Calcitonin targets extracellular signal-regulated kinase signaling pathway in human cancers

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Abstract

The mitogen-activated protein kinases (MAPKs) signaling pathway is a potential target in cancer therapy. Constitutive phosphorylated extracellular signal-regulated kinase (ERK1/2), which is one of the MAPKs has been detected in a variety of tumors. Calcitonin (CT) is a polypeptide hormone secreted by the thyroid gland and has been used to treat the osteoporosis and humoral hypercalcemia of malignancy. We report that CT decreases ERK1/2 phosphorylation in cancer cells showing constitutive phosphorylated ERK1/2. In MDA-MB-231 cells, a breast cancer cell line showing constitutive phosphorylated ERK1/2, CT phosphorylated c-Raf at Ser259 via the protein kinase A pathway, resulting in suppression of ERK1/2 phosphorylation. CT significantly reduced the tumor volume of MDA-MB-231 cells showing constitutive phosphorylated ERK1/2 compared with saline buffer. However, CT did not exert any significant effects on the proliferation of MCF-7 cells, a breast cancer cell line, showing non-constitutive phosphorylated ERK1/2. These novel findings indicate that CT may be used to target ERK in the treatment of cancer.

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Introduction

In molecular cancer therapy, the target which is selected and how it is developed for therapy is important. Most target molecules are oncogenes, growth factors, transcription factors, or cytokines involved in signaling pathways, DNA replication/repair, cell cycle, morphogenesis, drug resistance/sensitivity, metastasis, immunity, differentiation, and apoptosis. The mitogen-activated protein kinases (MAPKs) pathway plays a central role in regulating the growth and survival of cells from a broad spectrum of human tumors (Johnson & Lapadat 2002). Many oncogenes constitutively activate the MAPK pathway, and it is this inappropriate activation that mediates the transformed phenotype (Lewis et al. 1998). Mitogen-activated protein/ERK kinase 1 (MEK1), when engineered to be constitutively active, can transform mammalian cells to a cancerous phenotype (Cowley et al. 1994, Mansour et al. 1994, Webb et al. 1998), whereas inhibition of MAPK activity can inhibit the growth of Ras-transformed cells in vitro (Dudley et al. 1995, Sebold-Leopold et al. 1999). In addition, the activated MAPK or increased levels of MAPK expression have been detected in a variety of human tumors (Oka et al. 1995, Sivaraman et al. 1997, Hoshino et al. 1999, Kim et al. 1999, Ahmed et al. 2002, Satyamoorthy et al. 2003, Liang et al. 2005, Takata et al. 2005). Thus, the MAPK signaling pathway is a potential target in cancer therapy (Duesbery et al. 1999, Tecle et al. 1999, Fang & Richardson 2005, Staehler et al. 2005).

Calcitonin (CT) is a 32 amino acid polypeptide hormone secreted from parafollicular cells of the thyroid gland. CT has been clinically used as a drug to treat osteoporosis and humoral hypercalcemia of malignancy (McDermott & Kidd 1987, Zaidi et al. 1990). The main physiological roles of CT are to regulate calcium metabolism, inhibit bone resorption by osteoclasts, and increase Ca2+ excretion via the kidney (Chambers & Magnus 1982). There is a growing body of evidence that CT is involved in cell proliferation, apoptosis, and cell differentiation and development (Wang et al. 1998, Zhu et al. 1998, Jagger et al. 1999, Plotkin et al. 1999). It has been shown that treatment with CT influences cell proliferation, apoptosis, and cell invasion in cultured cancer cells (Ng et al. 1983, Shah et al. 1994, Lacroix et al. 1998, Chigurupati et al. 2005, Thomas & Shah 2005, Han et al. 2006).

Previously, we found that CT suppresses constitutive phosphorylation of extracellular signal-regulated kinase (ERK1/2) in DU145 prostate cancer cells (Segawa et al. 2001). However, the precise pathways...
involved are currently unclear. The purpose of this study was to determine the mechanism of the effects of CT on ERK1/2 phosphorylation in breast cancer cell line and to further investigate the therapeutic usefulness of CT targeting ERK1/2.

Materials and methods

Cell lines and cell cultures
MDA-MB-231 and T47D human breast carcinoma cell lines, MDA-MB-435 human melanoma cell line (Rae et al. 2007), and TT human thyroid cancer cell line were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). MCF-7 human breast carcinoma cell line was obtained from the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Tohoku University (Miyagi, Japan), and FTC-133 human follicular thyroid carcinoma cell line was from the European Collection of Cell Cultures (ECACC, Wiltshire, UK). KP-1NL and KP-3 human pancreatic carcinoma cell lines, CaR-1 human rectum carcinoma cell line, CCK81 human colon cancer cell line, VMC-RCW human renal cell carcinoma cell line, MRK-nu-1 human breast cancer cell line, and T24 human urinary bladder carcinoma cell line were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan). The DU145 human prostate cancer cell line was derived as previously reported (Segawa et al. 2001). MDA-MB-231, T47D, MCF-7, and MRK-nu-1 were maintained in Dulbecco’s modified Eagle’s medium (DMEM). MDA-MB-435 cells were maintained in Leibovitz L-15 medium supplemented with 1 mg/ml insulin. VMC-RCW, DU145, TT, KP-3, KP-1NL, and CCK-81 cells were maintained in RPMI164 medium. T24 and CaR-1 cells were maintained in Eagle’s minimal essential medium, and FTC-133 cells were maintained in the mixture DMEM and Ham’s F12K (1:1). All media contained 2 mM glutamine, 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA), and 100 units/ml penicillin and streptomycin. All cells were maintained at 37°C with 5% CO2.

Animals
Female, athymic BALB/c nu/nu mice, 5–6 weeks of age (CLEA Japan Inc., Tokyo, Japan) were used. Animals were maintained in a specific pathogen-free environment under controlled light and humidity conditions. All protocols for in vivo studies were approved by the Institutional Animal Care and Use Committee of Wakayama Medical University. These experiments also conformed to UK legal requirements.

Antibodies and other reagents
Salmon CT was purchased from Peninsula Laboratories Inc. (Belmont, CA, USA). Antibodies against phosphorylated and nonphosphorylated ERK1/2 and Ser259-phosphorylated c-Raf were purchased from Cell Signaling Technology (Beverly, MA, USA). PD98059, a MEK1 inhibitor, and calphostin C (CC), a protein kinase C (PKC) inhibitor, were obtained from Calbiochem (La Jolla, CA, USA). The protein kinase A (PKA) inhibitor H89 was obtained from Seikagaku Corp. (Tokyo, Japan) and dimethyl suloxide from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Forskolin (FK), phorbol 12-myristate 13-acetate (PMA) and anti-β-actin antibody were from Sigma.

Western blot analysis
Cells were grown as described above to 80% confluence and then serum starved (incubated for 18 h in medium supplemented with 0-5% FBS). Cells were treated for various times with various reagents in serum-starvation medium, then lysed in lysis buffer (Nakamura et al. 2002). Cell lysates (20 μg total protein) were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 12% gel. Western blotting was performed as previously described (Nakamura et al. 2002). Quantitative analysis of the autoradiograph was performed using the NIH Image densitometry program, with the levels of β-actin being used for normalization.

RT-PCR analysis
Total RNA was extracted using the Ultraspec RNA Isolation system (Biotecx Labs Inc., Houston, TX, USA). Total RNA (5 μg) was transcribed to cDNA using the Super Script RT-PCR kit (Life Technologies Inc). cDNAs of human CT receptor (CTR) mRNA transcripts were amplified using the following primers: forward 5’-CTATGACCAGTGACCCAGGTCA-3’; reverse 5’-ATGTTGAGACGGTGGTA-3’; PCR amplification was accomplished with an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. RT-PCR analysis with β-actin primers (forward 5’-AAGAGGACATCTCAACCTG-3’ and reverse 5’-TACATGGCTGGGTTGAA-3’) was used as an internal RNA control. The conditions of PCR were the same as that used for analysis of the CTR gene. PCR products were analyzed by electrophoresis on a 2% agarose gel containing ethidium bromide.

cAMP assay
MDA-MB-231 and LLC-PK1 cells were cultured in 24-well plates. After serum starvation (as described above), the cells were treated with 10−8 M CT for 30 min.
Intracellular cAMP content was measured using a cAMP enzyme immunoassay system (cAMP EIA Kit, Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer’s instructions.

**Tumor cell inoculation and CT injection**

MDA-MB-231 cells or MCF-7 cells were injected into the subaxillary mammary fat pads of mice at both sites (5 × 10^6 cells/site). Animals were randomly divided into two groups immediately after tumor cell inoculation, with the experimental group (n = 9) receiving subcutaneous injections of 70 μl CT (12 ng/kg per day), and the control group (n = 9) receiving comparable injections of isotonic saline solution. The sizes of all tumors and body weights were measured weekly. Tumor volumes were estimated from caliper measurements using the standard formula (length × width^2)/2 (Sabbisetti et al. 2005). Nine weeks after inoculation, all mice were killed 3 h after injection of CT or isotonic saline solution. Excised tumors were measured and fixed in 10% neutral-buffered formalin for 24 h and embedded in paraffin for immunohistochemistry.

**Immunohistochemistry**

Four micrometer thick sections were cut for phosphorylated ERK staining as described above. Immunohistochemical staining was performed using the standardized labeled streptavidin–biotin kit (DakoCyto- mation, Carpinteria, CA, USA) according to the manufacturer’s instructions. The pretreatment procedure used for antigen retrieval involved autoclaving in 0.01 M citrate buffer (pH 6.0) at 121 °C for 10 min. Slides were incubated overnight with primary antibody at 4 °C. For visualization, 3,3-diaminobenzidine tetrahydrochloride was used as a chromogen and methyl green was used as a counterstain. For a negative control, primary antibody was replaced by PBS.

**Statistical analyses**

Data are presented as means ± s.d.. Statistical analyses were performed by two-way repeated measures ANOVA for tumor volume and by student’s t-test for tumor weight. P values <0.05 were considered significant.

**Results**

**CT suppresses phosphorylation of ERK1/2 in cancer cells showing constitutive phosphorylated ERK1/2**

In MDA-MB-231, MDA-MB-435, T24, DU145, CaR-1, and VMC-RCW cancer cell lines, in which ERK1/2 was constitutively phosphorylated, CT suppressed ERK1/2 phosphorylation. In MCF-7, T47D, MRK-nu-1, TT, FTC-133, KP-1NL, KP-3, and CCK81 cancer cell lines, in which ERK1/2 was not constitutively phosphorylated, CT had no effect on ERK1/2 phosphorylation (Fig. 1A). There was an obvious upregulation of total ERK1/2 after CT treatment in MDA-MB-231, MDA-MB-435, and VMC-RCW cells. This suggests that CT not only decreases phosphorylation, but also reverses the relationship between ERK1/2 phosphorylation and expression in those cells. CTR mRNA is expressed in all cell lines (Fig. 1B). In conclusion, CT suppresses phosphorylation of ERK1/2 in cancer cells showing constitutive phosphorylated ERK1/2 (MDA-MB-231, MDA-MB-435, T24, DU145 CaR-1, and VMC-RCW), although it has no effect on ERK1/2 levels in cancer cells that do not show phosphorylation of ERK1/2 (MCF-7, T47D, MRK-nu-1, TT, FTC-133, KP-1NL, KP-3, and CCK81 cells).

**The effect of CT on ERK1/2 phosphorylation shows transient inhibition through the MEK1 pathway in MDA-MB-231 cells**

Time-course analysis of the effect of 10^-8 M CT on ERK1/2 phosphorylation in serum-starved MDA-MB-231 cells showed transient inhibition, with maximum effects after 15–30 min of treatment (Fig. 2A). PD98059 (50 μM) almost abolished phosphorylation of ERK1/2 in MDA-MB-231 cells (Fig. 2B), indicating MEK1 activity is required for constitutive phosphorylation of ERK1/2. Constitutive phosphorylation of p38 or JNK/SAP was not detected in MDA-MB-231 cells and CT had no effect on their phosphorylation (data not shown).

**Effects of adenylate cyclase (AC) and PKC activators on ERK1/2 phosphorylation in MDA-MB-231 cells**

The effects of the AC activator, FK, and the PKC activator, PMA, on ERK1/2 phosphorylation were investigated in MDA-MB-231 cells. FK eliminated ERK1/2 phosphorylation in CT-treated, untreated cells, or PKC inhibitor, CC-treated cells, whereas PMA increased basal ERK1/2 phosphorylation, and this increase was suppressed by CT (Fig. 3A). These results suggest that FK mimics or augments the effects of CT, while PMA antagonizes them in MDA-MB-231 cells.

**CTR increases cAMP levels in MDA-MB-231 cells**

CTR has been known to couple to Gs and Gq, activating AC and phospholipase C respectively (Chabre et al. 1992, Force et al. 1992, Offermanns et al. 1996, Shyu et al. 1996). To confirm that the CTR is linked to the cAMP pathway in MDA-MB-231 cells, we measured intracellular cAMP concentrations after 30 min of treatment with 10^-8 M CT. CT substantially increased intracellular cAMP levels in MDA-MB-231 cells. This suggests that CT not only decreases phosphorylation, but also reverses the relationship between ERK1/2 phosphorylation and expression in those cells. CTR mRNA is expressed in all cell lines (Fig. 1B). In conclusion, CT suppresses phosphorylation of ERK1/2 in cancer cells showing constitutive phosphorylated ERK1/2 (MDA-MB-231, MDA-MB-435, T24, DU145 CaR-1, and VMC-RCW), although it has no effect on ERK1/2 levels in cancer cells that do not show phosphorylation of ERK1/2 (MCF-7, T47D, MRK-nu-1, TT, FTC-133, KP-1NL, KP-3, and CCK81 cells).
cAMP (expressed as pmol/10^5 cells) from 10.3 ± 3.9 to 20.5 ± 0.7 (twofold) in MDA-MB-231 cells (Fig. 3B).

**PKA significantly contributes to a CT-induced suppression of constitutive ERK1/2 activation in MDA-MB-231 cells**

We investigated whether the PKA pathway is involved in CT inhibition of ERK1/2 phosphorylation. Serum-starved MDA-MB-231 cells were treated with 10^{-8} M H89, a PKA inhibitor, or 5 × 10^{-7} M CC, a PKC inhibitor, with or without 10^{-8} M CT, for 30 min. As shown in Fig. 3C, H89 diminished the effect of CT on ERK1/2 phosphorylation. CC does blunt the effect of CT somewhat, but not to the same degree as H89. These data suggest that the PKA pathway, but not the PKC pathway, primarily contributes to CT inhibition of ERK1/2 phosphorylation in MDA-MB-231 cells.

**Phosphorylation of the Ser^{259} residue of c-Raf contributes to PKA-dependent CT suppression of constitutive ERK1/2 activation in MDA-MB-231 cells**

c-Raf mediates activation of MAP kinases by GTP-Ras, but this activity is inhibited when it is phosphorylated by PKA at Ser^{259} and other sites (Dumaz & Marais 2003). We investigated the potential role of c-Raf in CT’s regulation of ERK1/2 phosphorylation. Time-course experiments in serum-starved MDA-MB-231 cells showed that 10^{-8} M CT increased phosphorylation of Ser^{259} in c-Raf, with maximal increases occurring after 15 min of treatment. However, 10^{-5} M H89 inhibited
the effect of CT (Fig. 3D). As mentioned above, H89 attenuates CT’s suppression of ERK1/2 phosphorylation in MDA-MB-231 cells. It is suggested that the effect of CT on ERK1/2 phosphorylation is partly mediated by phosphorylation of c-Raf by PKA.

CT suppresses tumor growth in nude mice injected with MDA-MB-231 cells, but not with MCF-7 cells

To further characterize the inhibitory effects of CT on ERK signaling in breast cancer cell proliferation, female nude mice were injected with MDA-MB-231 cells showing constitutive phosphorylated ERK1/2 or MCF-7 cells showing non-constitutive phosphorylated ERK1/2, and then treated with CT or saline buffer every day. CT had no effect on body weight in mice treated with either MDA-MB-231 or MCF-7 cells (data not shown). CT significantly reduced the tumor volume and tumor weight of MDA-MB-231 cells, compared with saline buffer (P<0.05; Fig. 4A). However, CT did not exert any significant effects on the tumor volume or tumor weight in MCF-7 cells (Fig. 4B).

Immunohistochemistry analysis of nude mice 9 weeks after treatment with CT or saline buffer was performed. In mice injected with MDA-MB-231 cells, phosphorylated ERK1/2 expression decreased 3 h after treatment with CT in the CT group compared with saline buffer treatment. In mice injected with MCF-7 cells, phosphorylated ERK1/2 expression was not changed between the CT and control groups (Fig. 4C).

Discussion

The duration of ERK activation is regulated by intracellular signals that cause the enzyme to be phosphorylated and dephosphorylated (Pearson et al. 2001). ERKs are activated by ligand binding at the cell surface, and inactivated by several dual-specificity phosphatases (Keyse 2000). It has been reported that the CTR couples to Gs and Gq, activating AC and phospholipase C (Chabre et al. 1992, Force et al. 1992, Offermanns et al. 1996, Shyu et al. 1996). In the present study, the cAMP/PKA pathway contributed to the CT-induced suppression of ERK1/2 activity in
Figure 3 CT activates c-Raf via the PKA pathway, resulting in suppression of ERK1/2 phosphorylation in MDA-MB-231 cells. (A) Effect of FK and PMA on ERK1/2 phosphorylation without or with CT. Serum-starved MDA-MB-231 cells were treated for 30 min as follows: H2O, same volume of H2O as CT; CT, 10^{-8} M CT; FK, 10^{-5} M FK; FK/CT, 10^{-5} M FK and 10^{-8} M CT; FK/CC, 10^{-5} M FK and 5 × 10^{-7} M CC; PMA, 10^{-8} M PMA; PMA/CT, 10^{-8} M PMA and 10^{-8} M CT. Cell lysates were prepared and assayed for phosphorylated ERK1/2 (upper panel) and total ERK1/2 (bottom panel) as described in the legend to Fig. 1. (B) Measurement of intracellular cAMP concentration in MDA-MB-231 cells. Serum-starved MDA-MB-231 cells were treated with 10^{-8} M CT for 30 min then assayed for intracellular cAMP as described in Materials and methods. Values represent means ± S.D. of two independent experiments, each with triplicate assays. (C) The effects of CT on ERK1/2 phosphorylation and effects of H89 or CC on ERK1/2 phosphorylation. Serum-starved MDA-MB-231 cells were treated for 30 min as follows: H2O, same volume of H2O as CT; CT, 10^{-8} M CT; H89, 10^{-5} M H89; H89/CT, 10^{-5} M H89 and 10^{-8} M CT; CC, 5 × 10^{-7} M CC; CC/CT, 5 × 10^{-7} M CC and 10^{-8} M CT. Western blotting for phosphorylated ERK1/2 (upper panel) and total ERK1/2 (bottom panel) was performed as described above. (D) Time-course analysis of the effect of CT on Ser^{259}-c-Raf phosphorylation. Serum-starved MDA-MB-231 cells were treated for the indicated times as follows: CT, 10^{-8} M CT alone; H89/CT, 10^{-5} M H89 and 10^{-8} M CT. At the indicated times, Ser^{259}-phosphorylated c-Raf was analyzed by western blotting (upper panel) after which the membranes were stripped and reprobed with anti-β-actin (middle panel). Quantitation of Ser^{259}-c-Raf phosphorylation by the NIH image densitometry program is shown in the bottom panel. Values represent means ± S.D. of two independent experiments.
MDA-MB-231 cells. This is consistent with our previous results in DU145 cells (Segawa et al. 2001).

c-Raf has been suggested as a candidate protein that may act to antagonize Ras-dependent activation of MAPK. ERKs are activated by the MAPKs, MEK1 and MEK2, which are activated by the MEK kinases, c-Raf, A-Raf, and B-Raf. Activation of c-Raf is a complex process (Avruch et al. 2001), the first step of which is the direct binding to active Ras (Ras.GTP), resulting in c-Raf recruitment to the plasma membrane. Phosphorylation of serine 338 (Ser338) and tyrosine 341 in the N-region, and threonine 491 and serine 495 (Ser495) in the activation segment, is essential for c-Raf activation (Fabian et al. 1993, King et al. 1998, Mason et al. 1999, Chong et al. 2001). All of these phosphorylation events occur at the plasma membrane, so Ras-mediated recruitment of c-Raf to the plasma membrane.
membrane is essential for its activation. In c-Raf regulation, 14-3-3 proteins also play a key role. These small (30 kDa) acidic dimers regulate the activity of many proteins by binding to short phosphorylated peptide motifs within the target proteins (Yaffe 2002). Two 14-3-3 motifs are present in c-Raf, centered on serine 259 (Ser259) and serine 621 (Ser621; Avruch et al. 2001). Binding to Ser621 appears to be essential for c-Raf activation, whereas binding to Ser259 appears to suppress activity. It was recently shown that binding of 14-3-3 to Ser259 antagonizes c-Raf recruitment to the plasma membrane and prevents its activation by the Ras-related proteins TC21 and R-Ras (Dhillon et al. 2002, Light et al. 2002). Dumaz & Marais (2003) have reported that when PKA is activated, it phosphorylates c-Raf and stimulates recruitment of 14-3-3, preventing c-Raf recruitment to the plasma membrane and subsequently blocking its activation. In the present study, we show that phosphorylation of c-Raf at Ser259 contributes to CT suppression of constitutive ERK1/2 activation in a PKA-dependent manner in MDA-MB-231 cells. Cell response-induced activation of the signaling cascade is varied and there are many questions to be answered on the activation of MAPK and cell responsibility. However, it is possible that ERK signaling plays the most central role in cell proliferation. In fact, the Ras and c-Raf-I genes, which lie upstream of the ERK pathway, have been identified as oncogenes. CT suppresses in vivo proliferation of cancer cells with constitutive ERK phosphorylation, but not cells with non-constitutive ERK phosphorylation. Thus, CT might be a useful drug targeting ERK in cancer.

In this article, we found that CT suppresses the in vivo proliferation of cancer cells showing constitutive ERK phosphorylation. However, there are some reports that CT increase tumorigenicity and invasiveness in the prostate cancer cell lines, PC-3, and LnCaP (Chen et al. 1999, Light et al. 2002, Chigurupati et al. 2005, Sabbisetti et al. 2005, Thomas & Shah 2005; Thomas et al. 2006). Both cell lines show no constitutive activation of ERK1/2, as seen with MCF-7 cells (Putz et al. 1999, Segawa et al. 2001, Culig et al. 2005). Previously, we also found that CT induced ERK phosphorylation in LLC-PK1 porcine renal cell line (Nakamura et al. 2002). Why does signaling by CT, similar to adrenomedullin and parathyroid hormone (Chaudhary & Avioli 1998, Swarthout et al. 2001, Kaiser & Chandrasekhar 2003), vary in different cells? The CTR is known to couple to Gq and Gs, activating AC and phospholipase C respectively (Chabre et al. 1992, Force et al. 1992, Offermanns et al. 1996, Shyu et al. 1999). Moreover, CTR can couple to Gi, but the inhibition of AC by Gz is negatively regulated by PKC (Chen et al. 1998, Shyu et al. 1999). A dominant negative mutant Gz containing three separate mutations affecting distinct functions has been characterized in HEK293 cells. The triple mutant prevents luteinizing hormone receptor and CTR from activating AC and blocks activation of phosphatidylinositol turnover by CTR (Berlot 2002). Differential regulation by CT may be correlated with the different abilities of the G proteins to compete with each other for binding to CTR, depending on cell type.

The urokinase plasminogen activator (uPA) is a serine protease that converts plasminogen to plasmin and it has been shown that uPA participates in the migration processes of normal and tumor cells (Dano et al. 1985). It is well established that the uPA system plays an important role in cancer invasion and metastasis (Ossowsky et al. 1991, Guo et al. 2000, Rabbani & Gladu 2002). In several cancers, mitogenesis and expression of uPA and uPA receptor (uPAR) are frequently activated through common signaling complexes and pathways (Aguirre et al. 1999). In MDA-MB-231 cells, there is a constitutive expression of uPA (Ma et al. 2001, Han et al. 2006). Ma et al. (2001) have shown that uPA–uPAR system acts within a positive feedback loop with phosphorylated ERK in MDA-MB-231 cells. Previously, we have reported that CT suppressed the expression of uPA and invasiveness in MDA-MB-231 cells through suppression of the ERK1/2 pathway (Han et al. 2006). In other reports, CT induced uPA expression in PC-3 and LnCaP cells (Wei et al. 1996, Sabbisetti et al. 2006, Thomas et al. 2006). Therefore, we propose that effects of CT on ERK1/2 phosphorylation in different cell types produce correspondingly contrary effects on the expression of genes whose products influence biological processes and phenotypes containing tumorigenesis, metastasis, and others. Finally, these systems may contribute to variability of the functions of the hormone.

In conclusion, CT decreased constitutive ERK phosphorylation in cancer cell lines. In addition, CT suppresses the in vivo proliferation of cancer cells with constitutive ERK phosphorylation. Our results suggest that if CT has clinical efficacy, it will be limited to patients with tumors that both express the CTR and respond to CT by downregulating ERK signals that promote the growth. CT would be a candidate for the treatment of cancer patients with accompanying osteoporosis and could also be used in combination with a complex hormone therapy.

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