Jpn. J. Cancer Res. 87, 146–152, February 1996

Establishment of a Hepatocyte Cell Line Producing Growth-promoting Factors for Liver-colonizing Tumor Cells

Takao Yamori,[1,6] Koji Shimada,[1,4] Hiroaki Kanda,[2] Yumiko Nishizuru,[1] Akiko Komi,[1] Kanami Yamazaki,[1] Keiko Asanoma,[1] Masako Ogawa,[1] Kimie Nomura,[2] Nobuo Nemoto,[3] Kaoru Kumada[4] and Takashi Tsuruo[1,5]

[1]Division of Experimental Chemotherapy, Cancer Chemotherapy Center, [2]Department of Pathology, [3]Department of Experimental Pathology, Cancer Institute, Japanese Foundation for Cancer Research, 1-37-1 Kami-Ikebukuro, Toshima-ku, Tokyo 102, [4]Department of Surgery, Fujigaoka Hospital, Showa University, 1-30 Fujigaoka, Midori-ku, Yokohama, Kanagawa 227 and [5]Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113

A hepatocyte-derived cell line designated MLE-15A2 was established from a primary culture of mouse hepatocytes. The MLE-15A2 cells appeared to retain the basic nature of hepatocytes in that they showed morphology of an epithelial cell type and secreted albumin into the culture medium. These cells were grown on collagen-coated plates and could be easily expanded to a large-scale culture. Therefore, MLE-15A2 cells may provide a more useful model for studying liver microenvironments than primary cultures of hepatocytes. We found that conditioned media from MLE-15A2 cells, as well as from primary cultures of hepatocytes, promoted the proliferation of highly liver-colonizing colon 26 NL-17 cells better than the poorly liver-colonizing colon 26 NL-4 cells. Moreover, the conditioned media stimulated the growth of some human colon cancer cell lines. These results indicate that MLE-15A2 cells secrete growth factors that selectively stimulate certain tumor cell types. Hepatocyte-derived growth factors may regulate selective survival and colonization of tumor cells in the process of liver metastasis. The growth-promoting activity was unaffected by dialysis, was stable at 80°C for 30 min and was bound to a heparin-Sepharose column. The major activity was eluted from the column with 0.7–0.75 *M* NaCl, and some minor activities eluted with lower concentrations of NaCl. These results suggest that the active components are heterogeneous heparin-binding proteins with lower affinity to heparin than platelet-derived and fibroblast growth factors.

Key words: Metastasis — Microenvironment — Hepatocyte — Liver-derived growth factor

Organ preference is a marked feature of tumor metastasis.[1,2] Organ microenvironments for tumor cells consist of host cells and various host-derived factors, e.g., adhesion molecules, chemotactic factors, extracellular matrices, cytokines and growth factors.[3–5] Organ preferential metastasis apparently results from interaction between the tumor cells and these factors. Growth factors derived from the secondary organ are important for tumor cells to survive and initiate secondary growth.[3]

Several researchers have found that paracrine growth factors produced by the host organ specifically stimulate the growth of tumor cells selected for organ-specific growth.[3,6–11] For example, cell growth of lung- and ovary-colonizing murine melanoma sublines in serum-limited medium was differentially stimulated by non-dialyzable factors from the target organ tissue.[11] We have shown that addition of lung tissue extract to serum-free medium stimulated the cell growth of highly lung-colonizing murine colon carcinoma sublines better than that of poorly lung-colonizing sublines.[12] Cavanaugh and Nicolson[13–15] have demonstrated that a lung-derived growth factor, similar in molecular structure to transferrin, is responsible for stimulating the growth of highly metastatic cell lines. We and others have implicated IGF-I[16,17] and TGF-β1[18–20] in the differential stimulation of highly versus poorly metastatic tumor cells.

In the present study, we focused on the liver, the major target organ of gastrointestinal cancer, because little has been reported on liver-derived tumor growth factors. To study these factors, we attempted to establish a hepatocyte cell line as a model of a liver microenvironment. We have successfully established a hepatocyte-derived cell line, MLE-15A2, and found that these cells secrete growth factors that stimulate proliferation of liver-colonizing cells. The factor was partially purified and proved to be a nondialyzable heparin-binding molecule.

MATERIALS AND METHODS

Animals, tumor cells and other materials C3H mice and BALB/c mice were purchased from CLEA Japan Inc. (Tokyo) and Charles River Japan (Yokohama), respectively. A highly metastatic cell line (NL-17) and a poorly metastatic one (NL-4) were both established in our

[6] To whom correspondence should be addressed.

laboratory from murine colon carcinoma colon 26.[12] Human colon cancer cell lines HT-29, HCC-2998, WiDr, HCT-15 and HCT-116 were kindly provided by Dr. Robert Shoemaker, NCI.[21] These tumor cells were maintained in RPMI 1640 supplemented with 5% fetal bovine serum (FBS). A mouse hepatic endothelial cell line (HSE), established by Belloni and Tressler,[22] was generously provided by Dr. G. L. Nicolson, University of Texas M. D. Anderson Cancer Center. The HSE cells were grown in MCDB 131 medium supplemented with 10% FBS and 50 μg/ml of endothelial cell growth supplement (Becton Dickinson, Bedford, MA). Antibiotic and antimycotic solution and gelatin were purchased from Sigma (St. Louis, MO). Columns for protein purification were obtained from Pharmacia (Uppsala, Sweden).

Hepatocyte cell line MLE-15A2 and a fibroblast cell line MLF An immortalized hepatocyte line was established essentially according to the procedure of Lee *et al.*[23] Briefly, a 6-week male C3H mouse was maintained in a plastic cage, given diet (CE-II, CLEA Japan Inc.) and water freely for over two years and then killed. There was no macroscopic tumor nodule in the liver. Hepatocytes were isolated by a collagenase perfusion technique. Viable hepatocytes were purified using an isodensity Percoll centrifugation method and plated onto 60-mm plastic dishes at a density of 2.5×10^5 hepatocytes per dish. The basal culture medium was Waymouth's (Flow Laboratory, Irvine, UK) supplemented with 10% FBS and 60 μg/ml kanamycin. The medium was changed twice each week, and cultures were maintained for 3 months. After 3 months, many hepatocyte and fibroblast colonies appeared. Some of the hepatocyte colonies were isolated with a cylinder, treated with 1 m*M* EDTA in phosphate-buffered saline and then with 0.25% trypsin solution (GIBCO, Grand Island, NY), detached by pipetting, and subcultured. Further colony cloning was performed over 5 times in order to eliminate contaminating fibroblasts. The established line was designated as MLE-15A2 (mouse liver epithelial-15, aged-2), and further subculture was performed in RPMI 1640 medium supplemented with 5% FBS. An immortalized fibroblast line, MLF (mouse liver fibroblast), was established from the same dish during the above procedures and maintained in RPMI 1640 medium supplemented with 5% FBS.

For the measurement of growth rate, MLE-15A2 cells (1×10^4 cells) were plated in 6-well plates coated with collagen, and the cell number was counted on days 1, 3, 5 and 7 with a Coulter counter (Coulter Electronics, Inc., Hialeah, FL) as described previously.[12]

Tumor growth in the liver Tumor cells (1×10^5), harvested from culture dishes following brief trypsin-EDTA (GIBCO) treatment, were directly injected through the peritoneum into the livers of anesthetized BALB/c mice. Two weeks later, the size of the tumor developing in the liver was noted.

Preparation of conditioned media of MLE-15A2 cells and hepatocytes The MLE-15A2 cells were grown to subconfluence in 100-mm culture dishes. Normal hepatocytes were prepared from the livers of 6- to 8-week-old BALB/c mice, as described above, plated onto 100 mm dishes and cultured for 24 h. The cells were washed twice with phosphate-buffered saline (PBS) and once with serum-free RPMI 1640 containing 15 m*M* HEPES buffer (SF-RPMI), and then cultured in 5 ml of fresh SF-RPMI. After 24 h the culture media (conditioned media) were collected. Detached cells and cellular debris were removed by centrifuging the medium at 250*g* for 5 min and then at 1000*g* for 10 min.

Albumin production Aliquots of conditioned media of MLE-15A2 cells and hepatocytes were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. The separated proteins were transblotted to a nitrocellulose membrane. The membrane was blocked with skim milk, and incubated first with 10 μg/ml of rabbit anti-mouse albumin IgG (Organon Teknika Corp., Durhum, NC) at room temperature for 4 h and then with a 1:2000 dilution of peroxidase-conjugated anti-rabbit IgG at room temperature for 1 h. Albumin was visualized with ECL western blotting detection reagents (Amersham, Buckinghamshire, UK).

Coculture of tumor cells with liver-derived cells The coculture was performed in Boyden chambers (Becton Dickinson). The bottom of the upper chamber was a microporous membrane with a pore size of 0.45 μm. The bottom of the upper and lower chambers was coated with collagen. The NL-17 cells (2×10^4) were inoculated into the upper chambers and cultured for 48 h without liver-derived cells in the lower chambers. In parallel, MLE-15A2, HSE, or MLF cells were inoculated into the lower chambers and grown to confluence without NL-17 cells in the upper chambers. The cells were washed with PBS and fed with fresh SF-RPMI, and coculture was started by placing the upper chambers containing NL-17 cells on the lower chambers where the liver-derived cells were growing. The cells were cocultured in SF-RPMI for another 48 h, and then [^{3}H]thymidine was added at a final concentration of 26 kBq/ml. The medium was removed 45 min later, and 10% trichloroacetic acid (TCA) was poured into the upper chambers to determine incorporation of [^{3}H]thymidine into the DNA of the NL-17 cells. After 30 min incubation on ice, the microporous membranes were cut out and dried. The TCA-insoluble materials were solubilized by incubation in 50 ml of 0.2 *M* NaOH-0.2% SDS at 37°C for 30 min. The mixture was neutralized with 25 ml of 2 *M* acetic acid and mixed

with 1 ml of ACS II scintillator (Amersham), and the radioactivity was measured.

Growth-promoting activity of conditioned medium Tumor cells (1×10^4 cells) were inoculated in collagen-coated 96-well plates (Iwaki Glass, Funabashi) and cultured in RPMI 1640 medium supplemented with 5% FBS for 24 h. The medium was removed, and the cells were washed with PBS, fed with RPMI 1640 supplemented with 0.1% FBS and cultured for 3 h. Then, conditioned media of MLE-15A2 cells and hepatocytes were added to the culture. The tumor cells were cultured for 18–24 h, labeled with [^{3}H]thymidine (3.7 kBq/well) for 45 min, washed once with PBS, and incubated in 10% TCA on ice for 30 min. The TCA-insoluble materials were solubilized, and the radioactivity was counted as described above. In some cases, growth-promoting activity was assessed by counting the cell number with a Coulter counter.

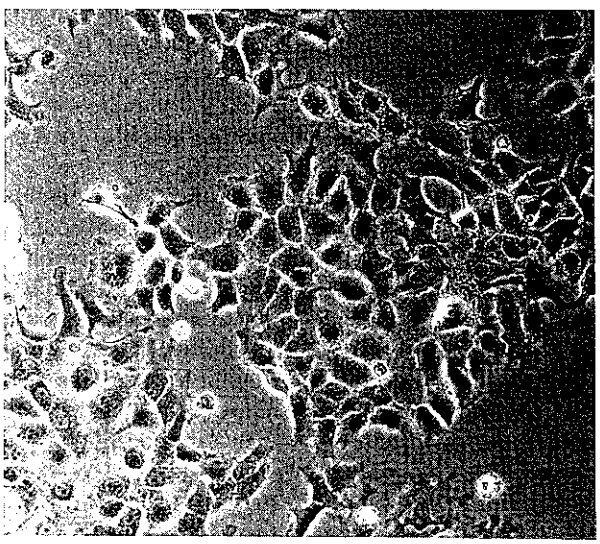

Fig. 1. Microscopic observation of MLE-15A2 cells. The cells showed epithelial cell-like morphology (phase contrast, $\times100$).

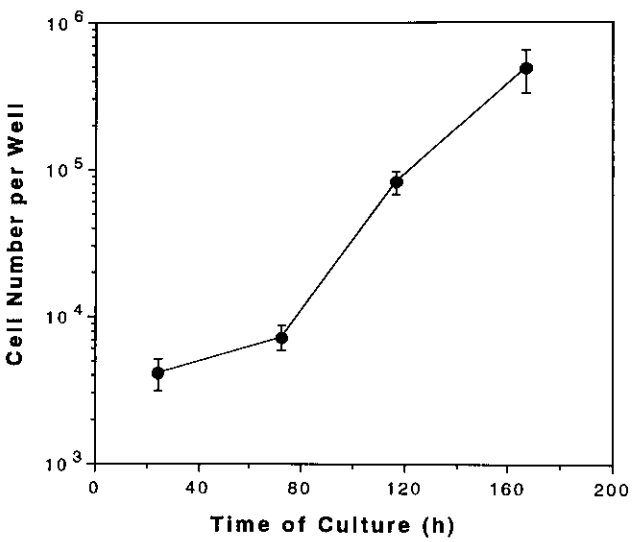

Fig. 2. Growth curve of MLE-15A2 cells. MLE-15A2 cells (1×10^4) were plated in 6-well plates coated with collagen, and the cell number was counted on days 1, 3, 5 and 7.

RESULTS

Characterization of MLE-15A2 cells The immortalized hepatocyte cell line MLE-15A2 was established from the livers of normal mice, as described in "Materials and Methods." The MLE-15A2 cells showed epithelial cell-like morphology (Fig. 1). These cells grew well on collagen-coated plates with a population doubling time of 21–23 h (Fig. 2). Saturation density was 200,000 cells/cm^2. To confirm that MLE-15A2 cells retain the features of hepatocytes, we examined their ability to secrete albumin. Conditioned medium of MLE-15A2 cells and hepatocytes were analyzed by using western blot analysis (Fig. 3). A 69-kDa band was detected with anti-albumin anti-

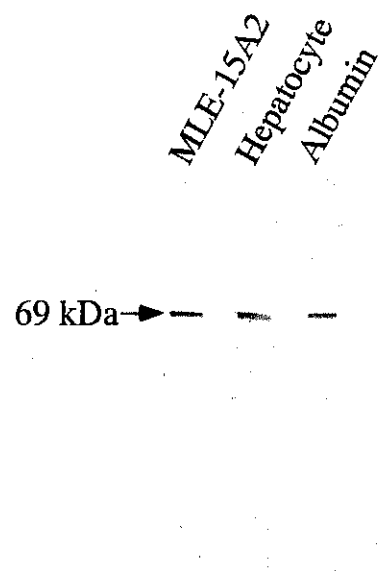

Fig. 3. Secretion of albumin by MLE-15A2 cells. Conditioned media of MLE-15A2 cells and hepatocytes were analyzed by western blot analysis as described in "Materials and Methods." Anti-albumin antibodies revealed a 69-kDa component at a position corresponding to that of albumin in either conditioned medium.

Table I. Development of Tumors after Direct Injection of Colon 26, NL-17 and NL-4 Cells into the Liver

Injected cells	No. of mice	Liver weight (g) Mean±SD
None	4	1.05±0.06
NL-17	7	2.38±0.91*
NL-4	7	1.44±0.50

Tumor cells (1×10^5) were directly injected into the liver. Liver weight was determined after 14 days.
* $P<0.02$, as compared with the control (None) by Student's two-tailed t test.

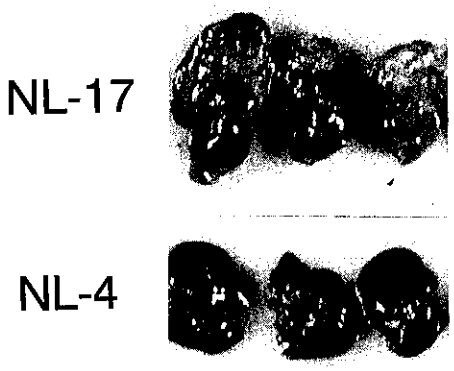

Fig. 4. Observation of tumors in the liver after intra-liver injection of colon 26 NL-17 and NL-4 cells. Tumor cells (1×10^5) were directly injected into the livers of BALB/c mice through the peritoneum, as described in "Materials and Methods." Two weeks later, tumors in the liver were observed. The NL-17 cells developed gross tumors in the liver, while NL-4 formed only small foci.

bodies in the conditioned medium of either MLE-15A2 cells or hepatocytes, demonstrating that MLE-15A2 cells as well as hepatocytes produce albumin.

Growth of tumor cells in the liver We compared two variants of colon 26 tumor for liver-colonizing ability by injecting the cells directly into the liver (Table I). The NL-17 cells developed gross tumors in the liver, while NL-4 formed only small foci (Fig. 4), thus demonstrating the high and low liver-colonizing ability of the two lines, respectively.

Growth-promoting factors secreted from MLE-15A2 cells We checked the possibility that MLE-15A2 cells secrete some factors promoting the growth of NL-17 cells by coculturing these cells in chambers separated by a microporous membrane. The presence of MLE-15A2 cells in the lower chamber stimulated the DNA synthesis of NL-17 cells in the upper chamber 2.5-fold, while other

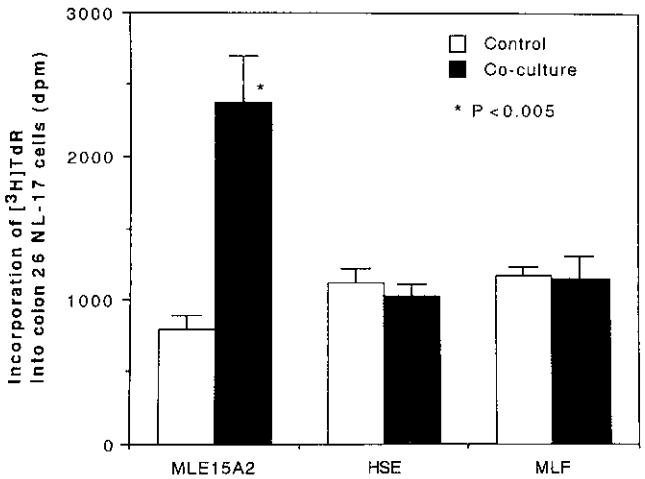

Fig. 5. Co-culture of tumor cells with liver-derived cells. The NL-17 cells were co-cultured with MLE-15A2, HSE, or MLF cells in chambers separated by a microporous membrane, as described in "Materials and Methods." The DNA synthesis of NL-17 cells in the upper chamber was stimulated to 2.5-fold in the presence of MLE-15A2 cells in the lower chamber. The HSE or MLF cells, however, showed no effect on the DNA synthesis of NL-17 cells in the co-culture.

Table II. Effect of Conditioned Media of MLE-15A2 Cells and Hepatocytes on the Growth of Colon 26 NL-17 and NL-4 Cells

Conditioned medium	NL-17		NL-4	
	Cell No.±SD	Ratio to control	Cell No.±SD	Ratio to control
Control	7298±655	1	4390±60	1
MLE-15A2	9865±823	1.4*	4018±154	0.9
Hepatocytes	11848±778	1.6*	4459±278	1.0

Tumor cells (5×10^3 cells) were inoculated into collagen-coated 24-well plates and cultured in 5% FBS-RPMI 1640 for 24 h. The cells were washed twice with PBS and cultured in serum-free RPMI 1640 for 3 h. Then, the medium was replaced with conditioned medium. The cells were trypsinized after 48 h and counted.
* $P<0.01$, as compared with the control (serum free RPMI-1640) by Student's two-tailed t test.

liver-derived cell types, HSE and MLF, had no effect (Fig. 5). These results definitely confirm the above hypothesis.

The cellular specificity of the growth-promoting activity was examined. The collected conditioned media of MLE-15A2 cells or hepatocytes were added to the serum-free culture of NL-17 and NL-4 cells. Both conditioned media significantly stimulated the proliferation of highly liver-colonizing NL-17 cells. In contrast, the conditioned

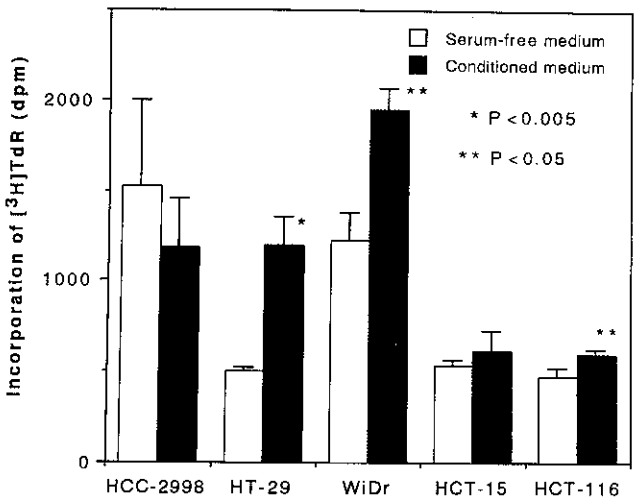

Fig. 6. Growth-stimulative effect of MLE-15A2-conditioned medium on human colon cancer cell lines. The MLE-15A2 conditioned medium was examined for growth-stimulative effect on several human colon cancer cell lines, as described in "Materials and Methods." The DNA synthesis was stimulated 2.3- and 1.6-fold in HT-29 and WiDr cells, respectively. Slight stimulation was observed in HCT-15 cells. No effect appeared in HCC-2998 or HCT-116 cells.

media did not stimulate the proliferation of poorly liver-colonizing NL-4 cells (Table II). The conditioned medium of MLE-15A2 slightly inhibited the growth of NL-4 cells. The MLE-15A2 conditioned medium was examined for growth-stimulative effect on several human colon cancer cell lines (Fig. 6). It increased the DNA synthesis in HT-29 and WiDr cells by 2.3- and 1.6-fold, respectively. A slight increase was noted in HCT-116 and no increase in HCC-2998 or HCT-15 cells. These results indicate that MLE-15A2-derived growth factors selectively stimulate certain cell types.

Partial purification of growth-promoting factors We then purified the growth-promoting factor of NL-17 cells. Conditioned medium was collected, and the active components of more than 30 kDa in size were concentrated by ultrafiltration. The crude materials were heated at 80°C for 30 min because the activity was stable under this treatment. The supernatant was applied to a heparin-Sepharose column after centrifugation. The growth-promoting activity was bound to the column and then eluted with NaCl (Fig. 7). The major activity was recovered in the fractions eluted with 0.7–0.75 *M* NaCl, and some minor activities in the fractions eluted with lower concentrations of NaCl. Overall recovery of the activity in the major active fractions was approximately 70%, and

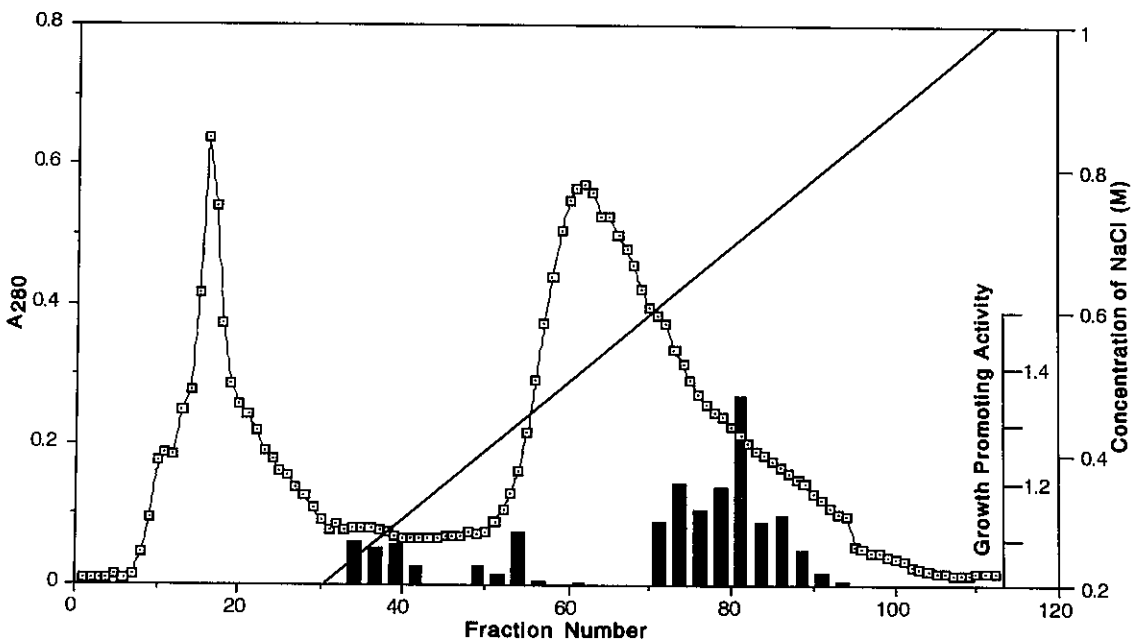

Fig. 7. Partial purification of MLE-15A2-derived growth-promoting factors by heparin-Sepharose. The crude materials containing heat-stable NL-17 cell growth-promoting activity were applied to a heparin-Sepharose column. The activity was bound and eluted with 0.7–0.75 *M* NaCl. Growth-promoting activity was calculated as Test (dpm)/Control (dpm); where Test (dpm) is incorporation of [^{3}H]thymidine into NL-17 cells cultured with fractionated materials and Control (dpm) is incorporation of [^{3}H]thymidine into NL-17 cells in serum-free conditions.

the specific activity was increased by 40-fold as compared with that of the crude conditioned medium.

DISCUSSION

Conditioned medium or extract of liver tissue is usually toxic to cultured tumor cells.[1] Therefore, it is not a good source for identifying liver-derived factors that are related to tumor-growth regulation. As hepatocytes are a major constituent of liver tissue, their primary culture is a good model to study microenvironments of the liver. However, expansion of the primary culture sufficiently to allow purification of trace amounts of cellular components is difficult. In the present study, we have established the MLE-15A2 cell line from a primary culture of mouse hepatocytes. These cells exhibited morphology of epithelial cell type and secreted albumin into the culture medium. These results indicate that MLE-15A2 cells retain the basic nature of hepatocytes. They grew well on collagen-coated plates, and could be easily expanded to a large-scale culture. We also established a liver-derived fibroblast cell line, MLF. These two newly established cell lines, together with the hepatic endothelial cell line HSE,[22] should provide useful models for studying liver microenvironments.

Conditioned media of MLE-15A2 cells as well as the primary culture of hepatocytes promoted the cell growth of NL-17 (highly liver-colonizing), but not that of NL-4 (poorly liver-colonizing) cells. Moreover, the conditioned medium of MLE-15A2 cells stimulated the growth of some human colon cancer cell lines. These results indicate that hepatocytes and MLE-15A2 cells secrete growth factors that selectively stimulate certain tumor cell types, probably depending on differential display of specific receptors on the cell surface. Hepatocyte-derived growth factors may regulate selective survival and colonization of tumor cells in the liver.

Little is known about liver-derived tumor growth factors compared with liver-derived chemotactic factors[24, 25] and adhesion molecules.[26, 27] Hart[1] reported that extract of liver tissue promoted the growth of liver-metastatic M5076 cells in the presence of arginine, which neutralizes the toxic effect of liver arginase. Nicolson[28] showed that conditioned medium of liver tissue stimulated the growth of liver-metastatic RAW117-H10 cells. Recently, Rusciano *et al.*[29] have found that a factor associated with liver plasma membrane is responsible for cell growth of liver-colonizing B16-LS cells. However, no one has identified liver-derived tumor growth factors.

We purified the tumor growth-promoting activity in the conditioned medium of MLE-15A2 cells. The activity was unaffected by dialysis, was stable at 80°C for 30 min and was bound to a heparin-Sepharose column. The major activity was eluted from the column with 0.7–0.75 *M* NaCl, and some minor activities were eluted with lower concentrations of NaCl. The concentrations of NaCl required for the elution of the active components are apparently lower than those for the elution of platelet derived growth factor (PDGF) and fibroblast growth factor (FGF), well known heparin-binding growth factors. These results suggest that the active components are heterogeneous heparin-binding proteins with lower affinity to heparin than platelet-derived and FGFs. Antibodies to PDGF, FGF, epidermal growth factor, IGF-I or transferrin failed to reveal any corresponding antigenic components in the above fractions by immunoblotting, and they failed to neutralize the growth-promoting activity (data not shown). Therefore, the active component did not seem to be any of these growth factors. Preliminary results using SDS-PAGE under non-reducing conditions suggest that a 34-kDa protein is responsible for the growth-promoting activity. Further purification is in progress in our laboratory to identify the active component.

In conclusion, we have established MLE-15A2, a new hepatocyte cell line, for studying the interaction of tumor cells with hepatic microenvironments. We have shown that MLE-15A2 cells secrete growth factors for liver-colonizing tumor cells.

ACKNOWLEDGMENTS

We thank Ms. K. Teshima and Ms. R. Hayashida for typing and editing the manuscript.

(Received September 18, 1995/Accepted November 20, 1995)

REFERENCES

1) Hart, I. R. 'Seed and soil' revisited: mechanism of site specific metastasis. *Cancer Metastasis Rev.*, **1**, 5–16 (1982).
2) Zetter, B. R. The cellular basis of site-specific tumor metastasis. *N. Engl. J. Med.*, **322**, 605–612 (1990).
3) Nicolson, G. L. Cancer progression and growth: relationship of paracrine and autocrine growth mechanisms to organ preference of metastasis. *Exp. Cell Res.*, **204**, 171–180 (1993).
4) Yeatman, T. J. and Nicolson, G. L. Molecular basis of tumor progression: mechanisms of organ-specific tumor metastasis. *Semin. Surg. Oncol.*, **9**, 256–263 (1993).
5) Netland, P. A. and Zetter, B. R. Organ-specific adhesion of metastatic tumor cells in vitro. *Science*, **224**, 1113–1115 (1984).

6) Horak, E., Darling, D. L. and Tarin, D. Analysis of organ-specific effects of metastatic tumor formation by studies *in vitro*. *J. Natl. Cancer Inst.*, **75**, 913–922 (1986).
7) Chackal-Roy, M., Niemeyer, C., Moore, M. and Zetter, B. R. Stimulation of human prostatic carcinoma cell growth by factors present in human bone marrow. *J. Clin. Invest.*, **84**, 43–50 (1989).
8) Gleave, M., Hsieh, J., Gao, C., von Eschenbach, A. C. and Chung, L. W. K. Acceleration of human prostate cancer growth *in vivo* by factors produced by prostate and bone fibroblasts. *Cancer Res.*, **51**, 3753–3761 (1991).
9) Manishen, W. J., Sivananthan, K. and Orr, F. W. Resorbing bone stimulates tumor cell growth: a role for the host microenvironment in bone metastasis. *Am. J. Pathol.*, **123**, 39–45 (1986).
10) Nicolson, G. L. Differential growth properties of metastatic large cell lymphoma cells in target organ-conditioned medium. *Exp. Cell Res.*, **168**, 572–577 (1987).
11) Nicolson, G. L. and Dulski, K. M. Organ specificity of metastatic tumor colonization is related to organ-selective growth properties of malignant cells. *Int. J. Cancer*, **38**, 289–294 (1986).
12) Yamori, T., Iida, H., Tsukagoshi, S. and Tsuruo, T. Growth stimulating activity of lung extract on lung-colonizing colon 26 clones and its partial characterization. *Clin. Exp. Metastasis*, **6**, 131–139 (1988).
13) Cavanaugh, P. G. and Nicolson, G. L. Purification and some properties of a lung-derived growth factor that differentially stimulates the growth of tumor cells metastatic to the lung. *Cancer Res.*, **49**, 3928–3933 (1989).
14) Cavanaugh, P. G. and Nicolson, G. L. Purification and characterization of a Mr 66,000 lung-derived paracrine growth factor that preferentially stimulates the *in vitro* proliferation of lung metastasizing tumor cells. *J. Cell. Biochem.*, **43**, 127–138 (1990).
15) Cavanaugh, P. G. and Nicolson, G. L. Lung-derived mitogenic activity for lung metastasizing tumor cells: identification as a transferrin. *J. Cell. Biochem.*, **47**, 261–267 (1991).
16) Koenuma, M., Yamori, T. and Tsuruo, T. Insulin and insulin-like growth factor I stimulate proliferation of metastatic variants of colon carcinoma 26. *Jpn. J. Cancer Res.*, **80**, 51–58 (1989).
17) Yamori, T., Iizuka, Y., Takayama, Y., Nishiya, S., Iwashita, S., Yamazaki, A., Takatori, T. and Tsuruo, T. Insulin-like growth factor I rapidly induces tyrosine phosphorylation of a Mr 150,000 and a Mr 160,000 protein in highly metastatic mouse colon carcinoma 26 NL-17 cells. *Cancer Res.*, **51**, 5859–5865 (1991).
18) Mooradian, D. L., Purchio, A. F. and Furcht, L. T. Differential effects of transforming growth factor $\beta 1$ on the growth of poorly and highly metastatic murine melanoma cells. *Cancer Res.*, **50**, 273–277 (1990).
19) Roberts, A. B., Anzano, M. A., Wakefield, L. M., Roche, N. S., Stern, D. F. and Sporn, M. B. Type β transforming growth factor: a bifunctional regulator of cellular growth. *Proc. Natl. Acad. Sci. USA*, **82**, 119–123 (1985).
20) Tucker, R. F., Shipley, G. D., Moses, H. L. and Holley, R. W. Growth inhibitor from BSC-1 cells closely related to platelet type β transforming growth factor. *Science*, **226**, 705–707 (1984).
21) Stinson, S. F., Alley, M. C., Kopp, W. C., Fiebig, H.-H., Mullendore, L. A., Pittman, A. F., Kenney, S., Keller, J. and Boyd, M. R. Morphological and immunocytochemical characteristics of human tumor cell lines for use in a disease-oriented anticancer drug screen. *Anticancer Res.*, **12**, 1035–1054 (1992).
22) Belloni, P. N. and Tressler, R. J. Microvascular endothelial cell heterogeneity: interactions with leukocytes and tumor cells. *Cancer Metastasis Rev.*, **8**, 353–389 (1990).
23) Lee, G.-H., Sawada, N., Mochizuki, Y., Nomura, K. and Kitagawa, T. Immortal epithelial cells of normal C3H mouse liver in culture: possible precursor populations for spontaneous hepatocellular carcinoma. *Cancer Res.*, **49**, 403–409 (1989).
24) Hamada, J., Cavanaugh, P. G., Miki, K. and Nicolson, G. L. A paracrine migration-stimulating factor for metastatic tumor cells secreted by mouse hepatic sinusoidal endothelial cells: identification as complement component C3b. *Cancer Res.*, **53**, 4418–4423 (1993).
25) Hamada, J.-I., Cavanaugh, P. G., Lotan, O. and Nicolson, G. L. Separable growth and migration factors for large-cell lymphoma cells secreted by microvascular endothelial cells derived from target organs for metastasis. *Br. J. Cancer*, **66**, 349–354 (1992).
26) Bresalier, R. S., Hujanen, E. S., Raper, S. E., Roll, F. J., Itzkowitz, S. H., Martin, G. R. and Kim, Y. S. An animal model for colon cancer metastasis: establishment and characterization of murine cell lines with enhanced liver-metastasizing ability. *Cancer Res.*, **47**, 1398–1406 (1987).
27) Wang, J., Fallavollita, L. and Brodt, P. Identification of an Mr 64,000 plasma membrane glycoprotein mediating adhesion of tumor H59 cells to hepatocytes. *Cancer Res.*, **51**, 3578–3584 (1991).
28) Nicolson, G. L. Cancer metastasis: tumor cell and host organ properties important in metastasis to specific secondary sites. *Biochim. Biophys. Acta*, **948**, 175–224 (1988).
29) Rusciano, D., Lorenzoni, P. and Burger, M. M. Paracrine growth response as a major determinant in liver-specific colonization by *in vivo* selected B16 murine melanoma cells. *Invasion Metastasis*, **13**, 212–224 (1993).