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Osteoblastic Differentiation of Vascular Smooth Muscle Cells

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Abstract

Atherosclerotic lesions are frequently associated with vascular calcification. Additionally, the process of atherosclerotic calcification shares a number of characteristics with skeletal tissue mineralization. At the onset of atherosclerotic lesions, we hypothesized that vascular smooth muscle cells might acquire osteoblastic characteristics. Using an in vitro calcification model, we examined the effect of dexamethasone (Dex), which is well known to be a potent stimulator of osteoblastic differentiation in vitro, on vascular calcification in this study. Dex increased the calcification of bovine vascular smooth muscle cells (BVSMCs) in a dose- and time-dependent manner, as we demonstrated. Alkaline phosphatase activity, procollagen type I carboxy-terminal peptide production, and cAMP responses to parathyroid hormone in BVSMCs were also enhanced by Dex in osteoblasts. In addition, we compared Dex's effects on BVSMCs and Saos-2 cells and examined its effects on human osteoblast-like (Saos-2) cells. BVSMCs were less affected by Dex than Saos-2 cells were by its effects on alkaline phosphatase activity and the cAMP response to parathyroid hormone. We found that Dex increased the gene expression of both transcription factors, and that Osf2/Cbfa1, a key transcription factor in osteoblastic differentiation, was expressed in both BVSMCs and Saos-2 cells. According to these findings, in vitro osteoblastic differentiation of BVSMCs may be enhanced by Dex.

Keywords: Atherosclerosis; Alkaline phosphatase; Core binding factor-α1

Introduction

We created an in vitro calcification system that allows for the induction of diffuse calcification by cultivating bovine vascular smooth muscle cells (BVSMCs) in the presence of -glycerophosphate (-GP). This allowed us to better understand how vascular calcification works. In this model, vascular calcification and the expression of osteopontin mRNA, which rises as calcification progresses, are dependent on alkaline phosphatase (ALP), one of the markers for osteoblastic differentiation. In addition, we discovered a local calcium-regulating system in which a peptide related to parathyroid hormone (PTH) serves as an autocrine and paracrine regulator of vascular calcification [1].

We hypothesized that VSMCs might acquire osteoblastic characteristics during the formation of atherosclerotic lesions based on recent evidence presented by us and other researchers [2].

From a committed progenitor cell of mesenchymal origin capable of proliferation to a postproliferative osteoblast expressing bone phenotypic markers, osteoblastic differentiation is a multistep process that progresses through distinct stages of maturation. However, it is still unclear what the molecular basis is for osteoblast-specific gene expression and differentiation. Osteoblast-specific transcription factor-2 / core binding factor-subunit 1 (Osf2/Cbfa1) has recently been identified as a crucial regulatory transcription factor in osteoblastic differentiation. Osteoblast- and T-cell-specific isoforms of two transcripts are produced by the Osf2/Cbfa1 gene. In contrast to the T-cell isoform in the mouse, the osteoblast isoform possesses a distinct 87-amino acid sequence at its amino-terminal end. However, it is still unclear how the two transcripts contribute to osteoblastic differentiation. The homozygous Osf2/Cbfa1 (-/-) mouse has no bone at all and keeps its partially calcified cartilaginous skeleton [3]. In humans, cleidocranial dysplasia is an autosomal dominant skeletal disorder caused by this gene's mutations.

Using an in vitro calcification model, we investigated how Dex affected vascular calcification in this study. Dex increased calcium deposition in a dose- and time-dependent manner, as we first demonstrated. Dex influenced cAMP responses to PTH and increased ALP activity, its mRNA expression, and production of procollagen type I C-peptide (PICP). Finally, we demonstrated that Dex increased Osf2/Cbfa1 gene expression. According to these findings, Dex may promote osteoblastic differentiation of VSMCs, which in turn may promote vascular calcification [4-7].

Results

We first examined the effect of Dex on BVSMC calcification. As previously described, β-GP induced calcium deposition in a time-dependent manner. In the presence of β-GP, Dex (10-7 mol/L) significantly increased calcium deposition compared with calcified controls at each time point (Figure 1). The calcium deposition in the Dex-treated group increased to 175% of the calcified control value on day 6. Likewise, Dex promoted calcium deposition in a dose-dependent manner on day 4, and the calcium deposition increased to 322% of the calcified control value at 10-7 mol/L. These results suggest that Dex increases BVSMC calcification [8].

ALP is known to be 1 of the phenotypic markers of osteoblastic differentiation. Because we reported that ALP plays an important role in this calcification system, we next examined the effect of Dex on ALP activity in BVSMCs. As a positive control of Dex's effect, we utilized human osteoblast-like (Saos-2) cells. In the absence of β-GP, Dex (10-7 mol/L) enhanced ALP activity in a time-dependent manner, and ALP activity had increased to 222% of controls on day 6. On day 4, Dex dose-dependently increased ALP activity in the absence of β-GP, and the maximal effect (236% of control) was observed at 10-7 mol/L. In

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Table 1: Effects of Dex on ALP Activities and PICP Production in BVSMCs and Saos-2 Cells.

BVSMCs	β-GP-	β-GP+	β-GP-	β-GP+
Ctl	0.66±0.04	0.50±0.02	0.41±0.02	0.42±0.03
Dex	1.59±0.03*	0.96±0.00*	0.60±0.05*	0.43±0.03
Saos-2 cells Ctl	3.96±0.94	2.87±0.86	0.47±0.05	0.20±0.01
Dex	29.35±5.21*	23.12±5.55*	0.45±0.02	0.24±0.01

the presence of β-GP, Dex also increased ALP activity in BVSMCs, but the response to Dex was less prominent than that in its absence (Table 1). Furthermore, a greater response was observed in Saos-2 cells, both in the absence and presence of β-GP. Next, we examined the effect of Dex on expression of the ALP gene in BVSMCs. Dex dose-dependently promoted the expression of ALP mRNA at 48 hours, and the maximal effect was observed at 10-7 mmol/L (180% increase of control). Taken together, these results suggest that Dex may accelerate BVSMC calcification partially through enhancing expression of the ALP gene and its activity [9-12].

Discussion

Because glucocorticoids are well known to be potent stimulators of osteoblastic differentiation, we utilized Dex, a potent synthetic glucocorticoid, to induce BVSMCs to acquire osteoblastic characteristics. As shown in this study, Dex enhanced not only in vitro calcification but also several phenotypic markers for osteoblastic differentiation in BVSMCs, such as ALP expression, type I collagen produc- tion, and cAMP responsiveness to PTH. Moreover, we confirmed the potency of Dex on osteoblastic differentiation by utilizing Saos-2 cells as a positive control. Regarding ALP activity and the cAMP response to PTH, the responsiveness of BVSMCs to Dex was less prominent than that of Saos-2 cells. The less potent effect of Dex on BVSMCs may be ascribed to a heterogeneous population of cells in the BVSMC culture. Interestingly, Dex exerted no effect on PICP production by Saos-2 cells, whereas Dex increased PICP secretion by BVSMCs in the absence of β-GP. Early phenotypic markers for osteoblastic differentiation such as PICP may not be affected by Dex in well-differentiated osteoblastic cells. However, the precise mechanism by which such differences of responsive- ness to Dex are induced remains to be clarified.

Several key factors in bone mineralization have been demonstrated in calcified lesions of arterial walls, such as matrix vesicles, BMP-2, osteopontin, matrix Gla protein, osteocalcin, and type I collagen. We previously dem- onstrated the significance of ALP, osteopontin, and PTH- related peptide in an in vitro model of vascular calcification by utilizing BVSMCs. Recently, a key regulatory factor in osteoblastic differentiation, Osf2/Cbfa1, has been identi- fied. BMP-7 induces expression of the osteoblastic isoform, followed by its enhancement of the osteocalcin gene in nonosteoblastic cells. Therefore, the Osf2/Cbfa1 gene is thought to be 1 of the "master genes" of as well as a molecular marker for osteoblastic differentiation. In this study, we showed the presence of the *Osf2*/*Cbfa1* gene in cultured BVSMCs as well as in Saos-2 cells. Additionally, we cloned a 5' partial sequence of the bovine osteoblast–specific *Osf2*/*Cbfa1* transcript by reverse transcription–polymerase chain reaction by using total RNA from BVSMCs in preliminary experiments. This evidence suggests that cultured VSMCs may be committed to differentiate into osteoblastic cells under certain conditions. However, whether the tran- script detected in BVSMCs is the osteoblast-specific isoform remains to be confirmed. Furthermore, Dex enhanced the gene expression of Osf2/Cbfa1 in a time dependent manner in BVSMCs. Therefore, it is likely that Dex may promote osteoblastic differentiation of VSMCs by increasing the expression of the Osf2/Cbfa1 gene.

Linkage of phenotypic gene induction to the downregulation of proliferation is the hallmark of differentiation in numerous cell types.40 Some agents inhibiting the proliferation of osteoblast lineage cells, such as hydroxyurea, can induce osteoblastic differentiation. It is therefore possible that antiproliferative agents of VSMCs may induce osteoblastic differentiation under certain conditions. Moreover, 17β- estradiol has been reported to promote osteoblastic differentiation of bovine vascular cells and in vitro calcification without affecting cell growth. In this study, we examined the hypothesis that Dex may inhibit the proliferative capacity of BVSMCs, resulting in osteoblastic differentiation. Because Dex did not affect DNA synthesis in the presence of 15% FCS, Dex may directly induce osteoblastic differentiation of BVSMCs without affecting their growth.

It is still not clear how glucocorticoids influence atherogenesis. Despite an increase in hypertriglyceridemia and hypercholesterolemia, glucocorticoids suppress the development of atherosclerosis in experimental animals when administered as anti-inflammatory drugs at high doses. Both the thrombin-induced expression of growth factors and the proliferation of cultured VSMCs have been shown to be inhibited by glucocorticoids. Then again, glucocorticoids are fit for diminishing the declaration of hepatic LDL receptors, invigorating the net amalgamation of apoB-100 and apoB-48 and diminishing their intracellular debasement. There is a strong correlation between an elevated serum cortisol level and the severity of coronary artery disease in humans, suggesting that these changes could be atherogenic. In this study, we demonstrated that by promoting osteoblastic phenotypes in BVSMCs, Dex promotes in vitro calcification. Dex may cause and exacerbate vascular calcification due to the fact that vascular calcification is frequently associated with osteoporosis and chronic glucocorticoid treatment induces osteoporosis. To better understand how long-term glucocorticoid use affects vascular calcification, particularly calcified atherosclerotic plaque lesions, more research is needed.

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