

## Calcium-binding proteins in human carcinoma cell lines

(parvalbumin/immunohistochemistry/two-dimensional polyacrylamide gel electrophoresis/high-performance liquid chromatography/peptide maps)

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**ABSTRACT** Elevated levels of  $\text{Ca}^{2+}$ -binding proteins are reported in transformed cells and thought to be involved in their uncontrolled proliferation and often increased motility. Therefore, three cell lines [LICR(Lond)-HN 1, -HN 2, and -HN 6] derived from human carcinomas displaying various degrees of locomotive activity were investigated for the presence of parvalbumin and related  $\text{Ca}^{2+}$ -binding proteins. By applying different immunohistochemical methods in conjunction with a monospecific anti-parvalbumin antiserum, an intense staining was seen in cells displaying translocative motility. Often in these cells, an association with filamentous structures located in the nuclear region was observed. Unique  $\text{Ca}^{2+}$ -binding proteins, absent from comparable normal tissue, were found in the malignant cell lines when analyzed by two-dimensional polyacrylamide gel electrophoresis and high-performance liquid chromatography. From these tumor cells a protein ( $M_r$ , 12,000; pI, 4.8) was isolated that crossreacted with anti-parvalbumin antiserum. Peptide maps of this protein revealed a further structural homology to parvalbumin ( $M_r$ , 12,000; pI, 4.9). In analogy to muscle, where there is evidence for a regulatory role of parvalbumin in the contraction-relaxation cycle, we speculate that this protein is tumor-associated and connected to the motile behavior of carcinoma cells.

Elevated levels of cytosolic  $\text{Ca}^{2+}$  (1, 2) and  $\text{Ca}^{2+}$ -binding proteins (3-6) are present in transformed cells and may be responsible for their uncontrolled proliferation and sometimes increased motility. Therefore, we analyzed for the presence of parvalbumin and structurally related  $\text{Ca}^{2+}$ -binding proteins three human epidermoid carcinoma cell lines [LICR(Lond)-HN 1, -HN 2, and -HN 6], known to express different modes of motility (7-9). Parvalbumin, first detected in muscle (for recent review, see ref. 10) and later also in several non-muscle tissues (11-14), has not been investigated in tumor cells. Calmodulin, a ubiquitous multifunctional  $\text{Ca}^{2+}$  receptor (for recent review, see ref. 15), is augmented in neoplastic cells (3-6). The S-100 proteins, a family of  $\text{Ca}^{2+}$ -binding proteins preferentially present in nerve (16, 17) and adipose tissue (18) have also been detected in cell lines of human malignant melanomas (19) and primary tumors of the nervous system of man (20) and rat (21). In contrast, another  $\text{Ca}^{2+}$ -binding protein, oncomodulin, is reported to be synthesized exclusively in neoplastic cells and tissues. Originally detected in rat hepatomas, it was also found in spontaneous or virally induced tumors of mouse, rat, and man (22, 23).

Here, we show by combined biochemical and immunohistochemical techniques that three tumor cell lines derived from human carcinomas express  $\text{Ca}^{2+}$ -binding proteins that are absent from comparable nonmalignant cells. These proteins are structurally and immunologically related to parvalbumin. Since parvalbumin is postulated to regulate muscle

relaxation (24), it is suggested that at least one of these proteins might be involved in the mediation of the motile behavior of the tumor cells investigated.

### MATERIALS AND METHODS

**Tumor Cells.** The three cell lines derived from human squamous carcinomas of tongue [LICR(Lond)-HN 1 and LICR(Lond)-HN 6] and larynx [LICR(Lond)-HN 2] have been described (26, 27).

**Normal Epithelial Cells (Controls).** Cells from human buccal mucosa were removed aseptically from four volunteers, washed with 0.9% NaCl, sedimented onto glass coverslips with a cytocentrifuge, model SCA-0025 (Chandon Elliott, Camberley, England;  $1000 \times g$ , 4 min, room temperature), and used for immunostaining (see below); 50% of the cells were viable when stained with 0.5% trypan blue in 0.9% NaCl prior to fixation. Fresh human skin removed from the thigh of a 40-year-old woman, consisted only of epidermis and was free of muscle fibers or gland tissue.

**Preparation and Characterization of the Antisera.** Monospecific antisera and the derived affinity-purified antibodies against rat muscle parvalbumin were raised in rabbits (12, 13). These antisera strongly crossreacted with human parvalbumin, which has  $M_r$ , pI, and hydrophobicity identical to rat parvalbumin (unpublished data).

**Indirect Immunofluorescence.** Cells were immunostained according to ref. 28 with modifications (29). Preabsorption of the antisera with the antigen (1  $\mu\text{M}$ ) was carried out as reported (29). Photographs were taken using Kodak Ektachrome 160 film.

**Peroxidase-Antiperoxidase Immunostaining.** LICR(Lond)-HN 6 cells were stained by applying the peroxidase-antiperoxidase technique (28). Cells were fixed (29), permeabilized with 0.1% Triton X-100 (10 min), and incubated with rabbit anti-parvalbumin antiserum (dilutions, 1:1000; 1:2000; 1:5000) for 24 hr (4°C) or with affinity-purified antibodies (0.1  $\mu\text{g}/\text{ml}$ ). Further incubations (1 hr, room temperature) followed, using goat anti-rabbit IgG (diluted 1:50; Nordic, Tilburg, Netherlands) and peroxidase-antiperoxidase complex (diluted 1:200; Sternberger-Meyer, Jarrettsville, MD). Peroxidase reaction was demonstrated with 3,3'-diaminobenzidine tetrahydrochloride (40 mg dissolved in 100 ml, consisting of 50 mM Tris-HCl/139 mM NaCl, pH 7.6) to which 60  $\mu\text{l}$  of a 30%  $\text{H}_2\text{O}_2$  solution was added immediately before use. Cultures were placed in Kaiser's glycerol/gelatin (Merck). Photographs were taken using Kodak Ektachrome 50 film. Sections of human skin were fixed in Bouin's fluid and stained for parvalbumin by the peroxidase-antiperoxidase technique (28).

**Preparation of Protein Extracts.** Cell cultures were harvested after 24 hr, washed 3 times with 0.9% NaCl, and extracted in 2 vol of 4 mM EDTA (pH 7.4) containing various protease inhibitors (29). After heat-treatment (30 min, 85°C) and centrifugation ( $40,000 \times g$ , 30 min, 4°C) the supernatants were analyzed for  $\text{Ca}^{2+}$ -binding proteins by two-dimensional PAGE and HPLC. Human skin was ground in liquid  $\text{N}_2$  and

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extracted identically. Protein concentrations were determined by the microbiuret method (30).

**Protein Standards.** Rat muscle parvalbumin (13), calf brain calmodulin (29), and the S-100 proteins (31) were used as standards for the biochemical analyses.

**Two-Dimensional NaDodSO<sub>4</sub>/PAGE.** Protein extracts were labeled *in vitro* by reductive methylation (32) with [<sup>14</sup>C]formaldehyde (specific activity, 47 mCi/mmol; 1 Ci = 37 GBq; New England Nuclear) and analyzed on two-dimensional gels (33). A mixture of ampholines (20% at pH 2.5–4 and 80% at pH 3.5–10) was used for isoelectric focusing. Second-dimension runs were carried out on 12.5% (wt/vol) polyacrylamide gels calibrated with <sup>14</sup>C-labeled proteins ( $M_r$ , 14,300–100,000; Amersham) and [<sup>14</sup>C]parvalbumin ( $M_r$ , 12,000). Proteins were visualized by fluorography (34).

**HPLC of Proteins.** Extracts were separated on an Aquapore RP-300 cartridge (particle size, 10  $\mu$ m; pore size, 300 Å; Brownlee Laboratories, Santa Clara, CA) as described (29, 31). The buffer systems used for gradient elution were as follows: buffer A, 25 mM Tris·HCl (pH 7.4) containing either 0.1 mM EGTA or 1 mM CaCl<sub>2</sub>; buffer B, same as buffer A but with 60% (vol/vol) acetonitrile (Baker). Parvalbumin, the S-100 proteins, and calmodulin were identified according to their distinct hydrophobicities (31). Each protein peak was collected separately, dialyzed against 1 mM CaCl<sub>2</sub> (48 hr), lyophilized, <sup>14</sup>C-labeled, and analyzed on one- and two-dimensional 12.5% polyacrylamide gels. Fractions containing protein bands in the  $M_r$  range of parvalbumin ( $M_r$ , 12,000) were further purified by HPLC rechromatography.

**Peptide Maps.** Purified proteins were digested for 7 hr (room temperature) with 8% (wt/wt) L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (Worthington; specific activity, 182 units/mg) as described (13).

**Dot-Immunobinding Assay.** This was performed according to ref. 35. Anti-parvalbumin antiserum was diluted 1:1000, and the peroxidase-labeled goat anti-rabbit IgG 1:1500 (Miles). The incubation with the first antibody was carried out overnight (4°C).

**Ca<sup>2+</sup> Analysis.** This was carried out according to ref. 13 on an IL atomic absorption spectrometer (Model 157/flameless atomizer 555) using a calcium standard solution (Sigma).

## RESULTS

### Immunocytochemistry with Anti-Parvalbumin Antiserum.

Upon indirect immunofluorescence, the LICR(Lond)-HN 1, -HN 2, and -HN 6 cell lines reacted strongly with antisera against parvalbumin (Fig. 1 *a–f*). In the LICR(Lond)-HN 1 cell line (Fig. 1 *a* and *b*), which is characterized by a nonconfluent type of growth *in vitro* (7–9), cells showed staining of the cytoplasm but mostly of the nuclear region. Immunoreactivity was found especially in cells displaying a polarized configuration (Fig. 1 *a*, arrows) and to a lesser degree in extended cells (Fig. 1 *a*, asterisk). LICR(Lond)-HN 2 cells (Fig. 1 *c* and *d*) form islands in culture (7–9). Reactive cells were generally found at the edge of the aggregates and, especially, in cells with long processes. In contrast, cells within the interior of the islands remained dark. In the LICR(Lond)-HN 6 cell line forming more loose islands than -HN 2 cells (7–9), staining was most intense at the edge of the islands or in single cells. Often, fluorescence was associated with net-like structures, especially in the nuclear area (Fig. 1 *e* and *f*).

When the peroxidase-antiperoxidase technique was applied to LICR(Lond)-HN 6 cells (Fig. 1 *k*) the distribution of label was identical to that observed in indirect immunofluorescence (Fig. 1 *e* and *f*).

Only background staining was found by incubation of all three cell lines with preimmune serum (Fig. 1 *g* and *h*) or with antiparvalbumin antiserum preabsorbed with parvalbumin (Fig. 1 *i* and *j*). Normal epithelial cells from the buccal

mucosa treated in the same manner as the tumor cells showed no immunostaining, and sections of human skin also remained unreactive in all layers of the epidermis (not shown).

**Two-Dimensional PAGE Analysis.** Heat-treated extracts of LICR(Lond)-HN 1, -HN 2, and -HN 6 cells were resolved into  $\approx$ 40 distinct proteins by two-dimensional PAGE (Fig. 2 *a–c*). All extracts contained a protein ( $M_r$ , 18,000; pI, 4.2) migrating identically to calmodulin (16). This protein separates into two components of NaDodSO<sub>4</sub> gels, a fact previously observed (36, 37). A protein of  $M_r \approx$ 12,000 and a pI value of 3.9 located at a position expected for oncomodulin ( $M_r$ , 11,500; pI, 3.9; see ref. 23) was detected in trace amounts in LICR(Lond)-HN 2 (Fig. 2 *b*) and as a major spot in -HN 6 cells (Fig. 2 *c*) but not in -HN 1 cells (Fig. 2 *a*). Similarly, the S-100a and S-100b proteins ( $M_r$ , 10,000; pI, 4.2–4.4), were found in LICR(Lond)-HN 2 and -HN 6 cells (Fig. 2 *b* and *c*) but again not in the LICR(Lond)-HN 1 cell line (Fig. 2 *a*). LICR(Lond)-HN 1, -HN 2, and -HN 6 contained two proteins labeled A ( $M_r$ , 12,000; pI, 4.8) and B ( $M_r$ , 11,000; pI, 4.9), migrating close to the position of added parvalbumin (Fig. 2 *a–d*). However, genuine parvalbumin ( $M_r$ , 12,000; pI, 4.9; see ref. 13) was absent from all three cell lines. This was unexpected because immunohistochemistry had given evidence of parvalbumin immunoreactivity. Human skin extract was devoid of the proteins A and B but, instead, contained a protein of  $M_r$  12,000 and pI 4.9 (Fig. 2 *e*) migrating identically to parvalbumin, as shown by coelectrophoresis (Fig. 2 *f*).

**HPLC Analysis.** HPLC (29, 31, 38, 39) was applied to investigate the tumor cell lines (Fig. 2 *g–j*) and human skin (Fig. 2 *k*) using a Tris·HCl/EGTA buffer system (pH 7.4). A protein with a hydrophobicity identical to calmodulin (eluted at 46.5% buffer B) was found in all extracts. This peak was reanalyzed by two-dimensional PAGE revealing  $M_r$  and pI values identical to calmodulin (not shown). LICR(Lond)-HN 6 extracts contained a protein that eluted at 12.5% buffer B (Fig. 2 *i*), present in minor amounts also in -HN 2 cells (Fig. 2 *h*) but absent from -HN 1 cells (Fig. 2 *g*). Purification by HPLC and subsequent analysis by two-dimensional PAGE showed the presence of a spot at the position of oncomodulin (Fig. 2 *l*). Similarly, the S-100 proteins, eluting at 51.5% (S-100a) and 57% (S-100b) buffer B were present in LICR(Lond)-HN 2 and -HN 6 but absent from the -HN 1 cells (Fig. 2 *g–i*). Neither extract contained a protein with the hydrophobicity of parvalbumin, which was shown to elute at 54% buffer B by cochromatography with LICR(Lond)-HN 1 cells (Fig. 2 *j*). This result was in agreement with the electrophoretic analyses (see above). However, in each cell line two protein peaks were found eluting at 22% (peak A<sub>1</sub>) and 24.5% (peak A<sub>2</sub>) buffer B, respectively. After rechromatography and analysis on two-dimensional PAGE (Fig. 2 *m* and *o*), both peaks gave a single protein spot with mutually identical  $M_r$  (12,000) and pI (4.8) values. Comigration experiments (Fig. 2 *n* and *p*) showed close spacing with parvalbumin ( $M_r$ , 12,000; pI, 4.9), indicating that the fractions A<sub>1</sub> and A<sub>2</sub> are components of the protein designated A (Fig. 2 *a–d*). The protein designated B ( $M_r$ , 11,000; pI 4.9) (Fig. 2 *a–d*) could not be detected by HPLC. In fact, this compound is retained on the column, because the total HPLC eluate was devoid of protein B when dialyzed, lyophilized, and reanalyzed by two-dimensional PAGE. Two-dimensional PAGE was also carried out on all the other isolated HPLC peaks; none of them contained proteins in the  $M_r$  range of parvalbumin.

Ca<sup>2+</sup>-binding proteins alter their hydrophobicities when separated by HPLC in the presence or absence of Ca<sup>2+</sup> ions (31, 38, 39). Therefore, all cell line extracts were also chromatographed in the presence of 1 mM CaCl<sub>2</sub>. Protein peaks corresponding to calmodulin, oncomodulin, S-100 proteins,

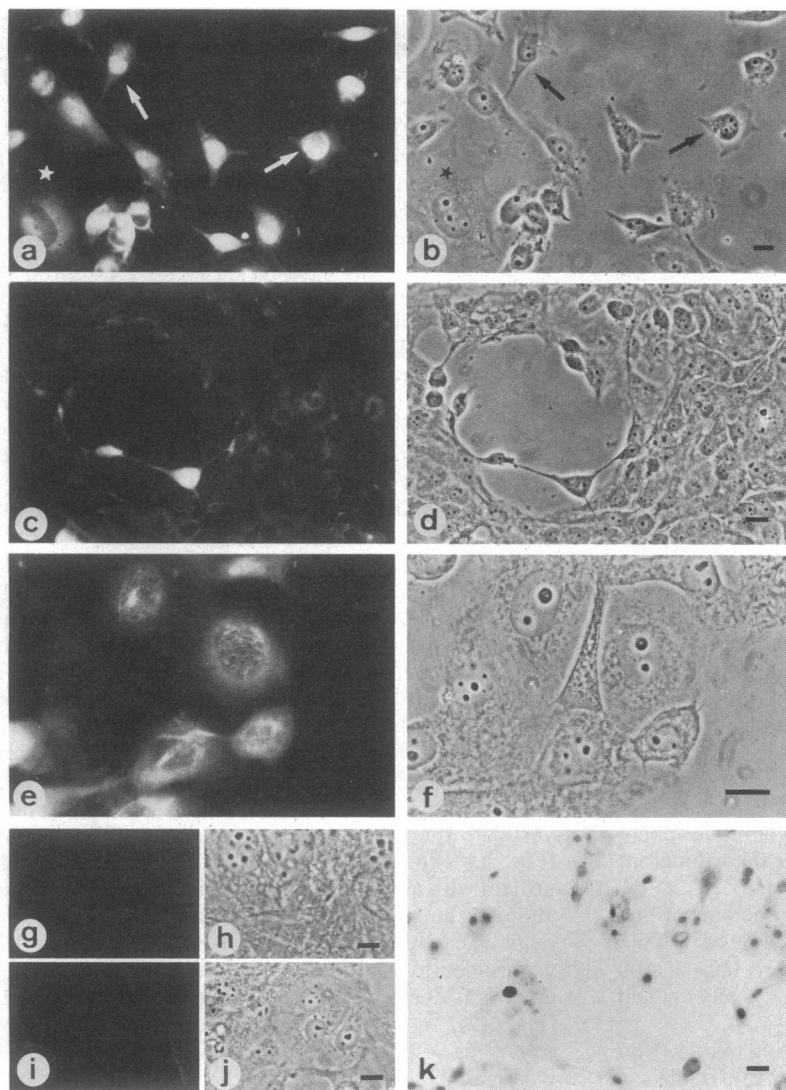


FIG. 1. Immunocytochemistry of human carcinoma cell lines with anti-parvalbumin antiserum (*a, c, e, g, i, and k*) and corresponding phase-contrast micrographs (*b, d, f, h, and j*). Indirect immunofluorescence with rabbit antiserum (*a, c, and e*), control serum (*g*), or rabbit antiserum preabsorbed with parvalbumin (*i*) diluted 1:30 and fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (diluted 1:350); (*k*) peroxidase-antiperoxidase immunostaining with rabbit antiserum diluted 1:1000. (*a* and *b*) LICR(Lond)-HN 1; (*c* and *d*) LICR(Lond)-HN 2; (*e-k*) LICR(Lond)-HN 6. In *a*, cells with different modes of motility are indicated: star denotes extended cell with stationary motility; arrows show transversely polarized cells. (Magnifications: *k*,  $\times 57$ ; *c, d, i, and j*,  $\times 143$ ; *a, b, g, and h*,  $\times 285$ ; *e* and *f*,  $\times 570$ .) [Bar =  $10 \mu\text{m}$  (*a, b, and e-h*);  $20 \mu\text{m}$  (*c, d, i, and j*);  $50 \mu\text{m}$  (*k*).]

protein fractions  $A_1$  and  $A_2$ , and parvalbumin (as a control) were shifted in their hydrophobicities, showing that, in fact, they represent  $\text{Ca}^{2+}$ -binding proteins. This was confirmed by measuring the  $\text{Ca}^{2+}$  content by atomic absorption. Protein fractions  $A_1$  and  $A_2$  showed the same  $\text{Ca}^{2+}$  content as muscle parvalbumin, averaging  $2 \pm 0.2$  mol of  $\text{Ca}^{2+}$  per mol.

An extract of human skin, when separated on HPLC under identical conditions, did not show protein peaks with hydrophobicities corresponding to oncomodulin or protein fractions  $A_1$  and  $A_2$  (Fig. 2*k*). This was in agreement with the electrophoretic (Fig. 2*e* and *f*) and immunocytochemical results (see above). In addition, there was no peak at the position of parvalbumin.

**Dot-Immunobinding Assay.** LICR(Lond)-HN 6 cells were extracted and the proteins separated by HPLC. Total extracts before and after heat treatment, as well as the individual HPLC fractions, were dotted onto nitrocellulose, reacted with anti-parvalbumin antiserum, and the immunoreaction was visualized by the peroxidase-antiperoxidase technique (Fig. 2*i*, *Inset*). Heat-treated and nontreated extracts as well as peaks  $A_1$  and  $A_2$  (compare Fig. 2*g-j*) crossreacted with the anti-parvalbumin antiserum. In contrast, total extract (heat-treated) of human skin as well as HPLC peaks corresponding to oncomodulin, calmodulin, and the S-100 proteins were immunologically unreactive.

**Peptide Maps.** Proteins  $A_1$  and  $A_2$  as well as oncomodulin were purified from the LICR(Lond)-HN 6 cell line by two cycles of HPLC, digested with L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin, and the resulting pep-

tides were analyzed by HPLC. The peptide patterns of proteins  $A_1$  and  $A_2$  were virtually indistinguishable (not shown). When compared to the peptide maps of oncomodulin and parvalbumin, certain similarities were revealed. However, proteins  $A_1$  and  $A_2$  clearly differed from these other  $\text{Ca}^{2+}$ -binding proteins, as exemplified by one major peptide unique to oncomodulin and three major peptides unique to parvalbumin. The peptide maps of proteins  $A_1$  and  $A_2$  were completely different from calmodulin (not shown).

## DISCUSSION

The three cell lines derived from human carcinomas of tongue and larynx with distinct growth characteristics *in vitro* display two forms of motility: stationary (carried out by nonpolarized cells) and translocative (coupled to a polarized configuration) as recorded by time-lapse cinematography (8). In contrast to nontransformed tissue (normal epithelial cells from human skin and human buccal mucosa), the three cell lines showed strong immunoreactivity with anti-parvalbumin antiserum. As already observed in muscle (40) and nerve tissue (12), not all cells were labeled. In our study, staining was most pronounced in single cells expressing translocative motility (Fig. 1*a* and *b*, arrows), or in cells located at the edge of an island (Fig. 1*c* and *d*). Cells in the interior of aggregates or those displaying stationary motility, recognizable by a more flattened or nonpolarized form (Fig. 1*a* and *b*, stars; see refs. 7-9), showed less reactivity. Mainly in LICR(Lond)-HN 6 cells, immunoreactivity was associ-

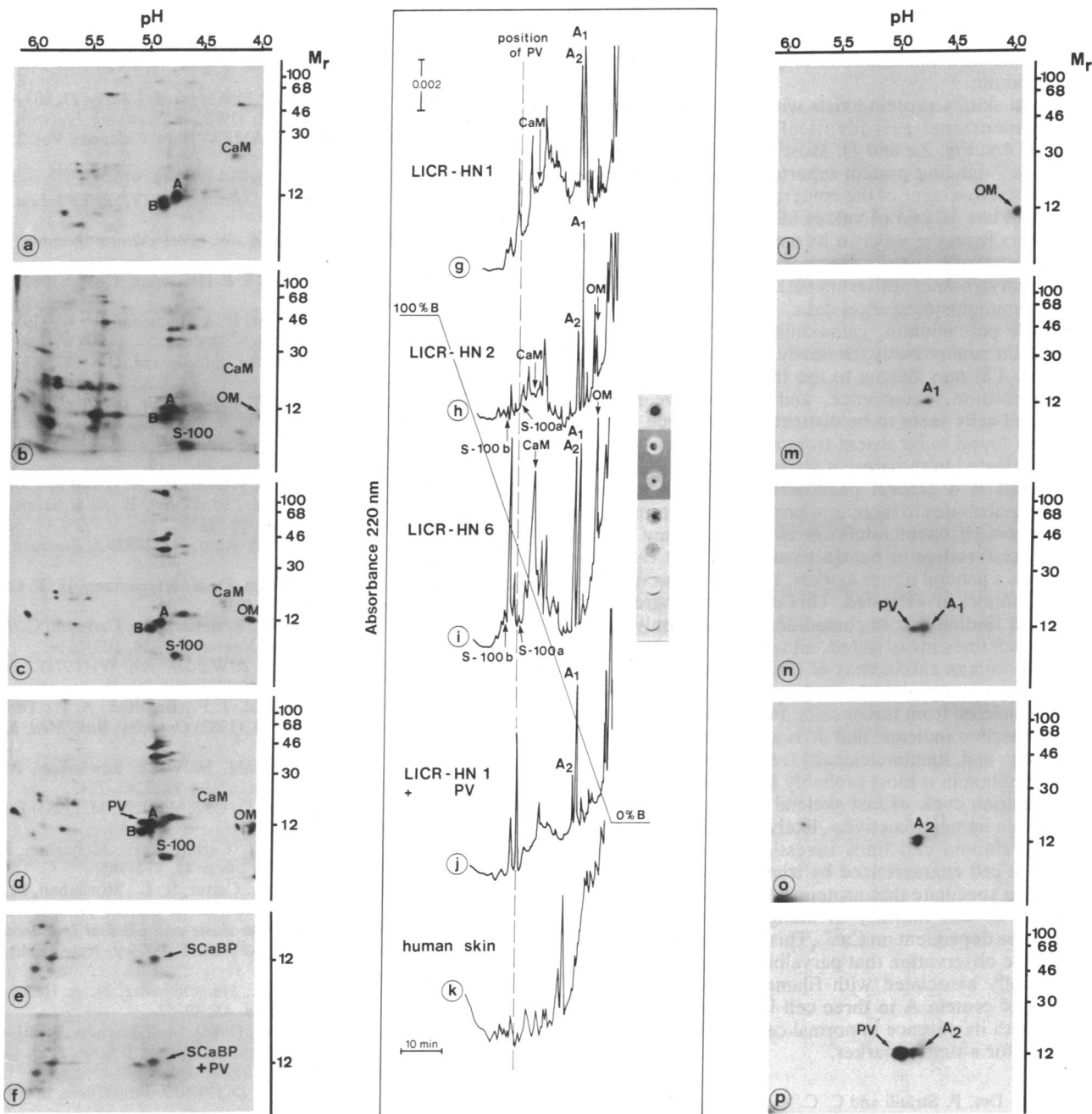


FIG. 2. Biochemical analyses of heat-treated 4 mM EDTA extracts of human carcinoma cell lines and control tissue. (a-f) Two-dimensional PAGE of <sup>14</sup>C-labeled extracts of (a) LICR(Lond)-HN 1; (b) LICR(Lond)-HN 2; (c) LICR(Lond)-HN 6; (d) comigration of c with rat parvalbumin (1 μg); (e) normal human skin; (f) comigration of e with rat parvalbumin (1 μg). In e and f, only the lower part of the pherograms are shown. Protein was applied onto the gels at 25–50 μg. (g-k) HPLC of heat-treated 4 mM EDTA extracts of (g) LICR(Lond)-HN 1; (h) LICR(Lond)-HN 2; (i) LICR(Lond)-HN 6; (j) cochromatography of g with parvalbumin (5 μg). In each run, 75–400 μg of protein was separated corresponding to 4–17 × 10<sup>5</sup> cells. (k) Human skin (100 μg). The Tris·HCl/EGTA, pH 7.4, buffer system was used. The gradient was 0–100% buffer B in 45 min. (i; Inset) Dot immunobinding assay with anti-parvalbumin antiserum showing, from top to bottom, parvalbumin (5 μg, control), total extract of LICR(Lond)-HN 6 cells before heat step (50 μg), heat-treated cell extract (5 μg), protein peak A<sub>1</sub>, protein peak A<sub>2</sub>, total extract (heat-treated) of LICR(Lond)-HN 6 cells. (l) Oncomodulin; (m) peak A<sub>1</sub>; (n) coelectrophoresis of m with parvalbumin; (o) peak A<sub>2</sub>; p coelectrophoresis of o with parvalbumin. <sup>14</sup>C-labeled proteins were identified by pI (scale on top) and  $M_r$  ( $\times 10^{-3}$ ) and were visualized by fluorography (2–4 days exposure). PV, parvalbumin; CaM, calmodulin; OM, position of oncomodulin; S-100, S-100 proteins; SCaBP, position of the skin Ca<sup>2+</sup>-binding protein; A, protein A ( $M_r$ , 12,000; pI, 4.8); B, protein B ( $M_r$ , 11,000; pI, 4.9).

ated with as yet undefined net-like structures in the nuclear area (Fig. 1 e and f).

The electrophoretic analyses revealed that all three cell lines contain two heat-stable proteins with  $M_r$  and pI values very similar to those of parvalbumin. One of these proteins, designated protein A ( $M_r$ , 12,000; pI, 4.8), could be further resolved by HPLC into two closely spaced protein peaks

(peak A<sub>1</sub> and peak A<sub>2</sub>, Fig. 2 g-j) with identical migration properties on two-dimensional gels (Fig. 2 m-p) and indistinguishable peptide maps (not shown). This indicates a very similar primary structure, and the slight shift in their hydrophobicities may arise from sequence microheterogeneity or post-translational modification. This view is further supported by immunological crossreactivity of both fractions with

anti-parvalbumin antiserum (Fig. 2i, *Inset*). Comparison of the peptide maps of components A<sub>1</sub> and A<sub>2</sub> showed structural similarities with oncomodulin and parvalbumin, but not with calmodulin.

In human skin, a protein exists with migration properties on two-dimensional gels identical to parvalbumin ( $M_r$ , 12,000; pI, 4.9; Fig. 2 e and f). Most probably, it represents the skin Ca<sup>2+</sup>-binding protein reported to be present in the basal proliferative layer of the epidermis of man and rat (41). This protein has  $M_r$  and pI values identical to parvalbumin, but it differs from the latter in its amino acid sequence and Ca<sup>2+</sup>-binding properties. Furthermore, it does not crossreact with anti-parvalbumin antiserum (41), in agreement with the present immunohistochemical data.

Although parvalbumin, calmodulin, the S-100 proteins, oncomodulin, and possibly the newly isolated protein A ( $M_r$ , 12,000; pI, 4.8) may belong to the troponin C superfamily, their distribution, occurrence, and presumptive roles in transformed cells seem to be distinct. For instance, parvalbumin was found to be absent from all three carcinoma cell lines investigated in the present study. It remains to be seen whether this is a general phenomenon. Calmodulin most probably contributes to increased proliferation in tumor cells (3, 6). Although oncomodulin is absent from any normal (adult or fetal) rodent or human tissue (23, 25), it cannot be regarded as a general tumor marker, because it was not present in all tumors investigated. This observation agrees with the present finding that oncomodulin occurs in only two of the three cell lines investigated, although all of them are derived from human carcinomas of similar origin.

In conclusion, a protein termed protein A ( $M_r$ , 12,000; pI, 4.8), was isolated from tumor cells. Its biochemical and antigenic properties indicate that it is a Ca<sup>2+</sup>-binding protein structurally and immunologically related to parvalbumin. Since parvalbumin is most probably involved in the contraction-relaxation cycle of fast skeletal muscle (24), and since parvalbumin immunoreactivity, likely to be due to protein A in the carcinoma cell lines investigated, was most pronounced in cell characterized by translocative activity, it is tempting to speculate that protein A might be related to the regulation of cell motility in malignant cells, a process known to be dependent on Ca<sup>2+</sup>. This conclusion is strengthened by the observation that parvalbumin immunoreactivity was partially associated with filamentous structures. The presence of protein A in three cell lines investigated, contrasting with its absence in normal cells, make it a plausible candidate for a tumor marker.

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