ABSTRACT

The antiproliferative action of 1,25-dihydroxyvitamin D₃ in osteosarcoma, breast carcinoma, and colon carcinoma cell lines has been described. In this study, the level of vitamin D receptor was analyzed in a panel of colon adenoma and adenocarcinoma cell lines and the receptor level was correlated with the response to treatment with 1,25-dihydroxyvitamin D₃. Ribonuclease protection and ligand-binding assays quantified the level of vitamin D receptor mRNA expression and the level of functional receptors, respectively. The more well-differentiated cell lines, such as VACO 330, showed higher levels of vitamin D receptor than less-differentiated cell lines, such as SW620. Proliferation assay, clonogenic assay, and growth curve study in HT29 and SW620 cell lines assessed the antiproliferative effect of 1,25-dihydroxyvitamin D₃ at concentrations ranging from 10⁻¹¹ to 10⁻⁴ M. HT29 showed significant (P < 0.05) growth inhibition at 10⁻⁸ to 10⁻⁹ M concentrations, but growth of SW620 remained unchanged. The amount of vitamin D receptor in 12 malignant colonic tumors was compared with that of adjacent normal tissue, and in 9 cases, the tumor expressed a lower vitamin D receptor level. Our results suggest that the level of vitamin D receptor correlates with the degree of differentiation in human colon cancer cell lines and may serve as a useful biological marker in predicting clinical outcome in patients.

INTRODUCTION

Vitamin D is a steroidal hormone which modulates calcium homeostasis via actions on kidney, bone, and gastrointestinal tract (1). The important role of vitamin D in cancer was first recognized in 1981 when Abe et al. (2) demonstrated induction of differentiation of M1 leukemia cells into macrophages. Subsequent studies have shown growth inhibition by vitamin D in osteosarcoma, breast carcinoma, and colon carcinoma cell lines (3, 4).

The effects of vitamin D are mediated by the binding of 1,25-dihydroxyvitamin D₃, the most active form, to a specific intracellular receptor (5, 6). The vitamin D receptor is a member of the steroid hormone receptor superfamily, with close homology to the estrogen, retinoic acid, and thyroid hormone receptors. After binding to its cognate ligand, the receptor regulates gene transcription through interaction with hormone response elements in the promoter regions of the genome (7). The vitamin D receptor has been demonstrated in melanoma, osteosarcoma, breast, lung, ovarian, and colon carcinoma cell lines (8).

In 1985, a study by Garland et al. (9) demonstrated an inverse correlation between dietary vitamin D and the incidence of colon cancer in a population of 1954 men in Chicago. Subsequent studies have shown a 3-fold decrease in the incidence of colon cancer in patients with serum 25-hydroxyvitamin D₃ levels >20 ng/ml (10). Death rates from colon cancer have been shown to inversely correlate with exposure to sunlight in 17 United States states (11). Epidemiological studies of the incidence of colorectal cancer in Europe have demonstrated a higher prevalence in the northern regions where there is less sunlight exposure (12). The incidence of colorectal cancer in Denmark was 24 cases in 100,000 population compared with 8 cases per 100,000 population in Greece. Vitamin D receptors were first identified in colon cancer cells by Frampton et al. (8) in 1982. We designed this study to assess the significance of vitamin D receptors in colon cancer cells and to evaluate the potential role of vitamin D in the treatment of colon cancer.

MATERIALS AND METHODS

Cell Culture

A panel of human colon cancer cell lines (Caco2, Colo205, WiDr, Caco-2, HCT15, HT29, SW480, SK-CO-1, LS180, SW620) available from the American Type Culture Collection (Rockville, MD) was used. The cells were grown as monolayers in RPMI 1640 (obtained from Biofluids, Rockville, MD) supplemented with 5% fetal calf serum, 2 mM glutamine, and 10 units/ml penicillin. VACO 235 and VACO 330 (gifts from James K. V. Willson, Case Western Reserve Medical Center, Cleveland, OH) human adenoma cell lines were grown on a collagen substrate (type I from rat tail; UBI Inc., Lake Placid, NY) in MEM (Gibco, Grand Island, NY) supplemented with 4 mM glutamine, 2% fetal calf serum, 0.1 mM nonessential amino acids, 50 µg/ml gentamicin, 10 µg/ml insulin, 2 µg/ml transferrin, 1 µg/ml hydrocortisone, and 5 × 10⁻⁹ M Na₂SeO₃, all of which were obtained from Biofluids (13). The collagen substrate was prepared by coating the flask surface with collagen solution (approximately 4 mg/ml) and exposing it to ammonium hydroxide fumes until solidified. One ml of collagen solution was used to coat a 75-mm flask. Each gel was rinsed 3-4 times with PBS, and then MEM with 8% fetal calf serum was added. Flasks were stored at 37°C until needed. Gels were preincubated with VACO growth medium, as described above, for 24 h prior to use.

Reagents

1,25-Dihydroxyvitamin D₃ (Duphar Co., Amsterdam, the Netherlands) was dissolved in absolute ethanol and stored in opaque glass vials at -20°C at a stock concentration of 10⁻⁴ M. The concentration was verified by UV spectrophotometry (Beckman DU-65 spectrophotometer) at 264 nm using the molar extinction coefficient of 18,300.

Vitamin D Receptor Assays

Ribonuclease Protection Assay. The cDNA sequence coding for the human VDR incorporated in a pGEM 7Zf (+) vector was provided by J. W. Pike (6, 14). This construct has been sequenced and shown to be capable of inducing transient high level expression of functional vitamin D receptor in human kidney cells (15). We created a 350-base pair riboprobe template by deleting the 849-base pair 3'-terminal segment of the cDNA and religating the vector. The vector was digested with NcoI, and a 319-base pair riboprobe was synthesized by RNA polymerase from the T7 start site. The probe corresponded to a 319-base pair protected fragment at the 3' end of the human VDR-coding sequence. The 5' and 3' termini of the construct were sequenced to confirm correct restriction enzyme digestion and orientation. Total cellular RNA was isolated from cell cultures using a cesium chloride gradient purification protocol (16). The probe was radiolabeled with ³²P and incubated overnight with 30 µg of purified RNA at 50°C. A radiolabeled probe (36B4) which detects a constitutively expressed human acidic ribosomal phosphoprotein was added to
the same samples to control for loading (17). The samples were incubated with RNase A at 25°C for 30 min to degrade the nonprotected sequences. The product was run on a 6.5% polyacrylamide gel with a size marker (Mspl-digested PBR322). Autoradiography was performed overnight at room temperature. The densities of the protected fragment bands were measured with a Quantity One (version 2.0 from PDI) densitometer and are presented as a fraction of the highest expressor which was arbitrarily assigned a value of 1. Assays were done twice. Values are reported ± 1 SD.

The ribonuclease protection assay was also done on tissue samples from 12 patients who underwent resection of malignant colonic tumors. In each case, tissue from the tumor and adjacent normal tissue (10-12 cm away from the tumor) were obtained from surgical pathology prior to any irrigation or fixation. Tumors were all histologically confirmed to be adenocarcinoma, and the adjacent tissue was confirmed to be normal in every case. Samples were placed on dry ice immediately and stored at -70°C. At the time of the assay, frozen tissue samples were pulverized using a mortar and pestle. Specimens were then homogenized using a stainless steel Dounce homogenizer. RNA was then isolated using the cesium chloride gradient purification protocol. Ribonuclease protection assay was performed as described above, and the density of the vitamin D receptor protected fragment are expressed as a fraction of the density of the band from the adjacent normal tissue.

It should be noted that the tumor and normal tissue have different structures with various amounts of stroma, smooth muscle, and epithelium. At the time of selection, there was no control for the amount of stroma. Hence, it is not possible to determine the amount of vitamin D receptor mRNA originating from stroma, epithelium, or muscle. This is a limitation of the study using the presented techniques. Immunohistochemical techniques will allow us to better compare receptor levels between tumor and normal tissue.

**Ligand-binding Assay.** For each of 6 colon carcinoma cell lines, 5 × 10^5 cells per well were plated in 24-well tissue culture plates (Costar Co., Cambridge, MA). The media used consisted of IMEM supplemented with 5% charcoal-stripped fetal calf serum, 2 mM glutamine, 10 units/ml penicillin, and 10 ng/ml insulin. An additional well was plated for protein content determination. After overnight growth, the cells were incubated in 1 nM [3H]1,25-dihydroxyvitamin D₃ with or without a 200-fold excess of unlabelled 1,25-dihydroxyvitamin D₃. Cells were incubated at 4°C for 4 h. They were then washed 3 times with PBS. Cells were lysed with 0.1 M NaOH. The lysates were then transferred to scintillation vials containing 10 ml scintillation fluid (Aquasol-2 universal liquid scintillation cocktail; DuPont Co., Boston, MA). Radioactivity was counted on a Beckman LS 7500 scintillation counter. Specific binding was calculated by subtracting nonspecific binding from total binding. Results were expressed as fmol receptor/mg cytoplasmic protein and plotted on a bar graph.

**Cell Growth Experiments**

**Proliferation Assay.** Growth studies were done on HT29 and SW620 cells. These two cell lines were chosen because they represented moderate and low expressors of vitamin D receptor, respectively. Cells (2 × 10^4 per well) were plated in 24-well tissue culture plates. After the cells grew overnight, media was changed to IMEM with 5% charcoal-stripped fetal calf serum, 5 mM glutamine, 10 units/ml penicillin, 10 ng/ml insulin, and increasing concentrations of 1,25-dihydroxyvitamin D₃ from 10⁻¹¹ to 10⁻⁶ M as a control, cells were grown in the same media with 0.1% ethanol (and no added 1,25-dihydroxyvitamin D₃). The level of 1,25-dihydroxyvitamin D₃ in the control was measured to be 2.4 × 10⁻¹⁴ M (determined by American Medical Laboratories, Chantilly, VA, using an extraction and purification sequestration saturation assay kit from Incstar Corp., Minneapolis, MN). Ninety-six h after incubation with treatment media, [3H]thymidine incorporation into DNA was measured. Cells were incubated with [3H]thymidine (10 μCi/ml) for 2 h at 37°C. After the cells were washed with PBS, they were trypsinized, lysed by sonication, and incubated with 10% trichloroacetic acid for 15 min at 4°C to remove the excess thymidine. The cell lysates were transferred to filters with 0.45-μm pores (Millipore, Bedford, MA). The filters were rinsed with 10% trichloroacetic acid. They were then placed in scintillation vials containing Aquasol-2 universal liquid scintillation cocktail. Radioactivity was counted by the Beckman LS 7500 scintillation counter. Thymidine incorporation was plotted as a percentage of control growth.

**Growth Curve Studies.** HT29 and SW620 monolayer cells (2 × 10⁴ cells per well) were plated in 6-well tissue culture plates. After the cells grew overnight, experimental conditions were achieved as described for the proliferation assay. On days 2, 4, and 6 after the addition of treatment media, cells were trypsinized and counted using a hemocytometer. Counts were obtained in duplicate at each time. Student's t-test was used to compare cell counts on day 6 of treatment.

**Soft Agar Assay.** HT29 and SW620 cells (2 × 10⁴) were plated in 35-mm tissue culture dishes in IMEM with 5% charcoal-stripped fetal calf serum and 2 mM glutamine (2.4%) added. After overnight growth, the cells were overlayed with experimental media as described for the proliferation assay. The experiment was done in triplicate and the SD was calculated. After 20 days, colonies were counted using a computerized image analyzer (Omnicon 3600 image analysis system) using a sized cutoff of 60 μm. Colony counts were compared using Student's t-test.

**RESULTS**

**Vitamin D Receptor Assays**

Ribonuclease protection assay demonstrated a wide range of vitamin D receptor mRNA expression in a panel of human colon carcinoma and two benign adenoma cell lines (Figs. 1 and 2A). LS180 showed the highest level of expression and was arbitrarily assigned a value of 1. The level of vitamin D receptor in the other cell lines was expressed relative to LS180. SK-CO-1, with a vitamin D receptor message level of 0.80 relative to LS180, showed higher expression than VACO 235 adenoma cells (0.52) but lower expression than VACO 330 adenoma cells (0.87). SW48 and HT29 were characteristic of moderate expressors (0.41, 0.40); Colo201 and SW620 showed the lowest level of vitamin D receptor message (0.13, 0.11).

The ligand-binding assay showed SK-CO-1 to have the highest expression of functional vitamin D receptors with a level of 128 fmol/mg protein (Fig. 2B). LS180 was slightly lower at 118 fmol/mg protein. The intermediate expressors by ribonuclease assay, Caco-2 and HT29, had levels of 63 and 59 fmol/mg protein, respectively. SW620 and Colo201 had low levels of receptor, 5 and 2 fmol/mg protein, respectively.

**Cell Growth Experiments**

**Proliferation Assay.** HT29 cells showed a dose-dependent inhibition of growth with increasing concentrations of 1,25-dihydroxyvitamin D₃ (Fig. 3). Uptake of tritiated thymidine decreased from 92.4% of control at 10⁻¹¹ M to 18.3% at 10⁻⁶ M 1,25-dihydroxyvitamin D₃. Growth inhibition at 10⁻⁷ and 10⁻⁶ M was statistically significant (P < 0.05).

SW620 did not show significant inhibition of growth with treatment by 1,25-dihydroxyvitamin D₃ at any concentration.

**Growth Curve Studies.** The antiproliferative effect of 1,25-dihydroxyvitamin D₃ on HT29 cells was dose dependent with cell counts on day 6 ranging from 97.6% of control at 10⁻¹¹ M to 24% of control at 10⁻⁴ M (Fig. 4A). Growth inhibition at 1,25-dihydroxyvitamin D₃ concentrations of 10⁻⁸ to 10⁻⁶ M was statistically significant (P < 0.05).

![Fig. 1. Ribonuclease protection assay gel of panel of human adenoma and adenocarcinoma cell lines.](image-url)
1.25-DIHYDROXYVITAMIN D3 RECEPTORS IN COLON CANCER CELLS

In SW620, the growth curve analysis showed no growth inhibition with 1,25-dihydroxyvitamin D3 treatment (Fig. 4B). On day 6, cell counts ranged from 96 to 104% of control.

Soft Agar Assay. 1,25-Dihydroxyvitamin D3 significantly inhibited the clonogenic growth of HT29 in a dose-dependent fashion (Fig. 5A). Colony counts ranged from 9% of control at 10^{-6} M to 29% at 10^{-1} M, 40% at 10^{-8} M, and 95% at both 10^{-9} and 10^{-10} M. No growth inhibition was seen at 10^{-11} M 1,25-dihydroxyvitamin D3. The growth inhibition at 10^{-6} and 10^{-8} M was statistically significant (P < 0.05).

The clonogenic assay of SW620 cells demonstrated no inhibitory effect when cells were treated with a range of concentrations of 1,25-dihydroxyvitamin D3 (Fig. 5B). All of the colony counts were higher than the control.

Vitamin D Receptor Assay in Human Tissue Samples

In tissue samples from 12 patients, 9 of 12 showed a lower vitamin D receptor expression in the tumor tissue as compared with the adjacent normal tissue (Fig. 6). The level of receptor expression is displayed as a fraction of the adjacent normal mucosa. Among these 9 patients, relative vitamin D receptor mRNA level ranged from 10–70% of the levels seen in normal tissue. Two patients had expressions of <20%, while 5 others had expressions of <50%. Two patients showed similar levels of vitamin D receptor mRNA in the tumor and normal mucosa. Only one patient had higher vitamin D receptor expression in tumor compared with normal tissue with a ratio of 1.23:1.

DISCUSSION

The vitamin D receptor was first isolated from the nuclei of chicken small intestinal cells in 1969 (18). The receptor has been cloned, sequenced, and expressed in COS-1 cells (14). Analysis of the deduced amino acid sequence revealed close homology with the members of the steroid hormone receptor superfamily (5). The vitamin D receptor is the smallest for a steroid hormone (5, 7). It contains a characteristic DNA-binding domain and hormone-binding domain. The DNA-binding domain is highly conserved and includes 2 zinc finger motifs rich in cysteine, lysine, and arginine residues. These are believed to modulate receptor-DNA interactions. The hormone-binding domain is located near the carboxy terminal of the receptor molecule.
In order to better understand the effect of vitamin D receptors on the growth and differentiation of colon cancer cells, we analyzed the vitamin D receptor level in a panel of well-characterized adenoma and adenocarcinoma cell lines. Such a comparison of vitamin D receptor levels among different colon cancer cells or between adenoma and adenocarcinoma cell lines has not been described. Previously published reports of the correlation between vitamin D receptor level and degree of differentiation of colon cancer cell lines not only showed conflicting data but was only performed in one cell line before and after differentiation. In a study of Caco-2 colon cancer cells, Guiliano et al. (19) showed a 2.4-fold increase in the level of vitamin D receptors as the cells underwent spontaneous differentiation. In investigations of HT29, Brehier and Thomasset (20) demonstrated results contradictory to those of Guiliano et al. They induced differentiation in HT29 cells by glucose deprivation. Removal of glucose from the media has been shown to induce differentiation as measured by the induction of brush border enzyme activity and characteristic morphological changes with polar growth. In their study, induction of differentiation was associated with lower levels of vitamin D receptor by...
ligand-binding assay compared to cells maintained in glucose-containing media. Harper et al. (21), in a similar study of HT29 in glucose-rich and glucose-deprived media, showed lower vitamin D receptor levels in the more differentiated cells (21). The loss of vitamin D receptor associated with glucose deprivation may represent a response of the cells to stressful growth conditions and adaptation to growth in disaccharides.

In the present study, vitamin D receptor mRNA was quantitated by ribonuclease protection assay. A radiolabeled ligand-binding assay was used as a measure of receptor function. As shown in Table 1 and Fig. 2A, receptor level varied widely. In Table 1, the colon adenoma and adenocarcinoma cell lines have been categorized in order of decreasing level of differentiation based upon their origin, Duke's stage, and level by ribonuclease protection assay (VDR). The correlation between the degree of differentiation and level of vitamin D receptor is seen particularly well in the well-differentiated and poorly differentiated cell lines. Analysis of the function of the receptors by ligand-binding assay showed close correlation with the ribonuclease protection assay results.

Despite extensive investigation of its action in mediating calcium homeostasis, the mechanism of action of vitamin D on cancer cells is unknown. In this study, we have compared the growth inhibitory effect of 1,25-dihydroxyvitamin D₃ in two colon cancer cell lines, one expressing moderate and one expressing low levels of vitamin D receptor. Even though 1,25-dihydroxyvitamin D₃ has been shown to act as an antiproliferative agent in colon cancer cells, previous studies have not correlated this effect with the level of receptor. We have demonstrated growth inhibition of HT29 in tissue culture by addition of 1,25-dihydroxyvitamin D₃, the active metabolite of vitamin D. HT29 is a moderately well-differentiated human colon cancer cell line (Table 1) which has been used extensively in studies of colon cancer growth and differentiation. The vitamin D receptor level in HT29 is 59 fmol/mg protein by ligand-binding assay. Measurement of vitamin D receptor mRNA by ribonuclease protection assay shows HT29 to be a median expressor with a level of 0.40 relative to the highest expressor (LSI80).

Table 1 Classification of human colon cancer cell lines based on level of differentiation

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>Duke's Stage</th>
<th>VDR</th>
<th>Tumor potential</th>
<th>Soft agar</th>
<th>Monopolar</th>
<th>Morphology</th>
<th>Alkaline phosphatase</th>
<th>CEA</th>
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<td>–</td>
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HT29 and SW48, originate from Duke's C tumors having lower CEA and alkaline phosphatase activities with higher tumorigenic potential. The low expressors, such as SW620 and Colo201, originate from Duke's D tumors with very high tumorigenic potential and little CEA activity. The results of our receptor assays showed SW620 to have the lowest level of vitamin D receptor mRNA with a value of 0.11 relative to the highest expressor, LSI80. In general, cells considered to be well differentiated demonstrated higher levels of vitamin D receptor than the poorly differentiated cells. Of note, VACO 330 and 235, which are benign adenoma cell lines, manifested moderately high vitamin D receptor mRNA levels (0.87 and 0.52). Two carcinoma cell lines (LS180 and SKCO1) showed comparable levels of vitamin D receptor mRNA. The correlation between the degree of differentiation and level of vitamin D receptor is seen particularly well in the well-differentiated and poorly differentiated cell lines.

Colon adenoma and adenocarcinoma cell lines have been categorized in order of decreasing level of differentiation based upon origin, Duke's stage, and level by ribonuclease protection assay (VDR). The median expressor with a level of 0.40 relative to the highest expressor (LSI80). SW620, which is a poorly differentiated human colon cancer cell line, derived from ascitic fluid, showed no growth inhibition by 1,25-dihydroxyvitamin D₃ at concentrations up to 10⁻⁷ M. SW620 cells had low levels of vitamin D receptors by ligand-binding assay (5 fmol/mg protein) and low levels of vitamin D receptor mRNA by ribonuclease protection assay (0.11 relative to the highest expressor (LSI80)).

Proliferation studies using tritiated thymidine incorporation into DNA as an index of growth yielded results which paralleled those of the growth curve analyses in HT29 and SW620. As a further measure of the effect of 1,25-dihydroxyvitamin D₃ on cell growth, clonogenic assays were performed. This technique measures the ability of cells to achieve anchorage-independent clonogenic growth in soft agar. Again,

Table 1 Classification of human colon cancer cell lines based on level of differentiation

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Fig. 6. Densitometry of ribonuclease assay comparing vitamin D receptor mRNA expression in colonic tumor tissue with adjacent normal mucosa in 12 patients. The vitamin D receptor level is expressed as a percentage of that of the normal tissue. Nine of 12 patients showed lower levels in the tumor tissue.
treatment with varying concentrations of 1,25-dihydroxyvitamin D₃ showed dose-dependent inhibition of growth in HT29 and no effect on SW620.

In all three growth assays, HT29, a moderate expressor of vitamin D receptor, showed significant inhibition, while SW620, a low expressor of vitamin D receptor, showed no change in growth pattern. This relationship is strongly suggestive of a receptor-mediated mechanism of growth inhibition by 1,25-dihydroxyvitamin D₃.

In view of the overall correlation between vitamin D receptor levels and the degree of differentiation in colon cancer cells, a comparison of vitamin D receptor level in malignant colonic tumors and adjacent normal tissue was undertaken using a ribonuclease protection assay. In 9 of 12 patients, vitamin D receptor mRNA was lower in the tumor than in normal mucosa (range, 10–70%); 2 patients showed equal expression, and one patient showed greater expression of vitamin D receptor mRNA in the tumor (120%). A previous study of vitamin D receptor level by Sandgren et al. (22) demonstrated higher levels in tumor than in normal adjacent mucosa in 8 of 10 patients by immunoradiometric assay. The ratio of vitamin D receptor in tumor to normal tissue was 1.87 in Duke’s B and 1.16 in Duke’s C tumors. In a related study, Lointier et al. (23) noted that vitamin D receptors were more frequently absent in left colonic tumors than in right-sided ones. In addition, they noted that the receptor levels were similar in the normal mucosa of patients with colon cancer and those with colonic tumors. Finally, by sucrose density gradient centrifugation, they found vitamin D receptors to be present more frequently in normal tissue than in tumors. While the immunoradiometric data of Sandgren et al. showed lower vitamin D receptor levels in colonic tumors compared with normal tissue, the sucrose gradient assay of Lointier et al. along with our ribonuclease protection assay demonstrated higher levels of receptor in normal tissue. As described before, in studies of tissues, it is difficult to determine the compartment from which the receptors originate, and the histological differences may explain some of the difference between the receptor level in normal and malignant tissue.

Epidemiological data support the role of 1,25-dihydroxyvitamin D₃ as a potentially important hormone in preventing the neoplastic progression of colon epithelium. The findings presented above allow one to speculate that either the loss of vitamin D receptor is one of the factors promoting the progression of normal epithelium to malignant epithelium or the loss of vitamin D receptor expression is merely a manifestation of epithelial dedifferentiation. If the former hypothesis is true, then vitamin D and the vitamin D receptor may be integral in the chemoprevention of colon carcinoma.

Our findings in colon cancer cells and tissue demonstrated a correlation between the levels of vitamin D receptor and degree of differentiation. The implication of this finding is that vitamin D receptor may serve as a useful biological marker in malignant colonic tumors. At present, there are no sensitive biological assays in colon cancer that reflect the degree of tumor aggressiveness. CEA, which is a cell surface glycoprotein, is produced in excess in a number of human malignancies and is only useful as a marker of colon carcinoma tumor recurrence (24). The only prognostic data currently used is the Dukes’ classification, which describes the pathological stage of the disease by determining the level of invasion of the bowel wall and presence of lymph node involvement (25). The utility of a sensitive biological marker in colon cancer would be instrumental in identifying very aggressive tumors at an early stage. At present, in the treatment of colon cancer, chemotherapy is given only to patients with evidence of lymphatic involvement. Based on Dukes’ classification, highly aggressive tumors cannot be identified until there is lymphatic spread. A marker of biological activity, such as vitamin D receptor level, may be helpful in identifying these aggressive tumors with high likelihood of metastatic progression and dictate the need for adjuvant treatment. This may improve the overall survival in colon cancer patients.

Prior reports have demonstrated the presence of vitamin D receptors in colon carcinoma cells and tissue and have compared the levels of these receptors in the same cell lines before and after induced differentiation. The antiproliferative effect of 1,25-dihydroxyvitamin D₃ in colon cancer cells has also been described. However, this study further expands our understanding of the vitamin D receptor by measuring the levels in different colon adenoma and adenocarcinoma cell lines and attempting to correlate receptor levels with the degree of differentiation. We have also correlated the antiproliferative effect of 1,25-dihydroxyvitamin D₃ with vitamin D receptor levels, shedding light on the mechanism of action. Results of this study provide evidence implicating vitamin D receptor as a marker of differentiation and mediator of growth inhibition in colon cancer.

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