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ELONGATED CELLS DERIVED FROM RAT MAMMARY CUBOIDAL EPITHELIAL CELL LINES RESEMBLE CULTURED MESENCHYMAL CELLS IN THEIR PATTERN OF PROTEIN SYNTHESIS

Roger Barraclough, Karin J. Dawson and Philip S. Rudland

Ludwig Institute for Cancer Research (London Branch),
Royal Marsden Hospital, Sutton, Surrey, U.K.

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Two-dimensional gel electrophoresis has been used to identify polypeptide patterns characteristic of rat mammary cuboidal epithelial cells or mesenchyme-derived cells. Elongated cells and cell lines derived from cloned cuboidal epithelial cells in culture possess a polypeptide pattern which resembles that of the cultured mesenchymal cells rather than that of the cuboidal epithelial cells from which they were derived. These elongated converts also resemble cultured mesenchymal cells in possessing a Triton-insoluble matrix in which vimentin and not prekeratin predominates.

The cloned cuboidal rat mammary epithelial cell line, Rama 25, spontaneously converts from a cuboidal to an elongated morphology in culture (1). This morphological change is associated with a redistribution of cytoskeletal components detected by immunofluorescence (2) and an increased and altered pattern of secretion of basement membrane proteins, type IV collagen and laminin, by the elongated cell converts (3, 4). The conversion of mammary epithelial cells to an elongated morphology is not confined to Rama 25 cells (4, 5, 6, 7, 8). In this paper the patterns of polypeptide synthesis of several cuboidal epithelial cell lines are compared with their elongated converts and with cultured fibroblasts. The differences in the patterns of synthesis are characterised.

MATERIALS AND METHODS

Isolation and Growth of Cells and Cell Lines Four previously-described cloned cuboidal epithelial cell lines were used: Rama 25 (1) & Rama 37 clone A3 (6) derived from mammary tumours, and Rama 41 (9, 10) & Rama 704 (7) derived from normal glands. Rama 41 cells do not convert in culture to elongated cells. Elongated convertant cell lines, Rama 29 (1), Rama 401 (5), Rama 37-E5 (6) and Rama 712 (7) were all derived from cuboidal epithelial cell cultures. Two mammary fibroblast cell lines, Rama 351 (11) and Rama 27 (1), were derived from cultures of ring-cloned cells which subsequently yielded fat cells in

Abbreviations: SDS, Sodium dodecylsulphate; S.D., Standard deviation; kd, Kilodaltons; TCA, Trichloro-acetic acid; N.D., Not determined.

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culture (11). Rat embryo fibroblasts were kindly supplied by Dr. J. Wyke (Imperial Cancer Research Fund, U.K.).

Secondary cultures of mammary fibroblasts were prepared from the abdominal mammary glands of 9-day-pregnant female rats as described previously (12), except that the digestion was performed overnight at 20°C and then continued for 2 h at 37°C. A "fast-sticking" stromal-enriched fibroblast fraction was obtained by allowing single cells to attach to petri dishes for 2 h. All these cells were grown in Dulbecco's modified Eagles' medium supplemented with 5% foetal calf serum, at 37°C in an atmosphere of 10% CO_2: 90% air. The epithelial cells were grown in the presence of hydrocortisone and insulin as previously described (1).

Labelling of Cultured Cells, Preparation of Extracts and Two-dimensional Gel Electrophoresis Cells were labelled with L-[^{35}S]methionine (50 µCi/10^7 cells) and extracts for electrophoresis were prepared as described previously (13, 14). The total TCA-insoluble counts/min incorporated were evaluated using a previously published method (15). Two-dimensional gel electrophoresis was carried out as previously described (13, 14). Gels were stained, destained (16), impregnated with diphenyloxazole (17), dried and exposed to Kodak X-Omat S Film at -70°C.

Quantitation of Incorporation of Radioactivity into Specific Polypeptides Separated by Gel Electrophoresis Regions of dried-down gels corresponding to either stained or radioactive proteins were rehydrated in destaining solution for 15 minutes. Each swollen gel fragment was heated for 7 h at 60°C in 0.5 ml of Soluene. Radioactivity was determined in a scintillation spectrometer (Packard) at least 9 h after addition of scintillation fluid (Instagel). ^{35}S was detected with an efficiency of 77%. Quantitative data were checked by visual comparison with the fluorographic film images.

Immunological Detection of Vimentin Triton-insoluble proteins were prepared by a modification (13) of the method of Gard et al. (18) and were analysed by electrophoresis on 12½% polyacrylamide gels containing SDS (19).

"Western" electrophoretic transfer of proteins onto nitrocellulose filters was carried out as described by Towbin et al. (20) and Burnette et al. (21), and the filters were incubated for 17 h with rabbit antivimentin serum or normal rabbit serum both diluted 1:500. Bound rabbit immunoglobulins were detected by incubating the filters with ^{125}I-labelled goat-anti-rabbit immunoglobulin (10 x 10^6 cpm; specific activity 1.1 µCi/µg) for 6 h at room temperature. Washed and dried filters (21) were subjected to autoradiography for 8 h. Molecular weights were determined from the migration of ^{125}I-labelled bovine serum albumin, pyruvate kinase, actin and soybean trypsin inhibitor.

RESULTS

Occurrence of Low-Molecular-Weight Polypeptides in Cultured Cell Lines

Fig. 1A shows the two-dimensional gel pattern of [^{35}S]methionine-labelled low-molecular-weight polypeptides isolated from Rama 29 cells, and illustrates 9 kd and 15.2 kd polypeptides (numbered 1 and 2 in Fig. 1), which have previously been shown to be absent in Rama 25 cuboidal epithelial cells, but present in both newly-converted elongated cells and the elongated cell line, Rama 29, derived from Rama 25 cells (13). These two polypeptides are defined by their characteristic apparent molecular weights and isoelectric points (Table 1).

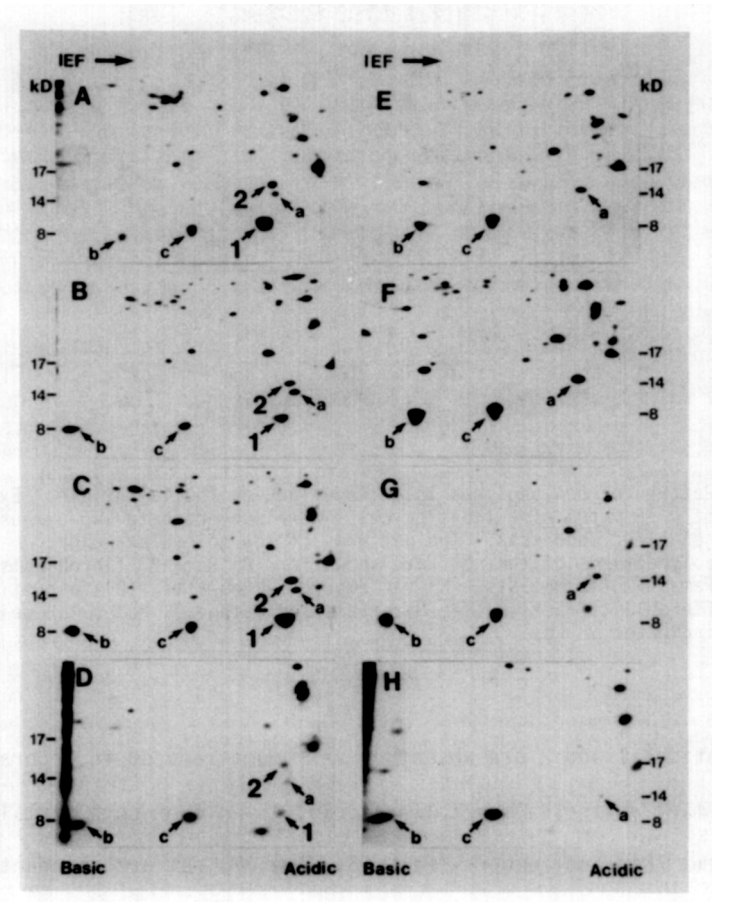

Fig. 1 Two-dimensional gel electrophoresis of low-molecular-weight polypeptides isolated from cultured rat mammary epithelial cells. Extracts of [^{35}S]methionine-labelled cells were subjected to two-dimensional gel electrophoresis. The patterns of radioactive low-molecular-weight polypeptides are shown for (A-D) elongated convertants, (E-H) cuboidal epithelial cells. (A) Rama 29 cells, (B) Rama 401 cells, (C) Rama 37-E5 cells, (D) Rama 712 cells, (E) Rama 25 cells, (F) Rama 41 cells, (G) Rama 37 CL-A3 cells, (H) Rama 704 cells. Molecular weights in kd are shown. a, b and c refer respectively to a 15 kd and two 8-9 kd polypeptides used for pattern recognition.

Table 1: Apparent molecular weights and isoelectric points of low molecular weight polypeptides

Polypeptide* Number	Molecular Weight (Daltons)	Isoelectric Point
1	9,000 ± 520 (13)	5.50 ± 0.27 (26)
2	15,200 ± 540 (27)	5.38 ± 0.28 (24)

Molecular weights and isoelectric points are expressed as mean ± S.D. calculated from the number of experiments shown in brackets. * Numbers refer to Fig. 1.

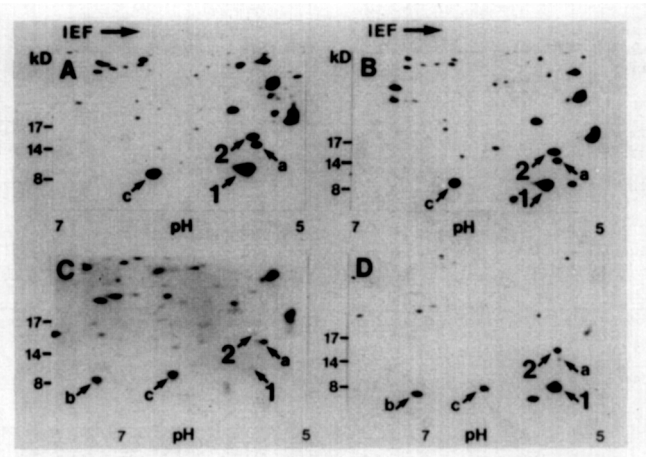

Fig. 2 Pattern of polypeptides in cells of non-epithelial origin. Extracts of [^{35}S]methionine-labelled cells were subjected to two-dimensional gel electrophoresis. The patterns of low-molecular-weight polypeptides of the gels are shown for (A) Rama 27 fibroblasts, (B) Rama 351 fibroblasts, (C) rat embryo fibroblasts, (D) fast-sticking fibroblast fraction (see Materials and Methods). Molecular weights are indicated in kd.

Polypeptides 1 and 2 are absent or very much reduced in separate cloned cuboidal mammary epithelial cell lines (Fig. 1E-H) from both normal glands (Rama 41, Rama 704) and tumours (Rama 25, Rama 37) but are abundant in elongated cell lines (Rama 29, Rama 37-E5, Rama 401, and Rama 712) which have been derived from these epithelial cells (Fig. 1A-D). The presence of small amounts of polypeptides 1 and 2 in Rama 37 cuboidal cell extracts probably reflects spontaneous conversion to elongated cells during culture (6).

Fig. 2 shows that polypeptides 1 and 2 are also abundant in extracts prepared from mammary elongated cell lines of non-epithelial origin (Fig. 2A-B) and present in much smaller radioactive amounts in rat embryo fibroblasts (Fig. 2C). Both low-molecular-weight proteins are found in the cells which grow from a fast-sticking fraction of rat mammary gland (Fig. 2D). Such cultures consist predominantly of fibroblasts that arise from the mesenchymal fraction (12).

Table 2 shows the abundance of the 9 kd and 15.2 kd proteins and shows that Rama 29 cells contain a 16-fold increase in these two proteins when compared with the cuboidal epithelial cell line, Rama 25.

Table 2: Quantitation of low molecular weight polypeptides in rat mammary cells

Cell line	Morphology	% Total radioactivity applied to gel		
		9,000 kd polypeptide	15,200 kd polypeptide	Actin
Rama 29	Elongated	0.26 ± 0.05§	0.078 ± 0.015§	3.5 ± 1.0§
Rama 401	Elongated	0.10 ± 0.04*	0.05 ± 0.03*	N.D
Rama 351	Fibroblast	0.19	0.1	4.8
Rama 27	Fibroblast	0.21	0.1	N.D
Fast-sticking fraction	Fibroblast	0.23	0.038	2.3
Rama 25**	Cuboidal	0.016 ± 0.009*	0.005 ± 0.004*	0.84 ± 0.4*

Radioactive polypeptide spots were cut from two-dimensional gels, digested as described in Materials and Methods and the radioactivity was determined. Values are expressed as mean ± S.D. § = Average of 4 separate determinations. * = Average of 5 separate determinations. ** = Gels were overloaded to detect trace polypeptides; some actin failed to enter the first dimension gels under these conditions.

The Triton-insoluble Proteins of Rat Mammary Cell Lines The cuboidal epithelial cells (Rama 25, Rama 37 CL-A3 and Rama 41 in Fig. 3) show a complex pattern of proteins consisting of actin and additional major polypeptides with molecular weights of 46 - 56 kd. In the case of epithelial Rama 25 cells three separate polypeptides with a molecular weight of 50 kd which differ in isoelectric point have previously been identified as prekeratins (13). Fig. 3B shows that in the preparations from cuboidal epithelial cells vimentin is a minor component of molecular weight 54 kd. The Triton-insoluble proteins of the mesenchyme-derived cell lines, Rama 27 and Rama 351, consist of one major protein, which has a molecular weight of 54 kd. Immunoblotting experiments show this to be vimentin (Fig. 3B). The elongated cell lines, derived from cuboidal mammary epithelial cells, contain a staining pattern of Triton-insoluble proteins identical to the pattern from the mesenchyme-derived cells (Fig. 3).

Patterns of Protein Synthesis in Rat Mammary Cells Fig. 4 shows the two-dimensional gel pattern of acidic polypeptides isolated from epithelial Rama 25 and its elongated convert, Rama 29. The arrows illustrate the major differences in polypeptides between the patterns from the two cell lines. Polypeptides 7 and 8 which are very much reduced in the elongated cell have

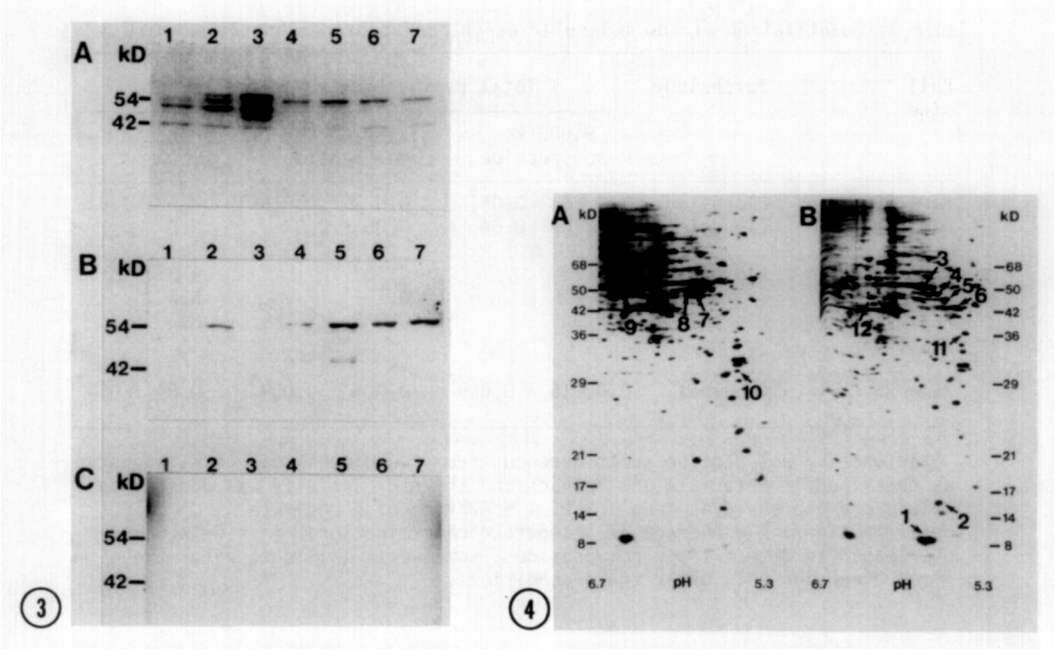

Fig. 3 Pattern of Triton-insoluble proteins from rat mammary cell lines. Triton-insoluble proteins were subjected to polyacrylamide gel electrophoresis in the presence of SDS. (A) The pattern of bands stained with Coomassie Blue. The protein bands were analysed, following electrophoretic transfer to nitrocellulose sheets, by reaction with (B) rabbit anti-vimentin or (C) normal rabbit serum. Bound iodinated antibodies were visualised by autoradiography for 8 h. Tracks 1-3, cuboidal epithelial cells; tracks 4-5, elongated convertants; tracks 6-7, fibroblasts. Track (1) Rama 25 cells, (2) Rama 37 CL-A3 cells, (3) Rama 41 cells, (4) Rama 29 cells, (5) Rama 401 cells, (6) Rama 351 cells, (7) Rama 27 cells. The Triton-insoluble proteins from about 1 x 10^5 cells were loaded onto each track. Molecular weights are indicated in kd.

Fig. 4 Comparison of the major polypeptide changes between cuboidal epithelial Rama 25 cells and elongated Rama 29 cells. Rama 25 and Rama 29 cells were labelled with [^{35}S]methionine. Cell extracts were subjected to two-dimensional gel electrophoresis. Only the acidic portions of each gel are illustrated. (A) Rama 25 cells, (B) Rama 29 cells. Arrows and numbers indicate polypeptide differences which are referred to in Table 3. Molecular weights are indicated in kd.

previously been identified as prekeratin and polypeptide 3 which is increased in relative abundancy in the elongated cells has been identified as vimentin (13). Table 3 shows that there are two broad patterns of synthesis in the cell lines examined. Firstly, the cuboidal epithelial cells synthesise polypeptides 7, 8 and 10 but do not express, or show reduced synthesis of, the elongated-cell specific polypeptides 1, 2, 11, 12. Secondly, the cultured fibroblasts from mammary stroma (Rama 351, Rama 27, fast-sticking fraction) and rat embryos synthesize polypeptides 1, 2, 11, 12, whereas polypeptides 7,

Table 3: The occurrence of polypeptides in extracts of elongated and cuboidal cells and cell lines

Cell	Morphology	Relative Intensity						
		Polypeptide no. (molecular weight (kd))						
		1 (9)	2 (15.2)	11 (37.5)	12 (45.3)	10 (31.0)	7 (50.0)	8 (50.0)
Rama 25	Cuboidal	-	-	++	+	+++	+++	+++
Rama 41	Cuboidal	-	-	++	-	+++	+++	+++
Rama 37	Cuboidal	+	+	++	+	+++	+++	+++
Rama 704	Cuboidal	-	-	++	-	+++	+++	+++
Rama 29	Elongated	+++	+++	+++	+++	-	+/-	+
Rama 37-E5	Elongated	+++	+++	++/+++	+++	-	-	-
Rama 712	Elongated	+++	++	+++	+++	+/-	-	-
Rama 401	Elongated	+++	+++	++	++	-	-	+
Rama 27	Fibroblast	+++	+++	+++	+++	-	-	-
Rama 351	Fibroblast	+++	+++	+++	+++	-	-	-
Rat embryo fibroblasts	Fibroblast	+/++	+++/++	+++	+++	-	-	-
Fast sticking fraction	Fibroblast	+++	+++	+++	+++	-	-	-

Extracts prepared from cells labelled with [^{35}S]methionine were subjected to two-dimensional gel electrophoresis and fluorography. The relative intensities of radioactive spots on the film were estimated by eye using a scale: (-) spot completely absent; (+) trace amount; (++) present at less than maximum intensity relative to surrounding polypeptides; (+++) present at maximum intensity for each particular polypeptide; (++/+) indicates a variation between separate experiments. Polypeptide numbers are the same as those used in Fig. 4.

8, and 10, characteristic of the epithelial cells, are absent. The elongated cells derived from the rat mammary epithelial cell lines (Rama 29, Rama 37-E5, Rama 401, Rama 712) show the pattern of synthesis characteristic of the fibroblastic rat cells.

DISCUSSION

The results presented in this paper suggest that, with regard to patterns of polypeptide synthesis and cytoskeletal components, the elongated cells derived in culture from cuboidal epithelial cells are more similar to cultured mesenchymal cells than epithelial cells. This interpretation is supported by several other lines of evidence. Firstly, Rama 29 cells resemble mesenchyme-derived cultured cells in their response to fibroblast growth factor (22), in the pattern of iodinated cell-surface proteins (2), in the synthesis of fibronectin (3) and in the arrangement of microfilamental

proteins in methanol and acetone-fixed cells (2, 23). In addition, the cell surface antigen Thy-1 is found on the surface of most elongated cells derived from mammary epithelial cells in culture and on the surface of some fibroblasts in vivo (10). The results are pertinent to the elongated convertants which have been shown previously to possess some properties of myoepithelial cells (1, 5). The patterns of protein synthesis of these cultured cells are now shown to resemble fibroblasts more closely than epithelial cells.

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