Journal of Basic & Clinical Medicine

http://www.sspublications.org/index.php/JBCM/index

The Two Functions of Calcitonin: Cell Type-specific Up- and Down-regulation of ERK Signaling and Urokinase-type Plasminogen Activator Expression

Misa Nakamura[1*], Hirotoshi Utsunomiya[2], Sachiko Nomura[2], Ryohei Kono[2], Kennichi Kakudo[3]

[1]Department of Rehabilitation, Osaka Kawasaki Rehabilitation University, Osaka; [2]Department of Strategic Surveillance for Functional Food and Comprehensive Traditional Medicine, Wakayama Medical University, Wakayama; and [3]Department of Pathology, Nara Hospital, Kinki University Faculty of Medicine, Nara, Japan

Journal of Basic & Clinical Medicine 2015, 4(1):25-31

Abstract

Calcitonin (CT) is a 32 amino-acid polypeptide hormone secreted by C-cells of the thyroid. Clinically, CT is administered to treat humoral hypercalcemia of malignancy and osteoporosis. Recently, an association between CT use and cancer incidence has been suggested. Extracellular signal regulated kinases 1 and 2 (Erk1/2) are ubiquitous protein kinases that are involved in functions including the regulation of cell proliferation, adhesion, and migration. Urokinase-type plasminogen activator (uPA) modulates cell adherence to the extracellular matrix and is involved in both normal tissue repair and malignancy. We previously reported that CT can either increase or decrease the phosphorylation of Erk1/2 and the expression of uPA, depending on the cell type. In the human breast cancer cell line MDA-MB-231, CT inactivates c-Raf *via* the protein kinase A pathway, thereby inhibiting Erk1/2 phosphorylation and decreasing uPA expression. However, in LLC-PK1 porcine renal cells, CT induces Erk1/2 phosphorylation through the protein kinase C pathway, and thus increasing the uPA expression. Furthermore, CT has different effects on tumor progression and metastasis in cancer cells. Thus, CT selectively regulates the Erk1/2 pathway in a cell type-specific manner, and these modes of regulation may play an important role in the actions of CT on various other types of cells. We focus on the mechanism of the opposite effects of CT on Erk1/2 phosphorylation and uPA expression, and review the differential regulation of CT depending on the tumor type.

Keywords: Calcitonin, cancer cell, Erk signaling, protein kinase A, protein kinase C, urokinase-type plasminogen activator

Introduction

Calcitonin (CT) is a peptide hormone comprising 32 amino-acid secreted by C-cells of the thyroid gland (1). The main physiological function of CT is the regulation of calcium metabolism through inhibition of bone resorption by osteoclasts, and enhancement of Ca^{2+} excretion by kidney (2). Therefore, CT is administered to treat diseases characterized by high bone turnover, including Paget's disease of the bone, humoral hypercalcemia of malignancy, and osteoporosis (3). Intranasal and injectable salmon CT are used worldwide to treat postmenopausal osteoporosis. Recently, salmon CT has been shown to be an effective drug for treating cherubism or disc degeneration (4, 5). Many reports have shown that proCT (PCT), the precursor of CT, is increased in the serum of septic patients and those with systemic inflammation, particularly in bacterial infections. The serum PCT levels are correlated with the mortality and severity of infection and persist for a relatively long period of time (6-8). Hence, PCT is an important marker of sepsis (9, 10). The CT receptor (CTR) is expressed in many types of tissues and cells (11-13), suggesting that CT/CTR may have other functions as well. There are many reports that CT is involved in the regulation of cell proliferation, adhesion, and migration (12-18). In cancer cells, their proliferation, tumorigenesis and metastasis are regulated by CT (19-26).

CTR belongs to the G protein-coupled seven transmembrane domain receptor (GPCR) superfamily, one of the largest families of membrane receptors. This superfamily includes the receptors for parathyroid hormone (PTH), luteinizing hormone (LH), and follicle stimulating hormone (FSH) (27). These receptors regulate the intracellular signaling cascades. The GPCRs interact with membrane-associated heterotrimeric GTP-binding proteins (G proteins) isoforms composed of α, β and γ subunits. It has been reported that CTR couples to Gs and Gq, resulting activation of adenylate cyclase (AC) and phospholipase C (Fig. 1) (28-34).

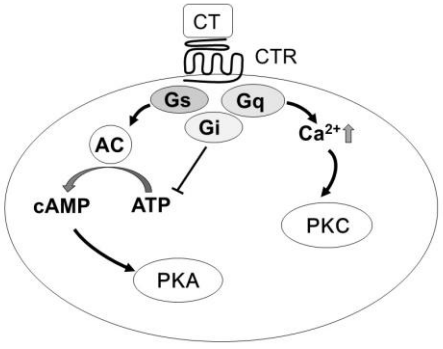

Fig. 1. Signaling pathways of CTR (28, 31-34, 85, 90).

GPCRs also control the activity of several members of the mitogen-activated protein kinase (MAPK) superfamily. Among these kinases are extracellular signal-regulated kinases 1 (Erk1) and 2 (Erk2) (or p44 MAPK and p42 MAPK), c-Jun NH(2)-terminal kinases (JNKs), p38 MAPK, and Erk5 (or BMK) (35). The Erks are ubiquitous protein kinases that are related with many cellular responses, including cell proliferation, adhesion, and

Received: July 23, 2015; Accepted: July 30, 2015
*Correspondence author: Misa Nakamura, Department of Rehabilitation, Osaka Kawasaki Rehabilitation University, 158 Mizuma, Kaizuka, Osaka 597-0104, Japan. Tel: +81-73-441-0635; Fax: +81-73-446-4825
E-mail address: nakamuram@kawasakigakuen.ac.jp

migration (36). GPCRs may signal to Erks *via* numerous signaling pathways that differ dramatically in different cell types or cell lines. Thus, GPCRs activate complex pathways that ultimately interconnect events at the plasma membrane to those in the nucleus, such as modulation of gene transcription *via* phosphorylation of transcription factors.

Previously, we performed cDNA subtractive hybridization experiments to study CT-induced changes in gene expression of the porcine kidney cell line LLC-PK1 (37). We identified urokinase-type plasminogen activator (uPA) as one of the genes whose expression was induced by CT stimulation, and that this up-regulation was mediated by phosphorylation of Erk1/2. Similarly, CT increases phosphorylation of Erk1/2 in human embryonic kidney cells (HEK293) transfected with rabbit CTR as well as in human nucleus pulposus (5, 38). Interestingly, we have found that CT decreases uPA expression by suppressing constitutive phosphorylation of Erk1/2 in breast cancer cells, MDA-MB-231 (39). These studies suggest that CT has opposite actions on Erk1/2 phosphorylation in different cells. In this review, we focus on the mechanism of the two functions of CT on Erk1/2 phosphorylation and uPA expression in LLC-PK1 cells and MDA-MB-231 cells in particular, and introduce an idea that this differential regulation of CT depends on the tumor type.

CT induces transient activation of Erk1/2 through the protein kinase C (PKC) pathway in LLC-PK1 cells

LLC-PK1 cells were established from the porcine kidney and are used as an *in vitro* model to study renal proximal tubule function (40, 41). The LLCPK1 cells have been used in many studies on CT/CTR function because the CTR was first cloned from the cells (42). We have demonstrated that CT induces Erk1/2 phosphorylation in LLCPK1 cells (37, 43). Time-course studies have shown that 10^{-8} M of CT maximally stimulates the phosphorylation of Erk1/2 after 30 min of treatment (Fig. 2A). H89, a protein kinase A (PKA) inhibitor, has no apparent effects on basal or CT-stimulated Erk1/2 phosphorylation. However, the PKC inhibitor, Calphostin C (CC), partially blocks the CT-induced Erk1/2 phosphorylation. These results suggest that CT-induced Erk1/2 phosphorylation is contributed by the PKC pathway, but not by the PKA pathway in LLC-PK1 cells. These results are consistent with those obtained from CTR-transfected HEK293 cells (38).

PKA contributes significantly to CT-mediated attenuation of constitutive Erk1/2 phosphorylation in MDA-MB-231 cells

The MAPK pathway plays a vital role in regulating the cell proliferation and differentiation (36). Constitutive phosphorylation of MAPK or elevated level of MAPK mRNA has been reported in a variety of tumors and to be associated with carcinogenesis (44-51). When the MAPK pathways are constitutively activated, normal mammalian cells are transformed into a cancerous phenotype (52-54), whereas blockade of MAPK activity inhibits the proliferation of Ras-transformed cells *in vitro* (55, 56). Thus, constitutive activation of MAPK promotes cell transformation, suggesting that the MAPK pathway is a therapeutic target for cancer (57-60).

Our previous studies have shown that CT inhibits phosphorylation of Erk1/2 in the cell lines of breast cancer (MDA-MB-231, MDA-MB-435, and T24), renal cell carcinoma (VMC-RCW), rectal carcinoma (CaR-1), and prostate cancer (DU145), while these lines exhibit constitutive phosphorylation of Erk1/2. In those cell lines of breast cancer (MCF-7, MRK-nu-1), thyroid cancer (TT, FTC-133), pancreatic carcinoma (KP-1NL, KP-3), and colon cancer (CCK81) without constitutive Erk1/2 activation, CT has no effects on the Erk1/2 phosphorylation (61). MDA-MB-231 cells are hormone-insensitive, highly invasive, and a representative of late-stage breast cancer. In our study, constitutive activation of JNK or p38 MAPK was not detected in MDA-MB-231 cells, and CT did not induce the Erk1/2 phosphorylation (61). As mentioned above, CT suppresses Erk1/2 phosphorylation in MDA-MB-231 cells (Fig. 2B). Because the CTR is capable of activating PKA and PKC (31, 33), we investigated whether these pathways were involved in attenuation of Erk1/2 phosphorylation by CT. When MDA-MB-231 cells were treated with a PKA inhibitor, H89, or a PKC inhibitor, CC, in the presence or absence of CT, H89 diminished Erk1/2 phosphorylation induced by CT, but CC had no effects (61). These results suggest that the PKA pathway, but not the PKC pathway, may contribute to the attenuation of Erk1/2 phosphorylation by CT in MDA-MB-231 cells, which is consistent with our previous studies on the prostate cancer cell line, DU145 (62).

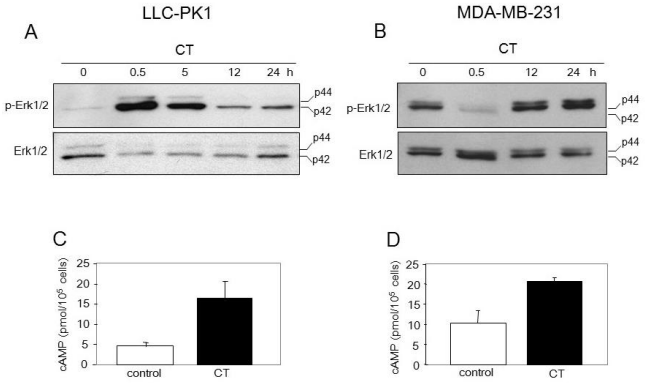

Fig. 2. The opposite effects of CT on phosphorylation of Erk1/2 in LLC-PK1 (A) and MDA-MB-231 (B) cells (37, 61). Serum-starved cells were incubated with 10^{-8} M CT for the indicated times. The upper and low panels in A and B show the results by Western blotting with anti-phosphorylated-Erk1/2 antibody and anti-total Erk1/2 antibody, respectively. LLC-PK1 (C) and MDA-MB-231 (D) cells were incubated with 10^{-8} M CT for 30 min. The value of intracellular cAMP is expressed as pmol/10^5 cells.

Activation of Ser259 of c-Raf is related to the CT-induced down regulation of constitutive Erk1/2 phosphorylation in a PKA-mediated manner in MDA-MB-231 cells

MAPK are regulated by intracellular phosphorylation cascades. In these cascades, Erk1/2 is activated by MAPK/ERK kinase 1, MEK1 and MAPK/ERK kinase 2, MEK2 (63). These MEKs are phosphorylated by MEKKs composed of A-Raf, B-Raf, and c-Raf (or Raf-1) (64). Essentially, c-Raf activation occurs by phosphorylation of Ser338 and Tyl341in the N-terminal region and Thr491 and Ser3495 (64-67), however MAPKs activity is suppressed when c-Raf is phosphorylated at Ser259 by PKA and other sites (68). 14-3-3 proteins, which are small acidic dimers (30 kDa), stabilize c-Raf in both low and high activity formations depending upon Raf phosphorylation status (63). Two 14-3-3 motifs are present on Ser259 and Ser621 in c-Raf (64). When 14-3-3 bound to Ser621 of c-Raf, it appears to activate of c-Raf. On the contrary, binding to Ser259 of c-Raf appears to inhibit of its activity. Therefore binding of 14-3-3 to Ser259 antagonizes c-Raf activity (65, 66). Dumaz *et al.* reported that activated PKA block c-Raf activation by stimulating 14-3-3 binding and preventing c-Raf interaction with Ras (67). We studied the contribution of c-Raf to

the regulation of Erk1/2 phosphorylation by CT in MDA-MB-231 cells was investigated (61). The results showed that CT enhanced phosphorylation of Ser259 of c-Raf. However, H89 inhibits this effect of CT, producing a statistically significant reduction in c-Raf phosphorylation. As noted above, H89 also attenuates the inhibition of Erk1/2 phosphorylation by CT in these cells. These results suggest that activation of Ser259 of c-Raf contributes to PKA-dependent attenuation of constitutive Erk1/2 phosphorylation by CT in MDA-MB-231 cells.

CT increases cAMP levels in both LLC-PK1 and MDA-MB-231 cells

To confirm that CTR is related to the cAMP pathway in MDA-MB-231 and LLC-PK1 cells, we measured the intracellular cAMP concentrations following CT treatment. The result showed that CT substantially increased cAMP level in both cell lines: 3.6-fold higher in LLC-PK1 cells, and 2-fold higher in MDA-MB-231 cells (Fig. 2C and D). However, increased cAMP level is contributed by the inhibition of Erk1/2 phosphorylation in MDA-MB-231 cells, but not in LLC-PK1 cells (37, 61).

The effects of AC and PKC activators on Erk1/2 phosphorylation in LLC-PK1 cells are similar to those in MDA-MB-231 cells, but the CT signaling is different in the two cell lines

The effects of the AC activator, forskolin, and the PKC activator, phorbol 12-myristate (PMA), on the Erk1/2 phosphorylation have been studied in LLC-PK1 cells and MDA-MB-231 cells (37, 61). In LLC-PK1 cells, PMA but not forskolin increases Erk1/2 phosphorylation. Furthermore, PMA augments the CT stimulation to Erk1/2 phosphorylation, while forskolin partially suppresses it. In MDA-MB-231 cells, PMA increases the basal Erk1/2 phosphorylation and blocks the suppression induced by CT, whereas forskolin eliminates the Erk1/2 phosphorylation in both CT-treated and untreated cells. These results suggest that the inhibitory effect of forskolin and the stimulatory effect of PMA on the Erk1/2 phosphorylation are similar in the two cell lines, but in the LLC-PK1 cells, PMA mimics or augments the effects of CT, whereas forskolin antagonizes these effects. The reverse is true in MDA-MB-231 cells: forskolin mimics or augments the effects of CT, whereas PMA antagonizes these effects.

The effects of CT on uPA expression in LLC-PK1 cells and MDA-MB-231 cells

We have found that CT induces expression of many genes including uPA, connective tissue growth factor (CTGF), NF-κB, NF-IL-3A, and interleukin-8 in LLC-PK1 cells. Of these genes, uPA and CTGF are induced by CT *via* the Erk1/2 pathway in LLCPK1 cells (37, 43). The uPA system consists of uPA, uPA receptor, and uPA inhibiting plasminogen activator inhibition types 1 and 2. Upon binding to uPA receptor, uPA breaks down various components of extracellular matrix, including collagen and fibronectin, through generation of plasmin converted from plasminogen (68). Thereby uPA participates in cellular invasion and migration (69-71). Ma *et al.* reported high levels of uPA expression through constitutive Erk1/2 phosphorylation in MDA-MB-231 cells (72). As shown in Figure 3, we previously reported that exogenous CT increased uPA expression about 18-fold in LLC-PK1 cells, but decreased it about 4-fold in MDA-MB-231 cells (37, 40). Consistent with its effects on uPA mRNA in MDA-MB-231 cells, CT decreased the uPA protein concentration in the medium to 67% of control levels. In the presence of the MEK1 inhibitor PD98509, CT-induced uPA expression was significantly decreased in LLC-PK1 cells, and constitutive uPA expression was also decreased in MDA-MB-231 cells. Our study has also shown that CT administration for 2 weeks to nude mice injected with MDA-MB-231 cells inhibits the expression of uPA mRNA in primary tumors by 25% compared to control (40). These results indicate that CT has opposite effects on uPA expression in these cell lines, which may be due to its opposite effects on Erk1/2 phosphorylation as shown the model of these system in Figure 4.

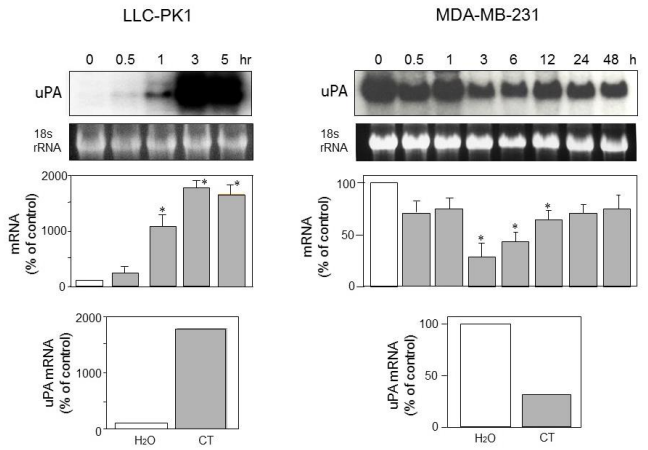

Fig. 3. CT has opposite effects on uPA expression in LLC-PK1 and MDA-MB-231 cells (37, 39). Serum-starved LLC-PK1 and MDA-MB-231 cells were incubated with 10^{-8} M CT for the indicated times, and then assayed for uPA mRNA using Northern blotting (top panels). The expression level of the uPA gene was normalized to the level of 18S rRNA (middle panels). To compare the expression levels among samples after 3 h incubation with CT, the expression level of uPA gene in the control cells was set to 100% (bottom panels). **P*<0.05 compared with the control.

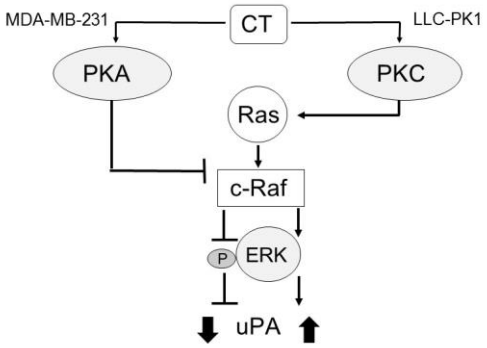

Fig. 4. A model of differential regulation by CT on Erk1/2 signaling and its downstream effectors in LLC-PK1 and MDA-MB-231 cells. In MDA-MB-231 cells, constitutive activation of Erk1/2 and constitutive uPA expression are present. CT inhibits c-Raf activation by phosphorylation of c-Ras at Ser259 via PKA, thus suppressing Erk1/2 activation and ultimately decreasing uPA expression. In LLC-PK1 cells, neither Erk1/2 activation nor uPA expression occurs constitutively. CT activates Erk1/2 via the PKC pathway, and thus induces uPA expression.

CT has opposite effects on biological functions

Consistent with the results from CTR-transfected HEK293 cells (39), our results suggest that CT induces Erk1/2 activation in LLC-PK1 cells by a PKC-dependent mechanism. Figure 3 depicts the differential regulation of Erk1/2 activity and uPA expression by CT in LLC-PK1 and MDA-MB-231 cells. In the MDA-MB-231

cells, constitutive activation of the Erk1/2 is suppressed by CT through the PKA pathway, while in the LLC-PK1 cells, activation of the Erk1/2 is induced by CT through the PKC pathway. We speculate that the PKA-mediated suppression of Erk1/2 phosphorylation by CT as observed in MDA-MB-231 and DU145 cells depends on the constitutive activation of Erk1/2 in these cells. Such contradictory effects have been reported for other peptide hormones. Adrenomedullin (ADM) is a 52 amino-acid peptide belonging to the CT gene superfamily (73). ADM is a tumor progression factor that acts in controlling angiogenesis (74). Conflicting results regarding ADM in vascular smooth muscle cells (VSMCs) have been reported. ADM has been reported to inhibit MAPK activity that is stimulated by epidermal platelet-derived growth factor, growth factor and endothelin in cultured rat glomerular mesangial cells and cultured rat VSMCs (75, 76). In contrast, ADM has also been reported to increase MAPK activity in serum-deprived rat VSMCs (77).

PTH, a polypeptide secreted by parathyroid glands, is a well-known mediator of bone remodeling and regulator of calcium homeostasis (78). PTH has catabolic effects, causing bone resorption by indirect activation of osteoclasts. PTH also has anabolic effects, leading to bone formation (79). PTH/PTH-related peptide (PTHrP) receptors comprise a distinct family of seven-transmembrane GPCRs to which CTR also belongs. Similar to the findings with CT, both stimulation (78, 80-81) and inhibition (82) of the MAPK pathway by PTH or PTHrP have been reported in osteoblasts. Verheijen *et al.* have demonstrated that PTH inhibits growth factor-induced Erk2 phosphorylation in osteosarcoma cells *via* the PKA pathway (83). On the other hand, this group has also reported that PTH induces transient activation of Erk1/2 in both Chinese hamster ovary R15 cells stably expressing high levels of rat PTH/PTHrP and parietal yolk sac carcinoma cells that endogenously express the receptor (83). They have concluded that PTH regulates MAPK activity in a cell type-specific manner. Chen *et al.* proposed that these opposite results might be due to the different cell systems used, e.g., osteosarcoma *versus* normal osteoblasts, and the various differentiation states of the cells (84).

Why does signaling by CT, like ADM and PTH signaling, vary in different cells? As noted above, CTR couples to Gs and Gq, resulting activation of AC and phospholipase C, respectively (31-34). Moreover, CTR can couple to Gi, but the inhibition of AC by GαI is negatively regulated by PKC (38, 85) (Fig. 1). A dominant negative mutant Gαs containing three separate mutations that affect distinct functions has been characterized in HEK293 cells. The results have shown that these triple mutants prevent CTR from activating AC and block activation of proteinase inhibitor turnover by CTR (85, 86). These results suggest that the different abilities of the G proteins to compete with each other for binding to CTR may influence differential signal transduction by CT, depending on the cell types. However, other factors must also play an important role, because CT increases cAMP in both LLC-PK1 and MDA-M-231 cells. Other possibilities are that CT may bind to different, unknown receptors and that CTR may interact with different receptors in LLC-PK1 and MDA-MB-231 cells.

The effects of CT on tumor progression and invasiveness

We have found that CT suppresses the tumor proliferation *in vivo* by inhibiting the constitutive Erk1/2 phosphorylation (60). Nude mice were treated with CT or saline every day after injection with MDA-MB-231 cells with constitutively phosphorylated Erk1/2 or MCF-7 cells without constitutively phosphorylated Erk1/2. In MDA-MB-231 cells, CT significantly reduced the tumor volume compared with saline. However, CT had no significant effects on tumor growth in MCF-7 cells. Furthermore, CT also inhibited the invasiveness of MDA-MB-231 cells by 37% in a matrigel invasion assay (39). These results suggest that CT suppresses cancer proliferation *in vivo* and invasiveness *in vitro*.

On the other hand, some reports have shown that CT increases tumorigenicity and metastatic potential in the prostate cancer cell lines PC-3, PC-3M and LNCaP (23, 86-91) and tumor tissues (24-26). None of these cell lines shows constitutive activation of Erk1/2, as seen in MCF-7 cells (62, 92, 93). In PC-3 and LNCaP cells, uPA expression induced by CT was reported (94). PC-3M cells were derived from a liver metastasis of PC-3 cells following intraspleen injection, and are androgen-refractory and have highly metastatic potential (95). LNCaP cells were established from lymph node metastasis in a prostate cancer patient, and are androgen-sensitive and have low metastatic potential (96). PC-3 cells, which were established from bone metastasis of a prostate cancer patient, are androgen refractory and moderately metastatic (97). DU145 cells, which were established from brain metastasis of a prostate cancer patient, are androgen refractory and have moderately metastatic potential (98). Shah *et al.* demonstrated that CT may induce the loss of cell-cell adhesion, an increase in the surface activity of or integrin's, and in the secretion of matrix metalloproteinases 2 and 9 and uPA (23). Therefore, CT may increase the tumorigenicity and invasiveness of prostate cancer cells thorough these molecular events. Regarding CT and CTR, LNCaP cells do not express CT, PC-3 cells do not express endogenous CTR, and PC-3M cells co-express CT and CTR (99). Overexpression of CT in LNCaP cells and CTR in PC-3 cells by transfection method increases their tumorigenicity and metastatic potential. On the contrary, silencing of CT expression in PC-3M cells does not only reduces their tumorigenicity, but also their metastatic potential (23).

Our results demonstrate that CT through its different effects on Erk1/2 phosphorylation depending on cell types performs correspondingly opposite effects on the gene expressions that influence biological processes including tumorigenesis, metastasis, and others. We propose that the clinical efficacy of CT will be useful in cancer that expresses the CTR and shows constitutive Erk1/2 phosphorylation. On the other hand, CT may induce malignancy in cancer cells that lack phosphorylated Erk1/2 signaling.

Conclusion

We introduced that the effects of CT on Erk1/2 phosphorylation and uPA expression are specific for cell types and are opposite in different cell types. These findings will assist in solving the problem of the variable functions of CT *via* CTR in many organs, tissues, and cells throughout the body. Furthermore, clarification of the detailed mechanisms of the action of CT is needed for its use as a drug in the future.

References

1. Zaidi MB, Moonga SP, Bevis J, Bascal ZA, Breimer LH. The calcitonin gene peptides: biology and clinical relevance. Crit Rev Clin Lab Sci 1990; 28:109-74.
2. Bijvoet OL, van der Sluys Veer J, de Vries HR, van Koppen AT. Natriuretic effect of calcitonin in man. N Engl J Med 1971; 284:681-8.
3. McDermott MT and Kidd GS. The role of calcitonin in the development and treatment of osteoporosis. Endocr Rev 1987; 8:377-90.

4. Etoz OA, Dolanmaz D, Gunhan O. Treatment of cherubism with salmon calcitonin: a case report. Eur J Dent 2011; 5:486-91.
5. Chen WH, Zeng R, Lo WC, Tina Chen SY, Lai TY, Williams DF, Deng WP. The role of the ERK1/2 pathway as an alternative to the aging-diminished cyclic AMP pathway in calcitonin- mediated chondrogenesis in human nucleus pulposus. Biomaterials 2012; 33:8256-64.
6. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 1993; 341:515-18.
7. Reinhart K, Bauer M, Riedemann NC, Hartog CS. New approaches to sepsis: molecular diagnostics and biomarkers. Clin Microbiol Rev 2012; 25:609-34.
8. Mehanic S, Baljic R. The importance of serum procalcitonin in diagnosis and treatment of serious bacterial infections and sepsis. Mater Sociomed 2013; 25:277-81.
9. Becker KL, Snider R, Nylen ES. Procalcitonin in sepsis and systemic inflammation: a harmful biomarker and a therapeutic target. Br J Pharmacol 2010; 159:253-64.
10. Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect Dis 2013; 13:426-35.
11. Kuestner RE, Elrod RD, Grant FJ, Hagen FS, Kuijper JL, Matthewes S, O'Hara PJ, Sheppard PO, Stroop SD, Thompson DL. Cloning and characterization of an abundant subtype of the human calcitonin receptor. Mol Pharmacol 1994; 46:246-55.
12. Sexton PM, Findlay DM, Martin TJ. Calcitonin. Curr Med Chem 1999; 6:1067-93.
13. Jagger C, Gallagher T, Chamgers T, Pondel M. The porcine calcitonin receptor promoter directs expression of a linked reporter gene in a tissue and developmental specific manner in transgenic mice. Endocrinol 1999; 140:492-9.
14. Wang J, Rout UK, Bagchi IC, Armant DR. Expression of calcitonin receptors in mouse preimplantation embryos and their function in the regulation of blastocyst differentiation by calcitonin. Development 1998; 125:4293-302.
15. Zhu LJ, Bagchi MK, Bagchi IC. Attenuation of calcitonin gene expression in pregnant rat uterus leads to a block in embryonic implantation. Endocrinol 1998; 139:330-9.
16. Plotkin LI, Weinstein RS, Parfitt AM, Roberson PK, Manolagas SC, Bellido T. Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. J Clin Invest 1999; 104:1363-74.
17. Jagger C, Chambers T, Pondel M. Transgenic mice reveal novel sites of calcitonin receptor gene expression during development. Biochem Biophys Res Commun 2000; 274:124-9.
18. Findlay DM, Raggatt LJ, Bouralexis S, Hay S, Atkins GJ, Evdokiou A. Calcitonin decreases the adherence and survival of HEK-293 cells by a caspase-independent mechanism. J Endocrinol 2002; 175:715-25.
19. Findlay D, Michelangeli VP, Eisman JA, Farmpton RJ, Moseley JM, Macintyre I, Whitehead R, Martin TJ. Calcitonin and 1, 25-dihydroxyvitamin D3 receptors in human breast cancer cell lines. Cancer Res 1980; 40:4764-7.
20. Ng KW, Livesey SA, Larkins RG, Martin TJ. Calcitonin effects on growth and on selective activation of type II isoenzyme of cyclic adenosine 3':5'-monophosphatedependent protein kinase in T47D human breast cancer cells. Cancer Res 1983; 43:794-800.
21. Shah GV, Rayford W, Noble MJ, Austenfeld M, Weigel J, Vamos S, Mebust WK. Calcitonin stimulates growth of human prostate cancer cells through receptor-mediated increase in cyclic adenosine 3',5'-monophosphates and cytoplasmic Ca^{2+} transients. Endocrinol 1994; 134:596-602.
22. Lacroix M, Siwek B, Body JJ. Breast cancer cell response to calcitonin: modulation by growth-regulating agents. Eur J Pharmacol 1998; 5:279-86.
23. Shah GV, Thomas S, Muralidharan A, Liu Y, Hermonat PL, Williams J, Chaudhary J. Calcitonin promotes in vivo metastasis of prostate cancer cells by altering cell signaling, adhesion, and inflammatory pathways. Endocr Relat Cancer 2008; 15:953-64.
24. Shah GV, Muralidharan A, Gokulgandhi M, Soan K, Thomas S. Cadherin switching and activation of beta-catenin signaling underlie proinvasive actions of calcitonin receptor axis in prostate cancer. J Biol Chem 2009; 284:1018-30.
25. Shah GV, Muralidharan A, Thomas S, Gokulgandhi M, Mudit M, Khanfar M, El Sayed K. Identification of a small molecule class to enhance cell-cell adhesion and attenuate prostate tumor growth and metastasis. Mol Cancer Ther 2009; 8:509-20.
26. Thakkar A, Bijnsdorp IV, Geldof AA, Shah GV. Profiling of the calcitonin receptor axis in primary prostate cancer: clinical implications and molecular correlates. Oncol Rep 2013; 30:1265-74.
27. Gorn AH, Lin, HY, Yamin M, Auro PE, Flannery MR, Tapp DR, Manning CA, Lodish HF., Krane SM, Goldring SR. Cloning, characterization, and expression of a human calcitonin receptor from an ovarian carcinoma cell line. J Clin Invest 1992; 90:1726-35.
28. Zaidi M, Datta HK, Moonga BS, MacIntyre I. Evidence that the action of calcitonin on rat osteoclasts is mediated by two G proteins acting via separate post-receptor pathways. J Endocrinol 1990; 126:473-81.
29. Chakraborty M, Chatterjee D, Kellokumpu S, Rasmussen H, Baron R. Cell cycle-dependent coupling of the calcitonin receptor to different G proteins. Science 1991; 251:1078-82.
30. Su Y, Chakraborty M, Nathanson MH, Baron R. Differential effects of the 3', 5'-cycle adenosine monophosphate and protein kinase C pathways on the response of isolated rat osteoclasts to calcitonin. Endocrinol 1992; 131:1497-502.
31. Chabre O, Conklin BR, Lin HY, Lodish HF, Wilson E, Ives HE, Catanzariti L, Hemmings BA, Bourne HR. A recombinant calcitonin receptor independently stimulates 3', 5'-cyclic adenosine monophospate and Ca^{2+}/inositol phosphate signaling pathways. Mol Endocrinol 1992; 6:551-6.
32. Force T, Bonventre JV, Flannery MR, Gorn AH, Yamin M, Goldring SR. A cloned porcine renal calcitonin receptor couples to adenylate cyclase and phospholipase C. Am J Physiol 1992; 262:F1110-5.
33. Shyu JF, Inoue D, Baron R, Horne WC. The deletion of 14 amino acids in the seventh transmembrane domain of a naturally occurring calcitonin receptor isoform alters ligand binding and selectively abolishes coupling to phospholipase C. J Biol Chem 1996; 271:31127-34.
34. Offermanns S, Iida-Klein A, Segre GV, Simon MI. G alpha q family members couple parathyroid hormone (PTH)/PTH-related peptide and calcitonin receptors to phospholipase C in COS-7 cells. Mol Endocrinol 1996; 10:566-74.
35. Johnson GL, Lapadat, R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 2002; 298:1911-2.
36. Nishida E, Gotoh Y. The MAP kinase cascade is essential for diverse signal transduction. Treands Biochem Sci 1993; 18:128-31.

37. Nakamura M, Yang Q, Ozaki T, Nakamura Y, Yamasaki H, Mori I, Kakudo K. Induction of uPA but not NF-IL3A by calcitonin is dependent on Erk1/2 phosphorylation in porcine renal cell line LLC-PK1. Biochem Biophys Res Commun 2002; 290:1483-8.
38. Chen Y, Shyu J-F, Santhanagopal A, Inoue D, David JP, Dixon SJ, Horne WC, Baron R. The calcitonin receptor stimulates Shc tyrosine phosphorylation and ERK1/2 activation. Involvement of Gi, protein kinase C, and calcium. J Biol Chem 1998; 273:19809-16.
39. Han B, Nakamura M, Zhou G, Ishii A, Nakamura A, Bai Y, Mori I, Kakudo K. Calcitonin inhibits invasion of breast cancer cells: involvement of urokinase-type plasminogen activator (uPA) and uPA receptor. Int J Oncol 2006; 28:807-14.
40. Caruso-Neves C, Pinheiro AA, Cai H, Souza-Menezes J, Guggino WB. PKB and megalin determine the survival or death of renal proximal tubule cells. Proc Natl Acad Sci U S A 2006; 103:18810-5.
41. Rangel-Filho A, Lazar J, Moreno C, Geurts A, Jacob HJ. Rab38 modulates proteinuria in model of hypertension-associated renal disease. J Am Soc Nephrol 2013; 24:283-92.
42. Lin HY, Harris TL, Flannery MS, Aruffo A, Kaji EH, Gorn A, Kolakowski LF, Jr Lodish HF, Goldring SR. Expression cloning of an adenylate cyclase-coupled calcitonin receptor. Science 1991; 254:1022-4.
43. Nakamura M, Ozaki T, Ishii A, Konishi M, Tsubota Y, Furui T, Tsuda H, Mori I, Ota K, Kakudo K. Calcitonin induces connective tissue growth factor through ERK1/2 signaling in renal tubular cells. Exp Mol Med 2009; 41:307-15.
44. Oka H, Chatani Y, Hoshino R, Ogawa O, Kakehi Y, Terachi T, Okada Y, Kawaichi M, Kohno M, Yoshida O. Constitutive activation of mitogen-activated protein (MAP) kinases in human renal cell carcinoma. Cancer Res 1995; 55:4182-7.
45. Sivaraman VS, Wang H, Nuovo GJ, Malbon CC. Hyperexpression of mitogenactivated protein kinase in human breast cancer. J Clin Invest 1997; 99:1478-83.
46. Hoshino R, Chatani Y, Yamori T, Tsuruo T, Oka H, Yoshid, O, Shimada Y, Ari-I, Wada H, Fujimoto J, Kohno M. Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors. Oncogene 1999; 18:813-22.
47. Kim SC, Hahn JS, Min YH, Yoo NC, Ko YW, Lee WJ. Constitutive activation of extracellular signal-regulated kinase in human acute leukemias: combined role of activation of MEK, hyperexpression of extracellular signal-regulated kinase, and downregulation of a phosphatase, PAC1. Blood 1999; 93:3893-9.
48. Ahmed N, Pansino F, Baker M, Ric, G, Quinn M. Association between alpha(v)beta6 integrin expression, elevated p42/44 kDa MAPK, and plasminogen-dependent matrix degradation in ovarian cancer. J Cell Biochem 2002; 84:675-86.
49. Satyamoorthy KLiG, Gerrero M, Brose MS, Volpe P, Weber BL, Van Belle P, Elder DE, Herlyn M. Constitutive mitogen activated protein kinase activation in melanoma is mediated by both BRAF mutations and autocrine growth factor stimulation. Cancer Res 2003; 63:756-9.
50. Liang B, Wang S, Zhu XG, Yu YX, Cui ZR, Yu YZ. Increased expression of mitogen-activated protein kinase and its upstream regulating signal in human gastric cancer. World J Gastroenterol 2005; 7:623-8.
51. Takata M, Goto Y, Ichii N, Yamaura M, Murata H, Koga H. Constitutive activation of the mitogen-activated protein kinase signaling pathway in acral melanomas. J Invest Dermatol 2005; 125:318-22.
52. Cowley S, Paterson H, Kemp P, Marshal IC. Activation of MAP kinase is necessary and sufficient for PC21 differentiation and for transformation of NIH 3T3 cells. Cell 1994; 77:841-52.
53. Mansour SJ, Matten WT, Hermann AS, Candia JM, Rong S, Fukasawa K, Vande Woude GF, Ahn NG. Transformation of mammalian cells by constitutively active MAP kinase. Science 1994; 265:966-70.
54. Webb CP, Van Aelst L, Wigler MH, Vande Woude GF. Signaling pathways in ras-mediated tumorigenicity and metastasis. Proc Natl Acad Sci USA 1998; 95:8773-8.
55. Dudley DT, Pang L, Decker SJ, Bridges AJ, Saltiel AR. A synthetic inhibitor of the mitogen-activated protein kinase cascade. Proc Natl Acad Sci USA 1995; 92:7686-9.
56. Sebolt-Leopold JS, Dudley DT, Herrera R, Van Becelaere K, Wiland A, Gowan RC, Tedle H, Barrett SD, Bridges A, Przybranowski S, Leopold WR, Saltiel AR. Blockade of the MAP kinase pathway suppresses growth of colon tumors in vivo. Nature Med 1999; 5:810-6.
57. Duesbery NS, Webb CP, Wounde GFV. MEK wars, a new front in the battle against cancer. Nat Med 1999; 5:736-7.
58. Fang JY, Richardson BC. The MAPK signaling pathways and colorectal cancer. Lancet Oncol 2005; 6:322-7.
59. Stealer M, Rohr Mann K, Haseke N, Stiff CG, Siebel's M. Targeted agents for the treatment of advanced renal cell carcinoma. Cur Drug Targets 2005; 6:835-46.
60. Astorgues-Xerri L, Riveiro ME, Tijeras-Raballand A, Serova M, Neuzillet C, Albert S, Raymond E, Faivre S. Unraveling galectin-1 as a novel therapeutic target for cancer. Cancer Treat Rev 2014; 40:307-19.
61. Nakamura M, Han B, Nishishita T, Bai Y, Kakudo K. Calcitonin targets extracellular signal-regulated kinase signaling pathway in human cancers. J Mol Endocrinol 2007; 39:375-84.
62. Segawa N, Nakamura M, Nakamura Y, Mori I, Katsuoka Y, Kakudo, K. Phosphorylation of mitogen-activated protein kinase is inhibited by calcitonin in DU145 prostate cancer cells. Cancer Res 2001; 61:6060-3.
63. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev 2001; 22:153-83.
64. Avruch J, Khokhlatchev A, Kyriakis JM, Luo Z, Tzivion G, Vavvas D, Zhang XF, Ras activation of the Raf kinase: tyrosine kinase recruitment of the MAP kinase cascade. Recent Prog Horm Res 2001; 56:127-55.
65. Dhillon AS, Pollock C, Steen H, Shaw PE, Mischak H, Kolch W. Cyclic AMP-dependent kinase regulates Raf-1 kinase mainly by phosphorylation of serine 259. Mol Cell Biol 2002; 22:3237-46.
66. Light Y, Paterson H, Marais R. 14-3-3 antagonizes Ras-mediated Raf-1 recruitment to the plasma membrane to maintain signaling fidelity. Mol Cell Biol 2002; 22:4984-96.
67. Dumaz N, Marais R. Protein kinase A blocks Raf-1 activity by stimulating 14-3-3 binding and blocking Raf-1 interaction with Ras. J Biol Chem 2003; 8:29819-23.
68. Angelucci A, D'Ascenzo S, Festuccia C, Gravina GL, Bologna M, Dolo V, Pavan A. Vesicle-associated urokinase plasminogen activator promotes invasion in prostate cancer cell lines. Clin Exp Metastasis 2000; 18:163-70.

69. Dano K, Andreasen PA, Gronddaho-Hansen K, Kirstensen P, Nielsen LS, Skrivers L. Plasminogen activators, tissue degradation and cancer. Adv Cancer Res 1985; 44:139-266.
70. Wei Y, Lukashev M, Simon DI, Bodary SC, Rosenberg S, Doyle MV, Chapman HA. Regulation of integrin function by the urokinase receptor. Science 1998; 273:1551-5.
71. Han B, Nakamura M, Mori I, Nakamura Y, Kakudo K. Urokinase-type plasminogen activator system and breast cancer. Oncol Rep 2005; 14:105-12.
72. Ma Z, Webb DJ, Jo M, Gonias SL. Endogenously produced urokinase- type plasminogen activator is a major determinant of the basal level of activated ERK/MAP kinase and prevents apoptosis in MDA-MB-231 breast cancer cells. J Cell Sci 2001; 114:3387-96.
73. Wimalawansa SJ. Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology, and therapeutic potentials. Endocr Rev 1996; 17:533-85.
74. Nakamura M, Han B, Nunobiki O, Kakudo K. Adrenomedullin: a tumor progression factor via angiogenic control. Curr Cancer Drug Targets 2006; 6:635-43.
75. Chini EN, Choi E, Grande JP, Burnett JC, Dousa TP. Adrenomedullin suppresses mitogenesis in rat mesangial cells via cAMP pathway. Biochem Biophys Res Commun 1995; 215:868-73.
76. Chini EN, Chini CC, Bolliger C, Jougasaki M, Grande JP, Burnett JC, Dousa TP. Cytoprotective effects of adrenomedullin in glomerular ell injury: central role of cAMP signaling pathway. Kidney Int 1997; 52:917-25.
77. Iwasaki H, Eguchi S, Shichiri M, Marumo F, Hirata Y. Adrenomedullin as a novel growth-promoting factor for cultured vascular smooth muscle cells: role of thyrosine kinase-mediated mitogen-activated protein kinase activation. Endocrinol 1998; 139:3432-41.
78. Swarthout JT, Doggett TA, Lemker JL, Partridge NC. Stimulation of extracellular signal-regulated kinases and proliferation in rat osteoblastic cells by parathyroid hormone is protein kinase C-dependent. J Biol Chem 2001; 276:7586-92.
79. Dempster DW, Cosman F, Parisien M, Shen V, Lindsay R. Anabolic actions of parathyroid hormone on bone. Endocr Rev 1993; 14:690-709.
80. Verheijen MH, Defize LH. Parathyroid hormone activates mitogen- activated protein kinase via a cAMP-mediated pathway independent of Ras. J Biol Chem 1997; 272:3423-9.
81. Kaiser E, Chandrasekhar S. Distinct pathways of extracellular signal-regulated kinase activation by growth factors, fibronectin and parathyroid hormone1-34. Biochem Biophys Res Commun 2003; 6:573-8.
82. Chaudhary LR, Avioli LV. Identification and activation of mitogen- activated protein (MAP) kinase in normal human osteblastic and bone marrow stromal cells: attenuation of MAP knase activation by cAMP, parathyroid hormone and forskolin. Mol Cell Biochem 1998; 178:59-68.
83. Verheijen MH, Defize LH. Parathyroid hormone inhibits mitogen-activated protein kinase activation in osteosarcoma cells via a protein kinase A-dependent pathway. Endocrinol 1995; 136:3331-7.
84. Chen C, Koh AJ, Datta NS, Zhang J, Keller ET, Xiao G, Fraceschi RT, D'Silva NJ, McCauley LK. Impact of the Mitogen-activated protein kinase pathway on parathyroid hormone-related protein actions in osteoblasts. J Biol Chem 2004; 28:29121-9.
85. Shyu J-F, Zhang S, Hernandez-Lagunas L, Camerino C, Chen Y, Inoue D, Baron R, Horne WC. Protein kinase C antagonizes pertussis- toxin-sensitive coupling of the calcitonin receptor to adenylyl cyclase. Eur J Biochem 1999; 262:95-101.
86. Berlot CH. A highly effective dominant negative alpha s construct containing mutations that affect distinct functions inhibits multiple Gs-coupled receptor signaling pathways. J Biol Chem 2002; 277:21080-5.
87. Chen T, Cho RW, Stork PJ, Weber MJ. Elevation of cyclic adenosine 3′,5′-monophosphate potentiates activation of mitogen-activated protein kinase by growth factors in LNCaP prostate cancer cells. Cancer Res 1999; 59:213-8.
88. Chigurupati S, Kulkarni T, Thomas S, Shah G. Calcitonin stimulates multiple stages of angiogenesis by directly acting on endothelial cells. Cancer Res 2005; 65:8519-29.
89. Sabbisetti VS, Chirugupati S, Thomas S, Vaidya KS, Reardon D, Chiriva-Internati M, Iczkowski KA, Shah GV. Calcitonin increases invasiveness of prostate cancer cells: role for cyclic AMP-dependent protein kinase A in calcitonin action. Int J Cancer 2005;17:551-60.
90. Thomas S, Shah G. Calcitonin induces apoptosis resistance in prostate cancer cell lines against cytotoxic drugs via the Akt/Survivin pathway. Cancer Biol Ther 2005; 4:1226-33.
91. Thomas S, Chigurupati S, Anbalagan M, Shah G. Calcitonin increases tumorigenicity of prostate cancer cells: evidence for the role of protein kinase A and urokinase-type plasminogen receptor. Mol Endocrinol 2006; 20:1894-911.
92. Putz T, Culig Z, Eder IE, Nessler-Menardi C, Bartsch G, Grunicke H, Uberall F, Klocker H. Epidermal growth factor (EGF) receptor blockade inhibits the action of EGF, insulin-like growth factor I, and a protein kinase A activator on the mitogen-activated protein kinase pathway in prostate cancer cell lines. Cancer Res 1999; 59:227-33.
93. Culig Z, Steiner H, Bartsch G, Hobisch A. Mechanisms of endocrine therapy responsive and -unresponsive prostate tumours. Endocr Relat Cancer 2005; 12:229-44.
94. Sabbisetti V, Chigurupati S, Thomas S, Shah G. Calcitonin stimulates the secretion of urokinase-type plasminogen activator from prostate cancer cells: its possible implications on tumor cell invasion. Int J Cancer 2006; 118:2694-702.
95. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest Urol 1979; 17:16-23.
96. Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Chu TM, Mirand EA, Murphy GP. LNCaP model of human prostatic carcinoma. Cancer Res 1983; 43:1809-18.
97. Stephenson RA, Dinney CP, Gohji K, Ordonez NG, Killion JJ, Fidler IJ. Metastatic model for human prostate cancer using orthotopic implantation in nude mice. J Natl Cancer Inst 1992; 84:951-7.
98. Stone KR, Mickey DD, Wunderli H, Mickey GH, Paulson DF. Isolation of a human prostate carcinoma cell line (DU 145). Int J Cancer 1978; 21:274-81.
99. Chien J, Ren Y, Qing Wang Y, Bordelon W, Thompson E, Davis R, Rayford W, Shah G. Calcitonin is a prostate epithelium-derived growth stimulatory peptide. Mol Cell Endocrinol 2001; 181:69-79.