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New Human Oral Squamous Carcinoma Cell Line and Its Tumorigenic Subline Producing Granulocyte Colony-stimulating Factor

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A new human carcinoma cell line, MISK81-5, was established from a metastatic lymph node of oral squamous cell carcinoma. Immunocytochemical and ultrastructural observations revealed an obvious epithelial origin of the cell line. Chromosome analysis revealed a hypertriploid karyotype with numerical and structural anomalies. MISK81-5 cells could form a tumor mass in the subcutaneous tissue of recipient BALB/c athymic mice only when coinjected with Matrigel. A stem cell assay revealed that conditioned medium (CM) of MISK81-5 contained granulocyte colony-stimulating factor (G-CSF) or interleukin-6 activity. Quantitation by ELISA disclosed a higher concentration of G-CSF in the CM of MISK81-5 than in the CM of other squamous and gastric carcinoma cell lines. The sMISK, that was derived from MISK81-5 as a subpopulation of the cell line having higher tumorigenicity, also showed a similar hematopoietic stimulating activity to that of MISK81-5. These characteristics of the MISK81-5 cell line and its subpopulation, sMISK will be useful for studying the biological behavior of oral squamous cell carcinomas and its relation to hematopoietic stimulating factors.

Key words: Squamous cell carcinoma — Carcinoma cell line — Oral mucosa — Granulocyte colony-stimulating factor — Tumorigenicity

A large number of cell lines from human solid tumors have been established and have contributed to research on various facets of tumor cell biology. Squamous cell carcinoma (SCC) is the most common type of cancer occurring at any site of the body, including the oral cavity. However, fewer SCC cell lines have been established and characterized from oral mucosa than from any other site, probably due to the lower prevalence of oral cancer and/or difficulty in cultivation because of contamination by oral bacterial flora. We established a new cell line, MISK81-5, from a metastatic lymph node of a poorly differentiated SCC that had originated in the buccal mucosa. The cell line produces high levels of granulocyte colony-stimulating factor (G-CSF). Recent studies have suggested that some hematopoietic stimulating factors, such as granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, interleukin-1 (IL-1), IL-6 and G-CSF, can stimulate the *in vivo* and *in vitro* proliferation, invasion, motility and metastasis of tumor cells.[1–5] Nevertheless, there have been only a few investigations into oral SCC cell lines that produce hematopoietic stimulating factors.[6,7] Here, we describe the establishment and characterization of the above-mentioned new oral SCC cell line, MISK81-5, that produces a high level of G-CSF, and its highly tumorigenic subline, survival MISK (sMISK).

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MATERIALS AND METHODS

Clinical history of the host The patient, an 81-year-old woman who had a 5-month history of an enlarging tumor mass in the right buccal mucosa, was admitted to the First Department of Oral Surgery of Kyushu University (Fukuoka) on June 10, 1991. She was diagnosed as having poorly differentiated SCC, and was treated by chemotherapy and radiotherapy followed by partial resection of the cheek with radical neck dissection. In spite of these therapies, the tumor developed rapidly and invaded the cervical region. The patient died of aspiration pneumonia on December 1 of the same year. The peripheral blood showed increased white cell counts up to 41,800/mm^3 with the progression of carcinoma, without any infectious foci. Almost all of the white cells in the peripheral blood were mature neutrophils.

Primary culture and cell cloning A metastatic lymph node obtained at radical neck surgery was cut into 1–2 mm cubes, placed on the bottom of sterile culture dishes containing 2 ml of α-minimum essential medium (α-MEM; Gibco Inc., Grand Island, NY) and 10% fetal calf serum (FCS; Bioproducts Inc., Walkersville, MD)

and 1% penicillin-streptomycin (Gibco), and incubated in a 5% CO_2 atmosphere at 37°C. After 1–2 weeks, polygonal epithelioid and spindle-shaped fibroblastoid cells had begun to propagate in the periphery of some explants. From these cells, epithelioid cells were selected and cloned by means of limiting dilution and the use of a fine Pasteur pipet attached to a micromanipulator (Narishige Co., Ltd., Tokyo), and the clonal epithelioid cell line, MISK81-5, was obtained. The cell line has been maintained for over 70 serial passages.

Light and electron microscopy Materials were fixed in 10% neutral formalin, embedded in paraffin and cut into 5 μm sections, which were stained with hematoxylin and eosin. Cultured cells collected by trypsinization were dispersed onto glass slides, fixed in absolute methanol, and stained immunohistochemically by means of avidin-biotin-peroxidase complex.[8] The primary antibodies used for immunocytochemistry are summarized in Table I.

Several confluent dishes were selected, prepared for transmission electron microscopy by means of routine techniques, and observed under a JEOL 200 CX (JEOL Co., Ltd., Tokyo) electron microscope.

Cell-doubling time Culture dishes (60 mm diameter) were inoculated with 10^4 cells in 5 ml of α-MEM containing 10% FCS. From day 4 to day 10 after inoculation, the number of cells harvested by trypsinization was counted. Growth curves were constructed from the cell counts and the doubling time was calculated.

Chromosome analysis Chromosomes of MISK81-5 cells at the 40th passage were analyzed by means of G-banding,[9] and described according to the ISCN (1985).[10]

Tumorigenicity Cultured MISK81-5 cells (1×10^7) were transplanted subcutaneously into both male and female BALB/c nu-nu mice (5 males and 5 females, 6–10 weeks old, Japan SLC Inc., Shizuoka). In another experiment, 0.1 ml of cold α-MEM containing $5\text{–}10\times10^6$ MISK81-5 cells was mixed with 0.1 ml of cold liquid basement membrane matrix, Matrigel (9.04 mg/ml, Becton Dickinson Labware, Bedford, MA), and immediately injected subcutaneously into nude mice. These animals were killed 4 weeks after transplantation, and examined for tumor growth, invasion and metastasis. A subline, sMISK was obtained from a rapid growing tumor of MISK81-5 cells implanted with Matrigel, 12 weeks thereafter. The tumorigenicity of sMISK was tested by means of re-implantation as described above.

Analysis of hematopoietic stimulating activity in conditioned media The conditioned medium (CM) of MISK81-5 and sMISK cells cultured for 4 days was harvested and centrifuged. The supernatant was frozen at −20°C until use. To analyze the hematopoietic activities in these CM, stem cell assay was performed using mouse bone marrow cells (BDF_1, 10–20 weeks old, female, Japan SLC). Details of this assay are described in the literature.[11–13] On day 7, granulocyte (G), granulocyte-macrophage (GM) and macrophage (M) colonies were counted under an inverted microscope, according to morphological criteria.[11–13] To confirm the G-CSF activity in CM, goat anti-hrG-CSF polyclonal antibody (Central Research Laboratories of Chugai Pharmaceutical Co. Ltd., Tokyo) was added to the stem cell cultures.

Quantitation of human G-CSF and IL-6 in the CM by means of an enzyme-linked immunosorbent assay (ELISA). Human G-CSF and IL-6 in CM of MISK81-5 were quantified by using ELISA systems (Amersham International plc, Buckinghamshire, UK). Other CM of six human carcinoma cell lines, HSC-2, HSC-3, HSC-4, MKN 28, MKN 45 and MKN 74 (supplied by the Japanese Cancer Research Resources Bank, Tokyo) were also prepared and quantified. The HSC and MKN series were human oral SCC and gastric cancer cell lines, respectively.

The *in vitro* effect of human recombinant G-CSF and IL-6 on the growth of MISK81-5 MISK81-5 cells were cultured in 5 ml of α-MEM containing 10% FCS with or without human recombinant G-CSF (hrG-CSF, Kirin Brewery Co. Ltd., Tokyo) or human recombinant IL-6 (hrIL-6, Central Research Laboratories of Ajino-

Table I. Immunohistochemical Staining of MISK81-5 Cells

Primary antibodies[a) No.	Designation	Immunized animal	Positive MISK81-5 cells (%)[b)
1.	Anti-epidermal growth factor (EGF) recetor	mouse, monoclonal	100
2.	Anti-human epithelial membrane antigen (EMA)	mouse, monoclonal	9.1
3.	Anti-keratin, wide spectrum screening[c)	rabbit, polyclonal	98.0
4.	Anti-vimentin	mouse, monoclonal	0
5.	Anti-desmin	mouse, monoclonal	0

a) No. 1 antibody was purchased from Oncogene Science Inc. (Uniondeal, NY) and No. 2–5 from the Dako Corporation (Carpinteria, CA).
b) Two hundred cells were observed, and the percentage of positive cells was calculated.
c) No. 3 is specific for epidermal keratin subunits of 60, 58, 56, 52, 51 and 48 kDa.

moto Co., Inc., Yokohama). Six days after cultivation, the trypsinized cells were harvested and the cells were counted.

Statistical analysis Statistical analysis was performed using Student's t test with Welch's correction. Differences between groups were considered significant if the P value was less than 0.05.

RESULTS

Morphology and growth of cultured cells The MISK81-5 cells growing on the bottom of culture dishes were polygonal, and proliferated in a pavement-like arrangement (Fig. 1a). Multiplication or piled-up foci were rarely observed. The growth profile of sMISK was similar to that of MISK81-5. However, multiplication and piled-up foci were often present in cultured sMISK. Ultrastructurally, the outline of the cells was almost smooth with poorly developed spinous processes (Fig. 1b). An intercellular bridge-like structure between each cell was occasionally observed. Cell junctional apparatus such as tonofibrils, zonula adherens and desmosomes, was also poorly developed. The cytoplasm contained a large number of poly-ribosomes and secondary lysosome-like intracytoplasmic structures.

Immunocytochemically, all MISK81-5 cells were positive for the EGF-receptor. Most MISK81-5 cells reacted with rabbit antisera containing polyclonal anti-keratin antibodies. The anti-EMA antibody reacted with 9.1% of the MISK81-5 cells. No immunoreaction to the anti-vimentin or anti-desmin antibody was detected (Table I).

The doubling time during the logarithmic growth phase was calculated to be 25 h.

Chromosome analysis The chromosome number varied within a hypertriploid range from 72 to 79 in a sample of 50 metaphases. The modal number was 77. Common changes in 10 cells were as follows. The numerical changes including no copies of the normal chromosomes 1, 2, 3 and 13, one copy of the normal X chromosome, three copies of the normal chromosome 18, and structural changes including 1p+, 1q−, 2p−, 3p+q−, 5q−, 8q+, 13p+, 14q−, 15p+, 16q+, 19p+ and Xp+, and 9–13 unidentified marker chromosomes were observed. In addition, one copy of chromosome 17, three of chromosome 7 and four each of chromosomes 6 and 9 were also detected in 9 of 10 cells. In 8 of 10 cells, i(5p) was observed. Furthermore, two structural profiles of 3p+q−, 10p+ and Xp+ were identified.

Tumorigenicity The MISK81-5 cells transplanted into the subcutaneous tissue of nude mice did not form any tumor masses (n=10). However, the cells formed small tumors (n=4, 8×5×3–5×3×2 mm) when coinjected with Matrigel. The sMISK cells proliferated without Matrigel in nude mice and formed larger tumors (n=5, 14×12×6–10×7×3 mm), and induced marked myeloid hematopoiesis in bone marrow, granulocytosis in the lung and/or granulocytic extra-medullary hematopoiesis in the liver, lymph node and spleen with or without splenomegaly. The histological findings of the tumors were similar to those in well-moderately differentiated squamous cell carcinoma with expansive growth, without invasion or matastasis.

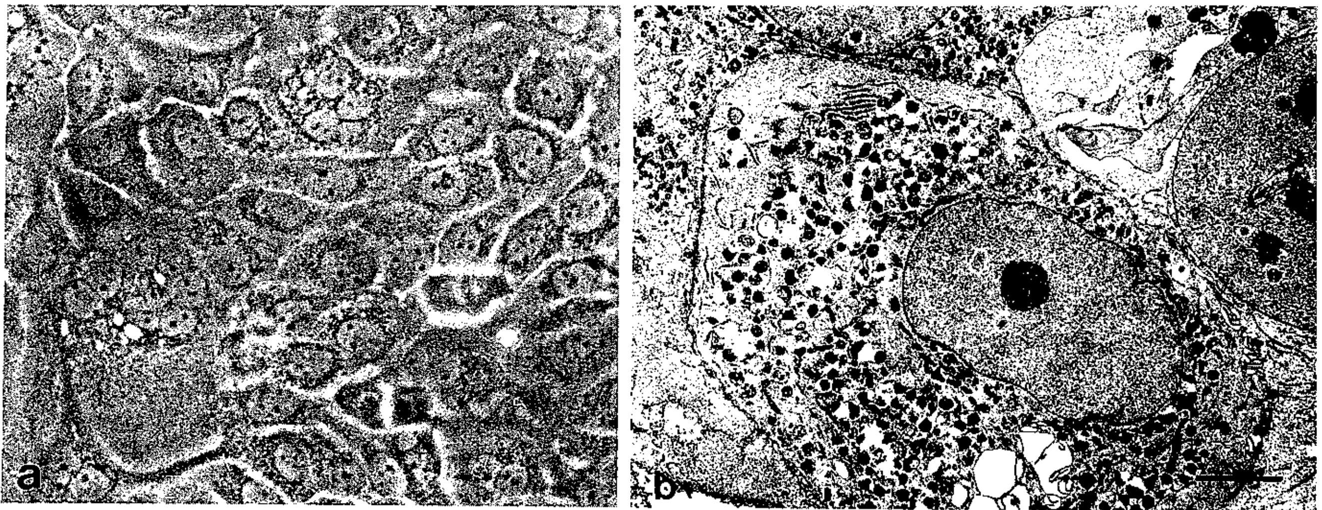

Fig. 1. Phase-contrast micrograph and electron microscopy of MISK81-5. Polygonal-shaped MISK81-5 cells are seen attached to the bottom of a culture dish, and proliferated in a pavement-like arrangement. Multiplication or piled-up foci are rare (a, ×330). The outline of the cytoplasm is relatively smooth, and poor in spinous processes. Large numbers of secondary lysosome-like dense materials are dispersed in the cytoplasm (b, ×2100, bar=5 mm).

Table II. Hematopoietic Stimulating Activity in MISK81-5 and sMISK CM by Stem Cell Assay

Samples	Anti-G-CSF antibody[a]	Number of colonies (mean±SE, n=4)		
		G	GM	M
None (control)	—	0.3±0.3	0	1.0±0.7
20 ng hrG-CSF	—	8.0±0.6[b]	0.3±0.3	3.0±0.4
	7 μl	0	0	0
20 ng hrIL-6	—	6.8±1.0[c]	3.0±0.7[d]	4.8±0.9[e]
	7 μl	6.3±1.5	3.3±0.5	8.3±1.0
10% MISK81-5 CM	—	11.8±1.0[b]	4.8±0.6[d]	5.8±1.1[e]
	7 μl	0	0	3.5±0.3
10% sMISK CM	—	13.0±0.4[b]	6.3±0.8[f]	7.0±0.4[b]
	7 μl	0	0	2.3±0.9

a) A volume of 7 μl of goat anti-human G-CSF polyclonal antibody was enough to neutralize 40 ng of G-CSF activity.
b) $P<0.001$, *c*) $P<0.01$, *d*) $P<0.05$, *e*) $P<0.02$, *f*) $P<0.005$, compared with control cultures.
Abbreviations of colonies; G, granulocyte; GM, granulocyte-macrophage; M, macrophage.

Table III. Evaluation of Human G-CSF and Human IL-6 in Conditioned Media

Samples	G-CSF (ng/ml) mean±1SD		IL-6 (ng/ml) mean±1SD	
Medium[a]	<0.01	(n=4)	<0.01	(n=4)
MISK81-5 CM	19.38±4.79	(n=4)	1.61±0.19	(n=4)
HSC-2 CM	2.59±0.09[b]	(n=2)	1.17±0.06[c]	(n=4)
HSC-3 CM	3.10±0.14[b]	(n=2)	0.35±0.03[d]	(n=2)
HSC-4 CM	0.03±0.01[e]	(n=2)	0.93±0.01[b]	(n=2)
MKN 28 CM	<0.01	(n=2)	0.01	(n=1)
MKN 45 CM	<0.01	(n=2)	0.03	(n=1)
MKN 74 CM	<0.01	(n=2)	0.01	(n=1)

Human G-CSF and IL-6 levels in various conditioned media were measured using human G-CSF and IL-6 ELISA kits (Amersham), respectively.
a) Medium (α-MEM) contained 10% fetal calf serum.
b) $P<0.01$, *c*) $P<0.05$, *d*) $P<0.001$, *e*) $P<0.005$, compared with the quantities of G-CSF and IL-6 in MISK81-5 CM.

Table IV. Effect of hrG-CSF or hrIL-6 on the Growth of MISK81-5

	Growth factor		
	None	hrG-CSF (20 ng/ml)	hrIL-6 (20 ng/ml)
Number of cells[a] ($\times 10^5$, n=4)	2.1±0.5	1.7±0.3	2.0±0.3

MISK81-5 cells (1.7×10^4) were cultured in α-MEM containing 10 % FCS with or without hrG-CSF or hrIL-6. On day 6, the number of trypsinized cells was counted.
a) The number of cells represents the mean±SE.

Hematopoietic stimulating activity in the conditioned medium MISK81-5 and sMISK CM both stimulated the formation of G, GM and M colonies by mouse bone marrow cells (Table II). These activities were similar to those of hrG-CSF and hrIL-6.[14–16] The G-CSF activity in CM was neutralized by goat anti-G-CSF antibody. However, M colony formation was not completely abolished by anti-G-CSF antibody.

Evaluation of human G-CSF and IL-6 in conditioned media The colony-forming activity of CM suggested that the MISK81-5 cells produced and released G-CSF or IL-6 into CM.[14–16] Therefore, we quantified these factors in MISK81-5 CM by ELISA. The concentrations of G-CSF and IL-6 in CMs of MISK81-5 were 19–32 ng/ml and 1.61–3.55 ng/ml, respectively. These values were significantly higher than those in the CMs obtained from other cell lines. Representative experimental data are shown in Table III.

Effect of hrG-CSF or hrIL-6 on the *in vitro* growth of MISK81-5 cells The *in vitro* growth of MISK81-5 cells was not modified by hrG-CSF or hrIL-6 at a concentration of 20 ng/ml (Table IV).

DISCUSSION

We established a new SCC cell line, MISK81-5, from a metastatic lymph node of a poorly differentiated buccal SCC, and maintained it for over 70 serial passages. The poorly differentiated characteristics of the primary carcinoma seemed to have been inherited by MISK81-5 as judged from morphological and immunohistochemical examinations.

The MISK81-5 cells injected into the subcutaneous tissue of nude mice were not tumorigenic. However, coinjection with Matrigel[17, 18] resulted in tumor formation. The major component of the Matrigel is laminin,

which promotes cell adhesion, migration, growth and type IV collagenase activity.[19–21] These properties of laminin in Matrigel may have augmented the tumorigenicity of MISK81-5 cells in nude mice. However, sMISK derived from the MISK81-5 cells had a higher tumorigenic potency without Matrigel. Therefore it is likely that an essential alteration of cellular properties had occurred in the sMISK cells.

Hematopoietic stimulating factors play important roles in the proliferation, invasion and metastasis of malignant tumors,[1–5] and several SCC cell lines produce and release some hematopoietic stimulating factors.[6, 7, 22–25] The present study disclosed that the MISK81-5 cells produce and release more G-CSF than other cell lines derived from SCC and adenocarcinomas. The host patient of the MISK81-5 cell line showed a developing granulocytosis consisting of mature neutrophils according to the progression of carcinoma tissue. This phenomenon may be related to the high G-CSF-producing ability of MISK81-5, and therefore the G-CSF productive potency seems to be an essential property of the primary carcinoma cells. However, little is known about the mechanism by which SCC cell lines produce excessive G-CSF.[25] G-CSF is coded by a single gene located on the q21-q22 region of chromosome 17.[26] The chromosomal analysis of MISK81-5 revealed only one copy of chromosome 17. Therefore, the numerical anomaly of chromosome 17 in MISK81-5 cells may be related to the high level of G-CSF production. Further analyses of the chromosomes as well as other factors in MISK81-5 should be performed to solve this issue.

Berdel *et al.* have reported that G-CSF directly stimulates the growth of some non-hematopoietic tumor cells *in vitro*.[1] However, the *in vitro* growth of MISK81-5 was not modified by hrG-CSF in the culture media at a concentration of 20 ng/ml, suggesting the lack of a direct effect of G-CSF on the growth of MISK81-5 cells. It has been suggested that neutrophils activated by G-CSF promote the growth, invasion and metastasis of tumor cells.[5, 7, 27–30] This promoting effect of G-CSF on the growth of malignant cells seems to be reflected by the rapid growth of carcinoma in the host patient of the MISK81-5 cells, with increasing granulocytosis. However, the promoting effect of G-CSF on the malignant phenotype has remained controversial. Colombo *et al.*[31] observed a suppressive effect of G-CSF on tumorigenicity. Their report proved that tumorigenic murine colon adenocarcinoma C-26 lost its tumorigenicity in nude mice after transfection with G-CSF gene and expression of it. This suppressive effect of G-CSF on tumorigenicity may be supported by the fact that MISK81-5 cells were weakly tumorigenic in nude mice. However, sMISK cells showed a higher tumorigenicity, although they produce similar levels of G-CSF to those of MISK81-5 cells. Therefore it will be of interest to compare the phenotypes and genotypes of MISK81-5 and sMISK cells.

The MISK81-5 cells also produced relatively higher amounts of IL-6 than other cell lines. Furthermore, MISK81-5 cells may produce macrophage-colony stimulating factor[14] and/or other cytokines, because macrophage colony formation was not completely abolished by anti-G-CSF antibody. MISK81-5 and sMISK cell lines should be useful for studying the tumor biology of oral SCC and the influence of hematopoietic stimulating factors, especially that of G-CSF, on the malignant phenotype of tumors.

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REFERENCES

1) Berdel, W. E., Danhauser-Riedl, S., Steinhauser, G. and Winton, E. F. Various human hematopoietic growth factors (interleukin-3, GM-CSF, G-CSF) stimulate clonal growth of nonhematopoietic tumor cells. *Blood,* **73,** 80–83 (1989).

2) Filderman, A. E., Bruckner, A., Kacinski, B. M., Deng, N. and Remold, H. G. Macrophage colony-stimulating factor (CSF-1) enhances invasiveness in CSF-1 receptor-positive carcinoma cell lines. *Cancer Res.,* **52,** 3661–3666 (1992).

3) Hamburger, A. W., Lurie, K. A. and Condon, M. E. Stimulation of anchorage-independent growth of human tumor cells by interleukin 1. *Cancer Res.,* **47,** 5612–5615 (1987).

4) Tamm, I., Cardinale, I., Krueger, J., Murphy, J. S., May, L. T. and Sehgal, P. B. Interleukin 6 decreases cell-cell association and increases motility of ductal breast carcinoma cells. *J. Exp. Med.,* **170,** 1649–1669 (1989).

5) Segawa, K., Ueno, Y. and Kataoka, T. *In vivo* tumor growth enhancement by granulocyte colony-stimulating factor. *Jpn. J. Cancer Res.,* **82,** 440–447 (1991).

6) Sato, N., Asano, S., Ueyama, Y., Mori, M., Okabe, T., Kondo, Y., Ohsawa, N. and Kosaka, K. Granulocytosis and colony-stimulating activity (CSA) produced by a human squamous cell carcinoma. *Cancer,* **43,** 605–610

(1979).
7) Yanoma, S., Watanabe, S., Utakoji, T., Kubota, A., Tsukuda, M. and Sawaki, S. Role of cytokines produced by head and neck tumor cell lines in tumor metastasis. *Proc. Jpn. Cancer Assoc., 50th Annu. Meet.*, 208 (1991) (in Japanese).
8) Hsu, S.-M., Raine, L. and Fanger, H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody PAP procedures. *J. Histochem. Cytochem.*, **29**, 577–580 (1981).
9) Seabright, M. A rapid banding technique for human chromosomes. *Lancet*, **ii**, 971–972 (1971).
10) ISCN 1985. "An International System for Human Cytogenic Nomenclature," ed. D. G. Harnden and H. P. Klinger (1985), published in collaboration with Cytogenet. Cell Genet., Karger, Basel.
11) Nakahata, T. and Ogawa, M. Identification in culture of a class of hemopoietic colony-forming units with extensive capability to self-renew and generate multipotential hemopoietic colonies. *Proc. Natl. Acad. Sci. USA*, **79**, 3843–3847 (1982).
12) Iscove, N. N. and Sieber, F. Erythroid progenitors in mouse bone marrow detected by macroscopic colony formation in culture. *Exp. Hematol.*, **3**, 32–43 (1975).
13) Matsuo, K., Oda, H., Fukushima, T., Matsuoka, J., Hashimoto, N., Tadokoro, K., Kohama, H., Sato, M., Jasmin, C., Urano, Y. and Tange, T. Detection of burst-promoting activity in spleens of myeloproliferative sarcoma virus-infected mice using serum-free cultures. *Acta Pathol. Jpn.*, **41**, 259–264 (1991).
14) Clark, S. C. and Kamen, R. The human hematopoietic colony-stimulating factors. *Science*, **236**, 1229–1237 (1987).
15) Wong, G. G., Witek-Giannotti, J. S., Temple, P. A., Kriz, R., Ferenz, C., Hewick, R. M., Clark, S. C., Ikebuchi, K. and Ogawa, M. Stimulation of murine hemopoietic colony formation by human IL-6. *J. Immunol.*, **140**, 3040–3044 (1988).
16) Suda, T., Yamaguchi, Y., Suda, J., Miura, Y., Okano, A. and Akiyama, Y. Effect of interleukin 6 (IL-6) on the differentiation and proliferation of murine and human hemopoietic progenitors. *Exp. Hematol.*, **16**, 891–895 (1988).
17) Fridman, R., Giaccone, G., Kanemoto, T., Martin, G. R., Gazdar, A. F. and Mulshine, J. L. Reconstituted basement membrane (Matrigel) and laminin can enhance the tumorigenicity and the drug resistance of small cell lung cancer cell lines. *Proc. Natl. Acad. Sci. USA*, **87**, 6698–6702 (1990).
18) Kleinman, H. K., McGarvey, M. L., Hassell, J. R., Star, V. L., Cannon, F. B., Laurie, G. W. and Martin, G. R. Basement membrane complexes with biological activity. *Biochemistry*, **25**, 312–318 (1986).
19) Terranova, V. P., Williams, J. E., Liotta, L. A. and Martin, G. R. Modulation of the metastatic activity of melanoma cells by laminin and fibronectin. *Science*, **226**, 982–985 (1984).
20) Liotta, L. A., Rao, C. N. and Wewer, U. M. Biochemical interactions of tumor cells with the basement membrane. *Annu. Rev. Biochem.*, **55**, 1037–1057 (1986).
21) Turpeenniemi-Hujanen, T., Thorgeirsson, U. P., Rao, C. N. and Liotta, L. A. Laminin increases the release of type IV collagenase from malignant cells. *J. Biol. Chem.*, **261**, 1883–1889 (1986).
22) Partridge, M., Chantry, D., Turner, M. and Feldman, M. Production of interleukin-1 and interleukin-6 by human keratinocytes and squamous cell carcinoma cell lines. *J. Invest. Dermatol.*, **96**, 771–776 (1991).
23) Danner, M. and Luger, T. A. Human keratinocytes and epidermoid carcinoma cell lines produce a cytokine with interleukin 3-like activity. *J. Invest. Dermatol.*, **88**, 353–361 (1987).
24) Matsuguchi, T., Okamura, S., Kawasaki, C., Shimoda, K., Omori, F., Hayashi, S., Kimura, N. and Niho, Y. Constitutive production of granulocyte colony-stimulating factor and interleukin-6 by a human lung cancer cell line, KSNY: gene amplification and increased mRNA stability. *Eur. J. Haematol.*, **47**, 128–133 (1991).
25) Nishizawa, M., Tsuchiya, M., Watanabe-Fukunaga, R. and Nagata, S. Multiple elements in the promoter of granulocyte colony-stimulating factor gene regulate its constitutive expression in human caricnoma cells. *J. Biol. Chem.*, **265**, 5897–5902 (1990).
26) Kanda, N., Fukushige, S.-I., Murotsu, T., Yoshida, M. C., Tsuchiya, M., Asano, S., Kaziro, Y. and Nagata, S. Human gene coding for granulocyte colony-stimulating factor is assigned to the q21-q22 region of chromosome 17. *Somatic Cell Mol. Genet.*, **13**, 679–684 (1987).
27) Segawa, K., Ueno, Y. and Kataoka, T. Growth enhancement of solid tumors by G-CSF administration. *Clin. Immunol.*, **24**, 295–301 (1992) (in Japanese).
28) Ishikawa, M., Koga, Y., Hosokawa, M. and Kobayashi, H. Augmentation of B16 melanoma lung colony formation in C57BL/6 mice having marked granulocytosis. *Int. J. Cancer*, **37**, 919–924 (1986).
29) Kariya, Y., Taga, T. and Uchida, A. Suppression of NK cell activity by recombinant human granulocyte colony stimulating factor (G-CSF). *Clin. Immunol.*, **25**, 1112–1117 (1993) (in Japanese).
30) Welch, D. R., Schissel, D. J., Howrey, R. P. and Aeed, P. A. Tumor-elicited polymorphonuclear cells, in contrast to "normal" circulating polymorphonuclear cells, stimulate invasive and metastatic potentials of rat mammary adenocarcinoma cells. *Proc. Natl. Acad. Sci. USA*, **86**, 5859–5863 (1989).
31) Colombo, M. P., Ferrari, G., Stoppacciaro, A., Parenza, M., Rodolfo, M., Mavilio, F. and Parmiani, G. Granulocyte colony-stimulating factor gene transfer suppresses tumorigenicity of a murine adenocarcinoma *in vivo*. *J. Exp. Med.*, **173**, 889–897 (1991).