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Alkaline Phosphatase as a Marker of Osteoinductive Cells

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Summary. Epithelial cells with osteoinductive potential (KB and WISH cell lines, transitional epithelium of several species) are rich in alkaline phosphatase activity. In contrast, cells devoid of osteoinductive ability are low in this enzyme activity. However, there were no differences between the two classes of cells with respect to acid phosphatase activity.

Key words: Bone induction — Alkaline phosphatase.

Ectopic cartilage and bone formation can be induced by a variety of means, e.g., by the transplantation of some epithelial cells of different origin: transitional epithelium of urinary tract, human amnion (K, FL, WISH established cell lines) and neoplastic cell lines (HeLa, Hep-2, HT-40, KB), or virally transformed fibroblasts (CLV-4, CLV-14, CLV-X, CLV-J_3, CLV-Var) [1–6]. Other epithelial cell lines (GMK, Vero, T-24) and fibroblastic primary cultures and established cell lines (L-929, 3T3, LNSV) are devoid of osteoinductive properties [7, 8].

The reason some cells are able to induce heterotopic osteogenesis while others are not is unknown as yet, but the similarity of the osteoinduction produced by grafting various epithelial cells obtained from different sources (amnion, urinary bladder, virus-transformed cells) suggests a possible identical mechanism for the induction of cartilage and bone differentiation of responding host cells. Consequently, one would expect that cells with osteoinductive properties may share some property other than their epithelial origin. Attempts to distinguish osteoinducing cells from nonosteoinducing ones by characterization of their receptors for lectins were not conclusive. No correlation was found between the presence of these receptors and the osteoinductive properties. However, among cell lines with osteoinductive potency, all of them exhibited receptors for Concanavalin A [5, 9].

Alkaline phosphatase was detected histochemically in the transitional epithelium of guinea pig [1, 10]. In a search for a biochemical marker of cells with osteoinductive potential we investigated whether this enzyme is present in osteoinductive cells other than the transitional epithelium of guinea pig. In addition, the acid phosphatase activity was examined in both categories of cells. We report here the positive correlation of alkaline phosphatase activity of cells with their osteoinductive potency.

Material and Methods

Cells

Alkaline and acid phosphatase activity was estimated in homogenates of following cells: KB—human oral epidermoid carcinoma cell line; WISH—human amnion cell line; uroepithelium of guinea pigs, rats, and mice; MK_2—monkey kidney cell line; HF-1510—human fibroblast cell line; 3T3—Swiss mouse embryo cell line; rat ameloblast epithelial cell line (provided by Dr. J. L. Christensen, Arhus, Denmark; M-MSV sarcoma cell line derived from Balb/C mouse sarcoma produced by Moloney sarcoma virus inoculation; cells from 2–7 *in vitro* passage; HT-29—human tumor epithelial cell line; murine bone marrow stromal cells cultured *in vitro* for 19–21 days.

All cell cultures were grown in RPMI 1640 medium supplemented with 10% calf serum (GIBCO) and antibiotics. Transitional epithelium of guinea pigs, rats, and mice were obtained either by trypsin digestion of inverted urinary bladders, or by

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stripping off an epithelial layer of mucosa by means of a scalpel. Isolated cells from 5–30 bladders were pooled and assayed.

Cultured cells were isolated by trypsin digestion. Cells were washed three times in cold saline. Cells were homogenized in ice-cold buffer containing 0.15 M NaCl and 0.003 M $NaHCO_3$ with a Polytron homogenizer [11]. Homogenates were centrifuged at 3000 rpm for 30 minutes at 4°C, aliquots of the supernatant were assayed for alkaline and acid phosphatase [12], and the protein content in the aliquots was determined according to Lowry et al. [13], using bovine serum albumin as standard.

Phosphatase

Enzyme activities were determined as described earlier [11, 12] using p-nitrophenyl phosphate as the substrate at 9.3 pH for alkaline and 5.0 pH for acid phosphatase. The optical density of the reaction product was read at 400 nm. One unit of enzyme activity was defined as the amount of enzyme required to hydrolyze 1.0 mole of the substrate during 30 minutes incubation at 37°C. The results were expressed as units per milligram of protein.

The osteoinductive properties of KB, WISH, and transitional epithelium of guinea pig, rat, and mice, and the lack of such properties by 3T3 cells were reported earlier [1, 5–7, 14]. In the present experiment, osteoinductive potency of MK_2, HT-29, HF-1510, M-MSV sarcoma cells, rat ameloblast established cell line, and murine bone marrow stromal cells were examined.

These cells were grafted into thigh muscles of Balb/C mice given 5 mg of cortisone acetate as an immunosuppressant. Such doses of cortisone do not interfere with heterotopic bone formation by epithelial cells [6, 7, 9, 14, 15]. Sites of cell grafting were examined histologically 8–28 days later for the presence of induced cartilage or bone. The mean values of enzyme activity were calculated for each cell type.

Results

The results of intramuscular implantation of cell lines into cortisone-treated mice are presented in Table 1. No bone induction was obtained when MK_2, HT-29, HF-1510, rat ameloblasts, M-MSV sarcoma, or mouse bone marrow stromal cells were implanted.

Table 1. Evaluation of selected cell lines for their osteoinductive potential in the thigh muscles of cortisone-treated Balb/C mice

Cells	Duration of implantation (days)	Implants	Bone induction
HF-1510	15	8	0
MK_2	15	8	0
HT-29	18	6	0
Rat ameloblasts	8–17	18	0
M-MSV sarcoma	10–28	32	0
Marrow stromal cells	14	8	0

Table 2. Alkaline and acid phosphatase activity of cells with and without osteoinductive potency

Cells	Number	Phosphatase activity (U/mg protein)	
		Alkaline mean ± SE	Acid mean ± SE
	Osteoinductive cells		
KB	8	2.85 ± 0.15	0.90 ± 0.06
WISH	27	4.34 ± 0.53	1.36 ± 0.09
Uroepithelium			
Guinea pig	8	0.68 ± 0.17	0.57 ± 0.11
Rat	19	2.31 ± 0.41	0.92 ± 0.21
Mouse	32	1.29 ± 0.17	0.85 ± 0.15
	Nonosteoinductive cells		
MK_2	13	0.07 ± 0.02	0.97 ± 0.11
Rat ameloblasts	7	0.09 ± 0.07	1.82 ± 0.25
HT-29	7	0.02 ± 0.01	1.17 ± 0.16
HF-1510	11	0.04 ± 0.01	0.54 ± 0.08
3T3	20	0.01 ± 0.01	0.77 ± 0.07
M-MSV sarcoma	8	0.04 ± 0.02	1.73 ± 0.32
Marrow stroma	1	0.08	1.21

Phosphatase Activity

The results of phosphatase isoenzymes activity of cells examined are summarized in Table 2. The group of cells having a potency to induce cartilage and bone (KB, WISH, transitional epithelium) is characterized by high activity of alkaline phosphatase (0.68–4.34 U/mg protein) and lower activity of acid phosphatase (0.57–1.36 U/mg protein). In contrast, nonosteoinductive cells (MK_2, HT-29, HF-1510, 3T3, rat ameloblasts, M-MSV sarcoma grown *in vitro* for 2–7 passages, bone marrow stromal cells) are characterized by extremely low activity of alkaline phosphatase (0.01–0.08 U/mg protein), while the activity of acid phosphatase is as high as in bone-inducing cells (0.54–1.82 U/mg protein).

Discussion

The high alkaline phosphatase activity is a newly found common biochemical feature of osteoinducing cells. The high activity of alkaline phosphatase is not specific for cells of epithelial origin. Nonosteoinductive cells comprised both the epithelial cells (MK_2, HT-29, rat ameloblasts) as well as fibroblastic cells (3T3, HF-1510, marrow stroma). The enzyme activity is not related to cell viability *in vitro*. Cells with low alkaline phosphatase activity *in vitro* are as viable following implantation as are those with high alkaline phosphatase levels.

Moreover, other experiments revealed that the low alkaline phosphatase cell lines remained viable in the host animal for 6–20 days (as evidenced by mitosis and growth), a period during which osteoinduction was evaluated for alkaline phosphatase positive cells.

So far the attempts to distinguish cells capable of inducing ectopic osteogenesis from noninducing ones by the analysis of their surface properties did not give positive correlation between the presence of concanavalin A and/or phytohemagglutinin receptors [5, 8]. However, as all osteoinductive cell lines have concanavalin A receptors, it is likely that cell surface properties are crucial in transmission of osteoinductive signal(s). Data presented in this communication corroborate the concept of the importance of surface contact of living cells with the host mesenchymal cells in the initiation of a chain of events leading to cartilage and bone induction [8]. Alkaline phosphatases are known to be membrane-bound enzymes [16].

Alkaline phosphatases have been implicated in bone formation and calcification [11, 17]. Robison postulated that alkaline phosphatase hydrolyzes hexosemonophosphate and increases the local concentration of calcium phosphate [18]. Fleish and Neuman [19] suggested that this enzyme hydrolyzes polyphosphates that are inhibitory to calcium phosphate nucleation. Alkaline phosphatase appearance precedes foci of heterotopic bone formation [1, 15, 17]. Some authors suggest, however, that although alkaline phosphatase may be important for the process of calcification, it is probably not a critical factor [20]. However, others deny a role for this enzyme in calcification [4].

Acid phosphatase is a lysosomal enzyme. This enzyme was found in normal bones and teeth [18] and in bone developed heterotopically by implantation of lyophilized dentin [22] or bone matrix [21]. Elevated activity of acid phosphatase in later phases of bone induction is connected with the process of bone resorption and remodeling [21]. The *in vitro* activity of acid phosphatase is similar in both inducing and noninducing cells. In conclusion, alkaline phosphatase is a useful marker for osteoinductive cells.

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References

1. Friedenstein AY (1968) Induction of bone tissue by transitional epithelium. Clin Orthop Rel Res 59:21–37
2. Hancox NM, Wlodarski K (1972) The role of host site in bone induction by transplanted xenogeneic epithelial cells. Calcif Tissue Res 8:258–261
3. Huggins CB (1931) The formation of bone under the influence of epithelium of the urinary tract. Arch Surg 22:377–408
4. Strates BS, Firschein HE, Urist MR (1971) Alkaline phosphatase and failure of calcification under the influence of diphosphonate. Biochem Biophys Acta 244:121–124
5. Wlodarski K, Ostrowski K (1979) Further investigations on possible correlation between agglutinability and osteoinductive properties of established cell lines. Arch Immunol Ther Exp (Warsz) 27:113–119
6. Wlodarski K, Poltorak A, Koziorowska J (1971) Species specificity of osteogenesis induced by WISH cell line and bone induction by vaccinia virus-transformed human fibroblasts. Calcif Tissue Res 7:345–352
7. Wlodarski K (1969) The inductive properties of epithelial established cell lines. Exptl Cell Res 57:446–448
8. Wlodarski K, Ostrowski K, Chlopkiewiez B, Koziorowska J (1974) Correlation between the agglutinability of living cells by concanavalin A and their ability to induce cartilage and bone formation. Calcif Tissue Res 16:251–255
9. Wlodarski K, Ostrowski K (1975) Investigations on the properties of established cell lines of human and animal origin to induce cartilage and bone and properties of human fibroblasts transformed by varicella virus. Materia Medica Polona 7:3–7
10. Kagawa S (1965) Enzyme histochemistry of bone induction by urinary bladder epithelium. J Histochem Cytochem 13:255–264
11. Reddi AH, Huggins CB (1972) Biochemical sequences in the transformation of normal fibroblasts in adolescent rats. Proc Nat Acad Sci USA 69:1601–1605
12. Reddi AH, Sullivan NE (1980) Matrix-induced endochondral bone differentiation: influence of hypophysectomy, growth hormone and thyroid-stimulating hormone. Endocrinology 107:1291–1299
13. Lowry OH, Rosebrough MJ, Farr AL, Randall RT (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275
14. Zaleski M, Moskalewski S (1963) Investigation on autologous transplants of urinary bladder wall in mouse. Bull Acad Pol Sci (Biol) 11:403–406
15. Ostrowski K, Wlodarski K (1971) Induction of heterotopic bone formation. In: Bourne GH (ed) Biochemistry and physiology of bone, vol. 3, 2nd ed. Academic Press, New York, London, pp 299–336
16. Dowben RM (1969) Biological membranes. Little, Brown and Co., Boston
17. Jaffe NR (1976) Alkaline phosphatase activity, characterization, and subcellular distribution during initial skeletogenesis in the prenatal rat limb. Calcif Tissue Res 21:153–162
18. Robison R (1923) The possible significance of hexosephosphoric esters in ossification. Biochem J 17:286–293
19. Fleisch H, Neuman WF (1961) Mechanism of calcification: role of collagen, polyphosphate and phosphatase. Am J Physiol 200:1296–1300
20. Nijweide PJ, Kawilarang de Haas EWM, Wassenaar AM (1981) Alkaline phosphatase and calcification, correlated or not? Metab Bone Dis Res Res 3:61–66
21. Rath NC, Hand AR, Reddi AH (1981) Activity and distribution of lysosomal enzymes during collagenous-matrix in-

duced cartilage, bone and bone marrow development. Dev Biol 85:89–98

22. Nilsen R, Magnusson BC (1981) Enzyme histochemical studies of acid phosphatase isoenzyme in induced heterotopic bone formation in guinea pig. Scand J Dent Res 89:485–490
23. Wlodarski K, Poltorak A, Zaleski M, Ostrowski K (1971) Bone induction evoked in mouse by xenogeneic grafts of the transitional epithelium. Experientia 27:688–689
24. Toverud SU, Hammarstrom LE, Kristoffersen UM (1975) Quantitative studies on acid phosphatase in developing rat bones and teeth during hypervitaminosis D. Arch Oral Biol 20:175–181

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