Journal of Steroid Biochemistry & Molecular Biology 136 (2013) 125–130

Contents lists available at SciVerse ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Review

Role of VDR in 1α,25-dihydroxyvitamin D_3-dependent non-genomic activation of MAPKs, Src and Akt in skeletal muscle cells

Claudia Buitrago*, Verónica Gonzalez Pardo, Ricardo Boland

Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, 8000 Bahía Blanca, Argentina

ARTICLE INFO

Keywords:
1α,25-dihydroxyvitamin D_3
VDR
Skeletal muscle
C2C12 cells
Non-genomic responses

ABSTRACT

1α,25-dihydroxyvitamin D_3 [1,25D] is recognized as a steroid hormone that rapidly elicits intracellular signals in various tissues. In skeletal myoblasts, we have previously demonstrated that one of the 1,25D-induced non-genomic effects is the upstream stimulation of MAPKs through Src activation. In this work, the data obtained suggest that the classical receptor of vitamin D (VDR) participates in non-transcriptional actions of 1,25D. We significantly reduced VDR expression by infection of C2C12 murine myoblasts with lentiviral particles containing the pLKO.1 plasmid with information to express a shRNA against mouse VDR. In these cells (C2C12-shVDR), Western blot analyses show that 1,25D-induced p38 MAPK activation and Src tyr416 phosphorylation were abolished. In addition, 1,25D-dependent activity of ERK1/2 was diminished in cells lacking VDR but to a lesser extent (∼−60%). Phosphorylation of Akt by 1,25D, recently demonstrated in C2C12 cells, in the present work also appeared to be partially dependent on VDR expression (∼50% in C2C12-shVDR cells). Our results indicate that VDR is involved in 1,25D-induced rapid events related to survival/proliferation responses in skeletal muscle cells, providing relevant information on the mechanism of initiation of the non-genomic hormone signal. The participation of a VDR-independent non-genomic mechanism of action should also be taken into consideration.

This article is part of a Special Issue entitled 'Vitamin D Workshop'.

Contents

1. Introduction

1α,25-dihydroxyvitamin D_3 (1,25D) is a steroid hormone which triggers actions in many tissues and organs [1–4]. Of interest, this hormone modulates skeletal muscle contractility and growth [5]. Besides regulating gene expression via its specific intracellular receptor VDR [6], 1,25D also exerts fast non-transcriptional responses involving an increase in intracellular calcium and stimulation of different kinases related to signal transduction pathways [7–9]. Robust evidence indicates that modulation of several 1,25D-dependent responses requires fast activation (phosphorylation) of MAPK cascades [10,11]. In agreement with this concept, we previously established that 1,25D rapidly stimulates ERK1/2 [12,13] and p38 MAPK [14] in proliferating skeletal muscle cells (myoblasts). Moreover, the activities of MAPKs are dependent on upstream 1,25D-regulation of Src tyrosine kinase in these cells [14,15].

* Corresponding author at: Departamento de Biología, Bioquímica & Farmacia, Universidad Nacional del Sur, San Juan 670, 8000 Bahía Blanca, Argentina.
Tel.: +54 291 4595100x2430; fax: +54 291 4595130.
E-mail addresses: cbuitrag@criba.edu.ar, cbuitrag@hotmail.com (C. Buitrago).

0960-0760/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jsbmb.2013.02.013

1,25D plays a role in the regulation of muscle cell proliferation and differentiation [16], stimulating thereby muscle growth. It has been established that stimulation of Akt is necessary in processes related to development and survival [17,18]. Recently, we established the involvement of Akt in 1,25D-induced proliferation and differentiation of C2C12 murine myoblasts. Furthermore, we evidenced that Src also participates in Akt activation by the hormone [19].

Although many rapid events of 1,25D in skeletal muscle have been clarified, the starting point of the intracellular signaling generated by the hormone remains unclear in this as well as in other tissues. On the one hand, the existence of a cell membrane receptor for 1,25D, different from VDR, was postulated which could be the responsible of transmembrane signaling events leading to short term cellular responses [20,21]. Nemere and collaborators proposed the presence of a novel vitamin D receptor called 1,25D-MARRS (membrane associated rapid response steroid binding) which would mediate non-genomic actions of 1,25D in chick intestinal basolateral membranes [22]. Recently, it was reported in osteoblast-like MC3T3-E1 cells that protein-disulfide isomerase-associated 3 (Pdia3), also named 1,25D3-MARRS and ERp57, initiates membrane responses to 1,25D [23]. On other hand, using chicken myoblasts, it was demonstrated that 1,25D promotes translocation of VDR from the nucleus to the cell membrane [24]. This information was corroborated in C2C12 myoblasts by immunocytochemistry studies which showed that the hormone induces localization of VDR in the proximity of the plasma membrane. Of importance, translocation of VDR to the plasma membrane after 1,25D treatment is abolished when caveolae structure was disrupted with MβCD [25]. Previously, we first reported the participation of VDR in non-genomic stimulation of tyrosine phosphorylation cascades by 1,25D in chicken myoblasts using antisense oligonucleotides against VDR to significantly reduce receptor expression [26]. Of relevance, recent evidence suggests that the classical nuclear VDR can mediate rapid, non-genomic responses, through binding of 1,25D to an alternative ligand-binding pocket A [27–29]. Moreover, other secosteroids can dock into the rapid-response A-pocket on the VDR as well as 1,25D [30].

In view of these evidences, in the present work we investigated the role of the VDR in the activation of Src, ERK1/2, p38 MAPK and Akt by 1α,25$(OH)_2D_3$, using as experimental model a C2C12 myoblast cell line with the VDR gene knocked down by stably expressing short hairpin RNA against the receptor.

2. Materials and methods

2.1. Materials

1α,25-dihydroxyvitamin D_3 and fetal bovine serum (FBS) were from Sigma Chemical Co. (St. Louis, MO, USA). Free-phenol red DMEM was from US Biological Inc. (Massachusetts, MA, USA). Anti-phospho p38, anti-phospho tyr416 Src, anti-actin and anti-tubulin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phospho ERK 1/2 antibody and anti-phospho-Akt (Ser473) antibody were from Cell Signaling Technology Inc. (Beverly, MA, USA). The Super Signal CL-HRP substrate system for enhanced chemiluminiscence (ECL) was from Perkin-Elmer (Boston, USA). The C2C12 cell line was recently obtained from ATCC (American Type Culture Collection, Manassas, VA). Puromycin was from Invitrogen (Invivogen, San Diego, CA). The plasmid to knock down the VDR was PLKO.1, clone ID TRCN0000027101 (Open Biosystems, Huntsville, AL). Lentivirus particles containing a pLKO.1 vector with the information to express a shRNA against VDR were kindly given by Dr. V. Gonzalez Pardo, Universidad Nacional del Sur. All other reagents were of analytical grade.

2.2. Cell culture

The mouse skeletal myoblastic cell line C2C12 was seeded at an appropriate density (120,000 cells/cm^2) in Petri dishes (100 mm diameter) with DMEM supplemented with 10% FBS and antibiotic–antimycotic solution. The cells were cultured at 37 °C under a humidified atmosphere of 95% air/5% CO_2. Under these conditions, myoblasts divide within the first 48 h and at day sixth these cells become differentiated into myotubes expressing morphological characteristics of adult skeletal muscle fibers. Cells cultured for two days (proliferative stage) were used for steroid hormone treatments. The cells were incubated in serum-free medium for 30 min prior to the addition of 1,25D.

2.3. Lentivirus infection and selection

To generate a stable (long-term) knockdown of VDR gene expression in C2C12 cell line, we infected these cells with lentivirus particles containing a pLKO.1 vector with the information to express a shRNA against mouse VDR. This plasmid also has a gene encoding puromycin resistance, thereby addition of puromycin allowed us to select cells that stably expressing shRNA against VDR (C2C12-shVDR). First, we determined the optimal puromycin concentration for the C2C12 cell line before initiating the experiments (titration assay). For this, we plated C2C12 cells in 3 cm plates and grown at 37 °C, 5% CO_2 overnight, until cells become approximately 80% confluent. Diluted puromycin in culture media (DMEM) was prepared at final concentrations of 1–4 μg/ml (with 1 μg/ml increments) and added to cells. We examined cells each day in an optical microscope and changed to fresh puromycin-containing media every other day. The minimum concentration of puromycin that results in complete cell death after 5 days was 2 μg/ml and we used this concentration for selection and maintenance of cells lacking VDR. To perform lentivirus infection of C2C12 cells, we used cells approximately 80% confluent. For this, we changed the cell medium to 1.5 ml of fresh culture media containing 4.5 μg of polybrene and 1.5 ml of virus followed by incubation of cells at 37 °C, 5% CO_2. We changed to fresh media 24 h after infection. To select the infected cells, we did not ad puromycin until 48 h after infection to allow for sufficient expression of the puromycin resistance gene. VDR knockdown was monitored by Western blot analysis and was achieved after five passages. We maintained one uninfected plate of cells in parallel. This plate served as a positive control for the puromycin selection.

2.4. Electrophoresis and Western blotting

Following treatment, the cells were lysed, the clarified lysates resolved by one-dimensional SDS-PAGE and then transferred to Immobilon P membranes as previously described [12]. The membranes were then probed with anti-phospho p38 MAPK, anti-phospho ERK1/2, anti-phospho tyr416 Src or anti-phospho Akt antibodies. The anti-phospho-antibodies were then stripped and the membrane was reprobed with anti-actin or anti-tubulin antibodies to account for equal loading. Autoradiograms were scanned with a Hewlett Packard densitometer to quantitate hormone signals by the ImageJ software program.

2.5. Statistical evaluation

The significance of the results was evaluated by Student's *t*-test [31].

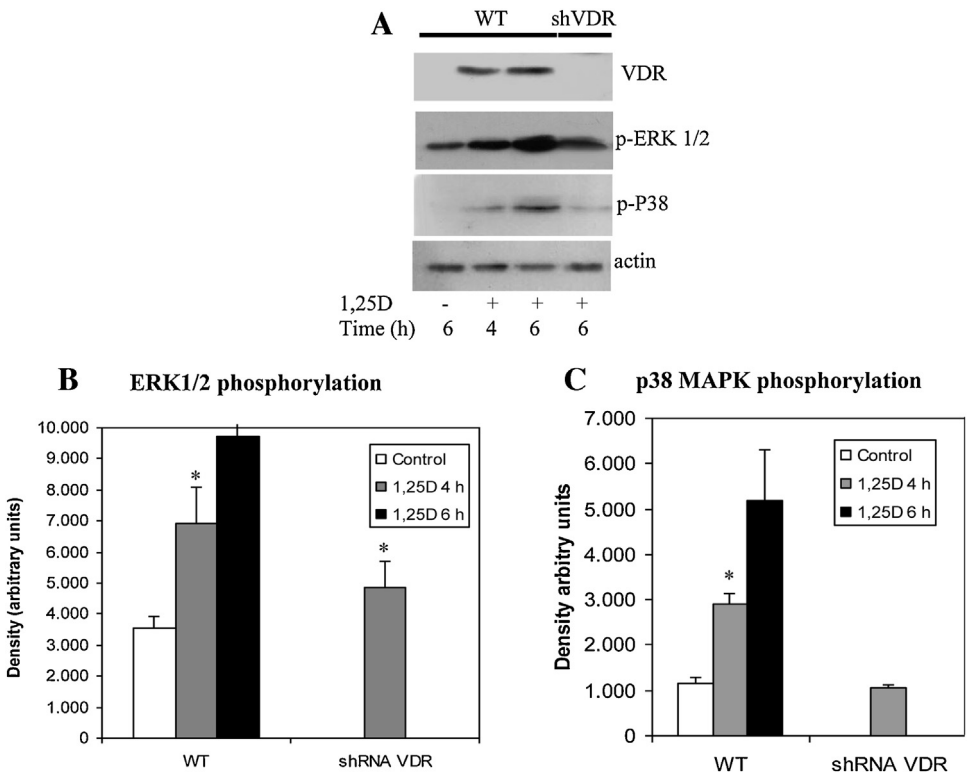

Fig. 1. Silencing of VDR expression in C2C12 cells suppress p38 MAPK phosphorylation and diminishes ERK1/2 activation induced by 1,25D. Cells infected with lentiviral particles containing a pLKO.1 plasmid with the information to express a shRNA against VDR were selected by puromycin resistance. These cells lacking VDR (shVDR) were treated with 1 nM 1,25D for 6 h. C2C12 wild type cells (WT) were stimulated with the hormone or vehicle (0.001% isopropanol) for 4 and 6 h. Western blot analysis of lysates was carried out using anti-VDR, anti-phospho ERK1/2 and anti-phospho p38 MAPK antibodies. The blotted membranes were re-probed with anti-actin antibody in order to ensure equal loading. (A) Representative immunoblots from three independent experiments. (B and C) Quantifications by scanning volumetric densitometry of blots (phospho ERK1/2 and phospho p38 MAPK, respectively) from three independent experiments showing averages ± SD. $^*p < 0.05$ respect to control.

3. Results and discussion

VDR expression has been clearly demonstrated in skeletal muscle of avian, murine and human [reviewed in Ref. [28]] although recently, studies carried out by Wang and DeLuca failed to detect the receptor protein in this tissue [32]. It is possible that the considerable lower basal levels of VDR in muscle than in duodenal cells (positive control of VDR expression widely utilized) did not allow detection of the receptor. It has been very recently reported that the VDR and CYP27B1 are expressed in C2C12 cells and regenerating skeletal muscle *in vivo* confirming that the VDR is present in skeletal muscle [33].

Evidences of VDR participation in the non-genomic action of 1,25D have been reported in the last year [34–36], in agreement with data from earlier studies supporting this concept. The presence of VDR in caveolae enriched plasma membrane of different tissues and cell lines was previously reported [37]. As mentioned in Section 1, we showed that the VDR localizes in the proximity of the plasma membrane after 1,25D treatment of skeletal muscle myoblasts only when caveolae structures of cells are intact [25]. In the present study we further inquired into the role of the vitamin D receptor in 1,25D non-genomic signal transduction using C2C12 cells with markedly reduced VDR expression through infection with lentivirus particles containing a pLKO.1 vector with the information to express a shRNA against mouse VDR. These cells (shVDR) selected by puromycin resistance showed by Western blot analyses 85% (average of different experiments) decrease of VDR expression and were used to evaluate the participation of the receptor in fast activation of key kinases. Cell treatments for 1, 4 and 6 h with 1 nM 1,25D enhanced VDR expression only in C2C12 wild type cells (WT) (Figs. 1–3, upper blots).

Within the mitogen-activated protein (MAP) kinase family are included the extracellular signal-regulated kinases 1 and 2 (ERK1/2) and p38 MAP kinases. These MAPKs are activated in various tissues via Src, a non-receptor tyrosine kinase [38,39]. In agreement with this concept, we previously established that Src is required for 1,25D-dependent activation of ERK 1/2 and p38 MAPK in skeletal muscle myoblasts [14,15]. In the present study, employing shVDR C2C12 cells, we observed that p38 MAPK phosphorylation by 1,25D was completely suppressed while phosphorylation of ERK1/2 was reduced by 61% (Fig. 1). The latter result is in agreement with that obtained in chicken myoblast cultures, where blockade of VDR expression with antisense oligonucleotides against VDR mRNA, partially affected ERK1/2 tyrosine phosphorylation by 1,25D [26,40]. This implies that both VDR-dependent and VDR-independent signaling mediate hormone stimulation of MAPKs. Regarding the second mechanism it is conceivable to envision that a receptor entity of 1,25D located in a membrane microdomain/caveolae other than the VDR also participates to promote hormone activation of ERK1/2 (Fig. 4). In line with this interpretation, studies in intestinal cells identified a 66 kDa protein named 1,25D3-MARRS/ERp57/PDIA3 as responsible of rapid actions of 1,25D, but this protein is not itself up-regulated by the hormone [41]. We consider that co-involvement of the 1,25D3-MARRS receptor in hormone-induced skeletal muscle rapid action is a relevant pending investigation. Another alternative may be related to the existence of a G protein coupled transmembrane receptor for 1,25D that would trigger the upstream activation of PLC/DAG/PKC, leading then to ERK1/2 phosphorylation (Fig. 4). Interestingly, cytoplasmic VDR coupling to protein kinases has been proposed to occur through G proteins in osteoblasts [42]. Clearly, further studies are necessary to elucidate the molecular

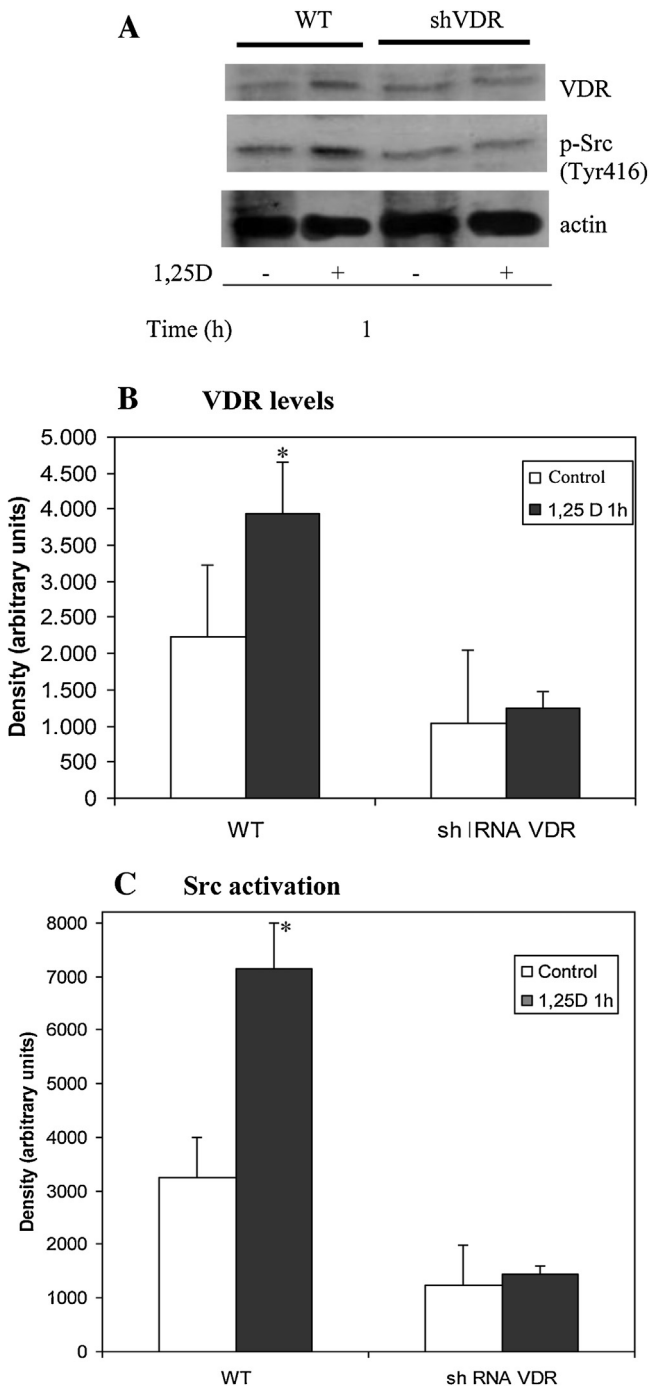

Fig. 2. 1,25D-Up regulated Src activation is dependent on VDR expression. Cells VDR (−) and C2C12 WT cells were treated with 1 nM 1,25D or vehicle (0.001% isopropanol) for 1 h. Western blot analysis of lysates was carried out using anti-VDR and anti-phospho tyr416 Src. The blotted membranes were re-probed with anti-actin antibody in order to ensure equal loading. (A) Representative immunoblots from three independent experiments. (B and C) Quantifications by scanning volumetric densitometry of blots from three independent experiments showing averages ± SD. *$p < 0.05$ respect to control.

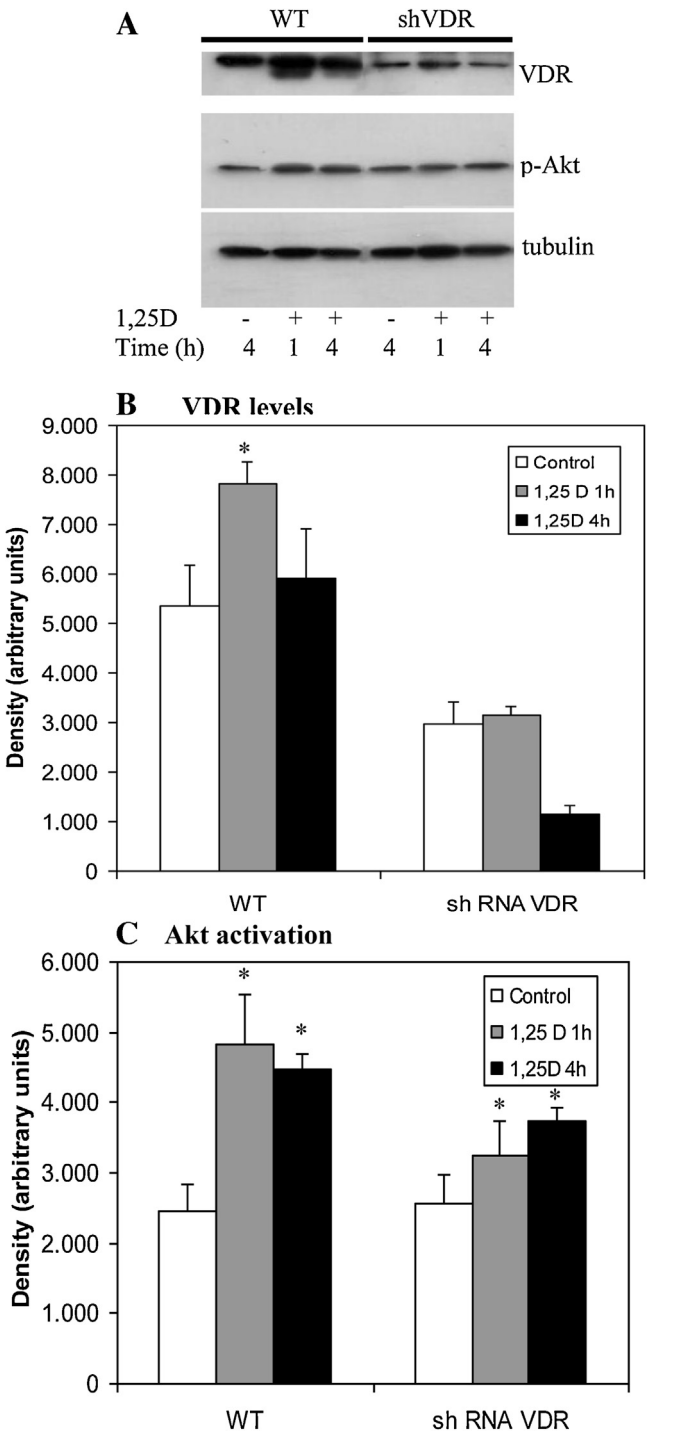

Fig. 3. VDR participates in Akt phosphorylation by the hormone. C2C12 shVDR and wild type cells were treated with 1 nM 1,25D or vehicle (0.001% isopropanol) for 1 or 4 h. Western blot analysis of lysates was carried out using anti-VDR and anti-phospho Akt antibodies. The blotted membranes were re-probed with anti-tubulin antibody in order to ensure equal loading. (A) Representative immunoblots from three independent experiments. (B and C) Quantifications by scanning volumetric densitometry of blots from three independent experiments showing averages ± SD. *$p < 0.05$ respect to the respective control.

mechanisms whereby 1,25D exerts fast non-genomic effects on MAPK activity in muscle cells.

Fast non-genomic Src activation by 1,25D has been previously reported in various cell types, including skeletal muscle myoblasts [15,43–45]. Furthermore, there is evidence that 1,25D induces rapid association of VDR with Src in skeletal muscle and osteoblastic cells [46,47]. Fig. 2 of this paper demonstrates that VDR expression is required for 1,25D-dependent Src tyr416 phosphorylation (i.e. activation) in C2C12 cells. These studies support experimentally the relationship between VDR and Src proposed by Fleet [48].

The PI3K/Akt pathway is one of the most critical molecular events involved in regulation of cell survival [49]. As mentioned in Section 1, we recently reported that 1,25D-induced Akt activation in skeletal muscle myoblasts is mediated by Src [19]. This

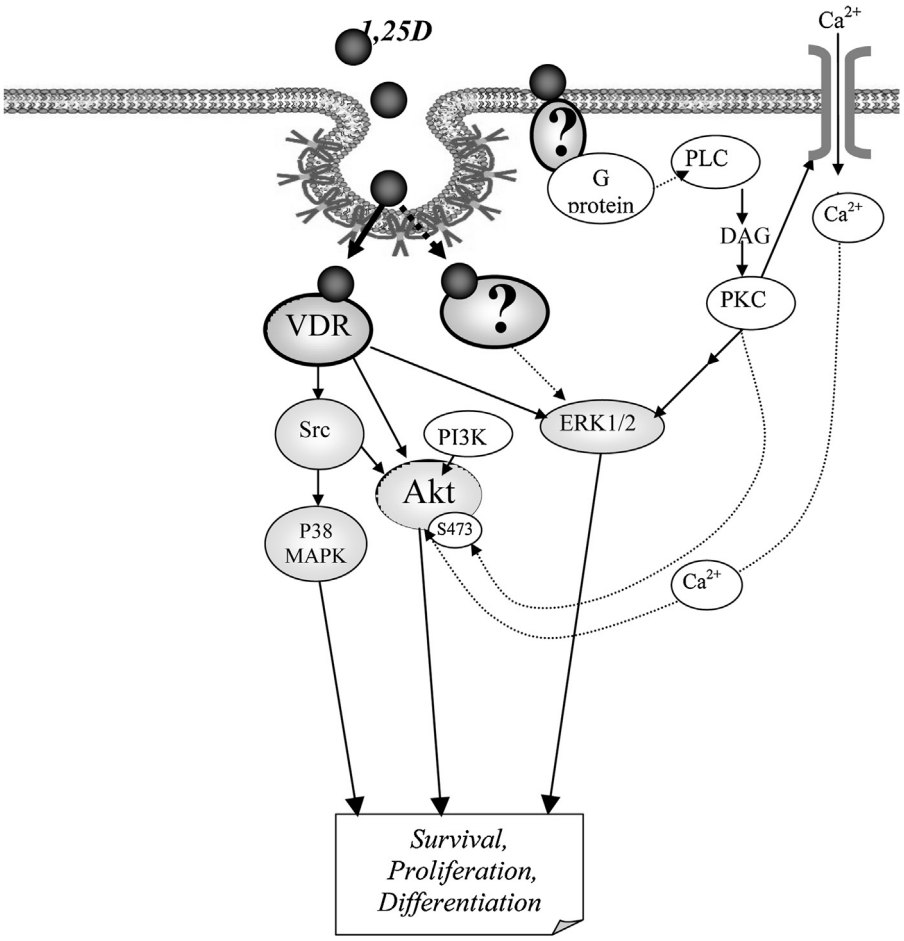

Fig. 4. Proposed mechanism of VDR-dependent and VDR-independent 1,25D non-genomic signaling in skeletal muscle cells. Our results show that 1,25D-induced p38 MAPK phosphorylation and Src activation are completely suppressed in C2C12 skeletal muscle cells lacking VDR expression. Nevertheless, ERK 1/2 phosphorylation and Akt activation by the hormone are partially dependent on VDR expression. This figure shows the participation of the VDR in rapid actions of 1,25D and also suggest the existence of others receptors, likely a membrane entity, which would intervene in mediating hormone-triggered fast effects via PLC/PKC, which could contribute to ERK1/2 and Akt activation finally leading to cellular response (survival, proliferation and differentiation).

study shows that the VDR also plays a role in the stimulation of Akt by 1,25D as the effects of the hormone on Akt phosphorylation in shVDR C2C12 cells diminished ~50% (Fig. 3). Interestingly, in osteoblasts there is evidence suggesting 1,25D activation of a VDR-dependent, PTX-sensitive PI3K/Akt pathway [50]. The fact that here the VDR is partially involved in 1,25D modulation of Akt activity in C2C12 muscle cells points out again the participation of another receptor in the hormone non-genomic action as discussed before for the mechanism of MAPK activation and illustrated in Fig. 4. In view of the functional relevance of Akt activation for the maintenance and survival of cells, and its regulation by 1,25D, the present data suggest that hormone-induced Akt phosphorylation through VDR-dependent and VDR-independent mechanisms are required to fulfill this role. It is possible that other signaling pathway intermediates may contribute to 1,25D regulation of Akt in C2C12 myoblasts as inferred from data obtained in other cell types. Thus, PKC-dependent phosphorylation of Akt-Ser473, crucial event in Akt activation, occurs in TCR induced T cells [51]. Connected to this observation, 1,25D has been shown to stimulate myoblast proliferation via PKC [16]. 1,25D-induced Akt activation by an elevation of intracellular calcium through L-type Ca^{2+} channels in osteoblasts and non-osteoblastic cells expressing different levels of VDR has also been described [52]. The participation of the PKC and Ca^{2+} signaling systems in 1,25D modulation of Akt in muscle cells is included in the schematic diagram of Fig. 4 (dashed arrows; needs to be confirmed).

4. Conclusions

It is concluded that the fast non-genomic 1α,25-dihydroxyvitamin D_3 signaling in skeletal muscle cells significantly involves the participation of the classical VDR located in caveolae, leading to the activation of Src, MAPKs and Akt, key kinases in the regulation of cell proliferation and differentiation. A role for other novel membrane receptors in the hormone rapid actions cannot be excluded in view of the evidences reported for other tissues. Our findings may help to understand the molecular mechanisms by which the hormone contributes to proliferation and survival of satellite myoblasts which account for its effects on skeletal muscle growth and regeneration of the injured tissue.

Acknowledgements

This research was supported by grants from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and Universidad Nacional del Sur, Argentina.

References

[1] H. Freake, C. Marcocci, J. Iwasaki, I. MacIntyre, 1,25-dihydroxyvitamin D_3 specifically binds to a human breast cancer cell line (T47D) and stimulates

growth, Biochemical and Biophysical Research Communications 10 (1981) 1131–1138.
[2] G. Murdoch, M. Rosenfeld, Regulation of pituitary function and prolactin production in the GH4 cell line by vitamin D, Journal of Biological Chemistry 256 (1981) 4050–4053.
[3] R. Gniadecki, Activation of Raf-mitogen-activated protein kinase signaling pathway by 1,25-dihydroxyvitamin D_3 in normal human keratinocytes, Journal of Investigative Dermatology 106 (1996) 1212–1217.
[4] A. Revelli, M. Massobrio, J. Tesarik, Nongenomic effects of 1alpha,25-dihydroxyvitamin D_3, Trends in Endocrinology and Metabolism 9 (1998) 419–427.
[5] R. Boland, Vitamin D and muscle, in: D. Feldman, W. Pike, F. Glorieux (Eds.), Vitamin D, 2nd edition, Elsevier Inc., Amsterdam, The Netherlands, 2005, pp. 883–897.
[6] M. Walters, Newly identified actions of the vitamin D endocrine system, Endocrine Reviews 13 (1992) 719–764.
[7] W. Tang, V. Ziboh, R. Isseroff, D. Martinez, Novel regulatory actions of 1 alpha,25-dihydroxyvitamin D_3 on the metabolism of polyphosphoinositides in murine epidermal keratinocytes, Journal of Cellular Physiology 132 (1987) 131–136.
[8] A. Bourdeau, F. Atmani, B. Grosse, M. Lieberherr, Rapid effects of 1,25-dihydroxyvitamin D_3 and extracellular Ca^{2+} on phospholipid metabolism in dispersed porcine parathyroid cells, Endocrinology 127 (1990) 2738–2743.
[9] A. de Boland, I. Nemere, Rapid actions of vitamin D compounds, Journal of Cellular Biochemistry 49 (1992) 32–36.
[10] E. Marcinkowska, A. Wiedłocha, C. Radzikowski, 1,25-dihydroxyvitamin D_3 induced activation and subsequent nuclear translocation of MAPK is upstream regulated by PKC in HL-60 cells, Biochemical and Biophysical Research Communications 241 (1997) 419–426.
[11] Z. Schwartz, H. Ehland, V. Sylvia, D. Larsson, R. Hardin, V. Bingham, D. Lopez, D. Dean, B. Boyan, 1alpha,25-dihydroxyvitamin D_3 and 24R,25-dihydroxyvitamin D_3 modulate growth plate chondrocyte physiology via protein kinase C-dependent phosphorylation of extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase, Endocrinology 143 (2002) 2775–2786.
[12] S. Morelli, C. Buitrago, R. Boland, A.R. de Boland, The stimulation of MAP kinase by 1,25$(OH)_2$-vitamin D_3 in skeletal muscle cells is mediated by protein kinase C and calcium, Molecular and Cellular Endocrinology 173 (2001) 41–52.
[13] A. Ronda, C. Buitrago, A. Colicheo, A. de Boland, E. Roldán, R. Boland, Activation of MAPKs by 1alpha,25$(OH)_2$-vitamin D_3 and 17beta-estradiol in skeletal muscle cells leads to phosphorylation of Elk-1 and CREB transcription factors, Journal of Steroid Biochemistry and Molecular Biology 103 (2007) 462–466.
[14] C. Buitrago, A. Ronda, A. de Boland, R. Boland, MAP kinases p38 and JNK are activated by the steroid hormone 1alpha,25$(OH)_2$-vitamin D_3 in the C2C12 muscle cell line, Journal of Cellular Biochemistry 97 (2006) 698–708.
[15] C. Buitrago, R. Boland, A. de Boland, The tyrosine kinase c-Src is required for 1,25$(OH)_2$-vitamin D_3 signalling to the nucleus in muscle cells, Biochimica et Biophysica Acta 1541 (2001) 179–187.
[16] D. Capiati, G. Vazquez, M.T. Tellez Iñón, R.L. Boland, Antisense oligonucleotides targeted against protein kinase C alpha inhibit proliferation of cultured avian myoblasts, Cell Proliferation 33 (2000) 307–315.
[17] M. Ceci, J. Ross Jr., G. Condorelli, Molecular determinants of the physiological adaptation to stress in the cardiomyocyte: a focus on AKT, Journal of Molecular and Cellular Cardiology 37 (2004) 905–912.
[18] D. Guttridge, Signaling pathways weigh in on decisions to make or break skeletal muscle, Current Opinion in Clinical Nutrition and Metabolic Care 7 (2004) 443–450.
[19] C. Buitrago, N. Arango, R. Boland, 1α,25$(OH)_2D_3$-Dependent modulation of Akt in proliferating and differentiating C2C12 skeletal muscle cells, Journal of Cellular Biochemistry 113 (2012) 1170–1181.
[20] D. Baran, Nongenomic actions of the steroid hormone 1 alpha,25-dihydroxyvitamin D_3, Journal of Cellular Biochemistry 56 (1994) 303–306.
[21] D. Baran, A. Sorensen, Rapid actions of 1 alpha-25-dihydroxyvitamin D_3 physiologic role, Proceedings of the Society for Experimental Biology and Medicine 207 (1994) 175–179.
[22] I. Nemere, S.E. Safford, B. Rohe, M.M. DeSouza, M.C. Farach-Carson, Identification and characterization of 1,25D_3-membrane-associated rapid response, steroid (1,25D_3-MARRS) binding protein, Journal of Steroid Biochemistry and Molecular Biology 89/90 (89) (2004) 281–290.
[23] J. Chen, R. Olivares-Navarrete, Y. Wang, T. Herman, B. Boyan, Z. Schwartz, Protein-disulfide isomerase-associated 3 (Pdia3) mediates the membrane response to 1,25-dihydroxyvitamin D_3 in osteoblasts, Journal of Biological Chemistry 285 (2010) 37041–37050.
[24] D. Capiati, S. Benassati, R. Boland, 1,25$(OH)_2$-Vitamin D_3 induces translocation of the vitamin D receptor (VDR) to the plasma membrane in skeletal muscle cells, Journal of Cellular Biochemistry 86 (2002) 128–135.
[25] C. Buitrago, R. Boland, Caveolae and caveolin-1 are implicated in 1alpha,25$(OH)_2$-vitamin D_3-dependent modulation of Src, MAPK cascades and VDR localization in skeletal muscle cells, Journal of Steroid Biochemistry and Molecular Biology 121 (2010) 169–175.
[26] C. Buitrago, G. Vazquez, A. de Boland, R. Boland, The vitamin D receptor mediates rapid changes in muscle protein tyrosine phosphorylation induced by 1,25$(OH)_2D_3$, Biochemical and Biophysical Research Communications 289 (2001) 1150–1156.
[27] M. Mizwicki, D. Keidel, C.M. Bula, J.E. Bishop, L.P. Zanello, J.M. Wurtz, D. Moras, A.W. Norman, Identification of an alternative ligand-binding pocket in the nuclear vitamin D receptor and its functional importance in 1alpha,25$(OH)_2$-vitamin D_3 signaling, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 12876–12881.
[28] R. Boland, VDR activation of intracellular signaling pathways in skeletal muscle, Molecular and Cellular Endocrinology 347 (2011) 11–16.
[29] M. Haussler, P.W. Jurutka, M. Mizwicki, A.W. Norman, Vitamin D receptor (VDR)-mediated actions of 1alpha,25$(OH)_2$vitamin D_3: genomic and non-genomic mechanisms, Best Practice and Research. Clinical Endocrinology and Metabolism 25 (2011) 543–559.
[30] T. Kim, J. Wangb, Z. Janjetovic, J. Chen, R. Tuckey, M. Nguyen, E. Tang, D. Miller, W. Li, A. Slominski, Correlation between secosteroid induced vitamin D receptor activity in melanoma cells and computer-modeled receptor binding strength, Molecular and Cellular Endocrinology (2012), April 21 (Epub ahead of print).
[31] G.W. Snedecor, G.W. Cochran, Statistical Methods, Iowa State University Press, Ames, IA, 1967.
[32] Y. Wang, H. DeLuca, Is the vitamin D receptor found in muscle, Endocrinology 152 (2011) 354–363.
[33] R. Srikuea, X. Zhang, O.K. Park-Sarge, K.A. Esser, VDR and CYP27B1 are expressed in C2C12 cells and regenerating skeletal muscle: potential role in suppression of myoblast proliferation, American Journal of Physiology: Cell Physiology 303 (2012) C396–C405.
[34] M. Blomberg Jensen, S. Dissing, Non-genomic effects of vitamin D in human spermatozoa, Steroids (2012), March 5 (Epub ahead of print).
[35] V. Sequeira, M. Rybchyn, W. Tongkao-On, C. Gordon-Thomson, P. Malloy, I. Nemere, A. Norman, V. Reeve, G. Halliday, D. Feldman, R. Mason, The role of the vitamin D receptor and ERp57 in photoprotection by 1α,25-dihydroxyvitamin D_3, Molecular Endocrinology 26 (2012) 574–582.
[36] H. Willems, E. van den Heuvel, G. Carmeliet, A. Schaafsma, J. Klein-Nulend, A. Bakker, VDR dependent and independent effects of 1,25-dihydroxyvitamin D_3 on nitric oxide production by osteoblasts, Steroids 77 (2012) 126–131.
[37] J. Huhtakangas, C. Olivera, J. Bishop, L. Zanello, A. Norman, The vitamin D receptor is present in caveolae-enriched plasma membranes and binds 1 alpha,25$(OH)_2$-vitamin D_3 in vivo and in vitro, Molecular Endocrinology 18 (2004) 2660–2671.
[38] Z. Li, Y. Hosoi, K. Cai, Y. Tanno, Y. Matsumoto, A. Enomoto, A. Morita, K. Nakagawa, K. Miyagawa, Src tyrosine kinase inhibitor PP2 suppresses ERK1/2 activation and epidermal growth factor receptor transactivation by X-irradiation, Biochemical and Biophysical Research Communications 341 (2006) 363–368.
[39] B. Thobe, M. Frink, M. Choudhry, M. Schwacha, K. Bland, I. Chaudry, Src family kinases regulate p38 MAPK-mediated IL-6 production in Kupffer cells following hypoxia, American Journal of Physiology: Cell Physiology 291 (2006) C476–C482.
[40] R. Boland, A. de Boland, C. Buitrago, S. Morelli, G. Santillán, G. Vazquez, D. Capiati, C. Baldi, Non-genomic stimulation of tyrosine phosphorylation cascades by 1,25$(OH)_2D_3$ by VDR-dependent and -independent mechanisms in muscle cells, Steroids 67 (2002) 477–482.
[41] B. Rohe, S. Safford, I. Nemere, M. Farach-Carson, Regulation of expression of 1,25D_3-MARRS/ERp57/PDIA3 in rat IEC-6 cells by TGF beta and 1,25$(OH)_2D_3$, Steroids 72 (2007) 144–150.
[42] V. Le Mellay, F. Lasmoles, M. Lieberherr, Gαq/11 and Gγ proteins and membrane signaling of calcitriol and estradiol, Journal of Cellular Biochemistry 75 (1999) 138–146.
[43] J. Chappel, F. Ross, Y. Abu-Amer, A. Shaw, S. Teitelbaum, 1,25-dihydroxyvitamin D_3 regulates pp60c-src activity and expression of a pp60c-src activating phosphatase, Journal of Cellular Biochemistry 67 (1997) 432–438.
[44] R. Gniadecki, Involvement of Src in the vitamin D signaling in human keratinocytes, Biochemical Pharmacology 55 (1998) 499–503.
[45] S. Khare, M. Bissonnette, R. Wali, S. Skarosi, G. Boss, F. von Lintig, B. Scaglione-Sewell, M. Sitrin, T. Brasitus, 1,25-dihydroxyvitamin D_3 but not TPA activates PLD in Caco-2 cells via pp60(c-src) and RhoA, American Journal of Physiology 276 (1999) G1005–G1015.
[46] C. Buitrago, G. Vazquez, A. Russo de Boland, R. Boland, Activation of Src kinase in skeletal muscle cells by 1,25-$(OH)_2$-vitamin D_3 correlates with tyrosine phosphorylation of the vitamin D receptor (VDR) and VDR–Src interaction, Journal of Cellular Biochemistry 79 (2000) 274–281.
[47] A. Vertino, C. Bula, J. Chen, M. Almeida, L. Han, T. Bellido, S. Kousteni, A. Norman, S. Manolagas, Nongenotropic anti-apoptotic signaling of 1alpha,25$(OH)_2$-vitamin D_3 and analogs through the ligand binding domain of the vitamin D receptor in osteoblasts and osteocytes. Mediation by Src, phosphatidylinositol 3-, and JNK kinases, Journal of Biological Chemistry 280 (2005) 14130–14137.
[48] J. Fleet, Rapid membrane-initiated actions of 1,25-dihydroxyvitamin D: what are they and what do they mean? Journal of Nutrition 134 (2004) 3215–3218.
[49] G. Song, G. Ouyang, S. Bao, The activation of Akt/PKB signaling pathway and cell survival, Journal of Cellular and Molecular Medicine 9 (2005) 59–71.
[50] X. Zhang, L. Zanello, Vitamin D receptor-dependent 1α,25$(OH)_2$-vitamin D_3-induced anti-apoptotic PI3K/AKT signaling in osteoblasts, Journal of Bone and Mineral Research 23 (2008) 1238–1248.
[51] L. Yang, G. Oiao, H. Ying, J. Zhang, F. Yin, TCR-induced Akt serine 473 phosphorylation is regulated by protein kinase C-alpha, Biochemical and Biophysical Research Communications 400 (2010) 16–20.
[52] X. Zhang, P. Biswas, M. Owraghi, L. Zanello, 1α,25$(OH)_2$-Vitamin D_3 membrane-initiated calcium signaling modulates exocytosis and cell survival, Journal of Steroid Biochemistry and Molecular Biology 103 (2007) 457–461.