The Role of Various Agents On the Development of Osteoclasts From Myeloid Progenitor Cells

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Abstract

Osteoclast cells break down bone during bone remodeling. Researchers aiming to study osteoclasts and their functions typically take a myeloid progenitor cell and expose it to a mixture of 10nm 1a,25$(OH)_2$D3 (Vitamin D3) and 50nm 12-O-Tetradecanoylphorbol-13-acetate (TPA) to induce differentiation of the progenitor cell into an osteoclast cell. This mixture of Vitamin D3 and TPA has been used extensively, though little is known on the exact function of these reagents. To determine the role that each agent has on osteoclast differentiation, HL60 progenitor cells were exposed to standard amounts of Vitamin D3 alone, TPA alone, and then together as the typical mixture used in labs. To analyze the effects of Vitamin D3 and TPA, RT-PCR of four specific genes were visualized through gel electrophoresis. These four genes are essential to osteoclast function and include RANK, MMP-9, TCIRG1 and CSF1R. Gel analysis of the RT-PCR products demonstrates the varying ability of Vitamin D3 or TPA alone to produce maximal gene expression in RANK, TCIRG1 and CSF1R, whereas the combined effect of the reagents is needed to produce any expression in MMP-9.

Introduction

As the major form of support throughout the body, bones must undergo the continual process of bone remodeling to maintain healthy density and to adapt to different stresses of the body. This process is balanced by osteoclasts, cells that break down bone, and osteoblasts, cells that deposit new bone material. Together, they are able to replace damaged or old bone with new tissue (Hadjakis, 2006). However, a prolonged disequilibrium between osteoclast and osteoblast function results in bone conditions, such as osteoporosis and osteopetrosis, which can affect an individual's ability to sustain daily activities.

Osteoporosis is a specific bone condition that causes patients to suffer from a decrease in bone density, making them susceptible to high-risk fractures and to frequent aches and pains. Most often, those that suffer from osteoporosis are postmenopausal women as a result of their sudden drop in estrogen. Therefore, typical therapies include drugs with similar estrogen functions. Other therapies include weight-bearing exercises that stress certain areas of the bone to stimulate an increase in bone rebuilding (Amin, 2012).

The fundamental cause of osteoporosis is the disequilibrium between osteoclasts and osteoblasts, wherein osteoclasts break down bone faster than osteoblasts are able to rebuild the bone. Since 1983, administration of vitamin D3 has been used to remedy the effects of osteoporosis, resulting in an increase in bone mineral density (Takahashi, 2011). Takahashi et. al postulated, however, that vitamin D3 *in vivo* induces osteoclast differentiation by stimulation of the osteoclast

progenitor cells, proposing that it is not vitamin D3 in osteoporotic therapies that suppresses osteoclast function but rather an indirect intermediate (Takahashi, 2011).

For *in vitro* studies involving the development of osteoclast cells, a mixture of vitamin D3 and TPA has been known to successfully induce osteoclast differentiation from HL60 progenitor cells (Figure 1). As mentioned above, *in vivo* uses of vitamin D3 are also able to stimulate development of osteoclast cells. Studies done by Hiura et. al have shown that there may even be a synergistic response of osteoclastogenesis when TPA is present in solution with vitamin D3 (Hiura, 2003). However, the roles of vitamin D3 and TPA as differentiating agents in vitro is not well understood, nor are the individual effects of vitamin D3 or TPA well-defined.

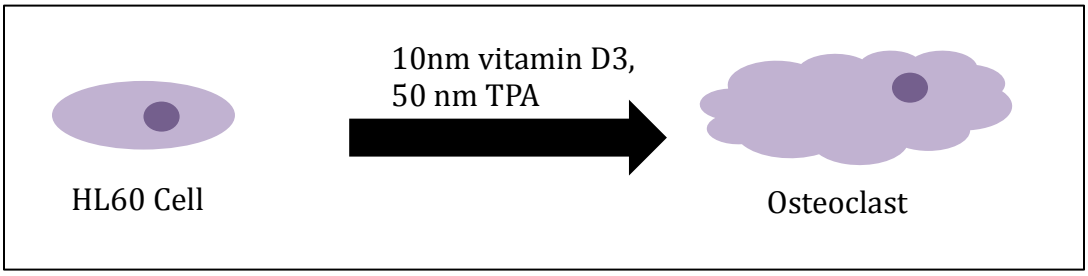

Figure 1. Standard method of differentiation of the HL60 progenitor cell by a mixture of vitamin D3 and TPA

Material and Methods

I. Cell lines

The HL60 cell line was kindly provided by Dr. Sylvia Anderson and Dr. Berish Rubin of the Department of Biological Sciences, Fordham University. The cells were then treated with 10 nm vitamin D3, 50 nm TPA or a mixture of 10 nm vitamin D3 and 50 nm TPA.

II. RNA preparation and reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was purified from the treated HL60 cells using RNeasy Plus Mini Kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. 10 ng of total RNA was then amplified in 20 uL RT-PCR reactions with 0.4 uM primers (Table 1) using OneStep RT-PCR kit (Qiagen) as follows:

50°C x 30 minutes
95°C x 15 minutes

94°C x 30s 57°C x 30s 72°C x 30s	40 cycles

72°C x 2 minutes

RT-PCR products were then analyzed on a 1% agarose gel.

III. PCR purification

8 uL of obtained PCR products were purified using QIAquick PCR Purification Kits (Qiagen) according to the manufacturer's instructions.

IV. Primers

Gene	Exon	Primer	Sequence (5' – 3')	Accession #
RANK	3	Forward	gtggcccggatgaatacttgg	NM_003839.3
	7	Reverse	ccacagacgcgaagagaagc	NM_003839.3
MMP-9	3	Forward	gacatcgtcatccagtttgg	NM_004994.2
	5	Reverse	ccaaaccgagttggaacc	NM_004994.2
TCIRG1	8	Forward	cagaagatccgcaagatcacg	NM_006053.3
	11	Reverse	cgtaggcatccacgatgc	NM_006053.3
CSF1R	4	Forward	cacaccaactactccttctcg	NM_005211.3
	6	Reverse	gtcagattgttgagggattgc	NM_005211.3

Table 1. 0.4 uM of each primer was used for RT- PCR.

Results

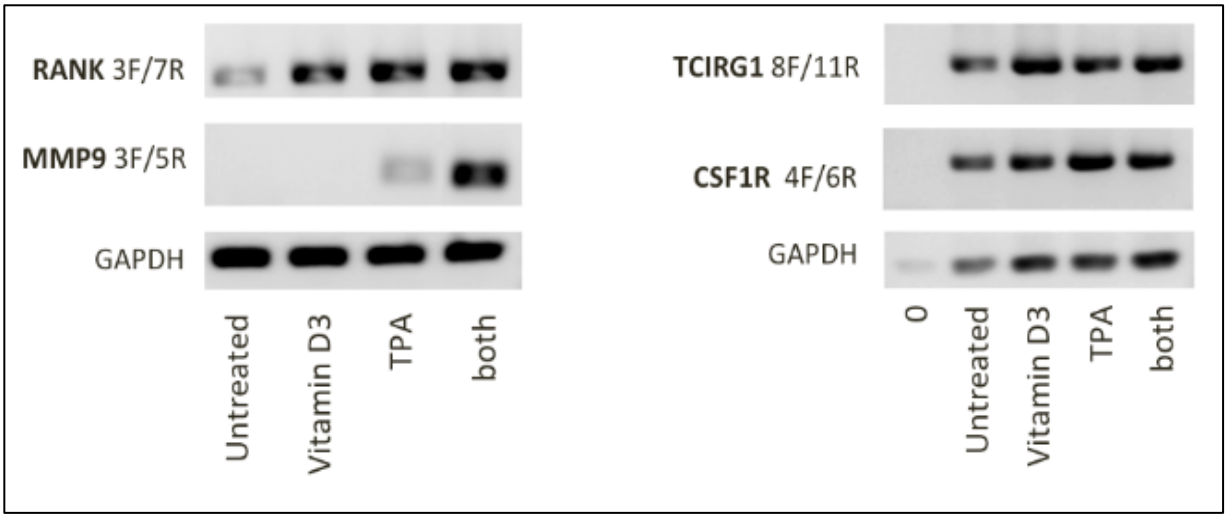

Figure 2. Gel electrophoresis results of RT-PCR from two separate days.

Data from the resultant gels reveals clear amplification of all four genes upon exposure to vitamin D3, TPA and/or the standard mixture; comparisons of the strength of the bands are indicative of how much gene products were obtained. Treatments of vitamin D3 or TPA work effectively in enhancing RANK, TCIRG1 and CSF1R gene expression comparable to the combined effect of vitamin D3/TPA that is typically used in the lab setting (Figure 3). Conversely, however, MMP9 does not show any bands upon treatment of vitamin D3 or TPA, and only shows a strong band upon exposure to the mixture of both vitamin D3 and TPA. It is worthy to note, also, that each gene is adequately amplified upon exposure to vitamin D3/TPA, as expected.

Total RNA were also confirmed to be the correct genes by PCR purifying the RT-PCR products and sending them out to GeneWiz, Inc. Resultant accession numbers can be found on Table 1, which originated from NCBI's Basic Local Alignment Search Tool (BLAST).

Discussion

For more than a decade, the standard mixture of 10nm vitamin D3 combined with 50 nm TPA has been used to induce differentiation of HL60 progenitor cells to functional osteoclast cells. Vitamin D3 has, interestingly, been used as therapy for osteoporotic patients to increase bone density, suggesting vitamin D3's possible ability to suppress osteoclast function *in vivo* as postulated by Takahashi et. al (Takahashi, 2011). However, through the analyses of gene expression presented in this study, it is evident that independent treatments of vitamin D3 or

TPA may *induce* sufficient differentiation of osteoclast function *in vitro*. Based on analyses of the presence and expression of genes specific to osteoclast differentiation and function, the ability of certain agents to produce maximal gene expression may also be indicative of their ability to induce differentiation.

Quantification of the gene expression of RANK, TCIRG1 and CSF1R, through gel electrophoresis, each have produced similar results showing that independent treatments of vitamin D3 or TPA has the ability to produce gene expression comparable to the standard treatment of vitamin D3/TPA commonly used in the lab to induce differentiation. The lack of MMP-9 gene product upon exposure to vitamin D3 or TPA and then the robust band given upon exposure to vitamin D3/TPA, however, reveals that gene expression is dependent on both vitamin D3 and TPA together.

Thus, the answer to how certain agents individually affect differentiation is a complicated one – there is no direct pattern between all four genes studied, demonstrating a variable ability of the reagents depending on the gene. The presence of gene products obtained in this study does not necessarily qualify total osteoclast differentiation, but does demonstrate the ability of certain compounds to enhance gene expression essential to osteoclast function, either individually or together. By determining the effects of vitamin D3 and TPA on gene expression, further studies can be taken on the individual effects of vitamin D3 and TPA on the overall differentiation of HL60 cells, and possibly even the mechanism through which these agents work to account for the variability between gene products.

References

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