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VitminD Treatment Change MTH1 and MYH Genes Expression in HUVEK cell

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Abstract:

Vitamin D (Vit D) ,as an antioxidant contributes to a wide range of diseases including obesity, type 2 diabetes, multiple sclerosis, and certain cancers that oxidative stress plays a vital role in their development. Excessive oxidative stress can damage to DNA and nucleotide pool. Base excision repair and house-cleaning enzymes can protect genome so that any disruption in expression of these genes indicates enhanced susceptibility risk for diseases like cancer. The present study was conducted aimed at evaluating the effect of Vit D on the expression of MYH and MTH1 as DNA repair genes, as well as effect of ViD treatment in Human Umbilical Vein Endothelial Cells (HUVEC) cell line. To do this, bioinformatics tools were used to predict the interaction of MTH1 and MYH with VDR as a specific transcription factor (TF) for Vit D. The cell line was treated with VitD. Next, viability was evaluated using MTT assay. The mRNA expression of MTH1 and MYH was assessed using real-time PCR at 48h post-treatment with Vit D.

Results of the study revealed that Vit D could regulate MTH1 and MYH transcript expression directly through its specific TF; VDR. In response to VitD treatment a different alteration was observed in DNA repair, and non-canonical nucleotide repair genes. Findings of this study showed a new regulation of DNA repair genes in Vit D signaling pathway, and it may be a new perspective for the therapeutic effect of Vit D on related diseases. Variation in interested genes may affect the vitD signaling and personalized medicine should be considered.

INTRODUCTION

It is believed that oxidative stress plays an essential role in pathogenesis of various diseases including cardiovascular conditions such as high blood pressure, atherosclerosis, and strokes, neurodegenerative and autoimmune diseases such as Alzheimer's disease, multiple sclerosis, diabetes, and inflammatory disorder (1). Cancer is developed due to DNA damage, genome instability, and cell proliferation induced by oxidative stress (2). The imbalance between antioxidant defense system and oxidative stress causes damage to lipids, proteins, and nucleic acids (3). Function of DNA repair pathways is responsible for the elimination of endogenous and exogenous mutagens. The ability of this system is vital to reduce damage in the genome and leads to modifying the effect of environmental exposures on disease risk. DNA repair system, especially the Base Excision Repair (BER) pathway, is involved in repairing DNA damages resulting from oxidative stresses. Documents have demonstrated that reduction in efficiency of the BER (4, 5) and house-cleaning enzymes (6) is associated with increased risk of cancer development and progression.

Vit D, as a microenvironment, has wide-ranging effects on different diseases including obesity, type 2 diabetes, hypertension, memory disorders, multiple sclerosis, osteoporosis, autoimmune diseases, and certain cancers. Vit D adequacy leads to the reduction of impairment of DNA repair and protects against oxidative DNA damage (7). Vit D is involved in controlling inflammation, aging process, and minimization of oxidative stress (8, 9). Moreover, Vit D could regulate genes expression via its specific transcription factor,VDR. Few studies have demonstrated the role of VitD in the regulation of expression of DNA repairs genes such as MTH1 and MYH contributing in removal and elimination of oxidative base from DNA (10). To the best of our knowledge, an important gap is whether Vit D is associated with DNA repair genes responding to oxidative stress conditions. In the present study,

we will consider that, whether it exerts its function through VDR. Regulatory effect of Vit D in MYH and MYH promoters that may mediate through VDR was assessed. To find a mechanism, expression of the interested genes were investigated in HUVEK cell line after treatment by VitD.

MATERIALS AND METHODS

Treatment the cell line with VitD

In this study, 1.5×10^5 Huvek cells were cultured in 6-well plate and treated with 100 nM 1, 25-DihydroxyVitD as effective concentration in the biological process(11, 12). After 48h total RNA was isolated and 3 µg of purified total RNA was used for cDNA synthesis (SinaClon First Strand cDNA synthesis Kit). Primers were designed (table.1) and the genes expression was carried out using Real-Time PCR. GAPDH was used as a reference gene. Fold change expression values were calculated using the $2^{-\Delta\Delta Ct}$ method as described by Livak (13). All tests were performed in triplicate.

Determination of cell viability

The MTT assay was used to evaluate viability of HUVEK cells following treatment with VitD. The cells were seeded in 96-well plates at a density of 3.5×10^4 per well and incubated for 48 h after VitD treatment at 37°C and 5% CO2 condition. MTT assay was carried out (Cat. 11465007001, Roche Applied Science, and Indianapolis, IN, USA) according to the manufacturer instructions. Optical intensity was read by a microplate ELISA reader at a wavelength of 570 nm.

Bioinformatics analysis

To identify potential binding sites for VDR in the MTH1 and MYH promoter genes, 1500 bp selected promoter region of MTH1 and MYH were used by ConSite(consite.genereg.net) and JASPAR CORE (http://jaspar.genereg.net/) databases. Screen were predicted with relative profile score threshold 80%.

Statistics analysis

Data analysis was performed using Graph Pad Prism 6 (Graph Pad Software, Inc., San Diego, CA, USA). The difference between groups was compared using either 2-tailed student's t-test or one-waay ANOVA test. P-values<0.05 was considered statistically significant, and data are shown as mean ± standard deviation (SD).

RESULTS

Vit D Treatment Effects on Genes Expression

Genes expression were analyzed treated with VitD . Results showed that expression of MTH1 (2.9 folds, P<0.001) significantly enhanced after VitD treatment, while MYH expression (2.04fold, P<0.05) decreased in comparison with the control cells. Difference in two genes response may due to variation in MYH gene regulatory elements or the difference in the response of its regulatory areas to the concentration of vitD that used in this experiment (figure1).

Cell viability was measured after treatment with VitD. Results showed a decrease by 75% in the growth of the HUVEK cell compared to control group, but it was not a statistically significant (figure2).

The MTH1, and MYH promoters were investigated for VDR binding site. Results of the analysis revealed that there was one VDRE site in the MTH1 promoter sequence located at +361 bp position. Also, in silico mapping analysis in the MYH promoter failed to find any VDRE site.

DISCUSSION

Vit D, as an antioxidant is capable of attenuating oxidative stress in the Central Nervous System (CNS) (14). VitD deficiency contributes to development of different diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis (15), cancer (16), hypertension, and cardiovascular diseases (17). Many of these diseases are related to oxidative stress, which may be regulated through function of VitD and NRF2 (17, 18). Sang -Bum Kim, et al. , in a study explained molecular mechanism of DNA protection against oxidative stress. Our study results revealed that VitD may indirectly regulate the MTH1 promoter directly by VDR TF. But, MYH downregulation may be the secondary or new effects of VitD , in HUVEK

Table 1. specific Primers list for qRT-PCR

Genes	Primer sequence	Product length bp
MYH	F: 5-GTATATGGGCTGGCCTTGGAAG-3 R: 5-CTGTTGGCCCTGATACACACG-3	**141**
MTH1	F: 5-GGGCCAGATCGTGTTTGAGTTCGT-3 R: 5-TCGTCGGGCCACATGTCCTTG-3	**159**
GAPDH	F : 5-CCATGAGAAGTATGACAAC-3 R: 5-GAGTCCTTCCACGATACC-3	**115**

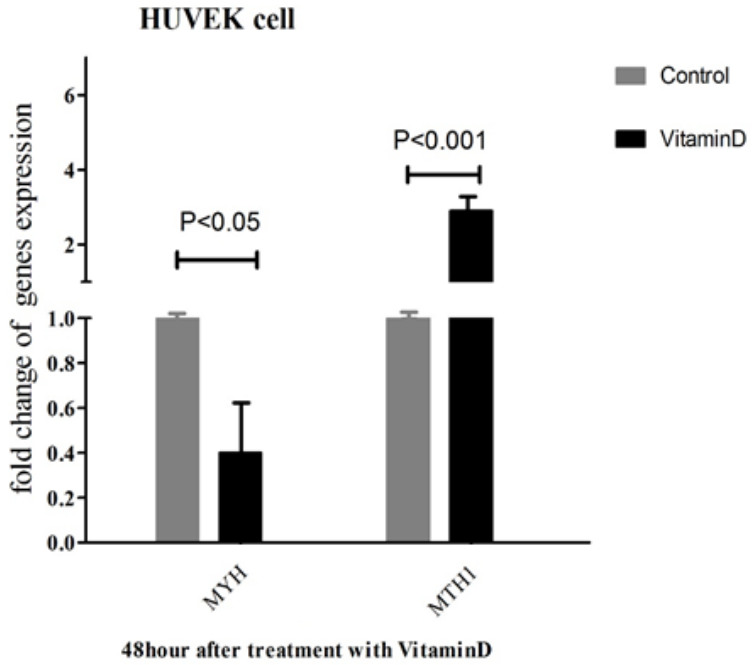

Figure1. The expression of MTH1 and MYH gene at 48 h following VitD treatment in HUVEK cell. Gene›s expression were analyzed by Real-Time PCR and normalized with GAPDH. Results presented as fold change. The presented results are from three biological replicates and three times

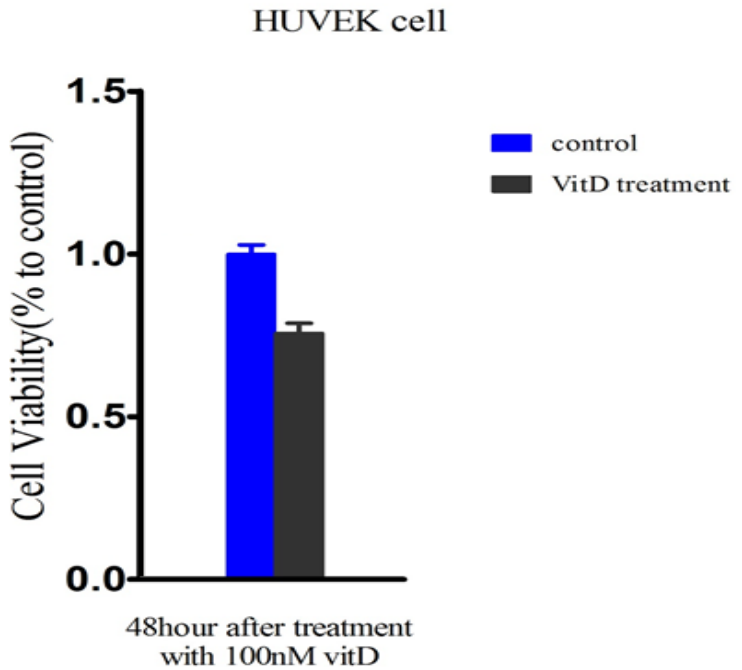

Figure2. Cell viability of Vit D treatment. Cell viability measured by MTT assay in HUVEK cell. After 48 h. The cells growth was inhibited at 48 h after treatment with VitD compare to control Data are mean± SD of three experiments.

cell. Moreover,the presence of putative VDRE in regulatory sequences of MTH1 can explain the direct up-regulation of MTH1 by Vitamin D treatment . These data suggest a possible regulation of MYH gene by another transcription factor or pathway that involve in VD signaling.

MTH1 is responsible for eliminating abnormal bases (19, 20) caused by oxidative stress. This study for the first time, elucidated the interaction between two nucleotide base repair genes and VitD. Our study results also showed that, activity of the interested genes were different at a concentration of 100 nM of Vit D. . In the current study, MYH mRNA levels significantly decreased after simultaneous with Vit D treatment. VDR polymorphisms and Vit D concentrations are important for regulation of ability of the ligand-bound

VDR to bind to VDREs in target gene promoters and initiate second messenger systems. Second messenger systems triggered with Vit D include many intracellular genomic activities and biochemical reactions such as reduction of oxidative stress. Results of a study demonstrated that, physiological concentration of Vit D has different effect on MYH and MTH1 expression. Low concentration of Vit D increases inflammation, and conversely, adequate concentration of Vit D decreases expression of inflammatory cytokines such as Tumor Necrosis Factor-α (TNF-α), and also reduces interpretation of the InsP3Rs and intracellular Ca^{2+} ,and results in acceleration of cellular damage ,apoptosis ,and aging(21). Vit D induces apoptosis in breast, clone, and glia cell lines (22). This result may be explained by the fact that anticancer effect of Vit D was demonstrated in this study, as supported in the study by Sungmin Baek, et al.. In this regard, they reported that a concentration of 10 μM of Vit D significantly reduced viability of studied cell lines; SNU1079, HUCCT1, SUN638, SUN1, whereas, a level of 0.01 μM significantly decreased cell viability just in SNU1079 cell line (23). This data demonstrated that Vit D influences in a dose-dependent manner in different cell lines. Contrary to studies mentioned above, Vertino , et al. demonstrated that Vit D prevents apoptosis in Hela, osteocyte, and keratinocyte cells (24), so, different function of VitD observed in two genes may indicate distinct response of cell in presence of selected concentration.

The previous study showed that Vit D has the anti-cancer effect and reduces cell proliferation (16). Vit D supports the cells against oxidative stress by maintaining normal mitochondrial functions. NRF2/PGC-1α-SIRT3 axis can be activated with Vit D (25). Vit D deficiency can increase oxidative stress. Consequently, accumulation of oxidative stress causes DNA damage and acceleration of cell death (26). Effect of Vit D may be profoundly dependent on the degree of oxidative damage in cell. Although vitamin D can change the expression of genes, the variation in the regulatory regions of genes can influence its signals, so personalized medicine has critical role in this field. Furthermore, the limitation of this study was that the effects of Vit D on expression of genes involved in cell growth, and other DNA repair pathways were not investigated, and future studies are suggested to conduct these analyses.

CONCLUSION

In general, results of the present study emphasized potential role of Vit D in expression of genes involved in DNA and nucleotide repair. These results indicate a new pathway regarding the effectiveness of Vit D in expression of DNA repair genes. In addition, these observations did not imply inconsistency in our study with others, but our findings showed a complementary perspective on function of Vit D.

CONFLICT

The authors have no conflict of interest to declare.

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