) project, we better understand general features of the histone modifications such as histone 3 lysine 9 acetylation (H3K9Ac) which denotes “active” regions at enhancers as well as promoters and histone 3 lysine 4 mono methylation (H3K4me1) that gives an indication of enhancers, whereas the tri-methyl form, H3K4me3, preferentially identifies the gene promoters that are active, as well as numerous other histone markers [5, 6].

In this short communication from the 16th Workshop on Vitamin D, we examine how the histone modifications can change during the treatment of marrow derived multipotential mesenchymal stem cells (MSCs) with 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃). In particular, we examine the genomic locus near the matrix metalloproteinase 13 (*Mmp13*) gene which is known to be up-regulated by 1,25(OH)₂D₃ in osteoblasts [7] and the MSCs as well as up-regulated during differentiation. Therefore, we hypothesize there will be either a multifunctional enhancer or several enhancers that bind the necessary transcription factors required for these activations. Indeed, we do find several enhancer regions within the *Mmp13* genomic locus that are denoted by distinct histone profiles. One such enhancer contains augmented 1,25(OH)₂D₃-induced H3K9Ac binding denoting the enhancer on which VDR acts the greatest at 10 kilobases upstream (–10kb) from the transcriptional start site (TSS).

2. Materials and Methods

2.1. Reagents

1,25(OH)₂D₃ was obtained from Tetrionics, Inc. (Madison, WI). Antibody to VDR (C-20, #sc-1008) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and acetylated H3 Lysine 9 (H3K9Ac, #06-942) was purchased from Millipore (Billerica, MA).

2.2 Cell Culture

MSC cells were derived from C57B6 mice as previously reported [8]. Cells were cultured in minimum Eagle's medium alpha modification supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin from Hyclone (Logan, UT).

2.3 Chromatin Immunoprecipitation sequencing (ChIP-seq)

MSC cells were treated for 3 h with vehicle or 10^{–7} M 1,25(OH)₂D₃ prior to Chromatin Immunoprecipitation which was performed as described previously [9–11]. ChIP-DNA was prepared and amplified using the Illumina ChIP-seq DNA preparation kit (1003473, #11257047 RevA), clusters formed and sequenced on the Illumina HiSeq2000 sequencers by the University of Wisconsin - Madison DNA Sequencing Facility in the University of Wisconsin- Madison Biotechnology Center [11]. Data were analyzed with HOMER [12] as previously reported [11].

3. Results and Discussion

The MSC cells have the ability to become several different cell types given the appropriate chemical stimulus. They are particularly adept at creating mature osteoblasts since they were derived from mouse bone marrow [8]. These cells are also responsive to treatment with $1,25(\text{OH})_2\text{D}_3$, regulating such osteoblast genes as osteopontin (*Spp1*), Rank ligand (*Tnfrsf11*), cystathionine-beta-synthase (*Cbs*) as well as the *Mmp13* gene among others [13]. We treated the MSC cells for 3 hours with ethanol vehicle or 10^{-7}M $1,25(\text{OH})_2\text{D}_3$ prior to ChIP assay as described in Materials and Methods. We immunoprecipitated with antibodies directed at the acetylation of histone 3 lysine 9 (H3K9Ac) as well as the VDR. After library preparation and sequencing, we found there was significant enrichment of the histone marker H3K9Ac around genes that were responsive to $1,25(\text{OH})_2\text{D}_3$ as detected by mRNA transcript regulation throughout the genome (data not shown). One such example can be seen in Figure 1 for the *Mmp13* gene locus. We found there was an enrichment of H3K9Ac sequencing (tag) density at three distinct regions upstream of the transcriptional start site denoted as -30kb , -20kb and -10kb . Furthermore, one of these regions (-10kb) displayed increased H3K9Ac enrichment after $1,25(\text{OH})_2\text{D}_3$ stimulation as indicated by the overlaid ChIP-seq data tracks or “hubs”, in which more “blue” indicates enrichment of tags after $1,25(\text{OH})_2\text{D}_3$ treatment (red indicate more in vehicle treated cells and purple is the overlaid/equivalent tag density).

Examination of the VDR binding at the *Mmp13* gene (also in Figure 1) maps the greatest VDR binding to the -10kb region. There does appear to be some VDR binding occurring at the -30 and -20kb region, however, those regions were unable to confer transcriptional activity (data not shown). Therefore, the -30 and -20kb regions must be controlled by different transcription factors and those factors may be important to the regulation of *Mmp13* during differentiation since they did not show significant up-regulation during $1,25(\text{OH})_2\text{D}_3$ treatment. These data demonstrate the ability of utilizing the ChIP-seq histone profiles for identification of VDR active regions after treatment with $1,25(\text{OH})_2\text{D}_3$. This is advantageous when cellular material is limited in supply as is the case with *in vivo* derived material. A histone marker ChIP-seq analysis may give at least the initial biological data and is needed for further biochemical and molecular biology investigations for mechanisms of gene regulation by $1,25(\text{OH})_2\text{D}_3$.

Acknowledgments

This work was supported by National Institutes of Health grant DK-072281 to J.W.P. We thank members of the Pike Lab for their helpful discussions and contributions to this manuscript. We also acknowledge the University of Wisconsin DNA Sequencing Facility supported by Marie Adams, John Alliet and Josh Hyman. We are very grateful for the helpful advice and HOMER software from Chris Benner in the Glass Lab at University of California at San Diego.

References

1. Solomon MJ, Varshavsky A. Formaldehyde-mediated DNA-protein crosslinking: a probe for *in vivo* chromatin structures. *Proc Natl Acad Sci U S A*. 1985; 82(19):6470–6474. [PubMed: 2995966]
2. Solomon C, Sebag M, White JH, Rhim J, Kremer R. Disruption of vitamin D receptor-retinoid X receptor heterodimer formation following ras transformation of human keratinocytes. *J Biol Chem*. 1998; 273(28):17573–17578. [PubMed: 9651351]

3. Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ. How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nat Struct Mol Biol.* 2007; 14(11): 1025–1040. [PubMed: 17984965]
4. Turner BM. Defining an epigenetic code. *Nat Cell Biol.* 2007; 9(1):2–6. [PubMed: 17199124]
5. Yavartanoo M, Choi JK. ENCODE: A Sourcebook of Epigenomes and Chromatin Language. *Genomics Inform.* 2013; 11(1):2–6. [PubMed: 23613676]
6. Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ, Zhao K. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet.* 2008; 40(7):897–903. [PubMed: 18552846]
7. Uchida M, Shima M, Chikazu D, Fujieda A, Obara K, Suzuki H, Nagai Y, Yamato H, Kawaguchi H. Transcriptional induction of matrix metalloproteinase-13 (collagenase-3) by 1 α ,25-dihydroxyvitamin D₃ in mouse osteoblastic MC3T3-E1 cells. *J Bone Miner Res.* 2001; 16(2):221–230. [PubMed: 11204422]
8. Case N, Xie Z, Sen B, Styner M, Zou M, O'Connor C, Horowitz M, Rubin J. Mechanical activation of β -catenin regulates phenotype in adult murine marrow-derived mesenchymal stem cells. *J Orthop Res.* 2010; 28(11):1531–1538. [PubMed: 20872592]
9. Meyer MB, Watanuki M, Kim S, Shevde NK, Pike JW. The human transient receptor potential vanilloid type 6 distal promoter contains multiple vitamin D receptor binding sites that mediate activation by 1,25-dihydroxyvitamin D₃ in intestinal cells. *Mol Endocrinol.* 2006; 20(6):1447–1461. [PubMed: 16574738]
10. Meyer MB, Goetsch PD, Pike JW. Genome-wide analysis of the VDR/RXR cistrome in osteoblast cells provides new mechanistic insight into the actions of the vitamin D hormone. *J Steroid Biochem Mol Biol.* 2010; 121(1–2):136–141. [PubMed: 20171278]
11. Meyer MB, Goetsch PD, Pike JW. VDR/RXR and TCF4/ β -catenin cistromes in colonic cells of colorectal tumor origin: impact on c-FOS and c-MYC gene expression. *Mol Endocrinol.* 2012; 26(1):37–51. [PubMed: 22108803]
12. Heinz S, Benner C, Spann N, Bertolino E, Lin Y, Laslo P, Cheng J, Murre C, Singh H, Glass C. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell.* 2010; 38(4):576–589. [PubMed: 20513432]
13. Kim S, Yamazaki M, Zella LA, Shevde NK, Pike JW. Activation of receptor activator of NF- κ B ligand gene expression by 1,25-dihydroxyvitamin D₃ is mediated through multiple long-range enhancers. *Mol Cell Biol.* 2006; 26(17):6469–6486. [PubMed: 16914732]

Figure 1.

The *Mmp13* gene locus contains three distinct enhancer regions. ChIP-seq tag density profiles (normalized to 10^7 tags) for the *Mmp13* gene locus for VDR and H3K9Ac. The vehicle treated cell tracks (red) are overlaid on the 1,25(OH)₂D₃ cell tracks (blue). Increased binding due to 1,25(OH)₂D₃ treatment will appear as blue, vehicle enriched as red and the overlapping regions purple. Genomic location (mouse mm9 genome) and scale are indicated in each track, max height of tag sequence density for the data track is indicated on Y-axis. Gene transcriptional direction is indicated by an arrow and exons by boxes.