Regulation of Transforming Growth Factor-β Type II Receptor Expression in Human Breast Cancer MCF-7 Cells by Vitamin D₃ and Its Analogues*

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In view of the tumor suppressor role of the transforming growth factor-β (TGFβ) type II receptor (RII), the identification and characterization of agents that can induce the expression of this receptor are of potential importance to the development of chemoprevention approaches as well as treatment of cancer. To date, the identification of exogenous agents that control RII expression has been rare. We demonstrated that proliferation of MCF-7 early passage cells (MCF-7 E), which express RII and are sensitive to TGFβ growth inhibition activity, was significantly inhibited by vitamin D₃ and its analogue EB1089. In contrast, proliferation of MCF-7 late passage cells (MCF-7 L), which have lost cell surface RII and are resistant to TGFβ, was not affected by these two compounds. TGFβ-neutralizing antibody was able to block the inhibitory effect on MCF-7 E cells by these compounds, indicating that treatment induced autocrine-negative TGFβ activity. An RNase protection assay showed approximately a 3-fold induction of the RII mRNA, while a receptor cross-linking assay revealed a 3–4-fold induction of the RII protein. In contrast, there was no change in either RII mRNA or protein in the MCF-7 L cells.

Transforming growth factor-β (TGFβ) is a family of hormone-like polypeptides that affects cell growth, adhesion, and differentiation (1). They act as growth inhibitors for most epithelial cells and some cancer cells. Two pathways are primarily involved in mediating effects of TGFβ on cell growth and differentiation. One pathway involves blockade of cell cycle transit, while the other involves alteration of the extracellular matrix environment.

TGFβ elicits their effects by binding to cell surface receptors. Three major types of receptors have been shown to be present in most TGFβ-responsive cell lines. They are designated as type I (RI), type II (RII), and type III (RIII), respectively. RII is a 280–330-kDa glycoprotein that has no functional signaling domain but rather serves as a ligand storage protein and presents TGFβ to the signaling receptors (2). RI and RII, which are glycoproteins of ~55 and 85 kDa, respectively, form a hetero-

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† The abbreviations used are: TGFβ, transforming growth factor-β; RA, retinoic acid; VDR, vitamin D receptor; RI, RII, and RIII, TGFβ receptor type I, II, and III, respectively.
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both in vitro and in athymic mice (23). However, a major drawback for its clinical application is that the doses effective for suppressing tumor growth often cause hypercalcemia. Consequently, analogues have been developed to reduce the calcemic effects while increasing the potency of inhibition of proliferation (23, 24). Two analogues, EB1089 and MC903, both of which are derived by modification of the C₁₇ side chain of vitamin D₃, have been shown to be effective against rat breast tumors in vivo (24) or as an antiproliferative agent when given topically for psoriasis as well as for cutaneous metastatic melanoma (25). However, the mechanisms of vitamin D₃-mediated growth inhibition and in particular its anti-tumor action remain largely unresolved.

In this report, we show a correlation between RII expression and vitamin D₃ inhibition in MCF-7 sublines that differ dramatically in their RII expression and hence their TGFβ sensitivity as well. We hypothesized that vitamin D₃'s mechanism of inhibition might involve induction of TGFβ autocrine activity through increased expression of RII. This hypothesis was confirmed by RNase protection assays showing approximately 3-fold induction of the RII mRNA and a 3–4-fold induction of cell surface RII protein. The increased inhibition by vitamin D₃ analogues was blocked by TGFβ-neutralizing antibodies, indicating an induction of negative autocrine TGFβ activity.

The use of an essential dietary nutrient with antiproliferative and anti-tumor properties represents an attractive approach for chemoprevention and/or therapy. This is particularly true of vitamin D compounds, since the high stress western style diet associated with colon and breast cancer is also associated with low levels of vitamin D and calcium (26). Thus, increased autocrine negative TGFβ activity mediated by vitamin D₃ compounds in MCF-7 E cells may provide a novel mechanism for blocking malignant progression by chemopreventive approaches.

MATERIALS AND METHODS

Cell Cultures—MCF-7 E cells (passage number 150) were kindly provided by Drs. Robert J. Pauley and Herbert D. Soole from the Michigan Cancer Foundation. MCF-7 L cells were obtained from the ATCC and used at a passage number greater than 500. These cell lines were cultured in McCoy's 5A medium supplemented with 10% fetal bovine serum, pyruvate, vitamins, amino acids, and antibiotics. Working cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂.

Vitamin D₃ Compounds—1,25-(OH)₂ vitamin D₃ as well as its analogues EB1089 and MC903 were generous gifts from Dr. Lise Binderup of LEO Pharmaceutical Products (Ballerup, Denmark). Stock solutions were prepared in isopropyl alcohol at 4 mM. Serial dilutions were made in absolute ethanol and stored at −20°C protected from light. These diluted solutions were added to the experimental culture media at a final ethanol concentration of 0.1%. Control cells received 0.1% ethanol vehicle, which had no effect on cell proliferation.

DNA Synthesis Assay—[³H]thymidine incorporation into DNA was measured as described previously to determine TGFβ and vitamin D₃ sensitivity (6). Briefly, MCF-7 cells were seeded in 24-well tissue culture plates at a density of 1.5 × 10⁶ cells/well in 1 ml of medium. Various concentrations of compounds (1,25-(OH)₂ D₃, EB1089, MC903, or TGFβ) were added after cell attachment (approximately 2 h). Following 4 days of incubation, cells received a 2-h pulse with [³H]thymidine (7 µCi, 46 Ci/mmol, Amersham Pharmacia Biotech). DNA was then precipitated with 10% ice-cold trichloroacetic acid, and the amount of [³H]thymidine incorporation was analyzed by liquid scintillation counting in a Beckman LS 7500 scintillation counter as described previously (6). To determine whether there is an increase in the inhibitory effects by TGFβ, following EB1089 treatment, MCF-7 E cells, which are TGFβ-responsive, were plated as described above. Various concentrations of EB1089 plus 0.1 ng/ml of TGFβ were added after attachment. Cells were incubated and [³H]thymidine incorporation was determined as described above.

TGFβ-neutralizing Antibody Assay—Cells were resuspended at a concentration of 1.5 × 10⁴ cells/ml and plated into 24-well tissue culture plates (1 ml/well) either untreated or in the presence of 10 µg/ml TGFβ₁ neutralizing antibody (R & D Systems) or control normal IgG. After 3 h of incubation, different concentrations of vitamin D₃ compounds were added as indicated. Cells were allowed to grow for 72 h without changing the media, followed by determination of [³H]thymidine incorporation as described above.

RNA Analysis—RNase protection assays were performed to determine RII RNA expression levels after vitamin D₃ treatment. A 476-base pair fragment of the RII cDNA within the cytoplasmic region was obtained by polymerase chain reaction with the following primers: 5'-TGACCCCTACTCTGTCTGTG-3' and 5'-TGTTAGGGAGCCGTCTTCA-3'. The fragment was subcloned into a pBSK (-) plasmid (Stratagene, La Jolla) for making the RII riboprobes. In vitro transcription using T3 RNA polymerase yields antisense riboprobes that protect a 476-base pair fragment of the RII cDNA within the cytoplasmic region was determined as described above.

Receptor Cross-linking—Simian recombinant TGFβ₁ was purified as described previously (27). Briefly, exponentially growing cells were treated with EB1089 at 1 × 10⁻⁷ M for the indicated time periods. Cells were solubilized in guanidine thiocyanate, and total RNA was obtained by cesium chloride gradient ultracentrifugation (28). 40 µg of total RNA was used for overnight hybridization with 32P-labeled antisense riboprobes. Following RNase A and T₁ treatment, the protected double-stranded RNA fragments were heat-denatured at 95°C and analyzed by urea-polyacrylamide gel electrophoresis, and the radioactive probes were visualized by autoradiography. Actin was used as an internal control for normalizing the amount of sample loading.

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FIG. 2. Effect of 1,25-(OH)₂ D₃ and EB1089 on DNA synthesis in MCF-7 cells. Cells were plated into 24-well plates at a density of 1.5 × 10⁴ cells/well. Different concentrations of vitamin D₃ (A), EB1089 (B), or MC903 (C) were added to tissue culture media after cell attachment. DNA synthesis was evaluated by measuring [³H]thymidine incorporation into DNA after a 2-h pulse. Results were presented as the percentage of incorporation of control with carrier ethanol alone. Each point is the mean ± S.E. of four replicates. ●, MCF-7 E; ○, MCF-7 L.
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**RESULTS**

**TGFβ Sensitivity of MCF-7 Cells**—Inconsistent response of MCF-7 cells to TGFβ has been observed in several laboratories (34, 35), probably due to growth selection during long term passage of cultures. Having obtained both early (150) and late (>500) passage MCF-7 cells, we decided to first test whether they responded differently to TGFβ (Fig. 1). MCF-7 E cells showed a significant dose-dependent inhibition by TGFβ with an IC$_{50}$ of 0.2 ng/ml. In contrast, MCF-7 L cells demonstrated complete resistance to TGFβ up to 25 ng/ml (Fig. 1). As described below, MCF-7 E cells expressed RII mRNA and protein in contrast to MCF-7 L cells, which had 5-fold less mRNA and no detectable cell surface protein.

**Vitamin D$_3$ Sensitivity**—Effects of 1,25-(OH)$_2$D$_3$ and its analogues on cell proliferation of MCF-7 cells were investigated by assessing [3H]thymidine incorporation following treatment by these compounds as described under “Materials and Methods.” MCF-7 E cells showed a dose-dependent inhibition by vitamin D$_3$ with an IC$_{50}$ of 5 × 10$^{-8}$ M. In contrast, MCF-7 L cells were not affected by vitamin D$_3$ (Fig. 2A). Vitamin D$_3$ analogues EB1089 and MC903 demonstrate similar growth-inhibitory patterns (Fig. 2, B and C). The overall potency of growth inhibition by EB1089 was approximately 2 orders of magnitude higher than vitamin D$_3$. MCF-7 E cells showed an IC$_{50}$ of 2.5 × 10$^{-10}$ M, and MCF-7 L cells did not respond to EB1089 treatment up to 1 × 10$^{-7}$ M.

**TGFβ Autocrine Activity**—The correlation between TGFβ and vitamin D$_3$ sensitivity suggested that vitamin D$_3$ may function through increasing TGFβ autocrine-negative activity in MCF-7 E cells. To test this hypothesis, TGFβ-neutralizing antibodies were used to determine whether they were capable of blocking the growth inhibition induced by these compounds (Fig. 3). At 10 μg/ml, TGFβ-neutralizing antibody reversed the inhibitory effect of vitamin D$_3$ and its analogues, generating an approximately 60% increase in DNA synthesis as compared with the normal chicken IgG treatment. In contrast, MCF-7 L cells did not respond to TGFβ-neutralizing antibody, indicating a lack of induction of autocrine TGFβ activity. These results indicate that the growth-inhibitory mechanism of vitamin D$_3$ involves induction of TGFβ autocrine-negative activity in MCF-7 E cells.

**Alteration of RII Expression**—Increased autocrine TGFβ activity could result from enhanced expression of TGFβ isoforms and/or their receptors. To test these possibilities, RNase protection assays were initially carried out on MCF-7 E cells to determine whether there were alterations of TGFβ isoform expression upon treatment with vitamin D$_3$ compounds. MCF-7 E cells expressed high levels of TGFβ$_1$ mRNA and low levels of TGFβ$_2$ and TGFβ$_3$ mRNA. Treatment with EB1089 did not generate altered mRNA expression for any of the three TGFβ isoforms (data not shown). In addition, enzyme-linked
immunosorbent assay analysis of the conditioned medium showed no significant increase in the levels of activated TGF\(\beta_1\) protein (data not shown). Since the levels of activated TGF\(\beta\) cannot be determined by enzyme-linked immunosorbent assay analysis, a growth inhibition bioassay on mink lung epithelial cells was performed. The condition medium from EB1089-treated and -untreated MCF-7 E cells was added to mink lung epithelial cells as described under "Materials and Methods." After exposure to either treated or untreated conditioned medium, no significant difference in growth inhibition was observed in the mink lung epithelial cells (Fig. 4). These results indicate that EB1089 treatment did not alter the activation of secreted growth and/or inhibitory peptides from MCF-7 E cells, one of which is likely to be TGF\(\beta_1\), as demonstrated by enzyme-linked immunosorbent assay analysis. Taken together, these results suggest that the enhanced TGF\(\beta\) autocrine activity by vitamin D\(_3\) did not result from modulation of ligand expression or activation.

The other possibility for increased autocrine TGF\(\beta\) activity upon treatment with vitamin D\(_3\) compounds was induction of receptor expression; therefore, we determined whether vitamin D\(_3\) analogue treatment modulated expression of RII mRNA. EB1089 (10\(^{-8}\) M) was utilized to determine the kinetic effects on RII expression. MCF-7 E cells expressed 5-fold higher RII mRNA than MCF-7 L cells. After exposure to EB1089, a 3-fold increase in the RII mRNA levels of MCF-7 E cells was observed. In contrast, no significant modulation was noted for the MCF-7 L cells after exposure to EB1089 (Fig. 5). EB1089 treatment did not effect the levels of RI or RIII mRNA (data not shown).

The increase in MCF-7 E RII mRNA expression led us to examine whether this corresponded to an increase in cell surface RII protein. This was tested by receptor cross-linking with \(^{125}\)I-labeled TGF\(\beta\) (Fig. 6A). The GEO cell line, which expresses all three types of TGF\(\beta\) receptors, was used as a positive control (lane 1). The specificity of cross-linking was demonstrated by competing with 100-fold cold TGF\(\beta_1\) (lane 2). MCF-7 E cells expressed all three types of receptors (lane 3). Treatment of these cells with the indicated concentrations of vitamin D\(_3\) or
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EB1089 resulted in a 3–4-fold induction of RII, while expression levels of RI remained relatively unchanged (Fig. 6A). Compared with the MCF-7 E cells, no cell surface RII protein was detected in MCF-7 L cells. Treatment with the vitamin D$_3$ compounds did not result in any change in receptor expression of these cells (Fig. 6B). To determine if the induction of RII protein in MCF-7 E cells was also time-dependent, a kinetic study was performed. Receptor cross-linking assays revealed a time-dependent increase of cell surface RII protein (Fig. 7A). Induction was detected as early as 8 h with a 3–4-fold increase after 24 h of treatment.

Responsiveness to Autocrine TGFβ—To evaluate whether RII induction by treatment with EB1089 enhanced autocrine TGFβ sensitivity, TGFβ-dependent promoter activity was analyzed using the TGFβ-responsive cyclin A luciferase reporter construct (32). TGFβ induces down-regulation of cyclin A promoter activity but requires a functional TGFβ type I and II receptor complex (32, 33). Thus, an increase in functional receptor levels would result in enhanced down-regulation of cyclin A promoter activity. The cyclin A reporter construct (−133/−2) contains only the activating transcription factor site, which has been shown to mediate down-regulation of cyclin A promoter activity by TGFβ1 in mink lung epithelial cells (32). This reporter construct was transiently transfected into MCF-7 E cells, which are sensitive to TGFβ, followed by treatment with TGFβ-neutralizing antibody and EB1089 as described under “Materials and Methods.” As expected, a decrease in luciferase activity was induced in MCF-7 E cells following treatment with EB1089. TGFβ-neutralizing antibody reversed the decrease in cyclin A luciferase activity by EB1089, increasing it by approximately 70%. TGFβ-neutralizing antibody alone had no significant affect on cyclin A luciferase activity (Fig. 8). The fact that EB1089 did not increase expression or activation of any of the three TGFβ isoforms indicates that enhanced responsiveness to autocrine TGFβ after EB1089 treatment is due to the increased expression of RII.

DISCUSSION

TGFβ signaling requires a heteromeric assembly of its two Ser-Thr kinase receptors, designated RI and RII, respectively (34). A recent model illustrating the physical and functional interactions between the two receptors proposes that upon ligand binding, the constitutively active RI recruits RI and transphosphorylates the RI, which subsequently initiates downstream cytoplasmic events (37). Defects in expression of either receptor would contribute to loss of response to exogenous as well as endogenous TGFβ. In particular, loss and/or lack of autocrine TGFβ response plays a major role in enhancing tumor progression. As was demonstrated previously, anti-

![Figure 5](image)

**FIG. 5. Regulation of TGFβ RII mRNA expression by EB1089.** Exponentially growing MCF-7 E and MCF-7 L cells were treated with EB1089 (10$^{-8}$ M) for 8, 16, and 24 h. Total RNA was collected, and RII mRNA expression levels were compared using RNase protection assays. Actin was used to normalize sample loading.

![Figure 6](image)

**FIG. 6. Effect of 1,25-(OH)$_2$ D$_3$ and EB1089 on expression of cell surface TGFβ receptors.** Receptor cross-linking assays were performed to determine modulation of cell surface TGFβ receptor expression by the vitamin D$_3$ compounds on MCF-7 E (A) and MCF-7 L cells (B). Cells were plated and allowed to grow to 70–80% confluency in 35-mm$^2$ tissue culture dishes and then subjected to treatment with the indicated concentrations of vitamin D$_3$ or EB1089 for 24 h. Monolayer of GEO cells (used as a positive control) and MCF-7 cells were incubated with 200 µM $^{32}$P-labeled TGFβ, alone or in the presence of 20 nM cold TGFβ, at 4°C for 4 h, followed by chemical cross-linking with disuccinimidyl suberate. 150 µg of total cell lysate protein was separated by electrophoresis using 4–10% gradient SDS-polyacrylamide gel electrophoresis gels. The autoradiographs are representative of three similar experiments.

sense TGFβ transfection in two early malignant colon carcinoma cell lines eliminated autocrine negative TGFβ activity but did not block response to exogenous TGFβ (27, 38). The TGFβ antisenese-transfected cells showed increased tumor growth and incidence in athymic nude mice, indicating that autocrine TGFβ plays a key role in blocking tumor progression. Reestablishment of autocrine TGFβ responsiveness leading to decreased tumorigenicity in cells with deficient TGFβ receptor function was achieved by stable transfection of RI in breast cancer MCF-7 L cells (6) and in the human colon carcinoma cell line HCT116 (8). Based on these studies, agents that can control the expression of TGFβ receptors may have therapeutic implications. In particular, agents that can enhance the expression level of RII in cells where it appears to be repressed may be an effective chemopreventive approach. To date, no such agents have been carefully and fully characterized for their ability to induce RII and subsequently enhance autocrine-
negative TGFβ activity. Characterization of these agents should lead to a better understanding of TGFβ-mediated growth inhibition and its anti-tumor effects.

In the present study, we demonstrated for the first time that an increase in autocrine TGFβ function by the active metabolite of vitamin D₃ is solely due to an increase in RII expression. However, the effective dose of active vitamin D₃, which induces negative autocrine TGFβ activity would also cause hypercalcemia, leading to unwanted side effects. To overcome this problem, analogues such as EB1089 have been developed that have increased potency and reduced hypercalcemic effects (39). We have shown here that the analogue EB1089 has similar effects to the parental compound at lower concentrations, which make it an attractive and potential chemopreventive agent. Thus, vitamin D₃ and its analogues can inhibit malignant cell growth through a novel mechanism of induction of negative autocrine TGFβ activity.

A number of studies have reported that expression of RI and RII protein can be regulated by factors such as cell density (40, 41); exposure to parathyroid, adrenal, or androgenic hormones (42–44); and TGFβ (45). However, these agents do not readily lend themselves to chemopreventive approaches. Moreover, these studies were restricted to cell surface analysis utilizing receptor cross-linking and did not determine biological effects with respect to potential autocrine activity changes. In this study, we demonstrated a 3-fold increase in steady state RII mRNA levels (Fig. 4) by vitamin D₃ treatment, which correlated with a 3–4-fold increase in cell surface RII protein (Figs. 5 and 6). This suggests that translational modifications were unlikely to be responsible for up-regulation of the RII protein. Induction of RII mRNA by vitamin D₃ may involve transcriptional and post-transcriptional mechanisms. Vitamin D₃ association with VDR can either increase the affinity of VDR binding to its target DNA sequence or cause conformational changes in the receptor leading to alterations in gene activation (46). VDR proteins were detected by Western analysis in both MCF-7 E and MCF-7 L cells (data not shown). The VD-VDR complex in combination with other steroid receptors could be directly involved in stimulation of the RII promoter activity or act indirectly by increasing the quantity or activity of related transcriptional activators. Examination of the recently characterized RII promoter region (47) did not reveal sequences analogous to the well established vitamin D₃-responsive elements, indicating that induction of RII mRNA in MCF-7 E cells by vitamin D₃ was unlikely to be a direct effect. The lack of RII induction in MCF-7 L cells suggests that the RII gene might be suppressed by factors or mechanisms that were not present in MCF-7 E cells and that certain transcriptional factors that were essential to activation of the VDR signaling pathway might be deficient in MCF-7 L cells. Unraveling these mechanisms may lead to novel approaches for re-activation of the RII tumor suppressor gene.

Modulation of TGFβ expression or secretion by vitamin D₃ has been shown in keratinocytes, chondrocytes, rat prostatic epithelial cells, and one human breast cancer cell line BT-20 (48–51). Danielpour (51) was able to demonstrate in a nontumorigenic rat prostate epithelial cell line that induction of TGFβ autocrine activity by vitamin D₃ was mediated by increases in all three isoforms of TGFβ. In addition, other steroid hormones have been shown to increase activation of latent TGFβ while not affecting total levels (52, 53). Interestingly, modulation of TGFβ levels or its activation by vitamin D₃ compounds was not observed in this study of this strain of human breast cancer cell line (MCF-7 E). A potential difficulty with chemopreventive approaches involving TGFβ ligand induction rather than receptor induction resides in the tumor-
enhancing effects associated with TGFβ overexpression, such as angiogenesis and immunosuppression (54). However, EB1089 may offer an advantage in that induction of autocrine-negative TGFβ activity occurs through RII and not its TGFβ ligand. Enhancement of autocrine-negative TGFβ activity, without the increase in TGFβ ligand and the potential tumor-enhancing effects associated with it, makes the use of these compounds an attractive approach by offering a potential novel mechanism for cancer prevention and/or therapy.

In addition to vitamin D₃, other related members of the steroid hormone family have been shown to modulate TGFβ receptor expression. In human neuroblastoma cells, RA increased RII mRNA levels and RI protein as well as increasing cell surface RII (55). However, cell surface RII was undetectable by receptor cross-linking in this study. The up-regulation of TGFβ₁ and the TGFβ receptors occurred only in the neuroblastoma cell line that was responsive to RA-induced growth arrest. RA treatment of RL human B lymphoma cells induced a 2-fold increase in total RII protein. However, the study failed to examine whether that correlated to increased cell surface RII (56). In addition to RA, vitamin E succinate and RA, indicating that treatment induced a TGFβ autocrine-negative loop. However, this study did not examine cell surface receptor levels; thus, the increase in negative TGFβ autocrine activity could be due to increased ligand levels. Both of these studies demonstrated the ability of other agents to induce RII mRNA or protein levels but failed to either detect or investigate RII cell surface levels. In addition, these compounds also enhance ligand expression, which may have adverse effects on surrounding tissue. The observation that vitamin D₃ induced autocrine negative TGFβ activity through increased cell surface RII and not TGFβ ligand may prove to be of significance in breast cancer therapy and/or prevention.

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REFERENCES

1. Roberts, A. B., and Sporn, M. B. (1990) *Peptide Growth Factors* 31, 613–43.
2. Park, K., Kim, S. J., Bang, Y. J., Park., J. G., Kim, N. Y., Roberts, A. B., and H. Y., Park, J.-G., Lynch, H. T., Roberts, A. B., and Sporn, M. B. (1994) *Antineoplastic Drugs* 45, 582–398.
3. Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L-Z., Lutterbaugh, J., Mark, J. W., and Coombes, R. C. (1993) *Cancer Res.* 53, 4429–4434.
4. Sun, L., Wu, G., Willson, J. K. V., Zborowska, E., Yang, J., Assef, J., and Miyazono, K. (1993) *Science* 260, 5359–5363.
5. Kyprianou, N., and Isaacs, J. T. (1988) *Cancer Res.* 48, 4266–4271.
6. Ross, T. K., Darwish, H. M., Moss, V. E., and Deluca, H. F. (1993) *J. Biol. Chem.* 268, 28374–28381.
7. Kolin, K., and Keski-Oja, J. (1995) *Cancer Res.* 55, 1540–1546.
8. Geiger, A. G., Burmester, J. K., Webbink, R., Roberts, A. B., and Sporn, M. B. (1992) *J. Biol. Chem.* 267, 2588–2593.
9. Geiser, A. G., Burmester, J. K., Webbink, R., Roberts, A. B., and Sporn, M. B. (1992) *J. Biol. Chem.* 267, 2588–2593.
10. Fransen, P., Ten Dijke, P., Ichijo, H., Yamashita, H., Schultz, P., Heldin, C-H., and Miyazono, K. (1993) *Cell* 75, 681–692.
11. Kodama, A., Arai, D., and Kan, H. Y., Park, J.-G., Lynch, H. T., Roberts, A. B., and Sporn, M. B. (1994) *J. Biol. Chem.* 269, 22259–22264.
12. Park, K., Kim, S. J., Bang, Y. J., Park., J. G., Kim, N. Y., Roberts, A. B., and H. Y., Park, J.-G., Lynch, H. T., Roberts, A. B., and Sporn, M. B. (1994) *Antineoplastic Drugs* 45, 582–398.
13. Fransen, P., Ten Dijke, P., Ichijo, H., Yamashita, H., Schultz, P., Heldin, C-H., and Miyazono, K. (1993) *Cell* 75, 681–692.
14. Liu, G. S., Wang, J. Y., Myeroff, L., Parsons, R., Sun, L-Z., Lutterbaugh, J., Mark, J. W., and Coombes, R. C. (1993) *Cancer Res.* 53, 4429–4434.
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