Vol. 93, No. 1, 1980
March 13, 1980

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 9-15

1,25-DIHYDROXYVITAMIN D_3 RECEPTOR IN A CULTURED HUMAN BREAST CANCER CELL LINE (MCF 7 CELLS)

J.A. Eisman, T.J. Martin, I. MacIntyre, R.J. Frampton
J.M. Moseley and R. Whitehead*

Department of Medicine, University of Melbourne
Repatriation General Hospital, Heidelberg, Victoria
Australia, and Department of Immunology*, Cancer
Institute, Melbourne

Received November 27, 1979

SUMMARY: MCF 7, a cultured human breast cancer cell line, is known to resorb bone independently of osteoclast action. In this report MCF 7 cells are shown to possess a receptor for 1,25-dihydroxyvitamin D_3 with a low capacity (8,000 sites/cell) and high affinity (Kd 1.1 x 10^{-11} M), and which sediments at 3.7S in sucrose density gradients. These characteristics and the relative affinity of other vitamin D and non-vitamin D steroids are very similar to those found for the well characterised chicken intestinal 1,25-$(OH)_2D$ receptor.

INTRODUCTION: Human breast cancer cells frequently establish as secondary growths in bone, causing dissolution of adjacent bone matrix. The breast cancer cell line, MCF 7 (1,2), which has been used extensively as a model system for the study of oestrogen, androgen and glucocorticoid receptors in human breast cancer (3-5), has been shown to cause resorption of devitalized bone (6). Since this would suggest that these cells possess an osteoclast-like activity, we considered the possibility that the cells might also have the capacity to respond to a steroid hormone which stimulates normal bone resorption. We report here that the MCF 7 cancer cell line possesses a specific, high affinity cytosol receptor for 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$).

0006-291X/80/050009-07$01.00/0

METHODS:

Preparation of cytosol: MCF 7 cells, obtained from Dr. C. McGrath, Michigan Cancer Foundation, were cultured to confluence in RPMI-1640 medium containing 10% foetal calf serum. For the twenty four hours before experiments, cells were grown in medium containing foetal calf serum which had been extracted with dextran-coated charcoal to remove steroid hormones (2). The $1,25\text{-}(OH)_2D$ level in the extracted serum determined by the method of Eisman et al. (7,8) was 14 pmol/l compared with the unextracted level of 104 pmol/l. Cells were rinsed twice with cold phosphate buffered saline and scraped into 1 ml per million cells of 0.05M potassium phosphate buffer pH 7.25 (buffer A). This buffer, which contained 0.15M potassium chloride, 0.002M EGTA, 0.002M dithiothreitol, 0.1% gelatin and 1000 KIU/ml of Trasylol, has been found to improve the stability of the chicken intestinal $1,25\text{-}(OH)_2D$ receptor (E. Sher & J.A. Eisman, unpublished observations). The cell suspension was sonicated for 60 seconds at 20 KHz and 50 Watts with a Rapidis sonicator (Australian Ultrasonics Ltd, Victoria) and then centrifuged for 30 minutes at 300,000 g max in the SW 50.1 head of a Beckman L5-75 ultracentrifuge. This high speed supernatant (cytosol) was usually studied immediately but on occasions was frozen at $-70^{\circ}C$ with no evidence of deterioration over some weeks. Each large culture flask ($100\,cm^2$) contained approximately 10^7 cells at confluence. Variations in this number were corrected with respect to the DNA content of the high speed pellet (9).

Sucrose Density Gradients: Sucrose density gradients were made in pH 7.4 buffer A from which Trasylol and gelatin had been omitted. Discontinuous gradients consisting of 0.7l ml each of 20%, 16%, 8% and 4% sucrose were poured at least 6 hours before use (10). Cytosol (0.25 ml) was incubated with 0.04-0.08 pmol $[23,24\text{-}^3H]\text{-}1,25\text{-}(OH)_2\,D_3$ of 110 or 82 Ci/mmole specific activity (The Radiochemical Centre, Amersham) and various additions for one hour at $25^{\circ}C$. Unbound radioactivity was removed by mixing each incubation with 0.1 ml of dextran-coated charcoal (0.2% dextran T-70 and 2% charcoal). After centrifugation at 2000 g for 15 mins at $4^{\circ}C$, 0.2 ml of the supernatant was applied to the preformed sucrose density gradients and centrifuged at 300,000 g max for 16-17 hours at $4^{\circ}C$ in the SW 50.1 rotor of a Beckman L5-75 ultracentrifuge. After centrifugation each gradient was fractionated into 0.1 ml portions from the bottom, and counted in a Packard Tri-Carb liquid scintillation counter, using Instagel as scintillant.

Binding Studies: 1 ml of cytosol was incubated with 0.04 pmol of $[23,24\text{-}^3H]\text{-}1,25\text{-}(OH)_2\,D_3$ of 82 or 110 Ci/mmol and varying concentrations of vitamin D_3 metabolites and other steroid hormones as indicated for 2 hours at $25^{\circ}C$. The incubations were cooled

to 4°C and 1.5 ml of 40% polyethylene glycol 4000 was added to each tube. After vigorous mixing by vortex, the tubes were centrifuged at 2000 g for 1 hour and the supernatant was discarded. The pellet was dissolved with 0.3-0.5 ml of Soluene 350 overnight at room temperature and counted with 4-8 ml of Dimilume scintillant.

At times Scatchard analysis of binding studies were performed using varying amounts of ^{3}H-1,25-(OH)$_2$D$_3$ from 0.005-0.05 pmol. Nonspecific binding tubes were identical except for the addition of 0.1 nmol of unlabelled 1,25-(OH)$_2$D$_3$. Incubations and determination of bound radioactivity were carried out as above.

RESULTS:

Initial experiments demonstrated a significant specific binding of ^{3}H-1,25-(OH)$_2$D$_3$ to a binding protein in the cytosol. In more detailed studies (Fig. 1) 25-(OH)-D$_3$ and 24,25-(OH)$_2$D$_3$ competed for the same binding site with much lower affinities, approximately 1/1000 and 1/4000 on a molar basis respectively. Furthermore oestradiol, progesterone, testosterone, dihydrotestosterone and cortisol were ineffective competitors at concentrations of up to 10^{-6}M. Scatchard analysis of the binding studies (Fig. 2) indicated a Kd of 1.1 x 10^{-11}M and approximately

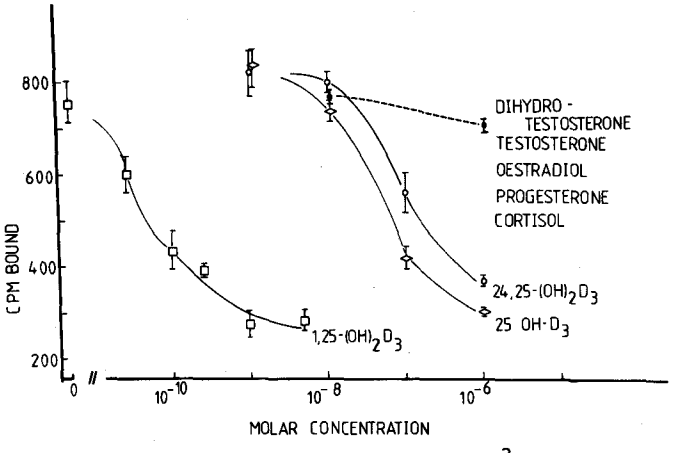

Fig. 1. Competitive binding curves of ^{3}H-1,25-(OH)$_2$D$_3$ and various vitamin D and non-vitamin D steroids for the MCF 7 cytosolic receptor.

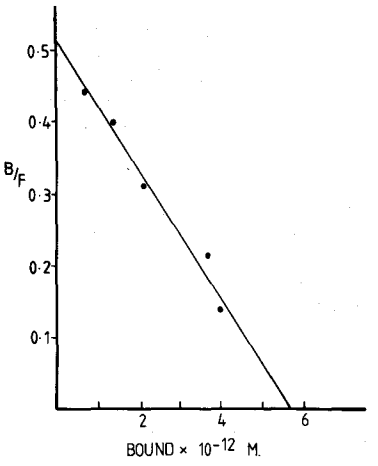

Fig. 2. Scatchard analysis of the binding of ^{3}H-1,25-$(OH)_2D_3$ to the MCF 7 cytosolic receptor.

8,000 binding sites per cell. Sucrose density gradient analysis of the binding protein revealed that the specific binding material sedimented at 3.7S and co-sedimented with the well characterised 1,25-$(OH)_2D$ receptor protein from chicken duodenal cytosol (Fig. 3). The 25-OH-D binding protein from serum, which contaminates all cytosol preparations, sedimented faster than the 1,25-$(OH)_2D$ binding protein and was quite distinct from it. Furthermore when cytosol was incubated with ^{3}H-1,25-$(OH)_2D_3$ and a 200 fold molar excess of unlabelled 1,25-$(OH)_2D_3$ there were no residual binding in the 1,25-$(OH)_2D_3$ binding region. However a 200 fold molar excess of 25-OH-D caused minimal reduction in ^{3}H-1,25-$(OH)_2D_3$ binding.

DISCUSSION:

The properties of this cytosol binding factor are very similar to those of the well characterised intestinal receptor (10-13) for 1,25-$(OH)_2D_3$, in that it is of high affinity and specificity, and on ultracentrifugal analysis has an approximate

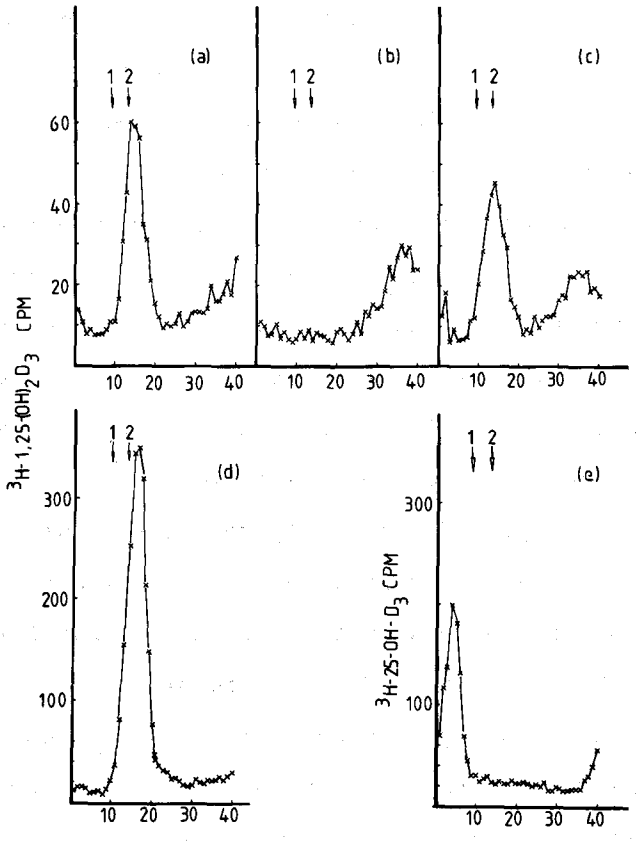

Fig. 3. Sucrose density gradient analysis of receptor binding. MCF 7 cytosol incubated with ^{3}H-1,25-(OH)$_2$D$_3$ and (a) no addition, (b) unlabelled 1,25-(OH)$_2$D$_3$ and (c) unlabelled 25-OH D$_3$. Chicken intestinal cytosol incubated with (a) ^{3}H-1,25-(OH)$_2$D$_3$ and (b) ^{3}H-25-OH D$_3$. 1 and 2 indicate sedimentation positions of bovine serum albumin and ovalbumin standards.

size of 3.7S, similar to that found for the chick intestinal receptor, and smaller than that for the receptor for 25-OH D. The relative affinities of 25-OH D$_3$ and 24,25-(OH)$_2$D$_3$ for binding are the same as those found in intestinal cells. A variety of other steroid hormones do not possess significant affinity for this receptor.

This is the first demonstration of a cultured cell line which possesses a 1,25-(OH)$_2$D receptor, and it is important in a number of respects.

First, it indicates that a tumor which is capable of directly mobilising bone, has at least a crucial part of the machinery which will allow it to respond to a calcium-mobilising hormone. Second, it indicates a new pathway of hormonal effect on human breast cancer cells, distinct from the well studied androgen, oestrogen and glucocorticoid receptors. Hence it clearly will be important to determine the spectrum of action of 1,25-$(OH)_2D$ on the tumor cell, and to evaluate its effect on the biology of the tumor. Third, it provides the only convenient, simple *in vitro* system of human origin in which to study biochemical events in the action of 1,25-$(OH)_2D_3$ and the properties of its receptor.

It is not yet known whether this receptor for 1,25-$(OH)_2$ in MCF 7 cells reflects an abnormality of the malignant cell, or is an expression of a normal property of the breast glandular cell. Preliminary data from rabbit breast tissue indicate that the latter is the case and this tissue is being investigated further.

ACKNOWLEDGEMENTS: This work was supported by grants from the National Health and Medical Research Council of Australia, the Victorian Anti Cancer Council and the Department of Veterans' Affairs.

REFERENCES:

1. Brooks, S.C., Locke, E.R. and Soule, H.D. (1973), J. Biol. Chem. 248, 6251-6253.
2. Lippman, M.E. and Bolan, D. (1975), Nature 256, 592-593.
3. Horwitz, K.B. and McGuire, W.L. (1975), Steroids 25, 497-505.
4. Goral, J.E. and Wittliff, J.L. (1975), Biochemistry 14, 2944-2952.
5. Bruckovsky, N., Sutherland, D.J.A., Meakin, J.W. and Minesita, T. (1975), Biochim. Biophys. Acta 381, 61-71.

6. Eilon, G. and Mundy, G.R. (1978), Nature 276, 726-728.
7. Eisman, J.A., Hamstra, A.J., Kream, B.E. and DeLuca, H.F. (1976), Arch. Biochem. Biophys. 176, 236-243.
8. Eisman, J.A., Hamstra, A.J., Kream, B.E. and DeLuca, H.F. (1976), Science 193, 1021-1023.
9. Setaro, F. and Morley, C.G.D. (1976), Analyt. Biochem. 71, 313-317.
10. Kream, B.E., Reynolds, R.R., Knutson, J.C., Eisman, J.A. and DeLuca, H.F. (1976), Arch. Biochem. Biophys. 176, 779-787.
11. Eisman, J.A. and DeLuca, H.F. (1977), Steroids 30, 245-257.
12. Proscal, D.A., Okamura, W.H. and Norman, A.W. (1975), J. Biol. Chem. 250, 8382-8388.
13. Wecksler, W.R., Mason, R.S. and Norman, A.W. (1979), J. Clin. Endocrinol. Metab. 48, 715-717.