RECEPTORS FOR 1,25(OH)$_2$-VITAMIN D$_3$ ENRICHED IN CLONED OSTEOBLAST-LIKE RAT OSTEOGENIC SARCOMA CELLS

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1. Introduction

Bone resorption is stimulated by parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D$_3$ (1,25-(OH)$_2$D$_3$) [1,2]. Cyclic adenosine 3′5′-monophosphate (cAMP) is implicated in this action ot PTH [3,4], although not in that of 1,25-(OH)$_2$D$_3$. Although the osteoclast is the major bone resorbing cell, studies with isolated bone cells point increasingly to the fact that the effects of PTH on cAMP production in bone result from actions upon osteoblasts rather than osteoclasts [5–9]. A transplantable osteogenic sarcoma in the rat, which has been shown to consist of cells with the features of osteoblasts [10–13] has retained for several years a PTH-responsive adenylate cyclase and high cellular level of alkaline phosphatase [11,14,15]. Here we report the development of clones of this osteogenic sarcoma, which have an active adenylate cyclase response to PTH and high alkaline phosphatase activity, and are enriched in a cytosol receptor for the bone resorbing steroid hormone, 1,25-(OH)$_2$D$_3$.

2. Materials and methods

The osteogenic sarcoma was propagated and cultured as in [9,10]. Single cell suspensions were prepared from adherent monolayer primary cultures by harvesting with pronase (0.01% Calbiochem) in phosphate-buffered saline and 0.02% EDTA for 10 min at room temperature. After washing, the cells were plated at 200 viable cells/35 mm dish in a double layer nutrient agar system [16]. The dishes were incubated for 14 days at 37°C in sealed plastic boxes gassed with 7% oxygen, 10% CO_2, 83% nitrogen, this low oxygen tension having been found to promote better colony growth. The cloning efficiency in this system ranged from 4–7.5%. Individual clones were picked up using sterile micropipettes and were reseeded into alpha modification of Eagle's medium with 20% fetal calf serum (Flow Labs.) and chlortetracycline (50 μg/ml) in 25 cm^2 plastic culture flasks (Costar). When adherence and spreading occurred, the cells were harvested and reseeded into 25 cm^2 flasks. Cultures were refed weekly until the cells reached 90% confluency, when they were subcultured.

Alkaline and acid phosphatase (EC 3.1.3.1 and EC 3.1.3.2, respectively) activities in lysed cells [8] and adenylate cyclase (EC 4.6.1.1) activity in homogenates of cultured cells [8,14] were assayed as described. Sources of parathyroid hormone, PGE_2, calcitonin and vitamin D metabolites have been reported [8,17].

For studies of 1,25-(OH)$_2$D$_3$ binding, cells at or near confluence were grown for at least 24 h in Eagle's medium containing 10^{-6} M cortisol and fetal calf serum (5%) which had been treated with dextran-coated charcoal to remove steroid hormones [17,18]. Cytosol preparations were prepared freshly as in [17] for cultured human breast cancer cells except that 0.4 M KCl was included in the extraction buffer. Sucrose gradient analysis was carried out on 4–20% sucrose gradients [17].

3. Results

From >20 clones grown originally in agar, 4

Table 1

Clone	Alkaline phosphatase (μmol . h^{-1} . mg protein^{-1})	Acid phosphatase	Adenylate cyclase (pmol cAMP . min^{-1} . mg protein^{-1})				1,25-(OH)$_2$D$_3$ receptor properties	
			Basal	Salmon calcitonin (10^{-7} M)	Parathyroid hormone (10^{-7} M)	PGE_2 (10^{-5} M)	Dissoc. const. (K_d) ($\times 10^{-11}$ M)	Receptor content (R_C) (fmol . mg protein^{-1})
UMR 104	59.05	4.29	2.1 ± 0.10	2.4 ± 0.10	3.4 ± 0.1[a]	3.4 ± 0.12[b]	1.7	69
UMR 105	71.11	5.93	8.4 ± 0.34	6.4 ± 0.24	14.2 ± 0.3[b]	12.2 ± 0.12[b]	1.3	38
UMR 106	185.48	6.44	1.9 ± 0.19	1.7 ± 0.12	23.8 ± 0.8[b]	6.7 ± 0.40[b]	1.1	54
UMR 108	100.69	4.83	5.9 ± 0.16	6.8 ± 0.25	21.2 ± 0.3[b]	14.0 ± 0.60[b]	1.3	43

The phosphatase activities are the means of duplicate measurements. Adenylate cyclase values are means ± SEM of triplicate measurements ([a]$p < 0.01$ vs basal; [b]$p < 0.005$) 1,25-(OH)$_2$D$_3$ receptor data is calculated from experiment of fig.2

(designated UMR 104, 105, 106, 108) were examined in detail. The enzyme activities in uncloned osteogenic sarcoma cell cultures up to the third passage were 26.45 ± 1.59 (alkaline phosphatase, 35 expt) and 3.04 ± 0.27 (acid phosphatase, 10 expt) μmol . h^{-1} . mg $protein^{-1}$ in experiments carried out at regular intervals over 2 years. The results in table 1 indicate that the clonal lines are enriched in alkaline phosphatase activity relative to the parent cultured cells. These are maintained during propagation in culture, as also are the relative activities in the different clones (data not shown). All clonal lines retain PTH-responsive adenylate cyclase, and in clone UMR 106 this is enhanced, with a 12-fold increase in activity in response to a maximally activating concentration of hormone.

In preliminary experiments with uncloned cells it was possible to demonstrate specific binding of ^{3}H-labelled 1,25-$(OH)_2D_3$ in cytosol preparations. However the large amounts of protein required to obtain sufficient bound radioactivity resulted in increased non-specific binding. Under these circumstances it is possible that erroneous estimates of receptor concentration and affinity are obtained [19]. In several clones there was substantial enrichment in the cytosol receptor for 1,25-$(OH)_2D_3$, which allowed competition curves and Scatchard analysis to be carried out using appropriately small amounts of cytosol protein. The cloned osteogenic sarcoma cell lines contained specific receptors for 1,25-$(OH)_2D_3$ (fig.1). Similar data was obtained with all 4 clones. Scatchard analysis of the 4 clones indicated a single class of high affinity receptors (fig.2) with dissociation constants and receptor concentrations (table 1) similar to those found with cultured human breast cancer cells [17]. Ultracentrifugal analysis (fig.3) revealed that the cytosol receptor was a 3.5 S macromolecule, as found for the receptor in human breast cancer [17] and rachitic chicken intestine [20].

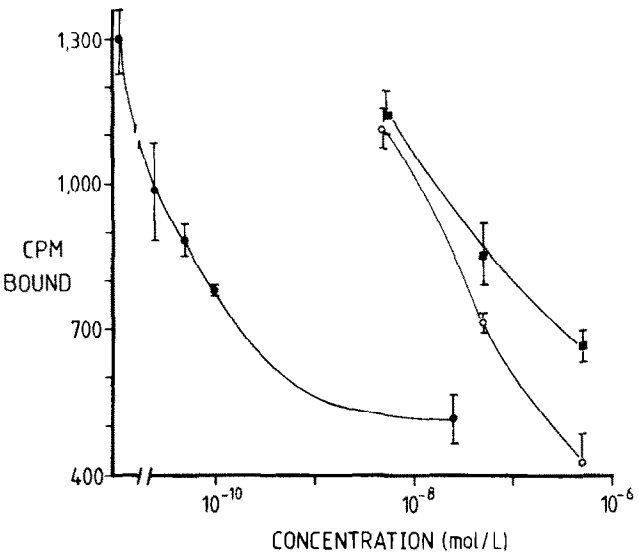

Fig.1. Competitive binding analysis of ^{3}H-labelled 1,25-$(OH)_2D_3$ binding in cytosol from UMR 108. Competing vitamin D metabolites; 1,25-$(OH)_2D_3$ (•), 25-OH-D_3 (○) and 24,25-$(OH)_2D_3$ (▪). Values are mean ± SEM of triplicate determinations. Testosterone, dihydrotestosterone, oestradiol, progesterone, cortisol, dexamethasone and aldosterone at $\leqslant 10^{-4}$ M did not compete with ^{3}H-labelled 1,25-$(OH)_2D_3$ binding.

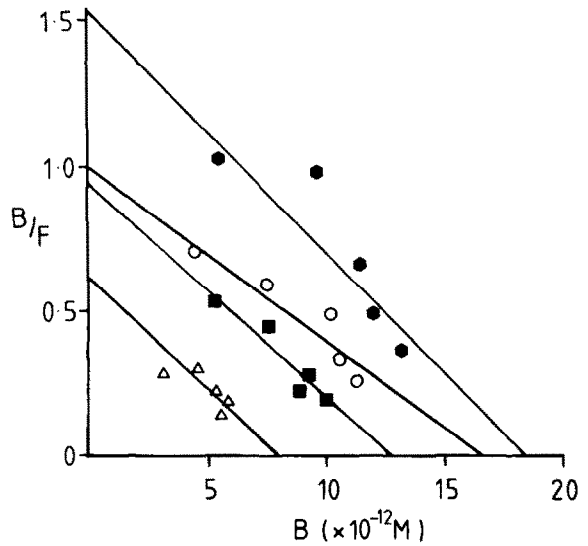

Fig.2. Scatchard analysis of 1,25-$(OH)_2D_3$ receptor binding in cloned cell lines. UMR 104 (○), UMR 105 (△) UMR 106 (●) and UMF 108 (▪).

4. Discussion

The malignant tumour from which these 4 clones were derived has been characterized extensively as osteoblast in nature [8,10–13]. Furthermore it is clear from the present data that the cloned cells are targets for the bone resorbing hormones, PTH and 1,25-$(OH)_2D_3$. The only available data on their action

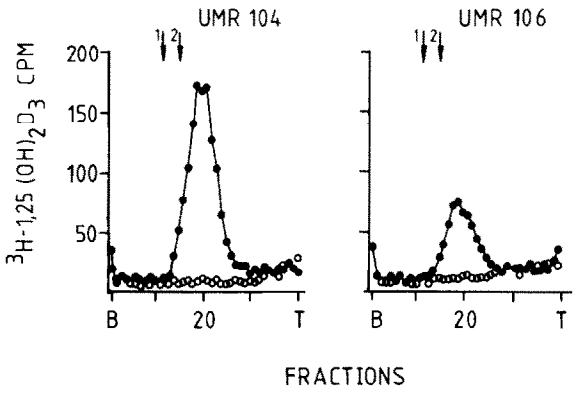

Fig.3. Sucrose density gradient analysis of ^{3}H-labelled 1,25-$(OH)_2D_3$ binding in two of the cloned cell lines. ^{3}H-labelled 1,25-$(OH)_2D_3$ binding in the presence (○) and absence (●) of 160-fold molar excess of unlabelled 1,25-$(OH)_2D_3$ are shown. Arrows indicate sedimentation positions of bovine serum albumin (1) and ovalbumin (2).

in normal osteoblast-rich populations indicates that both hormones decrease alkaline phosphatase, citrate decarboxylation and collagen synthesis [21]. However none of these effects can be definitely related to the stimulation of bone resorption by the hormones. It will be of considerable interest to define the nature of the actions of these hormones on the cloned osteoblast-like cell populations, and to determine whether this relates in any way to physiological bone resorption.

A receptor for 1,25-$(OH)_2D_3$ has been reported in cytosol prepared from bone [22]. In view of the stimulatory action of 1,25-$(OH)_2D_3$ on osteoclastic bone resorption, the osteoclast might be expected to have a 1,25-$(OH)_2D_3$ receptor. This has yet to be demonstrated, but these results establish that osteoblast-like cells possess such a receptor. Furthermore although cAMP is suggested as an intermediate in PTH stimulation of bone resorption, only osteoblastic bone cells have been demonstrated to respond to PTH with a cAMP increase and activation of cAMP-dependent protein kinase [9]. There is no evidence that osteoclast cAMP is changed by PTH. These data provide support for the hypothesis that the osteoblast might act as a transducer of bone resorbing stimuli to the osteoclast [8], besides providing a convenient target cell in which to study molecular mechanisms of 1,25-$(OH)_2D_3$ action.

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