

1,25-Dihydroxyvitamin D₃ Receptor Measurement in Primary Renal Cell Carcinomas and Autologous Normal Kidney Tissue¹

Torleif Trydal,² August Bakke, Lage Aksnes, and Dagfinn Aarskog

Department of Pediatrics [T. T., L. A., D. A.] and Department of Surgery [A. B.], Haukeland Hospital, University of Bergen, N-5021 Bergen, Norway

ABSTRACT

Recently it was reported that 1- α ,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃] inhibited cell growth in a cell line derived from a metastasis from renal cell carcinoma. We have examined samples from 23 primary renal cell carcinomas for 1,25-(OH)₂D₃ receptor content, and compared it with the concentrations in autologous normal kidney tissue. Nineteen of 23 (83%) renal cell carcinomas had detectable (above 1 fmol/mg protein) 1,25-(OH)₂D₃ receptor levels, and 15 of 23 (65%) had levels above 5 fmol/mg protein. Mean value for the renal cell carcinomas was 8.2 fmol/mg protein (range, 0–28 fmol/mg protein), and the mean value for autologous normal kidney tissue was 23.1 fmol/mg protein (range, 6.6–53.7 fmol/mg protein). The 1,25-(OH)₂D₃ receptor levels in the renal cell carcinomas were significantly lower than in the autologous normal kidney tissue ($P < 0.001$). The 1,25-(OH)₂D₃ receptor was characterized by sucrose gradient analysis and DNA-cellulose chromatography. The features found for renal cell carcinoma were similar to the 1,25-(OH)₂D₃ receptor in normal human tissue. No correlation of 1,25-(OH)₂D₃ receptor levels to clinical parameters was found. This study shows that carcinomas originating from the kidney, the major vitamin D regulating organ, usually contain the 1,25-(OH)₂D₃ receptor. The receptor may have a cellular function in the transformed cell.

INTRODUCTION

The biologically most active vitamin D compound 1,25-(OH)₂D₃³ binds intracellularly to a receptor, and subsequently the 1,25-(OH)₂D₃-receptor complex exerts its effect on gene transcription. The 1,25-(OH)₂D₃ receptor has been demonstrated in several normal tissues including the intestine, bone, kidney, parathyroidea, skin, pancreas, spleen, brain, ovary, testes, breast, and hematopoietic cells (1–3). Much attention has focused on the ubiquitous distribution of the 1,25-(OH)₂D₃ receptor. In addition to its presence in normal tissue, the 1,25-(OH)₂D₃ receptor has been demonstrated in several malignant cell lines (4–8). Cell lines originating from malignant melanoma (9), breast carcinoma (10), osteogenic sarcoma (11), and acute myelogenous leukemia (12–14) may be inhibited in growth by the addition of 1,25-(OH)₂D₃. Acute myelogenous leukemia (M1, HL-60 cells) may also differentiate to monocyte-macrophage-like cells upon 1,25-(OH)₂D₃ administration to the cell culture (12–14). Addition of 1,25-(OH)₂D₃ to HL-60 cells reduces the expression of *c-myc* oncogene (15–17). Recently the 1,25-(OH)₂D₃ receptor was demonstrated in a cell line established from a pulmonary metastasis of a human renal cell carcinoma, and it was found that 1,25-(OH)₂D₃ induced an inhibitory effect on cell growth in the cell culture (18). Several groups have demonstrated 1,25-(OH)₂D₃ receptor in tissue specimens from human breast carcinoma (19–24). There is no report of 1,25-

(OH)₂D₃ receptor content in tissue specimens from other human tumors, except some few from a pilot study of various tumor samples, including three renal cell carcinomas, two of which contained threshold values of the 1,25-(OH)₂D₃ receptor (23). To investigate a possible role of 1,25-(OH)₂D₃ in renal cell carcinoma, we have studied the frequency of the 1,25-(OH)₂D₃ receptor in renal cell carcinoma and compared the findings to those in autologous normal kidney tissue.

MATERIALS AND METHODS

Tissue Samples. The material was derived from twenty-three patients aged 38–88 years (mean, 61 years), who were operated on for primary renal cell carcinoma. During surgery samples were taken from both tumor tissue and the remaining normal cortex of the kidney tissue, immediately frozen on liquid nitrogen, and stored at -70°C . The tumors were renal cell carcinoma confirmed by histology (courtesy of Department of Pathology, the Gades Institute, University of Bergen). No patient had signs of major renal impairment, and all patients had normal serum creatinine levels except on patient whose value was 150 μM (upper normal range = 120 μM).

Preparation of Cytosol. The tissue was homogenized by an Ultra-Turrax homogenizer (Janke & Kunkel, Ika-Werk, Staufen, West Germany) in a hypertonic TED buffer (10 mM TRIS, 1 mM EDTA, pH 7.4), with 0.3 M KCl, 10 mM Na₂MoO₄, and 1000 kallikrein inhibitor units/ml Trasylol (Bayer, Leverkusen, Germany). All buffers used throughout the study contained 2 mM dithiothreitol (Bio-Rad Laboratories, Richmond, CA). The amount of buffer was 10.0 ml/g tissue (wet weight) in binding assays and DNA-cellulose chromatography, and 5.0 ml/g tissue (wet weight) in sucrose density gradient analysis. To extract the receptor from the nucleus, the homogenate was allowed to stand for 30 min at 4°C before centrifugation at $180,000 \times g$ for 60 min at 4°C (Beckman L-8 55) (25). The cytosols used were free from lipids and always kept at $0-4^{\circ}\text{C}$ during analysis, except as otherwise indicated. The protein concentration was estimated by the Coomassie blue dye method using bovine serum albumin as standard (26).

Binding Assay. Two hundred μl cytosol were added to each of seven tubes (except in 9 of 46 assays where less cytosol was available), containing 20 μl of [³H]1,25-(OH)₂D₃ (176–180 Ci/mmol; Amersham International, Buckinghamshire, England) giving 40–646 pM final concentration of ligand. It was incubated on ice for 16 h. To estimate nonspecifically bound [³H]1,25-(OH)₂D₃, parallel incubations contained 200-fold more unlabeled 1,25-(OH)₂D₃ (F. Hoffman-LaRoche and Co., Ltd., Basel, Switzerland). One h prior to the termination of the incubations, 1 μM (final concentration) of nonradioactive 25-hydroxyvitamin D in 2 μl ethanol was added to all tubes which effectively displaced 1,25-(OH)₂D₃ from vitamin D binding protein (27). Bound and free ligand were separated by the hydroxylapatite method (25, 28). Specific binding was considered as the difference between total and nonspecific bound [³H]1,25-(OH)₂D₃. The nonspecific binding of the cytosols was always less than 1.7% of the total added 1,25-(OH)₂D₃, when measured at the highest dose of ligand used in the binding assays. As a control for the normal and tumor cytosol binding assays, aliquots of cytosol from a normal human kidney tissue sample were incubated with [³H]1,25-(OH)₂D₃ (final concentration, 646 pM) in duplicate in the absence or presence of 200-fold excess unlabeled ligand. The variation coefficient of the specific binding of the one point 1,25-(OH)₂D₃ receptor control was 5.7% ($n = 13$).

Sucrose Density Gradient Analysis. Three hundred μl of cytosol were incubated with [³H]1,25-(OH)₂D₃ (1291 pM) in 30 μl of ethanol for 1 h at 24°C . To remove free ligand the incubation mixtures were transferred

Received 8/5/87; revised 12/29/87; accepted 1/29/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by the Norwegian Cancer Society, Norwegian Research Council for Science and the Humanities, the legacies of the Family Blix, and Astrid and Edvard Riisøen.

² Research fellow of the Norwegian Cancer Society. To whom requests for reprints should be addressed.

³ The abbreviations used are: 1,25-(OH)₂D₃, 1- α ,25-dihydroxyvitamin D₃; [³H]1,25-(OH)₂D₃, 1- α ,25-dihydroxy[26,27-methyl-³H]vitamin D₃.

to tubes containing preformed pellets of 400 μ l dextran and albumin coated charcoal (29). After centrifugation 200 μ l supernatant were layered on the top of a 5–20% sucrose gradient and spun at 280,000 \times g for 15 h at 4°C. The tubes were harvested from the bottom, and fractions of eight drops were counted for radioactivity. Sedimentation markers, [¹⁴C]bovalbumin (3.7S) and [¹⁴C]bovine serum albumin (4.4S) (Amersham International) were run in parallel gradients.

DNA-Cellulose Chromatography. Calf thymus DNA was coupled to cellulose (Cellex-N-1; Bio-Rad) (30). Five hundred μ l "high-salt cytosol" were incubated with 676 pM of [³H]1,25-(OH)₂D₃ in 25 μ l ethanol for 3 h at 4°C. A parallel incubation with 200-fold excess unlabeled 1,25-(OH)₂D₃ was also performed. Incubated cytosol was diluted with TED to a final KCl concentration of 50 mM and applied to a small DNA-cellulose column. Free ligand was washed out by 10 ml of 0.3% Triton X-100 in a TED buffer followed by 5 ml of the TED buffer. The receptor was eluted by a 20-ml linear gradient of 0–0.4 M KCl in TED buffer. Fractions of 10 drops were collected and counted for radioactivity. The KCl concentration was determined by analyzing the concentration of Cl⁻ on a Corning Chloride Analyzer 925 (Corning Limited, Halstead, Essex, England).

Statistical analysis was done by the Wilcoxon signed rank test.

RESULTS

Twenty-three samples from renal cell carcinomas and neighboring autologous normal kidney tissue were investigated for 1,25-(OH)₂D₃ receptor content. Scatchard plot analysis (31) of the specific binding in renal cell carcinoma and normal kidney cytosols yielded a straight line (Fig. 1), indicating a 1,25-(OH)₂D₃ specific receptor with high affinity and low capacity. The results are shown in Table 1.

Nineteen of 23 (83%) had measurable 1,25-(OH)₂D₃ receptor content defined as levels above 1 fmol/mg protein, and 15 of 23 (65%) had 1,25-(OH)₂D₃ receptor concentration above 5 fmol/mg protein. In paired samples, none of the carcinomas

Table 1 1,25-(OH)₂D₃ receptor concentration and dissociation constant

Cytosol from 23 primary renal cell carcinomas and autologous normal kidney tissue was incubated with [³H]1,25-(OH)₂D₃ in the presence or absence of 200-fold nonradioactive hormone. Bound and free ligand was separated by the hydroxylapatite method. Scatchard plot analysis was used to generate the receptor concentration and the dissociation constant of the specific binding.

Samples	Concentration, fmol/mg protein (range)	Dissociation constant pM (range)
Normal kidney tissue	23.1 \pm 11.2 ^a (6.6–53.7)	200 \pm 58 (108–292)
Renal cell carcinoma	8.2 \pm 6.9 ^b (0–27.8)	131 \pm 61 ^{c, d} (40–316)

^a Mean \pm SD.

^b $P < 0.001$.

^c $P < 0.01$.

^d Number = 19.

had receptor levels higher than the autologous normal kidney tissue. The 1,25-(OH)₂D₃ receptor concentration as compared to mg of protein and the equilibrium dissociation constant for 1,25-(OH)₂D₃ binding to receptor in the cytosols were all significantly lower in the renal cell carcinomas than in the autologous normal kidney tissues ($P < 0.001$ and < 0.01 , respectively, two-tailed tests). The protein concentrations of the cytosols from the normal kidney tissues were 2.0–5.9 mg/ml (mean, 4.3 mg/ml; SD = 1.1), and from the renal cell carcinomas 1.7–6.4 mg/ml (mean, 4.1 mg/ml; SD = 1.2) except in one heavily bloodstained cytosol, excluded in the mean, where the concentration was 15.8 mg/ml. There was no significant difference in the protein concentration between the cytosols from renal cell carcinoma and autologous normal kidney. The 95% confidence interval of the differences between the protein concentrations was –0.75–0.80 mg/ml (using Walsh averages), $P = 0.88$.

In sucrose density gradient analysis, the 1,25-(OH)₂D₃ receptor from tumor cytosol sedimented to 3.7S (Fig. 2A). The peak representing the specific binding was totally abolished by adding 200-fold more nonradioactive 1,25-(OH)₂D₃. To further characterize the 1,25-(OH)₂D₃ receptor from renal cell carcinoma, diluted cytosol incubated with [³H]1,25-(OH)₂D₃ was applied on a DNA-cellulose column (Fig. 2B). The 1,25-(OH)₂D₃ receptor eluted as a single peak at 0.15 M KCl when a linear gradient of KCl in a TED buffer was applied to the column. Cytosol incubated with [³H]1,25-(OH)₂D₃ in the presence of 200-fold more unlabeled ligand did not exhibit any peak. The control experiment with a cytosol from normal kidney tissue showed that normal 1,25-(OH)₂D₃ receptor eluted at the same concentration of KCl as receptor from renal cell carcinoma, indicating similar DNA-binding properties.

No correlation between 1,25-(OH)₂D₃ receptor levels and postsurgical primary tumor-regional node-distant metastasis staging or other clinical parameters was found, whether the 1,25-(OH)₂D₃ receptor levels were related to tissue weight, mg protein of cytosol, or ratio between tumor and normal kidney tissue 1,25-(OH)₂D₃ receptor levels.

DISCUSSION

In a previous pilot study, it was found that two of three renal cell carcinomas examined contained threshold concentrations (defined as 0.3 fmol/mg protein) of the 1,25-(OH)₂D₃ receptor (23). Our study confirms and extends the demonstration of 1,25-(OH)₂D₃ receptor in renal cell carcinoma. The frequency and the concentration of 1,25-(OH)₂D₃ receptor in renal cell carcinoma were comparable to that previously reported for breast carcinoma in which 75 and 41% of the tumors had receptor concentration above 1 and 8 fmol/mg protein, respectively (24). Several studies have demonstrated the 1,25-(OH)₂D₃ receptor in breast carcinoma. No correlations between the 1,25-

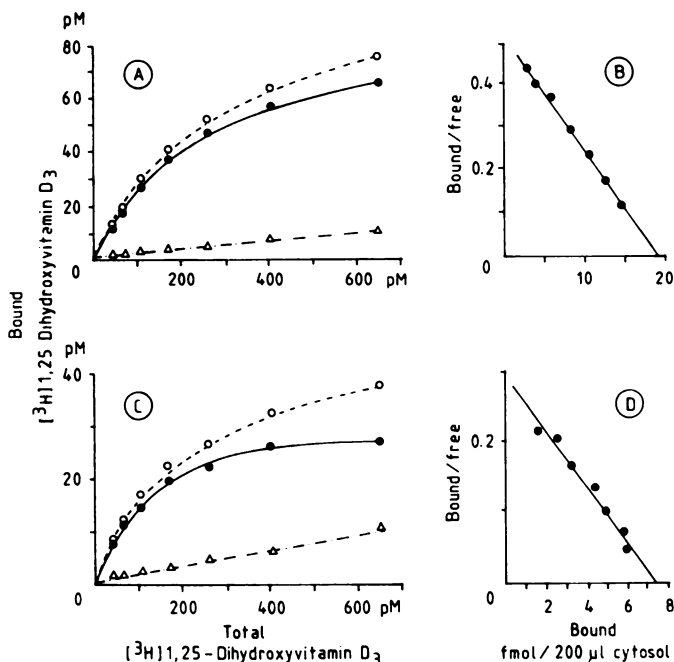
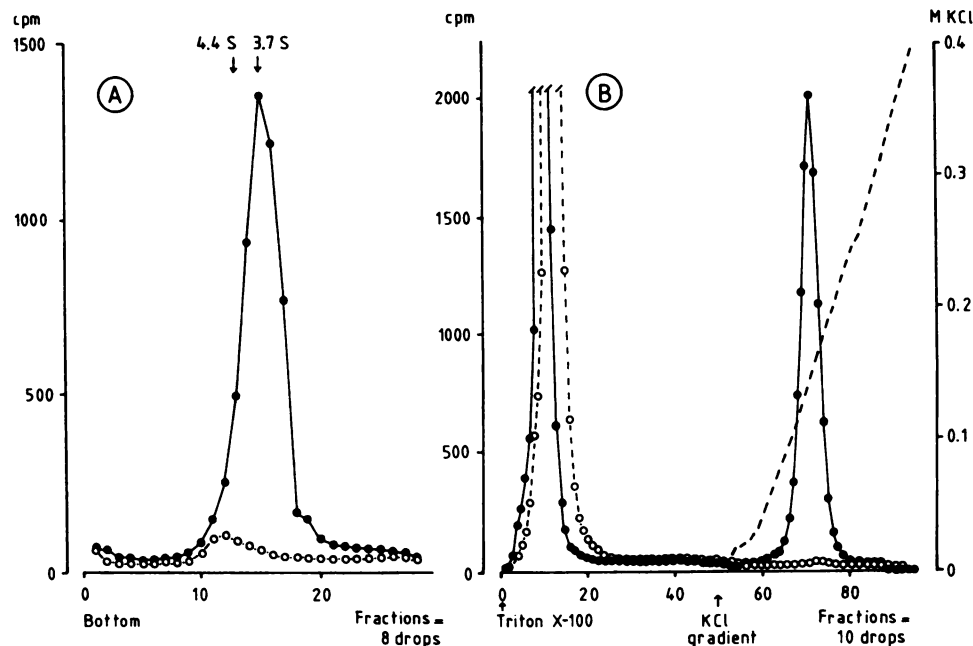


Fig. 1. Saturation binding assay for autologous normal human kidney tissue (A) and renal cell carcinoma (C) in one patient. Cytosol was incubated with increasing amounts of [³H]1,25-(OH)₂D₃ in the absence or presence of 200-fold excess nonradioactive ligand. Specific bound 1,25-(OH)₂D₃ (●) was considered as the difference between total (○) and nonspecific (Δ) bound ligand. Scatchard plot analysis indicated a high affinity receptor binding with low capacity in the normal human kidney tissue (B) ($N_{max} = 18$ fmol/mg protein; $K_d = 170$ pM), and in the renal cell carcinoma (D) ($N_{max} = 6$ fmol/mg protein and $K_d = 120$ pM).

Fig. 2. *A*, Sucrose density gradient analysis, 5–20%, of [³H]1,25-(OH)₂D₃ binding in cytosol prepared from renal cell carcinoma. The cytosol was incubated with [³H]1,25-(OH)₂D₃ in the absence (●) or presence (○) of 200-fold excess nonradioactive ligand for 1 h at 24°C. Free ligand was removed by albumin and dextran coated charcoal supernatant layered on top of the gradient and centrifuged for 15 h at 280,000 × *g*. Bovine serum albumin (4.4S) and ovalbumin (3.7S), used as markers, were run in parallel gradients. *B*, DNA-cellulose chromatography. Cytosol from renal cell carcinoma was incubated with [³H]1,25-(OH)₂D₃ (●) on ice for 3 h, diluted to 50 mM KCl final concentration, and applied to a DNA-cellulose column. A parallel incubation (○) contained 200-fold cold 1,25-(OH)₂D₃ in excess of radiolabeled ligand. Triton X-100 was used to wash out free ligand. 1,25-Vitamin D receptor was eluted by using a 20-ml linear KCl gradient (—).



(OH)₂D₃ receptor content and clinical or biochemical parameters have been reported (21–24), except that Eisman *et al.* (32) recently found a possible correlation between the 1,25-(OH)₂D₃ receptor content in the primary tumors and the late development of lymph node metastases. Our study of a relatively small series of patients did not exhibit any correlation with clinical parameters. The 1,25-(OH)₂D₃ receptor demonstrated in the renal cell carcinoma had biochemical properties indistinguishable from the receptor present in normal human tissue as demonstrated by sucrose density gradient centrifugation and DNA-cellulose chromatography.

The renal cell carcinomas had significantly lower 1,25-(OH)₂D₃ receptor concentration than their normal counterparts. The lower 1,25-(OH)₂D₃ receptor concentration may be due to the lack of differentiation or altered functional capabilities of the malignant transformed renal cell. However, there are several other factors which may influence the receptor concentration in the tumors, such as variation in the receptor content from cell to cell, contribution of noncellular proteins, heterogeneity of the tumor tissue, and extent of necrosis. Furthermore, DNA content, as analyzed by flow cytometry, shows heterogeneity in regard to ploidy in the same tumor (33). Therefore, to get a representative tissue sample, either a large sample or multiple samples from renal cell carcinoma should be examined.

The kidney is considered to be the main organ in the regulation of the vitamin D metabolism and is certainly a target organ for 1,25-(OH)₂D₃. The localization of the 1,25-(OH)₂D₃ receptor has mainly been found in the distal nephron, including the cortical and medullary thick ascending loop of Henle, the distal convoluted tubules, and to some extent in the glomeruli (34–38). By using defined single nephron segments the 1,25-(OH)₂D₃ receptor has also been demonstrated in the proximal nephron (39). The topographical localization of the 1,25-(OH)₂D₃ receptor in the kidney accords with the functions of 1,25-(OH)₂D₃ in handling calcium and phosphorous and in regulating the enzymes 25-hydroxyvitamin D-1 α -hydroxylase and 25-hydroxyvitamin D-24-hydroxylase.

In recent years it has been demonstrated that the 1,25-(OH)₂D₃ receptor also may have a function in the differentiation

of normal cells. Thus it has been shown that 1,25-(OH)₂D₃ may play a role in the differentiation of skin during fetal and neonatal periods (40) and in modulating the differentiation of hematopoietic cells (3). In addition, studies of malignant human cell cultures *in vitro*, including one cell line obtained from renal cell carcinoma metastasis, indicate that inhibition of cell growth could be induced by addition of 1,25-(OH)₂D₃ in physiological to supraphysiological doses to the media (9–14, 18). The effects of 1,25-(OH)₂D₃ is thought to be receptor mediated (1–3, 11, 14, 18, 41). It has also been shown that the 1,25-(OH)₂D₃ receptor may be lost during differentiation of a colonic carcinoma cell line (42). Therefore, the presence of the 1,25-(OH)₂D₃ receptor in renal cell carcinoma or other tumors may indicate that the tumors can be influenced by 1,25-(OH)₂D₃. On the other hand, the receptor may be a bystander passive marker of renal tubular cell origin without functional capabilities.

Other steroid receptors have been demonstrated in renal cell carcinoma. Thus, estrogen, progesterone, androgen, and glucocorticoid hormone receptors have been found with different frequencies (43–45). In a recent study, the dihydrotestosterone receptor was present in all renal cell carcinoma samples examined (20 of 20) and in 13 of 14 normal renal parenchyma samples (45). Testosterone receptor was found in slightly fewer samples, and estrogen receptor was present in low frequencies in both normal and renal cell carcinoma tissue. The progesterone receptor was found in 40 and 30% of normal and carcinomatous tissue, respectively. It was of interest that a significantly higher level of the dihydrotestosterone receptor was found in high-stage compared to low-stage tumors. Inhibition of tumor growth by progesterone, androgen, antiandrogen, and antiestrogen therapy has been reported. However, critical assessment seems to indicate a possible effect of these hormones on tumor growth in less than 2–6% of the treated patients (44).

Whether the active metabolite of vitamin D or analogues may influence renal cell carcinoma *in vivo*, and whether the presence of 1,25-(OH)₂D₃ receptor may have any prognostic implication for patients, remains to be seen. However, this demonstration of the presence of the 1,25-(OH)₂D₃ receptor in samples from renal cell carcinoma, and the ability of the receptor to bind to DNA, should encourage further exploration of

the effect of vitamin D on tumors of the kidney, the main vitamin D regulating organ.

ACKNOWLEDGMENTS

We thank Ole Didrik Laerum for a critical review of the article.

REFERENCES

- Haussler, M. R., Donaldson, C. A., Kelly, D. J., Mangelsdorf, D. J., Marion, S. L., and Pike, J. W. Functions and mechanism of action of the 1,25-dihydroxyvitamin D₃ receptor. *In*: A. W. Norman, K. Schaefer, H.-G. Grigoleit, and D. v. Herrath (eds.), *Vitamin D. Chemical, Biochemical and Clinical Update*, pp. 83–92. Berlin: Walter de Gruyter, 1985.
- Pike, J. W. Intracellular receptors mediate the biologic action of 1,25-dihydroxyvitamin D₃. *Nutr. Rev.*, **43**: 161–168, 1985.
- Suda, T., Miyaura, C., Abe, E., and Kuroki, T. Modulation of cell differentiation, immune responses and tumor promotion by vitamin D compounds. *Bone Miner. Res.*, **4**: 1–48, 1986.
- Eisman, J. A., Martin, T. J., MacIntyre, I., and Moseley, J. M. 1,25-Dihydroxyvitamin-D receptor in breast cancer cells. *Lancet*, **2**: 1335–1336, 1979.
- Manolagas, S. C., Haussler, M. R., and Deftos, L. J. 1,25-Dihydroxyvitamin D₃ receptor-like macromolecule in rat osteogenic sarcoma cell lines. *J. Biol. Chem.*, **255**: 4414–4417, 1980.
- Freake, H. C., Spanos, E., Eisman, J. A., Galasko, C. S. B., Martin, T. J., and MacIntyre, I. Specific binding of 1,25-dihydroxyvitamin D₃ in the VX₂ carcinoma. *Biochem. Biophys. Res. Commun.*, **97**: 1505–1511, 1980.
- Colston, K., Colston, M. J., Fieldsteel, A. H., and Feldman, D. 1,25-Dihydroxyvitamin D₃ receptors in human epithelial cancer cell lines. *Cancer Res.*, **42**: 856–859, 1982.
- Framton, R. J., Suva, L. J., Eisman, J. A., Findley, D. M., Moore, G. E., Moseley, J. M., and Martin, T. J. Presence of 1,25-dihydroxyvitamin D₃ receptors in established human cancer cell lines in culture. *Cancer Res.*, **42**: 1116–1119, 1982.
- Colston, K., Colston, M. J., and Feldman, D. 1,25-Dihydroxyvitamin D₃ and malignant melanoma: the presence of receptors and inhibition of cell growth in culture. *Endocrinology*, **108**: 1083–1086, 1981.
- Framton, R. J., Omond, S. A., and Eisman, J. A. Inhibition of human cancer cell growth by 1,25-dihydroxyvitamin D₃ metabolites. *Cancer Res.*, **43**: 4443–4447, 1983.
- Dokoh, S., Donaldson, C. A., and Haussler, M. R. Influence of 1,25-dihydroxyvitamin D₃ on cultured osteogenic sarcoma cells: correlation with the 1,25-dihydroxyvitamin D₃ receptor. *Cancer Res.*, **44**: 2103–2109, 1984.
- Abe, E., Miyaura, C., Sakagami, H., Takeda, M., Konno, K., Yamazaki, T., Yoshiki, S., and Suda, T. Differentiation of mouse myeloid leukemia cells induced by 1 α ,25-dihydroxyvitamin D₃. *Proc. Natl. Acad. Sci. USA*, **78**: 4990–4994, 1981.
- Miyaura, C., Abe, E., Kuribayashi, T., Tanaka, H., Konno, K., Nishii, Y., and Suda, T. 1 α ,25-Dihydroxyvitamin D₃ induces differentiation of human myeloid leukemia cells. *Biochem. Biophys. Res. Commun.*, **102**: 937–943, 1981.
- Tanaka, H., Abe, E., Miyaura, C., Kuribayashi, T., Konno, K., Nishii, Y., and Suda, T. 1,25-Dihydroxycholecalciferol and a human myeloid leukemia cell line (HL-60): the presence of a cytosol receptor and induction of differentiation. *Biochem. J.*, **204**: 713–719, 1982.
- Reitsma, P. H., Rothberg, P. G., Astrin, S. M., Trial, J., Bar-Shavit, Z., Hall, A., Teitelbaum, S. L., and Kahn, A. J. Regulation of *myc* gene expression in HL-60 leukemia cells by a vitamin D metabolite. *Nature (Lond.)*, **306**: 492–494, 1983.
- Matsui, T., Takahashi, R., Mihara, K., Nakagawa, T., Koizumi, T., Nakao, Y., Sugiyama, T., and Fujita, T. Cooperative regulation of *c-myc* expression in differentiation of human promyelocytic leukemia induced by recombinant gamma-interferon and 1,25-dihydroxyvitamin D₃. *Cancer Res.*, **45**: 4366–4371, 1985.
- Brelvi, Z. S., and Studzinski, G. P. Inhibition of DNA synthesis by an inducer of differentiation of leukemic cells, 1 α ,25-dihydroxyvitamin D₃, precedes downregulation of the *c-myc* gene. *J. Cell. Physiol.*, **128**: 171–179, 1986.
- Nagakura, K., Abe, E., Suda, T., Hayakawa, M., Nakamura, H., and Tazaki, H. Inhibitory effect of 1 α ,25-dihydroxyvitamin D₃ on the growth of the renal carcinoma cell line. *Kidney Int.*, **29**: 834–840, 1986.
- Eisman, J. A., MacIntyre, I., Martin, T. J., Framton, R. J., and King, R. J. B. Normal and malignant breast tissue is a target organ for 1,25-(OH)₂ vitamin D₃. *Clin. Endocrinol.*, **13**: 267–272, 1980.
- Christakos, S., Sori, A., Greenstein, M. S., and Murphy, T. F. Sucrose density gradient analysis of 1,25-dihydroxyvitamin D₃ binding in human breast tumors. *J. Clin. Endocrinol. Metab.*, **56**: 686–691, 1983.
- Ulmann, A., Bami, M., Corcos, D., Bader, C., Delarue, J. C., and Contesso, G. Systematic search for 1 α ,25-dihydroxyvitamin D₃ receptors in human breast carcinomas. *Biomed. Pharmacother.*, **38**: 204–208, 1984.
- Guillemand, S., Eurin, J., and Spyrtos, F. Study of 1,25-(OH)₂D₃ receptors in human primary breast cancers using polyacrylamide gel electrophoresis (PAGE). *In*: A. W. Norman, K. Schaefer, H.-G. Grigoleit, and D. v. Herrath (eds.), *Vitamin D. Chemical, Biochemical and Clinical Update*, pp. 897–900. Berlin: Walter de Gruyter, 1985.
- Eisman, J. A., Suva, L. J., Sher, E., Pearce, P. J., Funder, J. W., and Martin, T. J. Frequency of 1,25-dihydroxyvitamin D₃ receptor in human breast cancer. *Cancer Res.*, **41**: 5121–5124, 1981.
- Freake, H. C., Abeyasekera, G., Iwasaki, J., Marcocci, C., MacIntyre, I., McClelland, R. A., Skilton, R. A., Easton, D. F., and Coombes, R. C. Measurement of 1,25-dihydroxyvitamin D₃ receptors in breast cancer and their relationship to biochemical and clinical indices. *Cancer Res.*, **44**: 1677–1681, 1984.
- Levy, F. O., Eikvar, L., Jutte, N. H. P. M., Frøysa, A., Tvermyr, S. M., and Hansson, V. Properties and compartmentalization of the testicular receptor for 1,25-dihydroxyvitamin D₃. *J. Steroid Biochem.*, **22**: 453–460, 1985.
- Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**: 248–254, 1976.
- Levy, F. O., Eikvar, L., Frøysa, A., and Hansson, V. "Differential displacement"—a simple method to study receptors for 1,25-dihydroxyvitamin D₃ in the presence of contaminating serum vitamin D binding protein. *Biochem. Biophys. Res. Commun.*, **130**: 1020–1026, 1985.
- Weckslar, W. R., and Norman, A. W. An hydroxylapatite batch assay for the quantitation of 1 α ,25-dihydroxyvitamin D₃-receptor complexes. *Anal. Biochem.*, **92**: 314–323, 1979.
- Aksnes, L. Quantitation of the main metabolites of vitamin D in a single serum sample. II. Determination by UV-absorption and competitive protein binding assays. *Clin. Chim. Acta*, **104**: 147–159, 1980.
- Moss, L. G., Moore, J. P., and Chan, L. A simple, efficient method for coupling DNA to cellulose. *J. Biol. Chem.*, **256**: 12655–12658, 1981.
- Scatchard, G. The attraction of proteins for small molecules and ions. *Ann. N. Y. Acad. Sci.*, **51**: 660–671, 1949.
- Eisman, J. A., Suva, L. J., and Martin, T. J. Significance of 1,25-dihydroxyvitamin D₃ receptor in primary breast cancers. *Cancer Res.*, **46**: 5406–5408, 1986.
- Ljungberg, B., Stenling, R., and Roos, G. DNA content in renal cell carcinoma with reference to tumor heterogeneity. *Cancer (Phila.)*, **56**: 503–508, 1985.
- Chandler, J. S., Pike, J. W., and Haussler, M. R. 1,25-Dihydroxyvitamin D₃ receptors in rat kidney cytosol. *Biochem. Biophys. Res. Commun.*, **90**: 1057–1063, 1979.
- Colston, K. W., and Feldman, D. Demonstration of a 1,25-dihydroxycholecalciferol cytoplasmic receptor-like binder in mouse kidney. *J. Clin. Endocrinol. Metab.*, **49**: 798–800, 1979.
- Simpson, R. U., Franceschi, R. T., and DeLuca, H. F. Characterization of a specific, high affinity binding macromolecule for 1 α ,25-dihydroxyvitamin D₃ in cultured chick kidney cells. *J. Biol. Chem.*, **255**: 10160–10166, 1980.
- Christacos, S., and Norman, A. W. Studies of the mode of action of calciferol. XXIX. Biochemical characterization of 1,25-dihydroxyvitamin D₃ receptors in chick pancreas and kidney cytosol. *Endocrinology*, **108**: 140–149, 1981.
- Stumpf, W. E., Sar, M., Narbaitz, R., Reid, F. A., DeLuca, H. F., and Tanaka, Y. Cellular and subcellular localization of 1,25-(OH)₂-vitamin D₃ in rat kidney: comparison with localization of parathyroid hormone and estradiol. *Proc. Natl. Acad. Sci. USA*, **77**: 1149–1153, 1980.
- Kawashima, H., and Kurokawa, K. Metabolism and site of action of vitamin D in the kidney. *Kidney Int.*, **29**: 98–107, 1986.
- Kuroki, T. Possible function of 1 α ,25-dihydroxyvitamin D₃, an active form of vitamin D₃, in the differentiation and development of skin. *J. Invest. Dermatol.*, **84**: 459–460, 1985.
- Mangelsdorf, D. J., Koeffler, H. P., Donaldson, C. A., Pike, J. W., and Haussler, M. R. 1,25-Dihydroxyvitamin D₃-induced differentiation in a human promyelocytic leukemia cell line (HL-60): receptor-mediated maturation to macrophage-like cells. *J. Cell Biol.*, **98**: 391–398, 1984.
- Brehier, A., Perret, C., and Thomasset, M. Human colon cell line HT-29: disappearance of 1,25-dihydroxyvitamin D₃ receptors during differentiation. *In*: D. V. Cohn, T. J. Martin, and P. J. Meunier (eds.), *Calcium Regulation and Bone Metabolism. Basic and Clinical Aspects*, Vol. 9, pp. 512–516. Amsterdam: Excerpta Medica, 1987.
- Jacmst, D. Steroid receptors and renal cell carcinoma. *Prog. Surg.*, **17**: 6–14, 1983.
- Ahmed, T., Benedetto, P., Yagoda, A., Watson, R. C., Scher, H. I., Herr, H. W., Sogani, P. C., Whitmore, W. F., and Pertschuk, L. Estrogen, progesterone, and androgen-binding sites in renal cell carcinoma. Observation obtained in phase II trial of flutamide. *Cancer (Phila.)*, **54**: 477–481, 1984.
- Noronha, R. F. X., and Rao, B. R. Increased dihydrotestosterone receptor levels in high-stage renal adenocarcinoma. *Cancer (Phila.)*, **56**: 134–137, 1985.