EXPERIENTIA

Volume 41/No. 11 Pages 1357–1490 15 November 1985

Reviews

Alkaline phosphatase isozymes in cultured human cancer cells

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Summary. Alkaline phosphatase, an ubiquitous enzyme is known to exist in several isozymic forms. At least three different isozymes have now been identified in humans. Alkaline phosphatase isozymes are among the substances synthesized ectopically by a variety of human tumors and many continuous cell lines derived from different cancers have retained the capacity to produce these membrane-located glycoproteins. This paper reviews the identification of alkaline phosphatase isozymes in cultured tumor cells and relates these findings with recent developments concerning these cell membrane located glycoproteins.
Key words. Alkaline phosphatase; isozymes; tumor cell lines; cultured cells; HeLa cells.

Introduction

Alkaline phosphatase (EC 3.1.3.1) is the trivial name for a family of isozymes with similar catalytic properties that hydrolyze a large variety of phosphomonoesters at a rather high pH, usually pH 8 to 10.7. The enzyme is widely distributed in nature and is found in plants, algae, bacteria, protozoa, invertebrates and vertebrates. Irrespective of source, alkaline phosphatases are metalloenzymes with zinc being an integral part of the molecule. The native enzyme has four zinc atoms, of which two seem to be associated with catalytic activity and the other pair with structural integrity. In similarity to other hydrolases, serine is present at the active site. Most mammalian enzymes contain relatively large amounts of carbohydrates, especially sialic acid. However, the adult intestinal enzyme is devoid of the latter. Inorganic phosphate appears to be an integral part of the enzyme. Most alkaline phosphatases are dimeric with mol. wts ranging from 70,000 to 180,000 (for details see McComb et al.[48]). Hydrolysis of phosphomonoesters proceeds via cleavage of O–P bonds with an activation energy of 6000–10,000 cal/mol following first order and zero-order kinetics. The enzyme reaction takes place in two steps: a) following formation of an enzyme-substrate (Michaelis) complex, the hydrolysis of the substrate yields phosphorylenzyme and product and b) the phosphorylenzyme is subsequently split into enzyme plus inorganic phosphate. The K_m value is dependent on the assay conditions employed, usually increasing with increasing pH and substrate concentration. The pH optimum is dependent on substrate and buffer concentrations. In addition to water, a variety of aminoalcohols (often used as buffers) act as phosphate acceptors. Thus, alkaline phosphatase can also be considered to act as a transphosphorylase[17,48].

In mammals, alkaline phosphatase is found in almost all tissues and organs, with liver, bone, kidney, intestine and placenta exhibiting the greatest activities[48]. Based on studies with the enzyme from these and other sources it is now well established that in humans at least three separate structural gene loci determine the protein portion of the various forms of alkaline phosphatase. One locus codes for the term-placental form, at least one (and possible two[51]) code(s) for the intestinal (fetal and adult) forms and one codes for the liver/bone/kidney or 'tissue unspecific' form[49,52,65]. Operationally these isozymes can be easily distinguished from each other by several parameters[70], including thermostability, inhibition by specific inhibitors, electrophoretic mobility and immunologic properties (table 1).

Many human cancers make substances not normally produced by the tissue from which the tumor originates. Among the ectopically produced substances are the various isozymic forms of alkaline phosphatase[42]. A variety of tumor-derived continuous cell lines have retained the capacity to produce these membrane-associated glycoproteins (for examples see table 2). This has permitted their use for in vitro studies of factors controlling alkaline phosphatase expression independent of the complex organismal influences of animal models[30]. In this communication we will present a short overview of the identification of the various alkaline phosphatase isozymes in cultured human tumor cells and shall relate these findings to some recent developments in this area.

Alkaline phosphatase in cultured cells. Alkaline phosphatase activity in cultured tissues or cells has been the subject of investigations for over 50 years. The first report on this topic is probably represented by the pioneering work of Fell and Robinson[16] on the relationship of enzyme activity and in vitro bone formation in chick embryo femur. Since then the field has expanded considerably as witnessed by the ever-increasing number of publications. A computer-aided literature search indicates that 46 arti-

Table 1. Some characteristics of the main human alkaline phosphatase isozymes

	Liver/bone/kidney (tissue unspecific)	Intestinal	Term-placental
Thermostability (56 °C, 20 min, pH 10.6)	–	–	+
Inhibitor sensitivity*:			
L-Phenylalanine	≫ 50.0	3.0	4.5
L-Homoarginine	3.7	≫ 50.0	≫ 50.0
L-Phe-Gly-Gly	≫ 50.0	14.0	0.5
Levamisole	0.09	11.0	2.0
1-Bromotetramisole	0.01	3.0	0.6
Electrophoretic mobility[70]	Fast	Slow	Intermediate (polymorphic)
Reaction with antisera to:			
Liver/bone/kidney isozyme	+	–	–
Intestinal isozyme	–	+	(–)
Term-placental isozyme	–	(–)	+
N-terminal sequence[70]	Leu-Val-Phe	Phe-Ile-Pro	Ile-Ile-Pro-Val
Molecular weight[70]	136,000–170,000	140,000–170,000	116,000–125,000

*Expressed as inhibitor concentration (in mM) producing 50% inhibition using p-nitrophenol phosphate as substrate in 2-amino-2-methyl-l-propanol-HCl buffer, pH 10.6.

cles appeared between 1971 and 1974; 89 between 1975 and 1979 and 132 between 1979 and 1983.

Although occasional papers on alkaline phosphatase activity in cultured cells, especially as related to the effects of viruses[2,3,43] and its histochemical demonstration[8,11,25,26] appeared prior to 1960, the initial major impetus of the field coincided with the discovery of enzyme induction by phosphate depletion in bacteria[36,74]. It is interesting to note that by the early 1960's it had been determined that alkaline phosphatase is located at the cell membrane[26], that cultured cancer cells were heterogeneous regarding enzyme expression[22,58], that the alkaline phosphatase of HeLa S3 cells was heat-stable[34] and that the specific activity can be altered by experimental manipulations of the cellular microenvironment[13,25,59,60].

Most of the early studies were done with HeLa cells, a continuous line derived from a human uterine cervical carcinoma[24] and with cell lines later indicted as being in fact HeLa[23,54]. In these investigations striking quantitative differences in the specific activity of alkaline phosphatase were noted among different strains of HeLa[13,57] and among cell lines of supposedly noncervical origin[57]. For example, the activity among strains varied as much as 1000-fold[13,57]. By contrast the specific activity of a variety of other enzymes, including β-glucosidase, β-galactosidase, acid phosphatase, catalase, glucose-6-phosphate dehydrogenase, leucylaminopeptidase did not differ[57]. The marked dissimilarities in alkaline phosphatase activity were also noted among clonal strains derived from single cell platings of the parental cell lines[58].

Table 2. Examples of cultured human tumor cells expressing different alkaline phosphatase isozymes

Cells	Origin	Isozymes produced	References
HeLa S3	Cervix	Term-placental	15, 20, 34
T 24	Bladder	Term-placental	31
HT-29	Colon	Intestinal	35
HeLa TCRC-2	(HeLa derivative)*	Liver/bone/kidney	66
78–160	Brain	Liver/bone/kidney	72
LoVo	Colon	Term-placental + intestinal	Herz, unpublished
SW-620	Colon	Term-placental + liver/bone/kidney	32
Detroit-98	(HeLa derivative)*	Intestinal + liver/bone/kidney	6

*See text.

Studies to characterize the enzyme with respect to localization, pH optimum, substrate specificity, K_m and effect of the then known inhibitors showed that, despite very wide differences in specific activity, these properties were similar in all cell lines and their clonal strains[34]. However, studies on the physicochemical properties revealed marked dissimilarities. The alkaline phosphatase of cells with relatively low specific activity (e.g. HeLa S3) was stable during preincubation at 56 °C, while that of cells with high specific activity (e.g. KB, Chang liver) showed an almost complete loss of activity under the same conditions. However, in the case of the latter, there was always a residual activity that withstood preincubation, indicating that these cells have two alkaline phosphatases, one heat-labile, which comprised the major portion and one minor, heat-stable portion[34,59]. By contrast, no thermostable activity was seen in normal fibroblasts[34], thus providing evidence that the culture conditions per se did not stimulate the expression of the heat-stable enzyme. The kinetics of thermal inactivation were compatible with monomolecular reactions, the rate of which was increased in the presence of alkaline buffers[29,34]. Experiments with cell-free mixtures and with partially purified enzyme preparations eliminated the possibility of an inhibitor or stabilizer which might have accounted for the differences in heat stability. Since these characteristics persisted for many generations it was concluded that the differences in enzyme activity and thermostability were genetically determined[34]. The observation that HeLa S3 cells produced a heat-stable alkaline phosphatase was the first instance in which such an enzyme form was identified in cells of human origin. Although multiple alkaline phosphatase forms had been suspected for years[9,12] and multiple electrophoretic forms had been demonstrated[10], the significance of the thermostable enzyme in cultured cancer cells was not understood at the time.

Term-placental alkaline phosphatase

Subsequent studies demonstrated that the human placenta had an alkaline phosphatase that also could be incubated at 56 °C without loss of activity[53]. It is inter-

esting to note that earlier studies[1] had shown that the activity of the enzyme from placentas was highest when the reaction was carried out at 55 °C, but no reference regarding its thermostability was made. The significance of the heat-stable alkaline phosphatase produced by HeLa S3 cells was first appreciated when Fishman et al.[20] discovered the 'Regan' isozyme in the serum and tumor tissues of a patient with bronchogenic carcinoma. The demonstration by a variety of additional criteria that this enzyme form is similar to the alkaline phosphatase of term-placenta and HeLa cells[15,19] provided new directions and meaning to the study of this oncodevelopmental glycoprotein in cancer cells.

Later studies have shown that cell lines derived from tumors of a variety of organs also produce the heat-stable, Regan isozyme. These include, ChaGo, SCH, HCT-8, and T24, derived from chorionic gonadotropin-producing lung carcinoma[47], gastric choriocarcinoma[38], colorectal adenocarcinoma[68] and urinary bladder carcinoma[31], respectively, as well as many others[5,55]. In addition to HeLa, the term-placental isozyme is also expressed by other continuous lines derived from uterine cervix tumors[5,33,41,63]. Production of altered forms of term-placental alkaline phosphatase, including an enzyme resembling the rare D variant (Nagao isozyme) has been reported to occur in certain choriocarcinoma-derived cell lines[56,69] and in an epidermoid cancer line[37].

Liver/bone/kidney alkaline phosphatase in cultured cancer cells

Following the demonstration that the early human placenta has an alkaline phosphatase distinct from the term-placenta[18,64,69], with properties resembling those of the liver/bone/kidney (tissue unspecific)[71] enzyme form, it became evident that some of the 'non-Regan' isozymes described in a variety of human tumors corresponded to this heat-labile, L-phenylalanine-insensitive and L-homoarginine-sensitive enzyme. Production of this isozyme has also been reported in cancer cell lines[32,73]. However, because of the ubiquity of the tissue unspecific alkaline phosphatase in humans[71], caution is indicated before considering it as tumor-derived in the same context as the term-placental isozyme. For instance the alkaline phosphatase of normal colon is of the intestinal type and some colon carcinomas have been shown to produce the tissue unspecific isozyme[50]. Thus, important examples of cultured cancer cells producing the latter are those derived from colon tumors[32,50]. However, it should be emphasized that cancer cell lines producing this isozyme may also synthesize the term-placental form[27,32,34,61].

A clone of HeLa cells (HeLa TCRC-2) expressing the tissue unspecific isozyme monophenotypically has also been identified[66]. The significance of the presence of tissue unspecific alkaline phosphatase in cultured tumor cells derived from tissues normally producing this isozyme[72,73] still remains to be clarified. It should be stressed that normal cells in culture, including fibroblasts[7,75] and epithelial cells (Herz, unpublished) produce the tissue unspecific isozyme. There is controversy regarding the type of alkaline phosphatase synthesized by virally-transformed human fibroblasts. Whereas there are reports indicating that SV40-transformed WI-38 fibroblasts produce the term-placental isozyme[14,39], other investigators could not demonstrate its presence in such cells[46].

Intestinal alkaline phosphatases in cultured cancer cells

With regard to intestinal alkaline phosphatases, these enzyme forms have been identified mostly in clones of HeLa origin[6,40,77] or in lines indicted[54] as being in fact HeLa, including CCL6[44], FL amnion[21], and KB[29,67]. However, two bonafide non-HeLa contaminated cell lines derived from colonic adeno-carcinoma have been shown to produce intestinal alkaline phosphatase, HT-29[35] and LoVo (Herz, unpublished). In view of the findings that colon carcinoma produce the tissue unspecific isozyme[50], the ultimate meaning of these observations remains to be elucidated.

Cultured tumor cells producing two alkaline phosphatases

It has been evident for a long time that certain continuous cancer cell lines and clones derived therefrom produce in addition to heat-stable alkaline phosphatase a heat-labile enzyme[34]. With the use of appropriate identification procedures (e.g. organ specific inhibitors, electrophoretic mobility, antibody reactivity) the heat-labile activity has been characterized as either representing the tissue unspecific or the intestinal isozymes[7,62]. The simultaneous expression of these two forms has been described in the HeLa-derived Detroit-98 and D98/AH-R lines[6]. However, the coincidental production of three human isozymes by one continuous cell line has heretofore not been reported. In FL amnion and KB cells, which produce an intestinal and the term-placental alkaline phosphatases, a 'hybrid' enzyme form has been identified[21,67]. This 'hybrid' shares the characteristics of both isozymes. More recent results have confirmed these observations by isolating from KB cells two nonidentical subunits, one having the same isoelectric point and molecular weight as the term-placental isozyme and the other corresponding to the glycosylated subunit of the adult intestinal enzyme[4]. This mixed subunit structure is also present in the human fetal intestinal alkaline phosphatase[4]. By applying monoclonal antibodies the presence of interlocus heterodimers of intestinal and term-placental isozymes has been demonstrated in extracts of HEp 2/5 cells, a HeLa derivative[76]. These findings, as well as the flow cytometric studies with Chang liver cells[45] and single cell cloning experiments with SW-620 cells[32], are further evidence that the coincidental expression of two different alkaline phosphatase isozymes may occur in a single cell[32,58]. This point is of importance since under the influence of enzyme inducing agents (e.g. glucocorticoids, hyperosmolality, sodium butyrate), the activity of only one isozyme may be augmented while that of the other may not be affected[30], thus indicating that the expression of each isozyme is independently controlled.

Concluding comments

Despite that more than seven decades have elapsed since its discovery[48], alkaline phosphatase remains an enzyme in search of a physiological function(s). Although many functions have been proposed, none has withstood rigorous testing. These include, a role in cellular metabolism

by making available sugar moieties and inorganic phosphate, in embryonic development and differentiation, in (active) transport of specific substances, in calcification, etc.[48].

Because of the uncertainty surrounding the function(s) of alkaline phosphatase no real conclusions can be reached why some human tumors express an isozyme not normally produced by its tissue of origin. It has been suggested that the different isozymes which appear during normal trophoblast development may be re-expressed by neoplastic cells. This notion would support the concept that cancer is a disease of gene regulation, involving loci that are characteristically active in normal fetal or trophoblast development[42]. However, it is not known if the phenotypic expression of these genes in the malignant cell is coincidental or whether their expression is associated with tumor initiation and with maintenance of the neoplastic state[42]. The recent demonstration of trace amounts of term-placental alkaline phosphatase in normal adult testes, lung and cervix would suggest that the expression of this isozyme in tumors derived from these and other tissues may be due to an amplification of a gene locus normally expressed at a low level or to the inordinate clonal proliferation of certain cells that in normal tissue express the isozyme to a much larger extent than other cells[28]. The role played by oncogenes in anomalous alkaline phosphatase expression in tumors remains to be established. The understanding of why and how alterations in a family of cell membrane glycoproteins take place would yield important information on the transformation of normal cells into cancer cells.

1 Ahmed, Z., and King, E.J., Kinetics of placental alkaline phosphatase. Biochim. biophys. Acta *45* (1960) 581–592.

2 Albrecht, W., Mittelstrass, H.K., and Sauthoff, R., Das Verhalten der alkalischen und sauren Phosphatase in Zellkulturen aus Affennieren nach Infektion mit ECHO-Virus Serotyp 9 und Poliovirus hominis Type 1. Z. Naturforsch. *13b* (1958) 71–74.

3 Bachtold, J.G., Deig, F.E., and Gebhardt, L.P., Phosphatase activity in normal and poliovirus-infected tissue cultures. Exp. Cell Res. *18* (1959) 29–44.

4 Behrens, C.M., Enns, C.A., and Sussman, H.H., Characterization of human foetal intestinal alkaline phosphatase. Comparison with the isoenzymes from the adult intestine and human tumour cell lines. Biochem. J. *211* (1983) 553–558.

5 Benham, F.J., Fogh, J., and Harris, H., Alkaline phosphatase expression in human cell lines derived from various malignancies. Int. J. Cancer *27* (1981) 637–644.

6 Benham, F.J., and Harris, H., Human cell lines expressing intestinal alkaline phosphatase. Proc. natn. Acad. Sci. USA *76* (1979) 4016–4019.

7 Benham, F.J., Povey, M.S., and Harris, H., Heterogeneity of alkaline phosphatases in different HeLa cells. Som. Cell Genet. *4* (1978) 13–25.

8 Bimes, C., Planel, H., and David, J.F., Recherches histochimiques sur la souche cancéreuse HeLa. C. r. Soc. Biol. *153* (1959) 347–350.

9 Bodansky, O., Are the phosphatases of bone, kidney, intestine and serum identical? The use of bile acids in their differentiation. J. biol. Chem. *118* (1937) 341–362.

10 Boyer, S.H., Alkaline phosphatase in human sera and placentae. Science *134* (1961) 1002–1004.

11 Chevremont, M., and Firket, H., Phosphatase alcaline et croissance des cultures de tissus. J. Cyto-embryol. Belgo-Néerland. *7* (1949) 23–26.

12 Cloetens, R., Identification de deux phosphatases 'alcalines' dans les organes animaux. Enzymologia *6* (1939) 46–56.

13 Cox, R.P., and MacLeod, C.M., Hormonal induction of alkaline phosphatase in human cells in tissue culture. Nature *190* (1961) 85–87.

14 Dolbeare, F., Vanderlaan, M., and Phares, W., Alkaline phosphatase and an acid arylamidase as marker enzymes for normal and transformed WI-38 cells. J. Histochem. Cytochem. *28* (1980) 419–426.

15 Elson, N.A., and Cox, R.P., Production of fetal-like alkaline phosphatase by HeLa cells. Biochem. Genet. *3* (1969) 549–561.

16 Fell, H.B., and Robison, R., The growth, development and phosphatase activity of embryonic avian femora and limb-buds cultivated in vitro. Biochem. J. *23* (1929) 767–784.

17 Fernley, H.N., Mammalian alkaline phosphatases, in: The Enzymes, vol. 4, 3rd edn., pp. 414–447. Ed. P.D. Boyer. Academic Press, New York 1971.

18 Fishman, L., Miyayama, H., Driscoll, S.G., and Fishman, W.H., Developmental phase-specific alkaline phosphatase isoenzymes of human placenta and their occurrence in human cancer. Cancer Res. *36* (1976) 2268–2273.

19 Fishman, W.H., Inglis, N.R., Green, S., Anstiss, C.L., Gosh, N.K., Reif, A.E., Rustigian, R., Krant, M.J., and Stolbach, L.L., Immunology and biochemistry of Regan isoenzyme of alkaline phosphatase in human cancer. Nature *219* (1968) 697–699.

20 Fishman, W.H., Inglis, N.R., Stolbach, L.L., and Krant, M.J., A serum alkaline phosphatase isoenzyme of human neoplastic cell origin. Cancer Res. *28* (1968) 150–154.

21 Fishman, W.H., and Singer, R.M., Placental alkaline phosphatase. Regulation of expression in cancer cells. Ann. N.Y. Acad. Sci. *259* (1975) 261–272.

22 Fortelius, P., Saksela, E., and Saxén, E., A HeLa cell clonal line lacking alkaline phosphatase activity. Exp. Cell Res. *21* (1960) 616–618.

23 Gartler, S.M., Apparent HeLa cell contamination of human heteroploid cell lines. Nature *227* (1967) 750–751.

24 Gey, G.O., Coffman, W.D., and Kubicek, M.T., Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. Cancer Res. *12* (1952) 264–265.

25 Gropp, A., Vergleichende enzymhistochemische Beobachtungen zum Phosphatasenachweis an einigen Carcinomzellstämmen in vitro sowie über milieubedingte Veränderungen der Enzymaktivität. Z. Krebsforsch. *63* (1959) 156–167.

26 Gropp, A., Bontke, E., and Hupe, K., Untersuchungen zum Nachweis der alkalischen Phosphatase an in vitro gezüchteten menschlichen Carcinomzellen mit besonderer Berücksichtigung ihrer Beziehung zu der Zelloberfläche. Exp. Cell Res. *15* (1958) 200–213.

27 Hamilton, T.A., Tin, A.W., and Sussman, H.H., Regulation of alkaline phosphatase expression in human choriocarcinoma cell lines. Proc. natn. Acad. Sci. USA *76* (1979) 323–327.

28 Harris, H., Multilocus enzyme systems and the evolution of gene expression: The alkaline phosphatases as a model example. Harvey Lect. *76* (1982) 95–124.

29 Herz, F., Alkaline phosphatase in KB cells: Influence of hyperosmolality and prednisolone on enzyme activity and thermostability. Archs Biochem. Biophys. *158* (1973) 225–235.

30 Herz, F., Induction of alkaline phosphatase expression in cultured cancer cells, in: Human Alkaline Phosphatases, pp. 139–166. Eds T. Stigbrand and W.H. Fishman. Alan R. Liss, Inc., New York 1984.

31 Herz, F., Barlebo, H., and Koss, L.G., Modulation of alkaline phosphatase activity in cell cultures derived from human urinary bladder carcinoma. Cancer Res. *34* (1974) 1943–1946.

32 Herz, F., and Halwer, M., Preferential alkaline phosphatase isoenzyme induction by sodium butyrate. Biochim. biophys. Acta *762* (1983) 289–294.

33 Herz, F., Miller, O.J., Miller, D.A., Auersperg, N., and Koss, L.G., Chromosome analysis and alkaline phosphatase of C4I, a cell line of human cervical origin distinct from HeLa. Cancer Res. *37* (1977) 3209–3213.

34 Herz, F., and Nitowsky, H.M., Alkaline phosphatase activity of human cell cultures: Kinetic and physical-chemical properties. Archs Biochem. Biophys. *96* (1962) 506–515.

35 Herz, F., Schermer, A., Halwer, M., and Bogart, L.H., Alkaline phosphatase in HT-29, a human colon cancer cell line: Influenceof sodium butyrate and hyperosmolality. Archs Biochem. Biophys. *210*(1981) 581–591.

36 Horiuchi, T., Horiuchi, S., and Mizuno, D., A possible negative feedback phenomenon controlling formation of alkaline phosphomonoesterase in *Escherichia coli*. Nature *183* (1959) 1529–1530.

37 Jemmerson, R., Shah, N., Takeya, M., and Fishman, W.H., Characterization of the placental alkaline phosphatase-like (Nagao) iso-

zyme on the surface of A431 human epidermoid carcinoma cells. Cancer Res. *45* (1985) 282–287.
38 Kameya, T., Kuramoto, H., Susuki, K., Kanjo, T., Oshikiri, T., Hayashi, H., and Itakura, M., A human gastric choriocarcinoma cell line with human gonadotropin and placental alkaline phosphatase production. Cancer Res. *35* (1975) 2025–2032.
39 Knaup, G., Pfleiderer, G., and Bayreuther, K., Human diploid lung fibroblast cell lines WI 26 and WI 38 exhibit isozyme shift of alkaline phosphatase after viral transformation. Clinica chim. Acta *88* (1978) 375–383.
40 Kottel, R.H., and Fishman, W.H., Modulation of HeLa alkaline phosphatase isoenzyme expression by prednisolone. Scand. J. Immun. *8* (suppl. 8) (1978) 571–574.
41 Kottel, R.H., and Fishman, W.H., Placental alkaline phosphatase isoenzyme expression by the non-HeLa DoT cervical-carcinoma cell line. Biochem. J. *200* (1981) 679–684.
42 Kottel, R.H., and Fishman, W.H., Developmental alkaline phosphatases as biochemical tumor Markers, in: Biochemical markers for Cancer, pp. 93–115. Ed. T.M.Chu. Marcel Dekker, Inc., New York 1982.
43 Kovács, E., Comparative biochemical studies on normal and on poliomyelitis virus infected tissue cultures. X. Enzyme changes in the host cells during arrested or abortive infection. Z. Vitam.- Horm.- Fermentforsch. *10* (1960) 348–360.
44 Kudo, S., Seike, H., Higashino, K., Ohtani, R., Yamamura, Y., Takeuchi, T., and Hirohashi, S., Occurrence of Kasahara isoenzyme in an established cell line, CCL 6 from human fetal intestine, in: Carcino-Embryonic Proteins: Chemistry, Biology, Clinical Application, vol. 2, pp. 665–672. Ed. F.-G. Lehmann, Elsevier/North Holland, Amsterdam 1979.
45 Ludueña, M.A., Iverson, G.M., and Sussman, H.H., Expression of liver and placental alkaline phosphatases in Chang liver cells. J. Cell Physiol. *91* (1977) 119–130.
46 Ludueña, M.A., and Sussman, H.H., Characterization of KB alkaline phosphatase. Evidence of similiarity to placental alkaline phosphatase. J. biol. Chem. *251* (1976) 2620–2628.
47 Ludueña, M.A., Sussman, H.H., and Rabson, A.S., Synthesis of human placental alkaline phosphatase in vitro by the ChaGo cell line. J. natn. Cancer Inst. *52* (1974) 1705–1709.
48 McComb, R.B., Bowers, Jr, G.N., and Posen, S., Alkaline Phosphatase. Plenum Press, New York 1979.
49 McKenna, M.J., Hamilton, T.A., and Sussman, H.H., Comparison of human alkaline phosphatase isoenzymes: Structural evidence for three protein classes. Biochem. J. *181* (1979) 67–73.
50 Morita, A., Tsao, D., and Kim, Y.S., Effect of sodium butyrate on alkaline phosphatase of HRT-18, a human rectal cancer cell line. Cancer Res. *42* (1982) 4540–4545.
51 Mueller, H.D., Leung, H., and Stinson, R.A., Different genes code for alkaline phosphatases from human fetal and adult intestine. Biochem. biophys. Res. Commun. *126* (1985) 427–433.
52 Mulivor, R.A., Hannig, V.L., and Harris, H., Developmental changes in human intestinal alkaline phosphatase. Proc. natn. Acad. Sci. USA *75* (1978) 3909–3912.
53 Neale, F.C., Clubb, J.S., Hotchkiss, D., and Posen, S., The heat stability of human placental alkaline phosphatase. J. clin. Path. *18* (1965) 359–363.
54 Nelson-Rees, W.A., Daniels, D.W., and Flandermeyer, R.R., Cross-contamination of cells in culture. Science *212* (1981) 446–452.
55 Neuwald, P.D., Anderson, C., Salivar, W.O., Aldenderfer, P.H., Dermody, W.C., Weintraub, B.D., Rosen, S.W., Nelson-Rees, W.A., and Ruddon, R.W., Expression of oncodevelopmental gene products by human tumor cells in culture. J. natn. Cancer Inst. *64* (1980) 447–459.
56 Neuwald, P.D., and Brooks, M., Altered form of placental alkaline phosphatase produced by JAR choriocarcinoma cells in culture. Cancer Res. *41* (1981) 1682–1689.
57 Nitowsky, H.M., and Herz, F., Alkaline phosphatase in cell cultures of human origin. Nature *189* (1961) 756–757.
58 Nitowsky, H.M., and Herz, F., Alkaline phosphatase activity of human cell cultures. Proc. Soc. exp. Biol. Med. *107* (1961) 532–534.
59 Nitowsky, H.M., and Herz, F., Hormonal regulation of alkaline phosphatase in dispersed cell cultures. Biochem. biosphys. Res. Commun. *11* (1963) 261–266.
60 Nitowsky, H.M., Herz, F., and Geller, S., Induction of alkaline phosphatase in dispersed cell cultures by changes in osmolarity. Biochem. biophys. Res. Commun. *12* (1963) 293–299.
61 Nozawa, S., Engvall, E., Kano, S., Kurihara, S., and Fishman, W.H., Sodium butyrate produces concordant expression of 'early placental' alkaline phosphatase, pregnancy-specific beta, -glycoprotein and human chorionic gonadotropin beta-subunit in a newly established uterine cervical cancer cell line (SKG-IIIa). Int. J. Cancer *32* (1983) 267–272.
62 Nozawa, S., and Fishman, W.H., Heat-stable alkaline phosphatase: Chemistry and biology, in: Pregnancy Proteins: Biology, Chemistry and Clinical Applications, pp. 121–153. Eds J.G. Grudzinskas, B. Teisner and M. Seppälä. Academic Press, Sydney 1982.
63 Nozawa, S., Udagawa, Y., Ohta, H., Kurihara, S., and Fishman, W.H., Newly established uterine cervical cancer cell line (SKG-III) with Regan isoenzyme, human chorionic gonadotropin β-subunit, and pregnancy-specific β_1-glycoprotein phenotypes. Cancer Res. *43* (1983) 1748–1760.
64 Sakiyama, T., Robinson, J.C. and Chou, J.Y., Characterization of alkaline phosphatase from human first trimester placentas. J. biol. Chem. *254* (1979) 935–938.
65 Seargent, L.E., and Stinson, R.A., Evidence that three structural genes code for human alkaline phosphatase. Nature *281* (1979) 152–154.
66 Singer, R.M., and Fishman, W.H., Characterization of two HeLa sublines: TCRC-1 produces Regan isoenzyme and TCRC-2, non-Regan isoenzyme. J. Cell Biol. *60* (1974) 777–780.
67 Singer, R.M., and Herz, F., Multiple effects of glucocorticoids and hyperosmolality on isoenzyme expression in cultured KB cells. J. natn. Cancer Inst. *60* (1978) 573–577.
68 Singer, R.M., Tompkins, W.A.F., White, L.J., and Perry, J.E., Co-production of Regan isoenzyme and carcinoembryonic antigen in HCT-8 cells. J. natn. Cancer Inst. *56* (1976) 175–178.
69 Speeg, K.V. Jr., Azizkhan, J.C., and Stromberg, K., Characteristics of alkaline phosphatase from two continuous lines of human choriocarcinoma cells. Exp. Cell Res. *105* (1977) 199–205.
70 Stigbrand, T., Present status and future trends of human alkaline phosphatase, in: Human Alkaline Phosphatases, pp. 3–14. Eds T. Stigbrand and W.H. Fishman. Alan R. Liss, Inc., New York 1984.
71 Stigbrand, T., Millán, J.L., and Fishman, W.H., The genetic basis of alkaline phosphatase isozyme expression, in: Isozymes, vol. 6, pp. 93–111. Eds M.C. Rattazzi, J.G. Scandalios and G.S. Whitt. Alan R. Liss, Inc., New York 1982.
72 Takahara, N., Herz, F., Singer, R.M., Hirano, A., and Koss, L.G., Induction of alkaline phosphatase activity in cultured human intracranial tumor cells. Cancer Res. *42* (1982) 563–568.
73 Tokumitsu, S.-I., Tokumitsu, K., Kohnoe, K., and Takeuchi, T., Characterization of liver-type alkaline phosphatase from human gastric carcinoma cells (KMK-2) in vitro. Cancer Res. *39* (1979) 4732–4738.
74 Torriani, A., Influence of inorganic phosphate on the formation of phosphatases by *Escherichia coli*. Biochim. biophys. Acta *38* (1960) 460–469.
75 Vanneuville, F.J., Leroy, J.G., and Van Elsen, A.F., Alkaline phosphatase: Further studies on its behaviour in human diploid fibroblasts. Archs int. Physiol. Biochim. *85* (1977) 205–206.
76 Wray, L., and Harris, H., Demonstration using monoclonal antibodies of inter-locus heteromeric isozymes of human alkaline phosphatase. J. immun. Meth. *55* (1982) 13–18.
77 Wray, L.K., and Harris, H., Monoclonal antibodies to an ectopically expressed alkaline phosphatase in a human malignant cell line. Cancer Res. *43* (1983) 758–762.

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