Keratin expression and its significance in five cultured melanoma cell lines derived from primary, recurrent and metastasized melanomas

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Abstract With the exception of two cases, keratin is not expressed in cultured human melanoma cells. Using 2D-PAGE, immunological and electron microscopic analyses, we found keratin subunits in five established cultured cell lines derived from primary, recurrent and metastasized melanomas. The keratin subunits were composed of K1, K5, K10, K14, K15 and K18 in all cell lines examined, together with vimentin. In addition, K8, K16 and K18 expression were demonstrated in recurrent and metastasized cell lines. The results of the present and our previous study [Katagata Y, et al. J Dermatol Sci 1996;13:219–227] indicate that expression of keratin in melanoma cells may be a universal phenomenon. A specific increase in the proportion of K5 among the keratin subunits was suggestive of the nature of melanoma cells. Moreover, we detected two polypeptides that migrated on 2D-PAGE at positions which did not correspond to those of any keratin subunit. The amino acid sequences of these two polypeptides were determined; one was the human ATP synthase α-chain but the other did not match any known polypeptide in our homology search.

Key words: Keratin; Melanoma cell; ATP synthase α-chain

1. Introduction

Keratin is a component of intermediate filaments, and the major structural protein of epithelial cells, including keratinocytes, showing the greatest heterogeneity of all intermediate filament proteins [1,2]. Keratin subunits have roles in the structure and formation of epithelial cells [3,4] and also in the reorganization of cells during mitosis [5–7]. On the other hand, human melanocytes are developmentally derived from neural crest cells and are localized in the basal cell layer of the epidermis [8]. The main functions of melanocytes are to produce melanin and to supply the melanin to the epidermis as a means of protection against ultraviolet irradiation.

Proliferation is the major characteristic of malignancy in many tissues [9]. It was reported previously that among the intermediate filaments only vimentin was expressed in six human and two murine melanoma cell lines, and co-expression of vimentin and keratin(s) was found only in the human melanoma cell line M5 [10]. The keratin subunits in these cells consisted of the so-called simple epithelial keratins (K7, K8, K17 and K18) and non-keratinized keratin (K13). Subsequently, a correlation was also reported between the co-expression of vimentin with simple epithelial K8 and K18 and the invasive and metastatic behavior of three human melanoma cell lines [11]. A high salt solution was employed to prepare keratin subunits in the above two studies, and we demonstrated that such a solution is not suitable for analysis of keratin subunits in cultured melanoma [12] or SCC cell lines [13–15].

In this report, we describe keratin subunits expressed in cultured melanoma cell lines derived from primary, recurrent, and metastasized melanomas. All of the cell lines employed in this study expressed a novel polypeptide that did not correspond to any keratin in terms of molecular weight (M_r) or isoelectric point (p*I*) and that was not present in the BLAST homology database. This polypeptide may play an important role in the development of melanoma cells.

2. Materials and methods

2.1. Cell lines and culture methods

Primary melanoma (PM)-WK, recurrent primary melanoma (RPM)-MC, and RPM-EP cell lines were established from three melanomas (one original and two recurrent). Malignant melanoma (MM)-LH and MM-RU cell lines were also established from melanomas that had metastasized to the lung and lymph nodes, respectively [16]. Each cell line was named using the patient's initials. These five cultured melanoma cell lines were gifts from Dr. R.H. Byers (Department of Pathology, Boston University School of Medicine, Boston, MA, USA). We maintained the cells in culture for no longer than 10 days during each set of experiments to ensure that their metastatic phenotype would not change as a result of prolonged passage in vitro. Continuous cell cultures were routinely grown as monolayers in plastic dishes (78.5 cm^2, Falcon Plastics, Becton Dickinson Labware, Franklin Lakes, NJ, USA) at 37°C in a humidified incubator with 5% CO_2 in air. We serially cultured all cell lines at plating densities of approximately 1.5×10^3 cells/cm^2 in Minimum Essential Medium supplemented with 10% fetal calf serum (Gibco Laboratories, Grand Island, NY, USA), penicillin (100 units/ml), streptomycin (50 μg/ml), kanamycin (50 μg/ml), and hydrocortisone (0.4 μg/ml). Cell numbers were determined using a hemocytometer. Normal human melanocytes were also cultured using human foreskin melanocyte cell kits (Morinaga Institute of Biological Science, Yokohama, Japan).

2.2. Extraction of keratin polypeptides

After several days, when the cultured cells had reached approximately 80% confluence, cells were quickly rinsed twice with calcium- and magnesium-free phosphate-buffered saline (CMF-PBS) and scraped off with a rubber policeman in 1 ml of the aqueous solution (10 mM Tris-HCl (pH 7.4)/10 mM EDTA/phenylmethylsulfonyl fluoride (PMSF, 10 μg/ml)). The cells were sonicated for 15 s using an ultrasonic disrupter (Tomy, UR-20p, Tokyo, Japan) at a power output setting of 7, and centrifuged at 10000 rpm for 10 min. The residue was then washed by centrifugation (10000×*g*, 10 min) 3 times to remove aqueous soluble materials. The residue was dissolved in 50 μl of 10 M urea/10% 2-mercaptoethanol at 37°C for 6 h [17]. After centrifugation, one-tenth of the supernatant was treated with the same volume of 2×SDS buffer [18] for 3 min at 100°C, then the treated solution was subjected to SDS-PAGE (10% polyacrylamide) gels. To minimize contamination, all experimental procedures were carried out using sterile apparatus and conditions.

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Abbreviations: 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; K, keratin; SCC, squamous cell carcinoma

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2.3. SDS-PAGE

SDS-PAGE was carried out by the standard method [18]. Analysis of keratin polypeptides by 2D-PAGE was performed as described [19] with ampholytes of pH 3.5–10 (LKB, Bromma, Sweden) using the remaining 45 μl of each fraction. Quantitative analyses of expressed keratins were carried out with an ATTO Densitograph AE6920-M using photographs of gels stained with Coomassie brilliant blue (CBB) R-250.

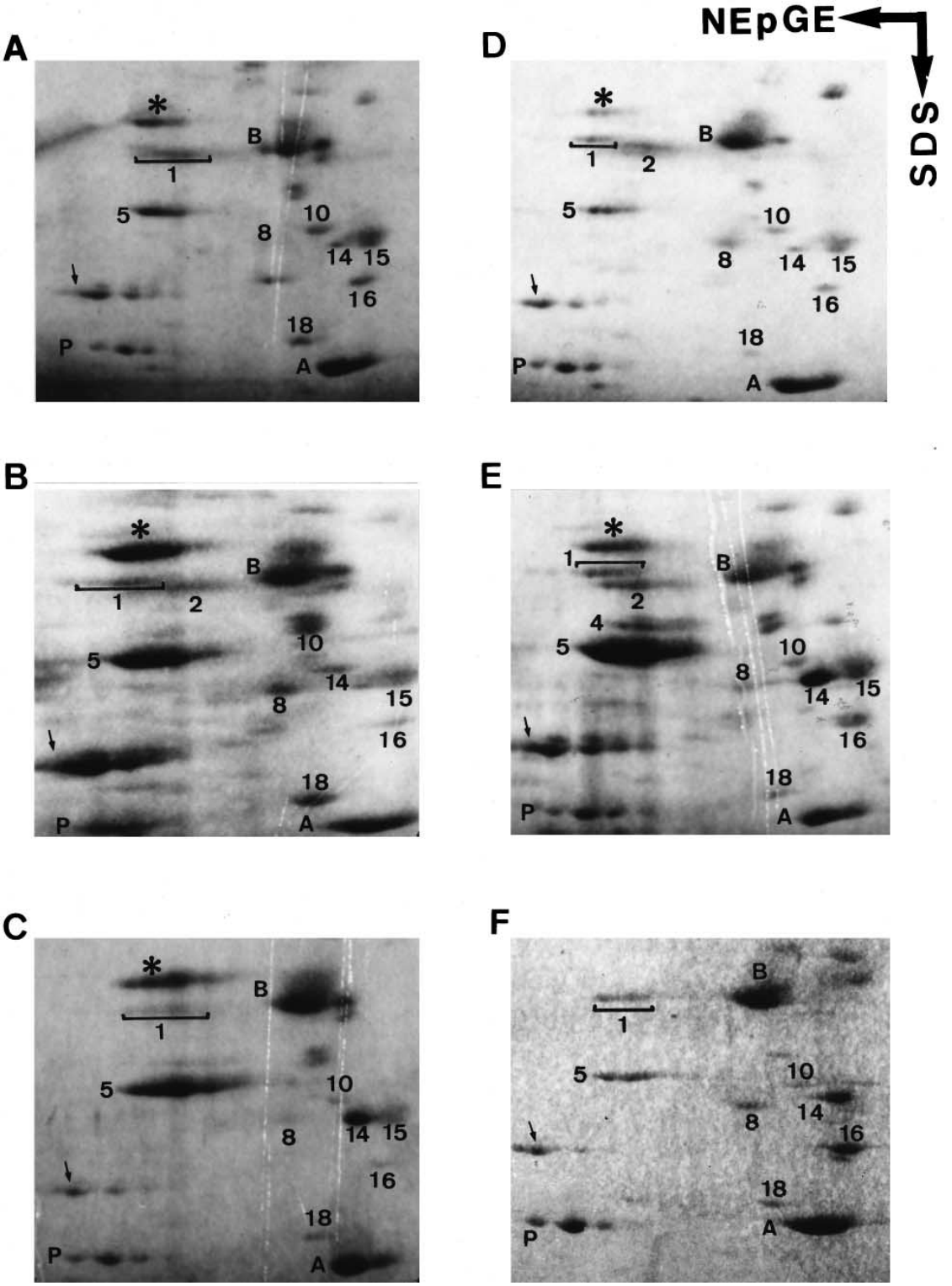


Fig. 1. 2D-PAGE of keratin subunits extracted from five cultured human melanoma and normal melanocyte cells. A: PM-WK; B: RPM-MC; C: RPM-EP; D: MM-LH; E: MM-RU; F: normal melanocyte cells. Standard proteins: P, 3′-phosphoglycerokinase (p*I* 7.4, M_r 43 kDa); B, bovine serum albumin (p*I* 6.35, M_r 68 kDa); A, rabbit α-actin (p*I* 5.4, M_r 42 kDa). The numbers denoted keratin nomenclature according to Moll et al. [2]. Separation of the proteins in the first dimension was by nonequilibrium pH gradient gel electrophoresis (NEpGE) and in the second dimension by 12.5% polyacrylamide gels containing sodium dodecyl sulfate (SDS).

Table 1
Summary of expressed keratin subunits in cultured melanoma cells

Melanoma cell line	Keratin subunit number									
	1	2	4	5	8	10	14	15	16	18
PM-WK	+	−	−	+	±	+	+	+	+	+
RPM-MC	+	+	−	2+	+	+	+	+	+	2+
RPM-EP	+	−	−	2+	±	+	2+	+	±	+
MM-LH	+	+	−	+	+	+	+	+	+	+
MM-RU	+	+	+	2+	+	+	2+	2+	2+	+

2.4. Immunoblotting analyses

After SDS-PAGE, proteins on the unstained gels were electroblotted onto polyvinylidene difluoride (PVDF) membranes (0.2 mm, Bio-Rad Lab., Hercules, CA, USA) at 2 mA/cm^2 for 1 h using the standard procedure [20]. To block nonspecific protein binding sites on the PVDF membranes, they were incubated with defatted milk for 1 h. Membrane strips were washed in CMF-PBS containing 0.005% Tween 20 and reacted with anti-keratin monoclonal antibodies (34βB4, Enzo Diagnostic, New York, NY, USA; 34βE12 and 35βH11, DAKO A/S, Glostrup, Denmark; LL002, Cymbus Bioscience Ltd., Southampton, UK; V9 (anti-vimentin), Boehringer Mannheim Biochemica, Mannheim, Germany) at a dilution of 1:50 in CMF-PBS at 37°C for 40 min. The strips were washed in CMF-PBS containing 0.005% Tween 20 and incubated with peroxidase-conjugated anti-mouse immunoglobulin rabbit serum (Dako Corp.) 1:300 at 37°C for 40 min. After washing with CMF-PBS containing 0.005% Tween 20, the strips were reacted in 0.03% 3,3′-diaminobenzidine/0.01% hydrogen peroxide/Tris-HCl buffer (pH 7.6) and washed in distilled water. Staining of standard proteins on the PVDF membranes was performed with 0.04% CBB R-250.

2.5. Electron microscopy

Cultured melanoma cells were prepared for electron microscopy by addition of 1 ml of 2.5% glutaraldehyde in 2 mM cacodylate buffer at pH 7.4 for 30 min and 1 ml of 2 mM osmium tetroxide to each dish at 4°C for 2 h. Then, the dishes were dehydrated by passage through a graded ethanol series and embedded in Quetol 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under an electron microscope.

2.6. Amino acid sequence

After 2D-PAGE, the gels were electroblotted onto PVDF membranes with a semidry apparatus (ATTO Co. Ltd., Tokyo, Japan) at 2 mA/cm^2 for 40 min. The blotted membranes were then stained with CBB R-250. Two spots that were not keratin polypeptides were cut out and their amino acid sequences determined using an automated protein sequencer (Applied Biosystems 470A, Perkin-Elmer Co., Rockville, MD, USA). When the N-terminal amino acid of the polypeptide was blocked, the sequence was determined with a protein N-terminal deblocking kit, according to the manufacturer's protocol (Takara Biomedicals Co. Ltd., Kyoto, Japan).

3. Results

3.1. SDS- and 2D-PAGE

Keratin subunits were prepared from cultured melanoma cell lines using an aqueous rather than a high salt solution which many researchers have employed in previous studies. The extracts were subjected to SDS-PAGE, and the resultant profiles indicated the presence of more than six keratin subunits with M_r values between 40 and 67 kDa [2] in five melanoma cell lines (data not shown).

To identify the keratin subunits extracted from each cell line, we performed 2D-PAGE. The results confirmed the presence of seven keratin subunits (K1, K5, K10, K14, K15, K16, and K18) in all cell lines examined and K8 was expressed in all but two cell lines (PM-WK and RPM-EP) (Fig. 1 and Table 1). Moreover, we detected two unknown spots (M_r ca. 80 kDa, pI 7.8; M_r ca. 48 kDa, pI 8.2) at the same positions in each cell line (labeled with asterisk and arrow, respectively, Fig. 1A–E). In normal melanocytes, only the former spot was detected (Fig. 1F). Vimentin expression was also confirmed with V9 anti-vimentin monoclonal antibody (data not shown).

3.2. Amino acid sequences

The amino acid sequences of the two unknown spots were determined with an automated amino acid sequencer using the extract from each membrane. The smaller spot (marked with an arrow; M_r ca. 48 kDa, pI 8.2) corresponded very closely (85.7%) with the just mature N-terminal region of ATP synthase α-chain [21]. The N-terminus of the larger spot (marked with an asterisk) was blocked; however, its amino acid sequence was elucidated (Table 2). From the results of a BLAST homology search, this sequence did not correspond to any known protein, and we have tentatively named it melanoma-related protein (MRP)-1.

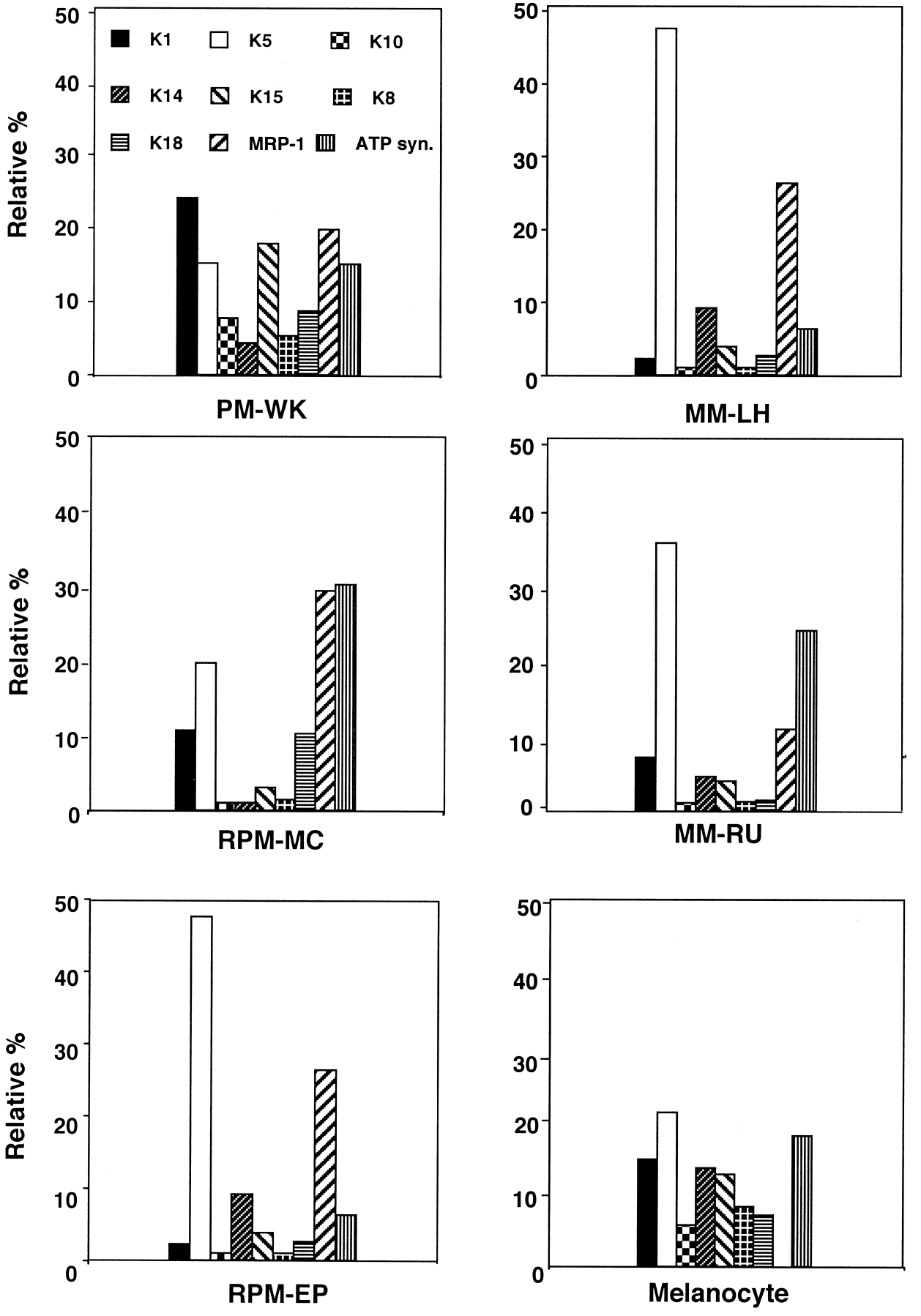
3.3. Quantitative analyses of keratin subunits, ATP synthase α-chain and MRP-1

To elucidate the relative amounts of each keratin subunit, ATP synthase α-chain and MRP-1, we analyzed gels stained

Table 2
The amino acid sequences determined of MRP-1 and ATP synthase α-chain[1]

MRP-1[2]	1	5	10	20	30
(Mr, Ca. 80 Kd, pI 7.8)	——————EGVVVR	VLSGILDDGG	RNLIYKLSID		
Arrow marks;			Q K T G T	A E M S F	I L E E V I L G N D T
(Mr, Ca. 48 Kd, pI 8.2)			· · · · · · · · ·	· · · ·	· · · · · · ·
ATP synthase α-subunits[3];	N L H A S N T	R L Q K T G T	A E M S S	I L E E R I	L G N D T S V D L E
	35	40	44	50	60 65

[1]Amino acid sequences are shown by one letter indication.
[2]Melanoma related protein-1 (asterisks in Fig. 1A–E). −, not detected.
[3]The sequence of ATP synthase α-chain [21].
Arrows in Fig. 1A–F.


K1
K5
K10
K14
K15
K8
K18
MRP-1
ATP syn.
Relative %
50
40
30
20
10
0
PM-WK
MM-LH
RPM-MC
MM-RU
RPM-EP
Melanocyte

with CBB R-250 using the Densitograph 3.1 program on a Macintosh computer. The densitographic results of each cell line are illustrated, showing relative amounts of each component (Fig. 2). Levels of K5 subunit were high in recurrent and metastasized melanoma cell lines, and MRP-1 and/or ATP synthase α-chain were also abundant in these cells.

3.4. Immunoblotting analyses

After SDS-PAGE, the electroblotted strips were reacted with several anti-keratin and anti-vimentin monoclonal antibodies. Only 34βB4 was also reacted to several bands, except for K1 (Fig. 3).

3.5. Electron microscopy

Electron micrographs of each cultured melanoma cell line showed large and dense bundles of tonofilaments in the cytoplasm. The tonofilaments were located close to the plasma membrane rather than in the perinuclear space (Fig. 4), in contrast to the results reported previously in human melanocytes [22].

4. Discussion

In this study, we employed an aqueous solution as a buffer to prepare keratin subunits from melanoma cell lines. Although highly concentrated salt and detergent (KCl, NaCl and Triton X-100) have been employed by many researchers to prepare keratin subunits from malignant neoplasms [23,24], cultured SCC [25,26] and melanoma cell lines [10,11], these reagents were originally used on native tissues or tumors to remove large amounts of subcutaneous fat tissues [27,28]. In fact, we demonstrated previously that this aqueous solution was more efficient than the high salt solution (1.5 M KCl/1% Triton X-100/150 mM NaCl/10 mM Tris-HCl (pH 7.4)/5 mM EDTA/PMSF (10 μg/ml)) [27] for detection of keratin subunits in cultured SCC [13–15] and melanoma cell lines [12]. Thus, we considered that high salt solution may be too harsh for cultured cells.

Torejdosiewicz et al. [10] reported that five keratin subunits (simple epithelial and non-keratinized types) were expressed together with vimentin in only the M5 human melanoma cell line, and no keratin was detected in five other human and two mouse melanoma cell lines. Since we estimated more than six kinds of keratin subunits in cultured melanoma cell lines by SDS-PAGE (data not shown), we performed 2D-PAGE to characterize them further because 2D-PAGE systems are superior for differentiation between the 21 kinds of keratin subunits with similar M_r (40–67 kDa) and pI (4.9–7.8). As shown in Fig. 1A–F, we confirmed the presence of stratified, hyperproliferative and other types of keratins, together with vimentin, as determined by the values of M_r and pI in all melanoma cell lines examined (Table 1). Small amounts of K8, a marker of late stage metastasis, and K18 [29,30] were present in specific melanoma cell lines, especially RPM-MC, MMs-LH and -RU. Combined with K13, which is a marker of early stage metastasis [31,32], these subunits are known to be related to tumor invasion and to changes in epithelial-mesenchymal interactions [33]. In addition to the above reports, Hendrix et al. [11] demonstrated that co-ex-

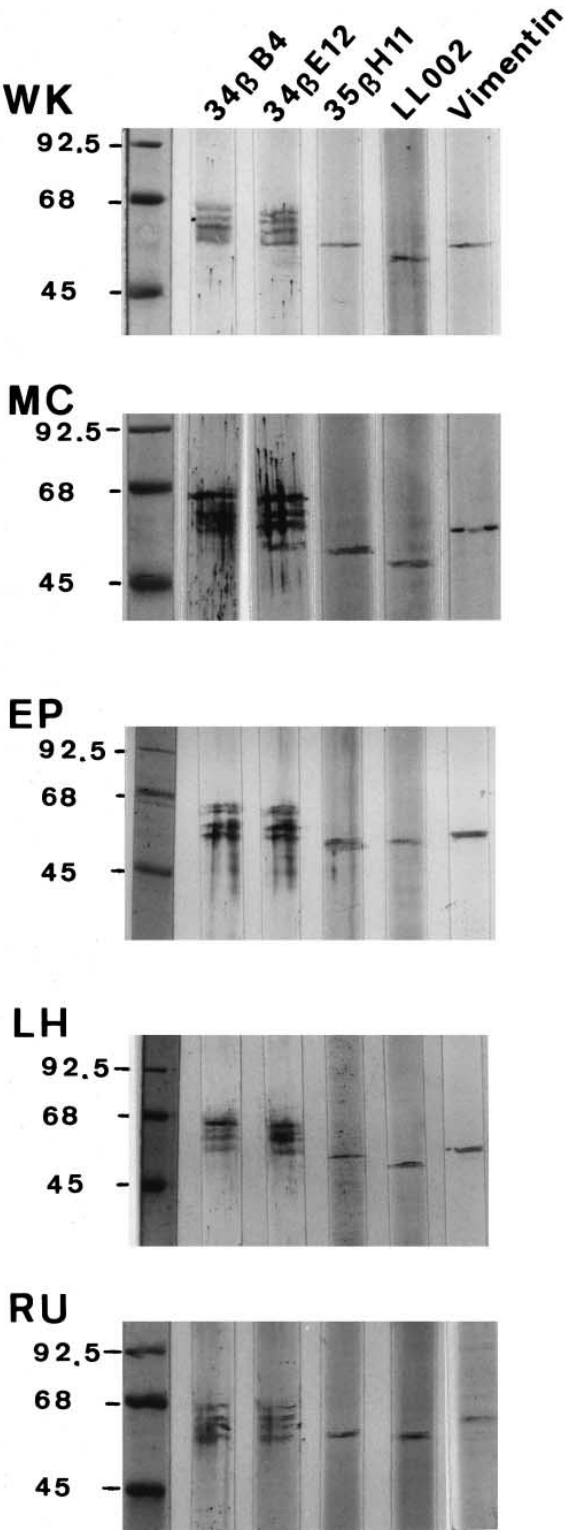


Fig. 3. Immunoblot analyses of keratin subunits extracted from five melanoma cells with keratin monoclonal antibodies: 34βB4 (against K1), 34βE12 (against K1, K5, K10 and K14), 35βH12 (against K8), LL002 (against K14), V9 (against vimentin). Standard proteins which stained with 0.04% CBB R-250 after transfer: 92.5, phosphorylase A; 68, bovine serum albumin; 45, ovalbumin (kDa×10^3).

←

Fig. 2. The relative percentages of keratin subunits, MRP-1, and ATP synthase α-chain expressed in five melanoma cell lines and normal melanocyte cells. In each panel, bars from left to right indicate K1, K5, K10, K14, K15, K8, K18, MRP-1, and ATP synthase α-chain, respectively.

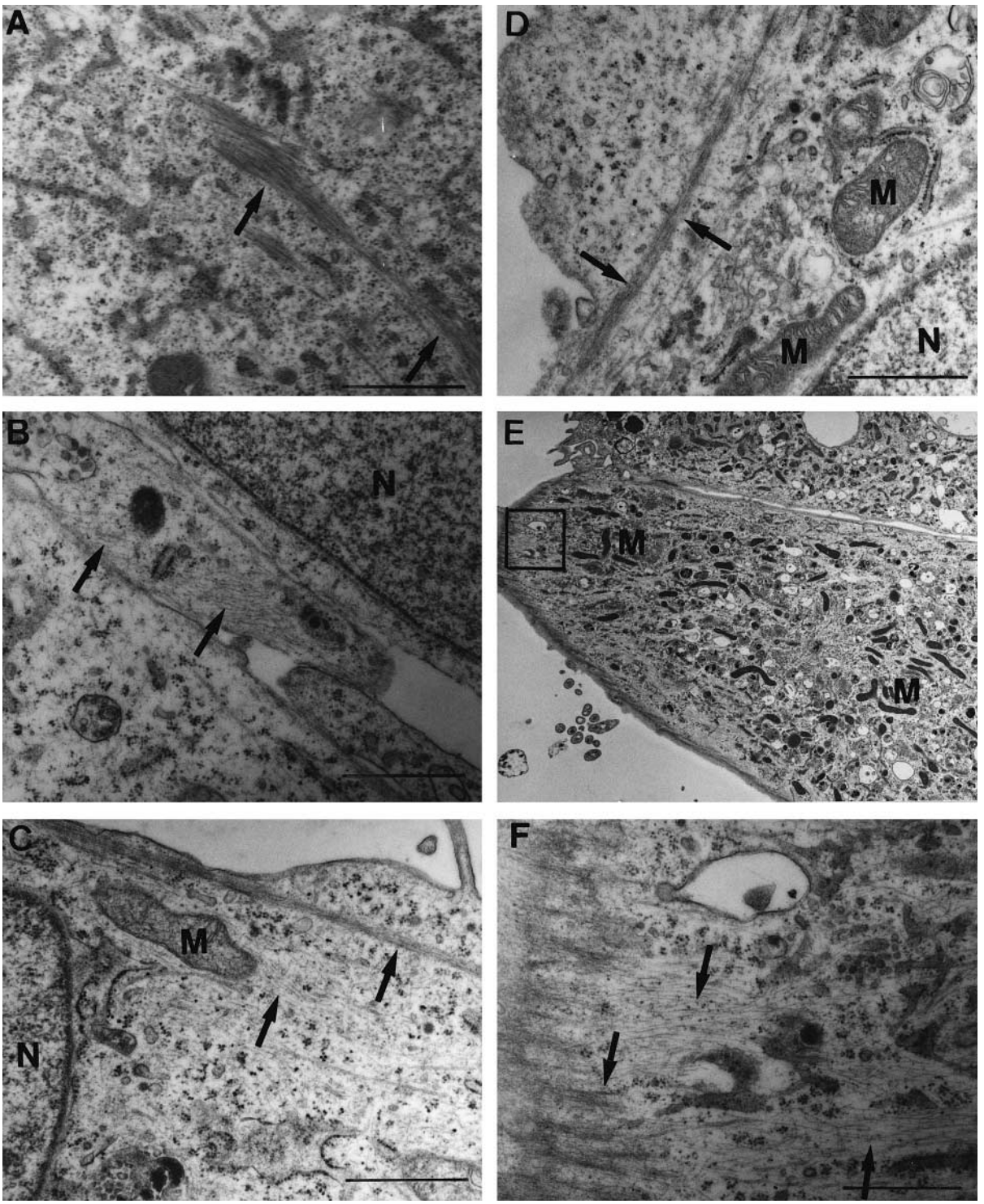


Fig. 4. Electron micrographs of the filaments in five melanoma cells. The filaments (arrow) are shown near the plasma membrane rather than perinuclear. A: PM-WK; B: RPM-MC; C: RPM-EP; D: MM-LH; E: MM-RU (×3000); F: MM-RU (×20000). A–D, F: magnification ×20000. Scale bar, 1 μm. E: magnification ×3000. Scale bar, 1 μm. F: high magnification (×20000) of the circled region in E. M, mitochondria; N, nucleus.

pression of vimentin with K8 and K18 subunits was highly correlated with the invasive and metastatic behavior of human melanoma cells. Our results were in accordance with those of the above reports, indicating the presence of keratin subunits in all the cell lines examined. Furthermore, the expression of ATP synthase α-chain may be common in cultured cells, because this polypeptide was reported to be expressed in six cultured SCC lines [13–15], eight melanoma cell lines [12], and cultured melanocytes (Fig. 1F), and was detected in five of the melanoma cell lines examined in this study. It seems reasonable to suppose that this component plays fundamental roles in cultured cells. The observation that MRP-1 was expressed in only five of the melanoma cell lines employed in this study, and was not expressed in normal melanocytes,

suggests a unique role of this molecule in cell behavior and function. All these findings were possible because we employed an aqueous instead of a high salt solution as a buffer for cultured melanoma cell lines. Many investigators may therefore have overlooked ATP synthase α-chain and MRP-1 due to the use of high salt solution.

The relative amounts of keratin subunits in PM-WK, which expressed MRP-1 and ATP synthase α-chain, were not significantly different. As this cell line was established and derived from a nodular melanoma, the relative amounts of keratin subunits may be similar to those of human melanoma cells in vivo. On the other hand, the keratin contents in recurrent (RPMs-MC and -EP) and metastatic (MMs-LH and -RU) cell lines were not constant and showed a characteristic value for each cell line. However, there was a tendency in these cell lines for higher levels of type II (K1 and K5 in this case) than type I (K10 and K14 in this case). As K5 and K14 are expressed only in the basal cells, this relationship may be reflected in the above phenomena. This may also be related to the observation that type II keratin gene induces type I keratin expression [34]. From the results presented above, we believe that the expression of MRP-1 and/or ATP synthase α-chain may also be necessary to act at the first step of recurrence or metastasis of melanoma cells.

To confirm the presence of keratin subunits in the melanoma cell lines, we performed immunological analyses using several anti-keratin monoclonal antibodies (Fig. 3). With the exception of 34βB4, the results indicated the co-expression of vimentin and keratin in all melanoma cell lines employed, similarly to many other cultured and tumor cells [11,35,36]. The co-expression of these molecules may be essential for maintenance of the epithelial cells themselves. Morphological analyses should be performed to elucidate cell structure and function. With the exception of one report [22] of 10 nm filaments in human melanocytes, there is no evidence that keratin filaments are present in melanoma cells. In the above study, the filaments shifted from the perinuclear area to the center of the dendritic processes in close association with melanosomes during different stages of UV-mediated melanin pigmentation. The discrepancy between our findings and theirs may be due to the melanoma cells employed. Keratin expression may be related to changes from melanocytes to melanoma cells, together with the expression of MRP-1, which is absent in melanocytes (Figs. 1 and 2).

In conclusion, the results of this study (Figs. 1–4) combined with those in our previous report [12] suggest that keratin subunits are universally present in cultured melanoma cells. In addition, our results indicated that ATP synthase α-chain and/or MRP-1 together with keratin subunits and vimentin may play significant roles in melanoma cell structure, metastasis, and recurrence.

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References

[1] M. Osborn, K. Weber, Cell 31 (1982) 303–306.
[2] R. Moll, W.W. Franke, D.L. Schiller, B. Geiger, R. Krepler, Cell 31 (1982) 11–24.
[3] P.D. Kouklis, E. Hutton, E. Fuchs, J Cell Biol 127 (1994) 1049–1060.
[4] E. Fuchs, K. Weber, Annu Rev Biochem 63 (1994) 345–382.
[5] B. Horwitz, H. Kupfer, Z. Eshhar, B. Geiger, Exp Cell Res 134 (1981) 281–290.
[6] W.W. Franke, E. Schmid, C. Grund, B. Geiger, Cell 30 (1982) 103–113.
[7] E.B. Lane, S.L. Goodman, L.K. Trejdosiewicz, EMBO J 1 (1982) 1365–1372.
[8] K.A. Holbrook, R.A. Underwood, A.M. Vogel, A.M. Gown, H. Kimball, Anat Embryol 180 (1989) 443–455.
[9] G. Poste, I.J. Fidler, Nature 283 (1980) 139–146.
[10] L.K. Trejdosiewicz, J. Southgate, J.T. Kemshead, G.M. Hodges, Exp Cell Res 164 (1986) 388–398.
[11] M.J.C. Hendrix, E.A. Seftor, Y.-W. Chu, R.E.B. Seftor, R.B. Nagle, K.M. McDaniel, S.P.L. Leong, K.H. Yohem, A.M. Leibovitz, F.L. Meyskens, D.H. Conaway Jr., D.R. Welch, L.A. Liotta, W. Stetler-Stevenson, J Natl Cancer Inst 84 (1992) 165–174.
[12] Y. Katagata, T. Aoki, Y. Hozumi, T. Yoshida, S. Kondo, J Dermatol Sci 13 (1996) 219–227.
[13] Y. Katagata, K. Aso, M. Sato, T. Yoshida, Biochem Biophys Res Commun 182 (1992) 1440–1445.
[14] Y. Katagata, J Dermatol 19 (1992) 781–785.
[15] Y. Katagata, T. Yoshida, FEBS Lett 316 (1993) 5–11.
[16] H.R. Byers, T. Etho, J.R. Doherty, A.J. Sober, M.C. Mihm Jr., Am J Pathol 139 (1991) 423–435.
[17] T. Achtstaetter, M. Hatzfeld, R.A. Quinlan, D.C. Parmelee, W.W. Franke, Methods Enzymol. 134 (1986) 355–371.
[18] U.K. Laemmli, Nature 227 (1970) 680–688.
[19] P.Z. O'Farrell, H.M. Goodman, P.H. O'Farrell, Cell 12 (1977) 1133–1142.
[20] H. Towbin, T. Staehelin, J. Gordon, Proc Natl Acad Sci USA 76 (1979) 4350–4354.
[21] H. Kataoka, C. Biswas, Biochim Biophys Acta 1089 (1991) 393–395.
[22] K. Jimbow, T.B. Fitzpatrick, J Cell Biol 65 (1975) 481–488.
[23] J. Viac, A. Reano, J. Thivolet, J Cutan Patol 9 (1982) 377–390.
[24] R. Moll, I. Moll, W.W. Franke, Arch Dermatol Res 276 (1984) 349–363.
[25] A.L. Rubin, N.L. Parenteau, R. Rice, J Cell Physiol 138 (1989) 208–214.
[26] P.G. Sacks, V. Oke, B. Amos, T. Vasey, R. Lotan, Int J Cancer 44 (1989) 926–933.
[27] W.W. Franke, D.L. Schiller, R. Moll, S. Winter, E. Schmid, I. Engelbrecht, J Mol Biol 153 (1981) 933–959.
[28] J. Schweizer, H. Winter, J Biol Chem 258 (1983) 13268–13272.
[29] C. Caulin, C. Bauluz, A. Gandarillas, A. Cano, M. Quintanilla, Exp Cell Res 204 (1993) 11–21.
[30] Y.-W. Chu, R.B. Runyan, R.G. Oshima, M.J.C. Hendrix, Proc Natl Acad Sci USA 90 (1993) 4261–4265.
[31] R. Nischt, D.R. Roop, T. Mehrel, S.H. Yuspa, M. Rentrop, H. Winter, J. Schweizer, Mol Carcinogen 1 (1988) 96–108.
[32] I. Gimenez-Conti, C.M. Aldaz, A.B. Bianchi, D.R. Roop, T.J. Slaga, C.J. Conti, Carcinogenesis 11 (1990) 1995–1999.
[33] A.C. Markey, E.B. Lane, L.J. Churchill, D.M. MacDonald, I.M. Leigh, J Invest Dermatol 97 (1991) 763–770.
[34] G.J. Giudice, E. Fuchs, Cell 48 (1987) 453–463.
[35] N. Azumi, H. Battifora, Am J Clin Pathol 88 (1987) 286–296.
[36] A. Om, T. Ghose, G. Rowden, Virchows Arch B 61 (1991) 81–87.