the conceptual and technical foundations for further developing HA formulations for the topical treatment of cutaneous malignant melanoma.

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References

[1] Brown TJ, Alcorn D, Fraser JR. Absorption of hyaluronan applied to the surface of intact skin. J Invest Dermatol 1999;113:740–6.
[2] Karjalainen JM, Tammi RH, Tammi MI, Eskelinen MJ, Agren UM, Parkkinen JJ, et al. Reduced level of CD44 and hyaluronan associated with unfavorable prognosis in clinical stage I cutaneous melanoma. Am J Pathol 2000;157:957–65.
[3] Brown MB, Hanpanitcharoen M, Martin GP. An in vitro investigation into the effect of glycosaminoglycans on the skin partitioning and deposition of NSAIDs. Int J Pharm 2001;225:113–21.
[4] Heijn M, Roberge S, Jain RK. Cellular membrane permeability of anthracyclines does not correlate with their delivery in a tissue-isolated tumor. Cancer Res 1999;59:4458–63.
[5] Mummert ME, Mohamadzadeh M, Mummert DI, Mizumoto N, Takashima A. Development of a peptide inhibitor of hyaluronan-mediated leukocyte trafficking. J Exp Med 2000;192:769–79.

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Letter to the Editor

Comparison of keratin expression in cultured human adenocarcinoma cell lines

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Adenocarcinoma is a cancer arising from various glandular tissues, possibly derived from epithelial cells of the breast, colon, lung, prostate, stomach, pancreas, cervix, or vagina. As implied by its developmental origins, epithelial tissues – keratins (Ks) – might be expressed in the adenocarcinoma or cultured cell lines. Although some K subunits might also be expressed in cultured adenocarcinoma cell lines, few reports have described K expression data [1].

The adenocarcinoma cell lines used for this study were derived as follows: RERF-LC-KO from the lung, HEC-1 [2] from the uterus, and SW-13 [3] from the adrenal cortex. The Health Science Research Resources Bank, Osaka, Japan, supplied these cell lines and SF-TY (human fibroblast cell line). Additionally, HaCaT, which was gifted by Dr. NE Fusenig was analyzed as normal keratinocyte cells [4].

The Ks were prepared from cultured cells using a particular medium. For the K preparation prepared from each cell line, we used an aqueous solution (10 mM Tris–HCl (pH 7.4)/10 mM EDTA/1 mM PMSF) [5], rather than the high-salt solution which had been used in earlier studies of cultured cells. The former solution proved to be useful for the effective extraction of keratin peptides in cultured cells [6]. The extracts were subjected to 2D-PAGE to identify the Ks present in the cultured cells of three kinds. As presented in Fig. 1, the Ks in RERF-LC-OK comprised K6, K8, K10, K14, K16, K17, K18, together with vimentin. In HEC-1, expressed Ks were almost identical to those of RERF-LC-OK, except for K19 (Fig. 1). On the other hand, a slightly different expression of Ks was detected in SW-13—only K7, K8, K16, and K17 were expressed (Fig. 1C). Neither K13 nor K19, so-called tumorigenic K markers, was expressed, as evidenced by the different expression patterns from those of the other two adenocarcinoma cell lines. The periodic acid Schiff staining results showed no keratins detected in three cultured adenocarcinoma cell lines and HaCaT cells (data not shown)—these data agree with our previously reported data of cultured squamous-cell carcinoma, malignant melanoma, and angiosarcoma cells [5].

Almost all anti-K monoclonal antibodies used for this study reacted well to Ks, as estimated from the results of 2D-PAGE in the three cultured adenocarcinoma cell lines described above. From numerous Western blotting data, a fine distinction of K expression was confirmed as presented in Fig. 1D. Particularly, K19 expression was found only in the HEC-1 cell line; a difference of K8/K18 expression was also confirmed. Consequently, K8/K18 expression was less in SW-13 and more in RERF-LC-OK. Vimentin expression respectively showed a definite level in the three cultured cell lines. These results were repeated several times and yielded good reappearance. The expressed Ks are presented in Table 1 along with the data of 2D-PAGE and Western blotting. In addition, four expressed K subunits – K8, K18, K13, and K19 – and actin/vimentin were extracted and then investigated in detail in relation to K and cell mobility. Although K8/K18, K13, and K19 have been reported as tumorigenic K markers [7,8], the SW-13 cell line showed a striking difference from other cell lines among the same adenocarcinoma cells. Furthermore, K19 overexpression decreases the invasion potential by diminishing their migrating capability in oral SCC [9]. Conversely, it is true that overexpression of K19 in hepatocellular carcinoma cells is related to metastatic behavior [10]—the relation of K expression and malignancy in tumor cells is insufficient to establish universal concepts. The data shown in Table 1 are useful for additional determination of the presence or absence of K expression. Based on the four Ks described above, the degree of malignancy in adenocarcinoma cells of three kinds was estimated temporarily as follows: HEC-1 > RERF-LC-OK > SW-13.

Experimental procedures of cell migration were performed according to the manufacturer's protocols. Each prepared cell

Abbreviations: 2D-PAGE, two-dimensional polyacrylamide gel electrophoresis; K, keratin; SDS, sodium dodecyl sulfate.

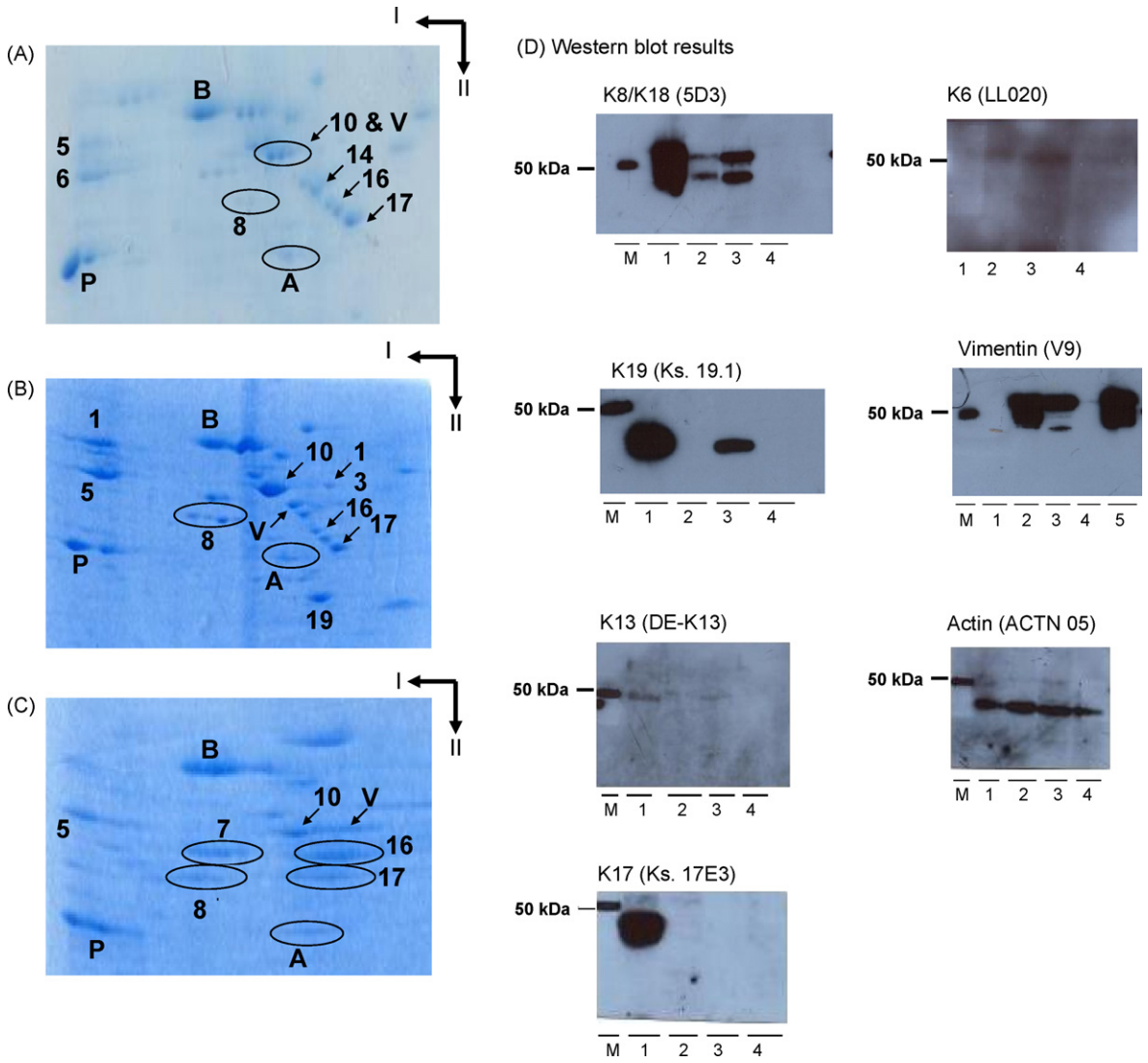

Fig. 1. 2D-PAGE and Western blot analyses of extracts prepared from cultured human adenocarcinoma cell lines of three kinds. 2D-PAGE: (A) RERF-LC-OK, (B) HEC-1, and (C) SW-13. Numbers were estimated as K subunits. Standard proteins in 2D-PAGE: (A) rabbit α-actin (pI 5.4, Mr 42 kDa) and (B) bovine serum albumin (pI 6.35, Mr 68 kDa); (P) 3′-phosphoglyceric phosphokinase (pI 7.4, Mr 43 kDa). Separation of proteins in the first dimension was by nonequilibrium pH gradient gel electrophoresis (I) and in the second dimension by 12.5% polyacrylamide gels containing sodium dodecyl sulfate (II). (D) Western blot analyses were conducted using several K-monoclonal antibodies: 5D3, K8/K18; Ks. 19.1, k19; DE-K13, K13; Ks.17E3, k17; LL020, K6; V9, vimentin; and ACTN05, actin. M, molecular marker. (1) HaCaT, (2) RERF-LC-OK, (3) HEC-1, (4) SW-13, and (5) SF-TY.

Table 1
Summary of keratin expressed in three kinds of adenocarcinoma cell lines from the data of 2D-PAGE and Western blotting.

Cultured cell		Keratin (K) number														
		K1	K5	K6	K7	K8	K10	K13	K14	K15	K16	K17	K18	K19	V	A
HaCaT	2D-PAGE	○	○	○	○	○	○	○	○	○	○	○	○	×	×	–
	WB	×	○	×	○	○	×	○	○	○	○	○	○	○	×	○
Adenocarcinoma																
RERF-LC-OK	2D-PAGE	×	×	○	○	○	○	○	○	○	○	○	○	×	○	–
	WB	–	△	△	○	○	–	△	○	–	○	○	○	×	○	○
HEC-1	2D-PAGE	×	○	×	○	○	○	○	○	×	○	○	×	○	○	–
	WB	–	△	△	○	○	–	○	△	–	○	○	○	○	○	○
SW-13	2D-PAGE	×	○	×	○	○	○	○	×	×	○	×	×	×	○	–
	WB	–	×	△	×	△	–	×	×	–	○	○	×	×	△	○

V, vimentin; A, action; WB, Western blotting; (○) present; (△) faint; (×) absent; (–) not detected.

Table 2
The migration activity of three kinds of adenocarcinoma cell lines.

	(A) NC migrated cell no. ($n = 4$)	(B) NC invasion (%)	(C) Tested migrated cell no.	(D) Tested cell invasion (%)	(E) Invasion index
RERF-LC-OK	1005	2.01	1226	2.45	1.219
HEC-1	900	1.8	508	1.02	0.567
SW-13	515	1.03	917	1.83	1.781
HaCaT	610	1.219	755	1.51	1.239

NC, negative control; spread cell no. (50,000/well); (A): average no. of four membranes in each control sample; (B): (A)/spread cell no. × 100; (C): average no. of four membranes in each tested sample; (D): (C)/spread cell no. × 100; (E) (D)/(B).

suspension (5×10^4 cells/0.5 ml) was set to a Falcon cell companion plate (inner chamber, 8 μm pore size membrane, 24 inserts each, BD Biocoat Matrigel Invasion chamber; Becton Dickinson Labware, Bedford, MA, USA) using four wells of each sample. Then, to falcon TC companion plates (outer chamber), 0.7 ml of tenascin (100 ng/ml) migration enhancement factor was added. As a negative control, each particular medium +0.1% FCS only was added to the falcon TC companion plate. The invasion chambers were placed in a 5% CO_2 incubator at 37 °C for 24 h. Using a hematocytometer, migrated cells of matrigel matrix were then counted with the matrigel matrix stained with hematoxylin and eosin. The Percentage Invasion and Invasion Index were derived using the following formulae

$$\%\,\text{Invasion} = \left(\frac{\text{Mean No. of cells invading through the Matrigel insert membrane}}{\text{Mean No. of spread cells}}\right) \times 100$$

$$\text{Invasion Index} = \frac{\%\,\text{Invasion test cells (+tenascin)}}{\%\,\text{Invasion control cells (−tenascin)}} \times 100$$

Therefore, according to the protocol given by Becton Dickinson Labware, Bedford, MA, USA, the index values mean the following: >1, high migration activity; <1, low migration activity; =1, even activity. Actually, SW-13 indicated the highest activity and was estimated as follows in the order of cell migration activities: SW-13 (invasion index, 1.781) > RERF-LC-OK (invasion index, 1.219) > HEC-1 (invasion index, 0.562). In addition, the invasion index of HaCaT, a control cell line, was 1.239 (Table 2).

It also appears that actin, its promoter factor, and extracellular environment might be necessary against a cell invasion. Furthermore, loss and down-regulation of K13, K14, and K16 are related to invasive and metastatic capabilities [8]. Considering the existence or non-existence of K (K13, K14, K19, and K8/K18) expression and other factors related to invasion (actin and its promoted factors, etc.) in each cultured cell, the order of migration activities might be reflected to a great degree. As reported in other reports in the relevant literature, these data concur with those of the invasion index, as indicated for K19 overexpression in HEC-1. These difficult problems must be resolved thoroughly through future studies.

References

[1] Sabia M, Fritze F, Geddert H, Weyherm C, Rosenberg R, Gellert K. Differentiation between pancreaticobiliary and upper gastrointestinal adenocarcinomas. Am J Clin Pathol 2007;128:255–9.
[2] Kuramoto H, Hamano M. Establishment and characterization of the cell line of a human endometrial adenocarcinoma. Eur J Cancer 1977;13:253–9.
[3] Leibovitz A, McCombs III WB, Johnston D, McCoy CE, Stinson JC. New human cancer culture lines. I. SW-13, small-cell carcinoma of the adrenal cortex. J Natl Cancer Inst 1973;51:691–7.
[4] Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. J Cell Biol 1988;106:761–71.
[5] Katagata Y, Hirayama T. Unexpected expression of Hsp47, a replacement of one amino acid (Val 7 Leu) in the amino terminal region, in cultured human tumorigenic cell lines. J Dermatol Sci 2008;49:33–8.
[6] Katagata Y, Takeda H, Ishizawa T, Hozumi Y, Kondo S. Occurrence and comparison of the expressed keratins in cultured human fibroblasts, endothelial cells and their sarcomas. J Dermatol Sci 2002;30:1–9.
[7] Hendrix MJ, Seftor EA, Chu YW, Trevor KT, Seftor RE. Role of intermediate filaments in migration and metastasis. Cancer Metastasis Rev 1996;15: 507–25.
[8] Morifuji M, Taniguchi S, Sakai H, Nakabeppu Y, Ohishi M. Differential expression of cytokeratin after orthotopic implantation of newly established human tongue cancer cell lines of defined metastatic ability. Am J Pathol 2000;156: 1317–26.
[9] Crowe DL, Milo GE, Shuler CF. Keratin 19 downregulation by oral squamous cell carcinoma lines increase invasive potential. J Dent Res 1999;78:1256–63.
[10] Ding SJ, Li Y, Tan YX, Jiang MR, Tian B, Liu YK, et al. From proteomic analysis to clinical significance. Mol Cell Proteomic 2004;3:73–81.

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