Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 89–90 (2004) 419–425
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Calcium as a mediator of 1,25-dihydroxyvitamin D_3-induced apoptosis☆

I.N. Sergeev*

Department of Chemistry and Biochemistry, South Dakota State University, P.O. Box 2202, SH 212, Brookings, SD 57007, USA

Abstract

Cellular calcium has been implicated in induction of apoptosis. We have shown that $1,25(OH)_2D_3$-induced apoptosis is associated with a sustained increase in concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) resulting from depletion of the endoplasmic reticulum (ER) Ca^{2+} stores and activation of the voltage-insensitive Ca^{2+} entry pathway [1,25-Dihydroxyvitamin D_3, intracellular Ca^{2+} and apoptosis in breast cancer cells, in: A.W. Norman, R. Bouillon, M. Thomasset (Eds.), Vitamin D: Chemistry, Biology and Clinical Applications of the Steroid Hormone, University of California, Riverside, 1997, pp. 473–474; Vitamin D and intracellular calcium, in: P. Quinn, V. Kagan (Eds.), Subcellular Biochemistry: Fat-Soluble Vitamins, Plenum Press, New York, 1998, pp. 271–297; 1,25-Dihydroxyvitamin D_3 and calcium signaling, in: A.W. Norman, R. Bouillon, M. Thomasset (Eds.), Vitamin D Endocrine System: Structural, Biological, Genetic and Clinical Aspects, University of California, Riverside, 2000, pp. 715–718; 1,25-Dihydroxyvitamin D_3 triggers calcium-mediated apoptosis in breast cancer cells, in: A.W. Norman, R. Bouillon, M. Thomasset (Eds.), Vitamin D Endocrine System: Structural, Biological, Genetic and Clinical Aspects, University of California, Riverside, 2000, pp. 399–402; Endocrine 9 (1998) 321]. This study was undertaken to investigate mechanism of $1,25(OH)_2D_3$-induced apoptosis in breast cancer cells and compare effects of the hormone on Ca^{2+} and apoptosis in cancer and normal human mammary epithelial cells. The treatment of MCF-7 breast cancer cells with $1,25(OH)_2D_3$ induced a sustained increase in $[Ca^{2+}]_i$ and activated the Ca^{2+}-dependent proapoptotic proteases, μ-calpain and caspase-12, as evaluated with antibodies to active (cleaved) forms of the enzymes and the calpain substrate. The selective inhibition of Ca^{2+} binding sites of μ-calpain decreased apoptotic indices in the $1,25(OH)_2D_3$-treated cells. $1,25(OH)_2D_3$ did not induce apoptosis in normal human mammary epithelial cells (HMECs), as evaluated by DNA fragmentation (TUNEL), loss of the plasma membrane asymmetry (Annexin V assay) and morphological criteria. In these cells, $1,25(OH)_2D_3$ triggered a transient Ca^{2+} response, which was not accompanied by the calpain and caspase activation. HMEC, but not MCF-7 cells expressed the Ca^{2+} binding protein calbindin-D_{28k} and buffered Ca^{2+} increases induced by a Ca^{2+} ionophore ionomycin. In conclusion, we have identified the novel apoptotic pathway in breast carcinoma cells treated with $1,25(OH)_2D_3$: increase in $[Ca^{2+}]_i \rightarrow$ μ-calpain activation → caspase-12 activation → apoptosis. Our findings also imply that differences of Ca^{2+} regulatory mechanisms in breast cancer versus normal mammary epithelial cells underlay resistance of normal cells and susceptibility of cancer cells to $1,25(OH)_2D_3$-induced Ca^{2+}-mediated apoptosis.

Keywords: Intracellular calcium; Apoptosis; Calpain; Caspase-12; MCF-7 cells; Human mammary epithelial cells

Dysregulation of apoptosis underlies pathophysiology of proliferative disorders [1]. A decrease in apoptotic cell death contributes to mammary tumorigenesis and increased risk for breast cancer in humans [2,3]. A family of intracellular cysteine proteases, the caspases, is believed to be responsible for most biochemical and morphological alterations during apoptosis, although the additional or alternative apoptosis initiation and execution pathways have been demonstrated (e.g. mitochondrion- or calpain-mediated apoptosis) [4,5].

Calcium, a key cellular regulator, has been implicated in induction of apoptosis and regulation of the apoptosis signaling pathways. We [5–10] and others [11–13] have shown that early and late increases in concentration of intracelluar Ca^{2+} ($[Ca^{2+}]_i$) occur in apoptosis. The critical characteristic of the apoptotic Ca^{2+} signal is a sustained increase in $[Ca^{2+}]_i$, reaching elevated, but not cytotoxic levels [7–9]. Although there is little doubt that such an increase in $[Ca^{2+}]_i$ triggers cell death via apoptosis, mechanisms of action of cellular Ca^{2+} in apoptotic pathways are not understood and, particularly, the apoptosis-related Ca^{2+} targets have not been identified. Caspases and Ca^{2+}-dependent neutral proteases, the calpains, are considered as possible targets [4,5,7]. Interestingly, oncoproteins in apoptosis-resistant cancer cells may function by preventing increases in $[Ca^{2+}]_i$, e.g. Bcl-2 protein inhibits release of Ca^{2+} from the endoplasmic reticulum (ER) stores [14]. On the other hand, Ca^{2+} release from the ER stores activates caspase-12 [15] via Ca^{2+}-dependent

☆ Presented at the 12th Workshop on Vitamin D (Maastricht, The Netherlands, 6–10 July 2003).

* Corresponding author. Tel.: +1-605-688-5465; fax: +1-605-688-6364.
E-mail address: igor_sergeev@sdstate.edu (I.N. Sergeev).

0960-0760/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.03.010

calpain [16,25]. Suppression of apoptosis by the Ca^{2+} binding protein calbindin-D_{28k} via inhibition of the caspase-3 activity has been also suggested [17].

Interaction of the Ca^{2+} signal with intracellular Ca^{2+} buffers may play a particularly significant role in the apoptotic process [7,11]. An important element of the cytosolic Ca^{2+} buffering system is the vitamin D-dependent Ca^{2+} binding protein calbindin-D_{28k}. We [18] and others [19] have shown that elevated levels of calbindin-D_{28k} dramatically increase the cytosolic Ca^{2+} buffering capacity. Remarkably, increased Ca^{2+} buffering due to enforced expression of calbindin-D_{28k} protect cancer cells against Ca^{2+}-mediated apoptosis [5].

We [5,6,9,10] and others [20,21] have demonstrated that $1,25(OH)_2D_3$ induces apoptosis in breast cancer cells. Recently, we have also shown that apoptosis induced by $1,25(OH)_2D_3$ in these cells is associated with the Ca^{2+}-dependent calpain activation [5]. This study was undertaken to compare $1,25(OH)_2D_3$-induced Ca^{2+} signaling and apoptosis in human breast cancer and normal cell. The hypothesis was that mechanisms of Ca^{2+} entry, Ca^{2+} mobilization and/or Ca^{2+} buffering are different in breast cancer and normal cells and that these differences underlay the cell's susceptibility to $1,25(OH)_2D_3$-induced Ca^{2+}-mediated apoptosis.

1. Materials and methods

1.1. Cells

The normal human mammary epithelial cells (HMECs) (Clonetics) and the human breast carcinoma cell line MCF-7 (ATTC) were used in this study. MCF-7 cells were cultured in RPMI-1640 medium supplemented with 5% fetal calf serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. HMEC were maintained in Mammary Epithelial Growth Medium (Clonetics) under the same conditions, as MCF-7 cells.

1.2. Intracellular calcium

Concentration of intracellular free Ca^{2+} ($[Ca^{2+}]_i$) was measured using Ca^{2+} indicator fura-2, as described previously [8,22]. Cells grown on coverslips were loaded with 1 μM fura-2/AM in Dulbecco's PBS (D-PBS) supplemented with 0.1% dimethylsulfoxide and 0.01% Pluronic F-127 for 40 min at 37 °C. The dynamics of intracellular Ca^{2+} was assessed with cells in the microincubation chamber (37.0 ± 0.2 °C) on a Nikon Eclipse TE-300 inverted microscope equipped for epifluorescence, ratiometric, digital imaging. The images were captured using SuperFluor 40× or 100× oil-immersion objectives and CoolSnapFX digital CCD camera (Photometrics), ratioed (340/380 nm excitation, 510 nm emission) on a pixel-by-pixel basis, and stored for analysis. The fluorescence signal of intracellular Ca^{2+} was calibrated in situ at the end of experiments. Image analysis was performed using MetaFluor 5.0 software (Universal Imaging).

The following treatments were used to evaluate the Ca^{2+} control mechanisms [22]: Ca^{2+} ionophore ionomycin (1 μM); mobilizer of the ER Ca^{2+} stores thapsigargin (1 μM); depolarization of plasma membrane with KCl (50 mM); agonist of voltage-dependent Ca^{2+} channels Bay K8644 (5 μM). To evaluate Ca^{2+} release from the ER, thapsigargin (1 μM) was added to cells after recording the basal $[Ca^{2+}]_i$ for 1–3 min, and peak values of the $[Ca^{2+}]_i$ increase were measured. To measure the Mn^{2+} entry rate as a reporter of indirect Ca^{2+} influx [21], the images were recorded at excitation of 360 nm (the fura-2 isosbestic point) and emission of 510 nm. After recording the basal level of the fluorescence intensity, Mn^{2+} was added to the final concentration of 2 mM. The rates of Ca^{2+} entry were estimated from the slope of the Mn^{2+} quench curves. Cytosolic Ca^{2+} buffering capacity was evaluated by measuring the maximum of $[Ca^{2+}]_i$ rises after exposure to ionomycin in the presence of a lowered concentration of extracellular Ca^{2+} [18]. Cells were placed in a nominally Ca^{2+}-free buffer (i.e. without $CaCl_2$ plus 0.1 mM EGTA), and immediately then 0.5 μM ionomycin and 0.5 mM Ca^{2+} were added.

1.3. Caspase and calpain activity and immunofluorescence labeling

Caspase activity was detected in living cells with the fluorochrome-labeled pan-caspase inhibitor FAM–VAD–FMK (Intergen), which binds to active caspases. The inhibitor was used at concentration 10 μM; loading time was 1 h. Calpain activation in the cells was evaluated using the cell-permeable fluorogenic substrate t–BOC–Leu–Met–CMAC (Molecular Probes) at concentration of 50 μM [23].

Monoclonal antibodies directed against caspase-12 (a gift from Dr. J. Yuan) and the μ-calpain large and small subunits (Chemicon) were used to evaluate activation of these proteases. A calpain inhibitor, selectively interacting with the Ca^{2+} binding sites of μ-calpain, was PD 151746 (10 μM) (Calbiochem). Calbindin-D_{28k} was detected with monoclonal anti-calbindin-D_{28k} antibodies (Sigma). For immunofluorescence labeling, the fixed and permeabilized cell preparations were pre-incubated with non-specific serum for 20 min, incubated for 1 h at 37 °C or overnight at 4 °C with the primary antibodies and 1 h at room temperature with secondary antibodies (Alexa Fluor-488 signal-amplification mouse antibodies (Molecular Probes) and FITC- or Texas Red-conjugated AffiniPure anti-rat and anti-mouse IgG (Jackson ImmunoResearch). Fluorescence microscopy was carried out as described for Ca^{2+} imaging. Image analysis and measurement of fluorescence intensity was performed using MetaMorph 5.0 software (Universal Imaging), as we described [24]. In some experiments, fluorescence of cells grown in the 96-well microplates and loaded with fluores-

cent probes was measured in the FLx800 reader with KC software (Bio-Teck).

1.4. Apoptosis

Apoptosis was measured by DNA fragmentation and membrane changes. Treatment of cells with 1,25 (100 nM), ionomycin (500 nM) or thapsigargin (100 nM) was used to evaluate the role of increased $[Ca^{2+}]_i$ in apoptosis. The DNA fragmentation was measured with terminal deoxynucleotidyl transferase and BrdUTP (APO-BrdU TUNEL Assay Kit; Molecular Probes). Annexin V assay (Alexa Fluor 488 Annexin V; Molecular Probes) was used for detection of the apoptotic plasma membrane (phosphatidylserine translocation). Utilization of the two techniques permits detection of the late- and early-stage apoptotic cells. Fluorescent dye-staining was used in concert with TUNEL and Annexin V assays to specify of onset of nuclear fragmentation (Hoechst 33258) and disruption of plasma membrane (YOPRO-1 labeling and propidium iodide uptake).

2. Results

2.1. Intracellular Ca^{2+}

$[Ca^{2+}]_i$ was measured in HMEC and MCF-7 cells treated with $1,25(OH)_2D_3$ (100 nM) for 1, 3 or 6 days. $1,25(OH)_2D_3$ induced a sustained increase in $[Ca^{2+}]_i$ in MCF-7 cells (Fig. 1A). In HMEC, $1,25(OH)_2D_3$ triggered a transient Ca^{2+} response, but failed to induce a sustained increase in $[Ca^{2+}]_i$ (Fig. 1B). Both MCF-7 cells and HMEC expressed

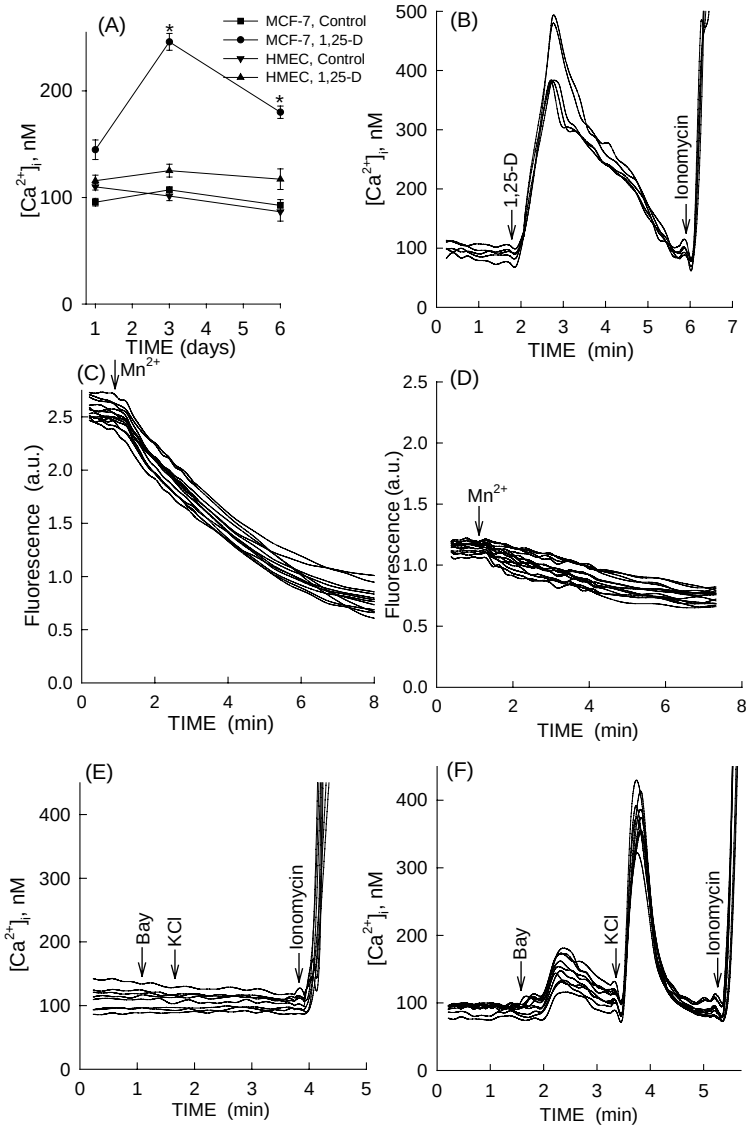

Fig. 1. Rapid and chronic effects of $1,25(OH)_2D_3$ on $[Ca^{2+}]_i$ and Ca^{2+} entry pathways in HMEC and MCF-7 cells. Cells were treated and $[Ca^{2+}]_i$ was measured as described in Section 1. On panels B–F, the traces represent responses of individual cells. Panels B, D and F: HMEC; panels C and E: MCF-7 cells.

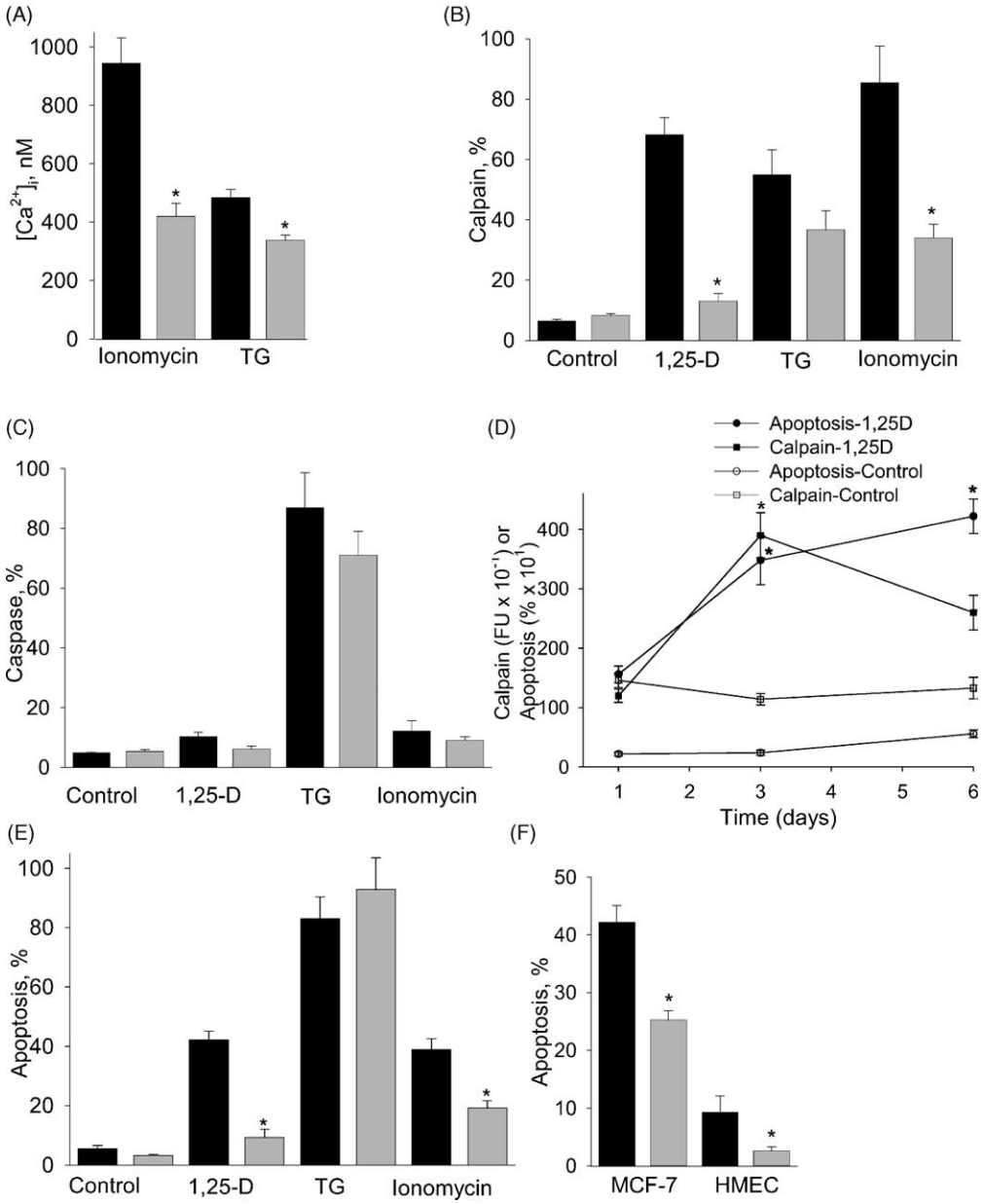

Fig. 2. Ca^{2+} buffering, Ca^{2+} mobilization, calpain and caspase activation, and apoptosis in HMEC and MCF-7 cells. Ca^{2+} buffering and Ca^{2+} mobilization are shown as the maximum $[Ca^{2+}]_i$ rises after addition of ionomycin or thapsigargin, respectively. Calpain and caspase activation and apoptosis were measured at day 6 and expressed as percentage of fluorescently labeled cells or relative fluorescence intensity (arbitrary fluorescence units). See Sections 1 and 2 for additional explanations. Note that mobilizer of the ER Ca^{2+} stores thapsigargin, which also causes the ER stress, induced significant apoptosis in HMEC. Black bars: MCF-7 cells; gray bars: HMEC.

the voltage-insensitive Ca^{2+} entry pathway, but its permeability was four-fold lower in HMEC (Fig. 1C and D). The voltage-dependent Ca^{2+} entry was evident in HMEC, but not in MCF-7 cells (Fig. 1E and F). Cytosolic Ca^{2+} buffering capacity, as evaluated with ionomycin, was 2.2-fold larger in HMEC than in MCF-7 cells (Fig. 2A). The Ca^{2+} mobilization response was 1.4-fold lower in HMEC than in MCF-7 cells (see Fig. 2A), which may also indicate differences in the Ca^{2+}-buffering capacity. HMEC expressed the cytosolic Ca^{2+} binding/buffering protein calbindin D_{28k}, which was absent in MCF-7 cells (not shown). These findings indicate significant differences in Ca^{2+} entry and Ca^{2+} buffering between normal and cancer human mammary epithelial cells.

2.2. Calpain and caspase-12

A sustained increase in $[Ca^{2+}]_i$ in MCF-7 cells treated with $1,25(OH)_2D_3$ was accompanied by activation of the Ca^{2+}-dependent apoptotic proteases μ-calpain and caspase-12. The Ca^{2+}-dependent calpain activation was demonstrated by the its cleavage (e.g. presence in cells of the calpain small subunit) and by cleavage of the fluorogenic

peptide calpain substrate Figs. 2B and 3B). Importantly, calpain activation by $1,25(OH)_2D_3$ was significantly lower in HMEC than in MCF-7 cells (see Fig. 2B). Caspase-12 was expressed in the $1,25(OH)_2D_3$-treated MCF-7 cells (Fig. 3A), but not in HMEC (not shown). However, $1,25(OH)_2D_3$-dependent caspase activation (as evaluated with the pan-caspase inhibitor binding to active caspases) was undetectable in MCF-7 cells and HMEC (Fig. 2C). The

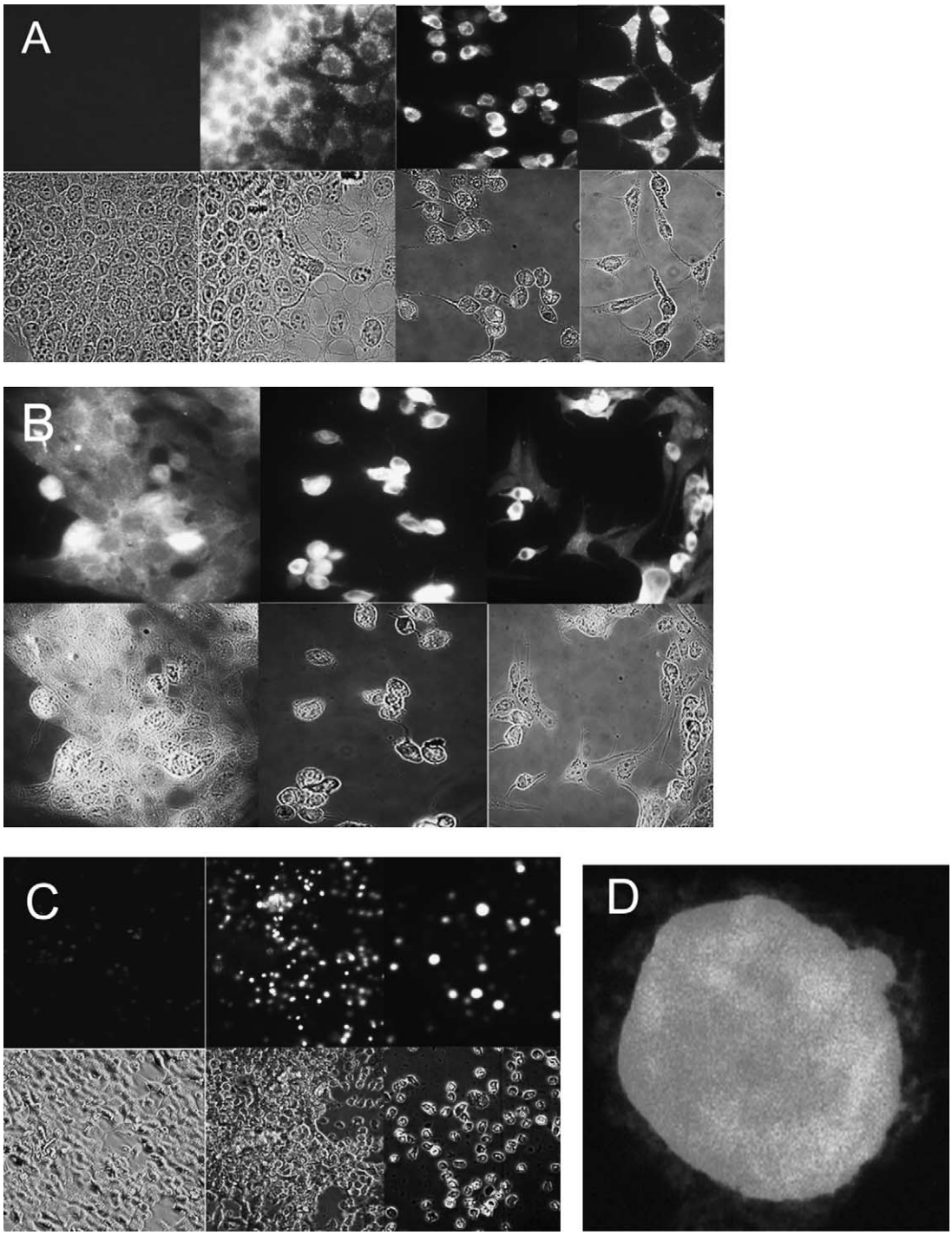

Fig. 3. Immunofluorescence detection of caspase-12, calpain, DNA fragmentation, and apoptosis in MCF-7 cells. Panel A: fluorescent (upper row) and light (lower row) images of caspase-12 in cells treated with vehicle, $1,25(OH)_2D_3$, thapsigargin, or ionomycin (left-to-right). Panel B: fluorescent and light images of the calpain small subunit in cells treated with $1,25(OH)_2D_3$, thapsigargin, or inomycin (left-to-right). The calpain cleavage was undetectable in control cells (not shown). Panel C: fluorescent and light images of the TUNEL-labeled MCF-7 cells treated with vehicle, $1,25(OH)_2D_3$ or thapsigargin (left-to-right). Panel D: the single apoptotic nucleus of a $1,25(OH)_2D_3$-treated MCF-7 cell labeled for fragmented (TUNEL) and total (propidium iodide) DNA. Areas of fragmented DNA are seen as more transparent on this B/W image.

$1,25(OH)_2D_3$-induced calpain activation in MCF-7 cells followed the time-course of the $[Ca^{2+}]_i$ increase and coincide with the onset of apoptosis (Fig. 2D; compare with Fig. 1A). These results imply that $1,25(OH)_2D_3$ induces the Ca^{2+}-dependent calpain activation in breast cancer cells. Caspase-12 activation in $1,25(OH)_2D_3$-treated MCF-7 cells was evident with anti-caspase-12 antibodies. Specific caspase-12 substrate or inhibitor are not available and, therefore, the protease's activity cannot be measured directly.

2.3. Apoptosis

A sustained increase in $[Ca^{2+}]_i$ in $1,25(OH)_2D_3$-treated MCF-7 cells was accompanied by induction of apoptosis (as evaluated by DNA fragmentation and the plasma membrane asymmetry), whereas $1,25(OH)_2D_3$-dependent apoptosis was insignificant in HMEC (Fig. 2E; Fig. 3C and D; Annexin V data not shown). Morphological criteria confirmed apoptosis in $1,25(OH)_2D_3$-treated MCF-7 cells, but not in HMEC (not shown). Importantly, treatment of MCF-7 cells with $1,25(OH)_2D_3$ in the presence of the μ-calpain inhibitor, which selectively blocks its Ca^{2+} binding sites, significantly reduced apoptotic indices in MCF-7 cells and HMEC (Fig. 2F). These results imply that induction of apoptosis with $1,25(OH)_2D_3$ in MCF-7 cells requires the Ca^{2+}-dependent calpain activation.

3. Discussion

An increase of intracellular Ca^{2+} induces apoptosis in various cell models [5,6,11,12]. Here, we report that the $1,25(OH)_2D_3$-induced sustained increase in $[Ca^{2+}]_i$ triggers apoptosis in human breast carcinoma cells, but not normal mammary epithelial cells. Apoptosis induced by $1,25(OH)_2D_3$ in MCF-7 breast cancer cells was associated with expression/activation the Ca^{2+}-dependent proapoptotic proteases, μ-calpain and caspase-12. Normal breast cells were resistant to $1,25(OH)_2D_3$-induced Ca^{2+}-mediated apoptosis. It appears that the large cytosolic Ca^{2+} buffering capacity is critical for maintaining such a resistance. Additionally, $1,25(OH)_2D_3$ may not activate the voltage-insensitive Ca^{2+} entry and/or the ER Ca^{2+} mobilization pathways in normal cells. Collectively, our results support the hypothesis that the Ca^{2+} entry and Ca^{2+} buffering mechanisms are different in breast cancer and normal cells. Ca^{2+} handling by HMEC, allowing only a transient, $1,25(OH)_2D_3$-induced Ca^{2+} response, and a large cytosolic Ca^{2+} buffering capacity of these cells seem sufficient to protect normal mammary epithelial cells from the $1,25(OH)_2D_3$-induced apoptosis.

4. Conclusion

We have identified the novel apoptotic pathway in breast carcinoma cells treated with $1,25(OH)_2D_3$: increase in $[Ca^{2+}]_i$ → μ-calpain activation → caspase-12 activation → apoptosis. This mechanism cannot be activated by $1,25(OH)_2D_3$ in normal breast cells, because they, apparently, are protected from the Ca^{2+}-mediated apoptosis via adequate buffering of Ca^{2+} increases and limited permeability of the Ca^{2+} entry pathway.

Acknowledgements

We thank Julie Colby for valuable technical assistance and Dr. J. Yuan from Harvard Medical School for a gift of caspase-12 antibodies.

References

[1] C.B. Thompson, Apoptosis in the pathogenesis and treatment of disease, Science 267 (1995) 1456–1462.
[2] D.J. Allan, A. Howell, S.A. Poberts, Reduction in apoptosis relative to mitosis in histologically normal epithelium accompanies fibrocystic change and carcinoma of the premenopausal human breast, J. Pathol. 67 (1992) 25–32.
[3] R. Strange, R.R. Friis, L.T. Bemis, F.J. Geske, Programmed cell death during mammary gland involution, Methods Cell Biol. 46 (1995) 355–368.
[4] E. Carafoli, M. Molinari, Calpain: a protease in search of a function? Biochem. Biophys. Res. Commun. 247 (1998) 193–203.
[5] I.S. Mathiasen, I.N. Sergeev, L. Bastholm, F. Elling, A.W. Norman, M. Jaattela, Calcium and calpain as key mediators of apoptosis-like death induced by vitamin D compounds in breast cancer cells, J. Biol. Chem. 277 (2002) 3078–30745.
[6] I.N. Sergeev, W.B. Rhoten, A.W. Norman, 1,25-Dihydroxyvitamin D_3, intracellular Ca^{2+} and apoptosis in breast cancer cells, in: A.W. Norman, R. Bouillon, M. Thomasset (Eds.), Vitamin D: Chemistry, Biology and Clinical Applications of the Steroid Hormone, University of California, Riverside, 1997, pp. 473–474.
[7] I.N. Sergeev, W.B. Rhoten, V.B. Spirichev, Vitamin D and intracellular calcium, in: P. Quinn, V. Kagan (Eds.), Subcellular Biochemistry: Fat-Soluble Vitamins, Plenum Press, New York, 1998, pp. 271–297.
[8] I.N. Sergeev, W.B. Rhoten, Regulation of intracellular calcium in breast cancer cells, Endocrine 9 (1998) 321–327.
[9] I.N. Sergeev, A.W. Norman, 1,25-Dihydroxyvitamin D_3 and calcium signaling, in: A.W. Norman, R. Bouillon, M. Thomasset (Eds.), Vitamin D Endocrine System: Structural, Biological, Genetic and Clinical Aspects, University of California, Riverside, 2000, pp. 715–718.
[10] I.N. Sergeev, J. Colby, A.W. Norman, 1,25-Dihydroxyvitamin D_3 triggers calcium-mediated apoptosis in breast cancer cells, in: A.W. Norman, R. Bouillon, M. Thomasset (Eds.), Vitamin D Endocrine System: Structural, Biological, Genetic and Clinical Aspects, University of California, Riverside, 2000, pp. 399–402.
[11] D.L. McConkey, S. Orrenius, The role of calcium in regulation of apoptosis, Biochem. Biophys. Res. Commun. 239 (1997) 357–366.
[12] M.J. Berridge, M.D. Bootman, P. Lipp, Calcium: a life and death signal, Nature 395 (1988) 645–648.
[13] E. Carafoli, L. Santella, D. Branca, M. Brini, Generation, control, and processing of cellular calcium signals, Crit. Rev. Biochem. Mol. Biol. 36 (2001) 107–260.
[14] S. Gil-Parrado, A. Fernandez-Montalvan, I. Assfalg-Macnleidt, O. Popp, F. Bestvater, A. Holloschi, W. Machleidt, Ionomycin-activated calpain triggers apoptosis: a probable role for Bcl-2 family members, J. Biol. Chem. 277 (2002) 27217–27226.

[15] T. Nakagawa, H. Zhu, N. Morishima, E. Li, J. Xu, B.A. Yankner, J. Yuan, Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amiloid-β, Science 403 (2000) 98–103.
[16] T. Nakagawa, J. Yuan, Cross-talk between two cysteine protease families: activation of caspase-12 by calpain in apoptosis, J. Cell Biol. 150 (2000) 887–894.
[17] T. Bellido, M. Huening, M. Raval-Pandya, S.C. Manolagas, S. Christakos, Calbindin-D_{28k} is expressed in osteoblastic cells and suppresses their apoptosis by inhibiting caspase-3 activity, J. Biol. Chem. 275 (2000) 26328–26332.
[18] W.B. Rhoten, I.N. Sergeev, Calbindin-D_{28k} appears to buffer intracellular Ca^{2+} in butyrate-treated rat insulinoma cells, Endocrine 2 (1994) 989–995.
[19] D. Reddy, A.S. Pollock, S.A. Clarl, K. Sooy, R.C. Vasavada, A.F. Stewart, T. Honeyman, S. Christakos, Transfection and overexpression of the calcium binding protein calbindin-D_{28k} results in a stimulatory effect on insulin synthesis in a rat β-cell line (RIN 1046-38), Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 1961–1966.
[20] J.E. Welsh, K. VanWeelden, L. Flanagan, I. Byrne, C.J. Narvaez, The role of vitamin D_3 and antiestrogens in modulating apoptosis of breast cancer cells and tumors, in: P. Quinn, V. Kagan (Eds.), Subcellular Biochemistry: Fat-Soluble Vitamins, Plenum Press, New York, 1998, pp. 245–270.
[21] K. Colston, in: D. Feldman, F.H. Glorieux, J.W. Pike (Eds.), Vitamin D and Breast Cancer: Therapeutic Potential of New Vitamin D Analogs, Academic Press, San Diego, 1997, pp. 1107–1123.
[22] I.N. Sergeev, W.B. Rhoten, 1,25-Dihydroxyvitamin D_3 evokes oscillations of intracellular calcium in a pancreatic β-cell line, Endocrinology 136 (1995) 2852–2861.
[23] B.G. Roser, G.J. Gores, Cellular in vivo assay of calpain activity using a fluorescent substrate, Methods Mol. Biol. 144 (2000) 2452–2466.
[24] I.N. Sergeev, W.B. Rhoten, M.D. Carney, Calbindins decreased after space flight, Endocrine 5 (1996) 335–340 (cover illustration).
[25] I.N. Sergeev, A.W. Norman, Calcium as a mediator of apoptosis in bovine oocytes and preimplantation embryos, Endocrine 22 (2003) 169–175.