Jpn. J. Cancer Res. 87, 153–160, February 1996

Establishment and Characterization of Human Gastric Carcinoma Lines with High Metastatic Potential in the Liver: Changes in Integrin Expression Associated with the Ability to Metastasize in the Liver of Nude Mice

Takahiro Yasoshima,[1] Ryuichi Denno,[1] Satoshi Kawaguchi,[2] Noriyuki Sato,[3] Yojiro Okada,[1] Hideki Ura,[1] Kokichi Kikuchi[3] and Koichi Hirata[1]

[1]*First Department of Surgery,* [2]*Department of Orthopedic Surgery,* [3]*First Department of Pathology, Sapporo Medical University School of Medicine, South 1, West 16, Chuo-ku, Sapporo 060*

There is a need to establish animal models which are suitable for investigation of human gastric cancer metastasis to the liver. To this end, a human gastric carcinoma line, AZ521 was injected into the spleens of nude mice. Cells from the few liver metastatic foci of injected AZ521 were expanded *in vitro* and subsequently injected into the spleens of nude mice. By repeating these procedures three times, we were able to obtain a cell line, designated as AZ-H3c, with high metastatic potential in nude mice. Liver metastasis developed in 15 of 21 (71%) animals injected with AZ-H3c, but in only 14% of those injected with parental AZ521. Further, AZ-H3c caused faster tumor development than did AZ521. However, the primary AZ-H3c tumors and liver metastatic AZ-H3c tumors showed essentially the same histological appearance. We also analyzed the cell surface expression of adhesion molecules. The data showed that the expression of VLA-1, VLA-2, VLA-3, VLA-4, VLA-5 was enhanced in AZ-H3c. In contrast, the expression of VLA-6, $\alpha v\beta 3$, E-cadherin, ICAM-1 and LFA-1 was reduced in this high-metastatic line. These results suggest that $\beta 1$ integrins play an important role in the liver metastasis of human gastric carcinoma cells. Our high-metastatic line should be useful for studies aimed at the prevention of liver metastasis.

Key words: Metastasis — Human gastric carcinoma — Integrin — Nude mouse

Recent studies have suggested that hematogenous metastasis requires a series of biological interactions of tumor cells with extracellular matrix (ECM) or with various adhesion molecules.[1,2] Indeed, these interactions are dependent on the expression of cell surface receptors of ECM and adhesion molecules. Some of these receptors belong to the integrin family.[3–6] The integrins are heterodimers consisting of noncovalently associated α and β subunits, and have been divided into three major subfamilies. At least six different subunits are associated with members of the $\beta 1$ subunit family (called very late antigen: VLA). VLA-1[7,8] and VLA-2[9] interact with both collagen and laminin. VLA-3 binds to fibronectin, collagen, and laminin, and also binds to a novel glycoprotein, epigrin, which is an ECM component of human foreskin keratinocytes.[10] VLA-5[11] and VLA-6[12] are receptors for fibronectin and laminin, respectively. $\alpha v\beta 3$ is a receptor for vitronectin. The cell surface expression of integrin molecules on tumors such as melanoma,[13] carcinoma of the bladder,[14] neuroblastoma,[15] colon cancer,[16,17] osteosarcoma,[18] and small lung cell carcinoma[19] has been reported, and it has been suggested that these molecules may be involved in the mechanism of tumor metastasis.

The inactivation of E-cadherin (epithelial cadherin), which is a Ca^{2+}-dependent cell-cell adhesion molecule, may trigger the release of cancer cells from cancer nests.[20,21] Suppression of the invasion function by E-cadherin has been observed in *in vitro* studies. Furthermore, E-cadherin expression was strong in well differentiated carcinomas, whereas it was generally reduced in undifferentiated ones showing a strong invasive character. ICAM-1 is a sialylated glycoprotein which binds to the leucocyte integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) to support cell-cell adhesion and induction and effector functions in the immune response.[22]

Liver metastasis is very often observed in human gastric carcinoma, which is one of the most frequent causes of cancer death in Japan. The establishment of relevant animal metastatic models of these tumors is very important in the search for new therapeutic agents of gastric carcinoma. Recently, an attempt to demonstrate metastatic activity in the liver from human cancers has been made using intrasplenic injections of cancer cells[23–26] or by using intact tissue orthotopic implant techniques.[27–29] However, animal models suitable for the investigation of gastric tumor metastasis to the liver have not been reported. In this paper, we describe a line, designated as AZ-H3c, with high metastatic potential in the liver. By comparing AZ-H3c with the parental AZ521, we also analyzed several features of the cell surface expression of adhesion molecules.

MATERIALS AND METHODS

Animals Female BALB/c nu/nu mice, which originated from the Central Institute for Experimental Animals (Kawasaki), were obtained from CLEA Japan, Inc. (Tokyo). Animals which were 6–7 weeks old and weighed 20–22 g were used.

Cell line and cell culture The human gastric carcinoma line AZ521 was obtained from the Japanese Cancer Research Resources Bank (Tokyo). The cell line was maintained in a culture of RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) (Sigma, St. Louis, MO), 2 m*M* L-glutamine, 50 units/ml penicillin, and 50 mg/ml streptomycin (GIBCO, Grand Island, NY). Cells were passaged and expanded by trypsinization of cell monolayers followed by replating every 4–5 days. The culture media were changed every 2–3 days. For mouse inoculation, cells in log-phase growth were harvested by trypsinization, and a medium containing 10% FCS was added. After washing three times with serum-free RPMI 1640, cells were resuspended in phosphate-buffered saline (PBS). Cells were kept at 37°C until inoculation into mice.

Selection of liver metastatic cell lines Human gastric carcinoma cell lines with high liver metastatic capability were established by *in vivo* stepwise selections according to the method described by Fidler *et al.*[25] Briefly, a human gastric carcinoma cell line, AZ521 (5×10^6) was injected into the spleens of nude mice. After 5 weeks, the mice were killed and the livers with a few metastatic foci of AZ521 cells were harvested. Single cell suspensions were made by mincing and trypsinization, and then cultured *in vitro*. The cells in this culture were designated as AZ-H1c. AZ-H1c cells were subsequently injected into the spleens of nude mice for the second cycle of selection. The livers were harvested, cells were cultured, and AZ-H2c was obtained. The same procedure was repeated using AZ-H2c cells, and AZ-H3c was established upon the third cycle of selection. Each resultant cell line was used for experiments at *in vitro* passage 3–7.

Evaluation of growth rate and metastatic potential of cell lines Cultured AZ521 and AZ-H3c cells (1×10^7 cells, passage 5) were inoculated subcutaneously into four 6-week-old nude mice. The resulting tumors were measured with calipers and their volume was estimated by using the formula $V=L\times W\times H/2$ (V, volume; L, length; W, width; H, height). The ability to form metastatic foci in the liver was determined following an intrasplenic injection as described by Fidler *et al.*[25] Briefly, AZ521 and AZ-H3c cells (5×10^6/0.1 ml in PBS) were injected into the spleens of nude mice using a 26-guage needle. The mice were killed approximately 5 or 10 weeks after the injection, and autopsied. The ability of cells to produce metastasis in nude mice was evaluated.

Antibodies and fluorescence-activated cell sorter (FACS) analysis Cell surface expression of adhesion molecules on AZ521 and AZ-H3c was analyzed by flow cytometry using various monoclonal antibodies (mAbs) listed in Table I. FACS analysis was carried out as previously reported.[30]

RESULTS

Tumorigenicity and metastasis We first examined the *in vivo* tumorigenicity of parental AZ521 and metastatic AZ-H3c lines. Nude mice were injected s.c. with AZ521 and AZ-H3c. As shown in Fig. 1, AZ-H3c cells grew much more rapidly than AZ521 cells. Mice were killed at 30 days after intrasplenic inoculation of tumor cells, and metastatic foci were observed. As shown in Fig. 2, mice injected with AZ-H3c showed multiple round metastases in the liver. The pathohistology of these foci is illustrated

Table I. Summary of the mAbs Used in the Study

Antigen	mAb name	Source	Ref.
Integrin $\alpha1$	Ts2/7 (Asc.)	Dr. Martin E. Hemler (Dana-Farber Cancer Institute, Boston, MA)	46)
Integrin $\alpha2$	IIE10 (Sup.)	Dr. Martin E. Hemler	47)
Integrin $\alpha3$	IIF5 (Asc.)	Dr. Martin E. Hemler	48)
Integrin $\alpha4$	B5G10 (Asc.)	Dr. Martin E. Hemler	49)
Integrin $\alpha5$	A5-PUJ2 (Sup.)	Dr. Martin E. Hemler	50)
Integrin $\alpha6$	A6-ELE (Sup.)	Dr. Martin E. Hemler	50)
Integrin $\beta1$	A-1A5 (Asc.)	Dr. Martin E. Hemler	51)
Integrin $\alpha v\beta3$	LM609	CHEMICON International Inc. (Temecula, CA)	
LFA-1	G43-25B	Pharmingen (San Diego, CA)	
E-cadherin	HECD-1	Takara (Tokyo)	
ICAM-1	HA58 (Asc.)	Dr. K. Imai (Sapporo Med. Univ. Sch. Med., Sapporo)	52)

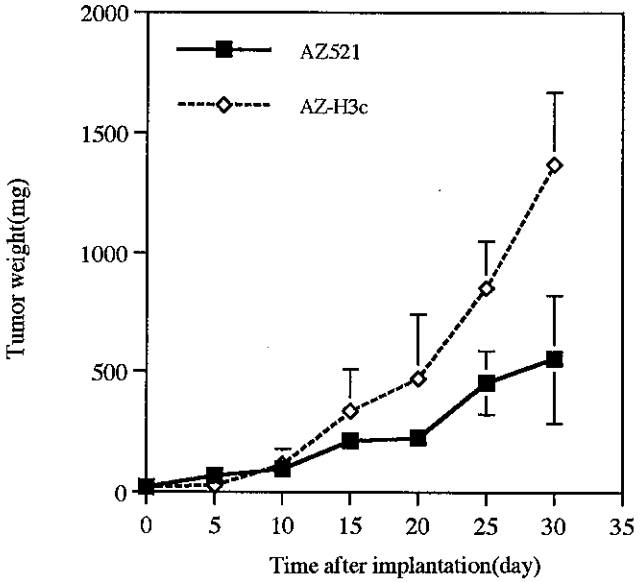

Fig. 1. The growth curves of the AZ521 and AZ-H3c lines implanted into subcutis of nude mice as cell suspensions. AZ-H3c cells grew much more rapidly than AZ521. Bars represent mean±SE.

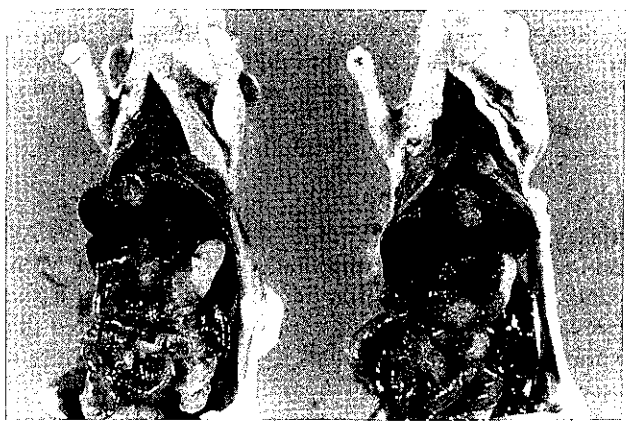

Fig. 2. Macroscopic views of a metastatic liver tumor after intrasplenic injection of AZ-H3c. Multiple round metastases in the liver are seen.

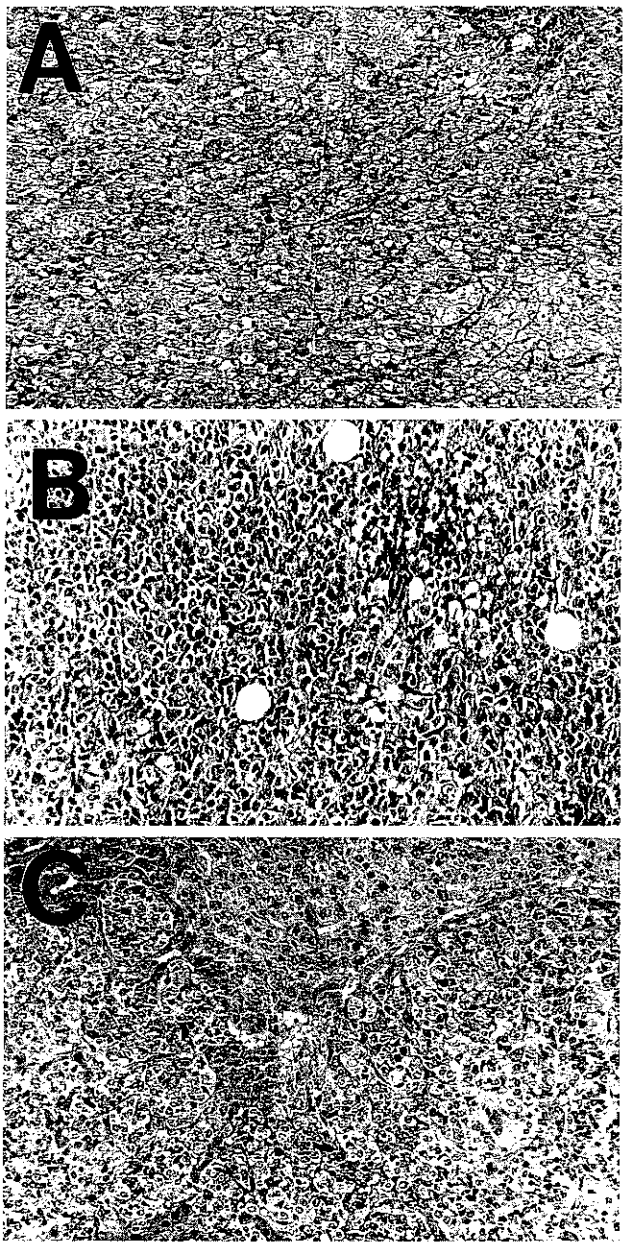

Fig. 3. Microscopic views of the subcutaneous tumors of AZ521 and AZ-H3c and the metastatic liver tumor after intrasplenic injection of AZ-H3c. A, subcutaneous tumor of AZ521; B, subcutaneous tumor of AZ-H3c; C, metastatic liver tumor after intrasplenic injection with AZ-H3c. Tissues were fixed, embedded, sectioned, and stained with hematoxylin and eosin using standard procedures. ×100.

in Fig. 3, showing poorly differentiated adenocarcinoma, with essentially the same appearance for parental AZ521 and AZ-H3c tumors.

To evaluate the potential of AZ-H3c to metastasize to the liver, we employed a greater number of mice. The metastatic potential of AZ-H1c and AZ-H2c was also determined. The data (Table II) indicate that although AZ-H1c and AZ-H2c showed enhanced metastatic potential as compared to that of parental AZ521, AZ-H3c was the most highly metastatic line. Since there was a possibility that the difference of metastatic potential between AZ521 and AZ-H3c may only reflect that of growth potential of the cells, we observed the liver metas-

Table II. Metastasis after Intrasplenic Injection of Gastric Carcinoma Lines

Gastric carcinoma line	Total mouse number	Liver metastasis at 5 weeks after inoculation		
		Experiment 1 (%)	Experiment 2 (%)	Total (%)
AZ521	14	0/8 (0)	2/6 (33)	2/14 (14)
AZ-H1c	5	3/5 (60)	ND	3/5 (60)
AZ-H2c	4	2/4 (50)	ND	2/4 (50)
AZ-H3c	21	9/14 (64)	6/7 (86)	15/21 (71)

ND: not determined.

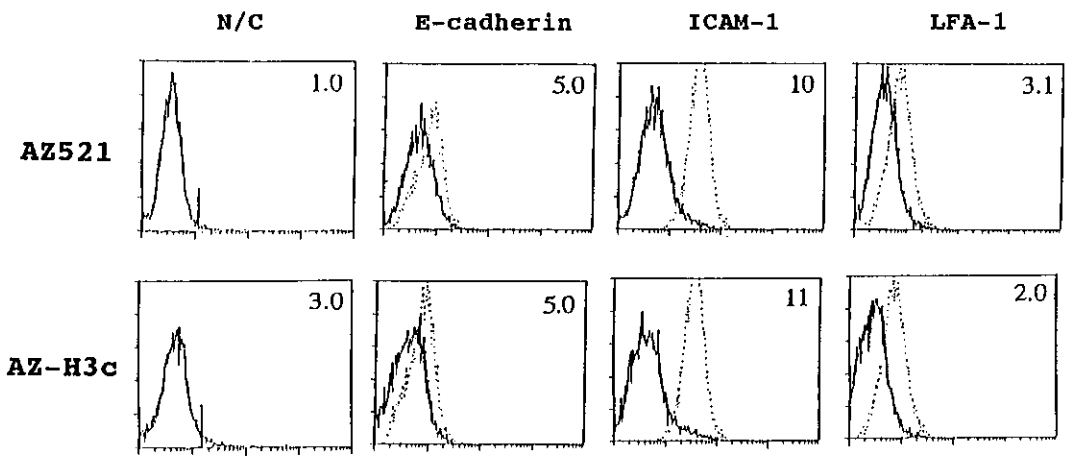

Fig. 4. FACS analysis of the cell surface expression of E-cadherin, ICAM-1, and LFA-1 on AZ521 and AZ-H3c. Abscissa; logarithm of fluorescence intensity. Ordinate; relative cell number. The dotted lines show positive controls. Each number indicates the actual number of positive cells (%). Cell samples were stained with the mAbs shown in Table I and analyzed by FACS.

tasis at 10 weeks after injection. Although the splenic tumors in AZ521-injected mice were similar in size to those in the case of AZ-H3c, only one out of eight mice showed liver metastasis (data not shown). These data seem to rule out the above possibility. It was also observed that AZ521, AZ-H1c, AZ-H2c and AZ-H3c all showed metastasis to mesenteric lymphnodes. However, none of the lines exhibited pulmonary metastasis (data not shown).

Expression of metastasis-related cell surface molecules on AZ521 and AZ-H3c lines It would be interesting to know the characteristics of metastasis-related cell surface antigens in the various cells. To this end, we employed parental AZ521 and the most highly metastatic line, AZ-H3c. To determine how integrin and other adhesion molecules are expressed on the cell surface of AZ521 and AZ-H3c lines, FACS analysis was performed using mAbs. As compared with the parental AZ521 line, decreased cell surface expression of E-cadherin, ICAM-1 and LFA-1 was observed in AZ-H3c line (Fig. 4). In contrast, this line showed an increase in the cell surface expression of VLA-1, VLA-2, VLA-3, VLA-4, VLA-5 and integrin $\beta 1$ subunit, especially VLA-1 (Fig. 5). VLA-6 and $\alpha v \beta 3$ expression was reduced. The results of successive FACS analysis (three more times) indicated that expression of these molecules is stable during culture of these cells.

DISCUSSION

In the present study, we established new lines of human gastric carcinoma with metastatic potential. Namely, the metastatic tumors of nude mice intrasplenically injected with parental AZ521 cells were harvested, and the cell line, AZ-H1c, was established *in vitro*. Two additional cycles of the selection procedure yielded another cell line, designated as AZ-H3c, with a high metastatic ability in the liver of nude mice. This is the first such *in vitro* line of human gastric carcinoma.

There are several mechanisms that confer upon cells a high liver metastatic capacity. It is well known that hematogenous metastasis occurs as a consequence of

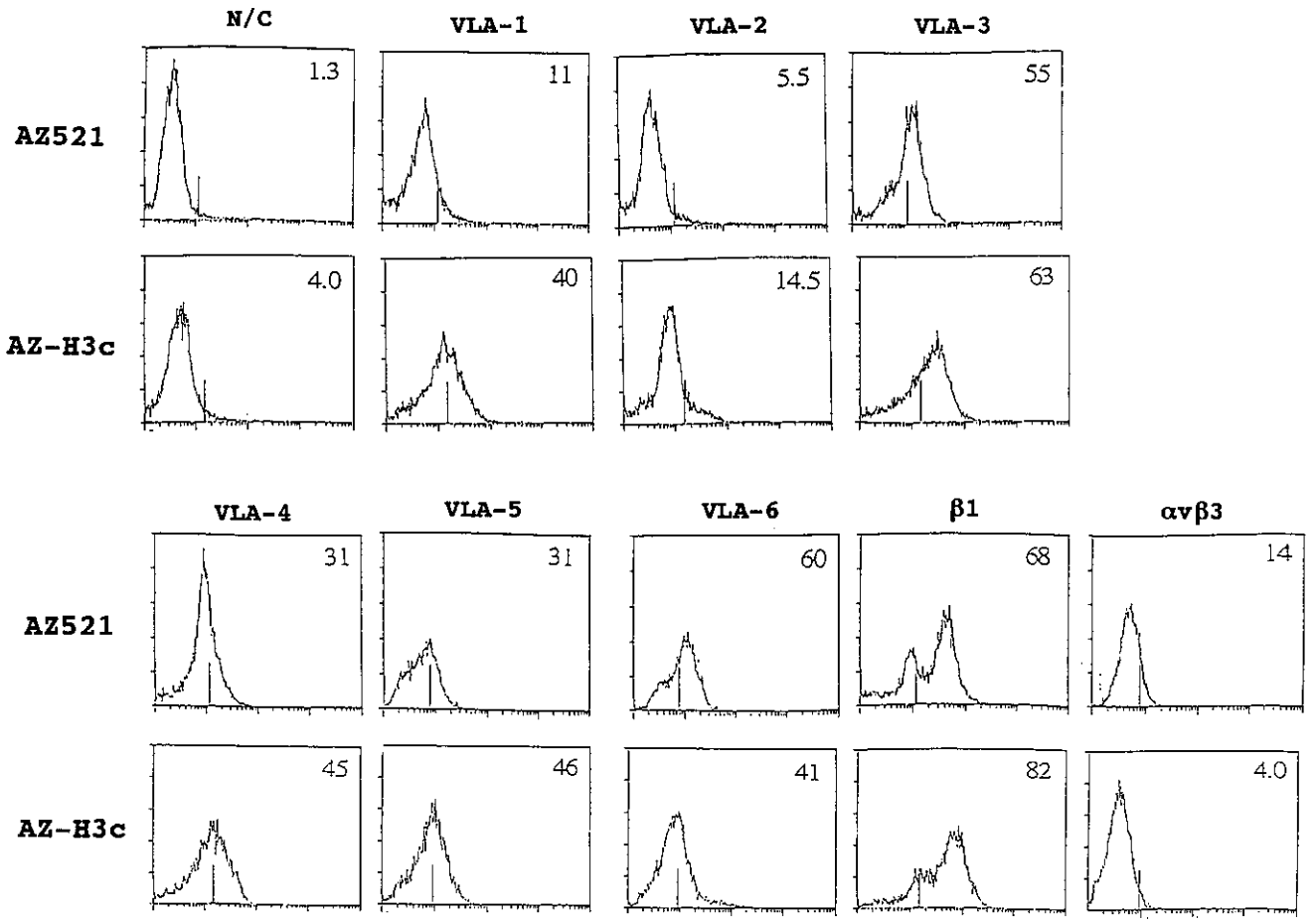

Fig. 5. FACS analysis of the cell surface expression of integrin family members on AZ521 and AZ-H3c. Abscissa; logarithm of fluorescence intensity. Ordinate; relative cell number. Each number indicates the actual number of positive cells (%). Cell samples were stained with the mAbs shown in Table I and analyzed by FACS.

sequential steps: (i) growth of neoplastic cells in the primary lesion, (ii) vascularization and local invasion, (iii) entrance into blood vessels, (iv) survival and circulation, (v) arrest in the capillary beds of a target organ, (vi) extravasation toward the organ parenchyma, and (vii) tumor cell growth.[31] Therefore, a mechanism that affects any of these steps can influence the metastatic potential of tumor cells. Since our model starts with an intrasplenic injection, it does not reflect the entire scope of hematogenous liver metastasis of gastric carcinomas. Nonetheless, it is highly likely that a step involving tumor cell growth is critical for the liver metastasis of gastric carcinoma, since AZ-H3c cells injected into the subcutis of nude mice grew much more rapidly than did AZ521 cells. At present, it is not known whether the differential growth ability in the subcutis reflects back to the primary lesion or is restricted to the metastatic lesion. In this regard, it would be worth attempting to inject those lines orthotopically into the stomach wall, as has been reported for other carcinomas using intact tumor tissue.[27, 32–34]

In all steps of hematogenous metastasis, a variety of cell adhesion molecules are known to be critically involved.[21, 35–39] Therefore, we next examined the expression of various integrins, immunoglobulins, and E-cadherin in AZ521 cells and AZ-H3c cells. It should be noted that AZ521 cells expressed all VLA integrins with a characteristic pattern of low levels of VLA-1 and VLA-2, moderate levels of VLA-4 and VLA-5, and higher levels of VLA-3 and VLA-6. So far no report has appeared on the expression of VLA integrins in gastric carcinoma. However, the above findings suggest that there may be a tumor-type-related VLA expression in gastric carcinoma. Supporting this idea, Miettinen *et al.*[17] reported that expression patterns of VLA integrins in various carcinomas except gastric carcinoma were quite different from

that observed for AZ521 cells. We are currently investigating VLA expression in gastric carcinoma tissues.

Comparison of AZ521 and AZ-H3c cells demonstrated that there are certain changes in the expression of adhesion molecules associated with high liver metastatic capacity. The expression level of all VLA integrins except VLA-6 was substantially higher in AZ-H3c cells, suggesting that these increased integrins may be important in the formation of liver metastasis. For example, it is possible that AZ-H3c cells are better able to interact with the capillary beds of the liver through increased surface VLA-4 that binds to VCAM-1 on the endothelial cells. The importance of VLA-4/VCAM-1 interaction in hematogenous metastasis has already been shown.[30] Also, increased VLA-1, VLA-2, and VLA-3 can support attachment of AZ-H3c cells to the subendothelial basement membrane by binding to their ligands, laminin and type IV collagen. VLA-5 is a classical fibronectin receptor recognizing the RGD amino acid sequence[4] and a crucial role of VLA-5 in metastasis has been demonstrated by the fact that RGD-containing synthetic peptides completely blocked the experimental lung metastasis of melanoma cells.[40] The role of VLA-6 in metastasis remains controversial, for example, in breast carcinoma.[41–43] Therefore, impaired expression of VLA-6 on AZ-H3c might favor metastasis. Because of the complexity of the metastatic cascade, the roles of the integrins can not be simply determined. Some authors have proposed a negative role of VLA-4 or VLA-5 in tumor metastasis.[44, 45] At present, it is not even clear that the altered expression of integrins in AZ-H3c cells necessarily reflects a change in their function. In this regard, we recently observed that an intravenous injection of GRGDTP-peptide (2 mg/mouse) completely inhibited the liver metastasis formation by AZ-H3c cells, supporting the idea that at least VLA-5 in AZ-H3c cells plays a significant role (data not shown).

In conclusion, we have established new lines of human gastric carcinoma that efficiently and preferentially metastasize to the liver when injected into the spleens of nude mice. A comparative analysis using high-metastatic cell lines and the low-metastatic parental line demonstrated that a difference in the growth potential is likely to be related to the acquired high metastatic capacity. Furthermore, there were substantial changes in the expression levels of adhesion molecules in the high-metastatic line, such as an increase in integrins VLA-1 to VLA-5, suggesting their possible contribution to the mechanism of liver metastasis. Our experimental model should be useful for research into metastasis of human gastric carcinoma.

ACKNOWLEDGMENTS

The authors thank Dr. Martin E. Hemler at the Dana-Farber Cancer Institute for providing antibodies to integrins. The authors also thank Dr. K. Imai at the First Department of Internal Medicine, Sapporo Medical University School of Medicine for providing antibodies to ICAM-1.

(Received July 20, 1995/Accepted November 10, 1995)

REFERENCES

1) Dedhar, S. Integrins and tumor invasion. *Bio Essays,* **12,** 583–590 (1990).
2) Liotta, L. A. Tumor invasion and metastasis — role of the extracellular matrix. *Cancer Res.,* **46,** 1–7 (1986).
3) Albelda, S. M. and Buck, C. A. Integrins and other cell adhesion molecules. *FASEB J.,* **4,** 2868–2880 (1990).
4) Hemler, M. E. VLA proteins in the integrin family: structures, functions, and their role on leukocytes. *Annu. Rev. Immunol.,* **8,** 365–400 (1990).
5) Hynes, R. O. Integrins; a family of cell surface receptors. *Cell,* **48,** 549–554 (1987).
6) Ruoslahti, E. and Pierschbacher, M. D. New perspectives in cell adhesion: RGD and integrins. *Science,* **238,** 491–497 (1987).
7) Ignatis, M. J. and Reichardt, L. F. Identification of a neural laminin receptor: an Mr 200/120 kd integrin heterodimer that binds laminin in a divalent cation-dependent manner. *Neuron,* **1,** 715–725 (1988).
8) Kramer, R. H. and Marks, N. Identification of integrin collagen receptors of human melanoma cells. *J. Biol. Chem.,* **264,** 4684–4688 (1989).
9) Elices, M. J. and Hemler, M. E. The human integrin VLA-2 is a collagen receptor on some cells and a collagen/laminin receptor on others. *Proc. Natl. Acad. Sci. USA,* **86,** 9906–9910 (1989).
10) Carter, W. G., Ryan, M. C. and Gahr, P. F. Epiligrin, a new cell adhesion ligand for integrin $\alpha 3\beta 1$ in epithelial basement membranes. *Cell,* **65,** 599–610 (1991).
11) Pytela, R., Pierschbacher, M. D. and Ruoslahti, E. Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. *Cell,* **40,** 191–198 (1985).
12) Sonnernberg, A., Modderman, P. W. and Hogervororst, F. Laminin receptor on platelets in the integrin VLA-6. *Nature,* **336,** 487–489 (1988).
13) Natali, P. G., Nicota, M. R., Bartolazzi, A., Cavaliere, R. and Bigotti, A. Integrin expression in cutaneous malignant melanoma: association of the $\alpha 3\beta 1$ heterodimer with tumor progression. *Int. J. Cancer,* **54,** 6757–6764 (1990).
14) Fradet, Y., Cordon-Cardo, C., Thomson, T., Daly, M. E., Whitmore, W. F., Lloyd, K. O., Melamed, M. R. and Old, L. J. Cell surface antigens of human bladder cancer

defined by mouse monoclonal antibodies. *Proc. Natl. Acad. Sci. USA,* **81,** 224–228 (1984).
15) Hemler, M. E. Adhesive protein receptors on hematopoietic cells. *Immunol. Today,* **9,** 109–113 (1988).
16) Pignatelli, M., Smith, M. E. F. and Bodmer, M. F. Low expression of collagen receptors in moderate and poorly differentiated colorectal adenocarcinoma. *Br. J. Cancer,* **61,** 636–638 (1990).
17) Miettinen, M., Castello, R., Wayner, E. and Schwarting, R. Distribution of VLA integrins in solid tumors: emergence of tumor-type-related expression patterns in carcinomas and sarcomas. *Am. J. Pathol.,* **142,** 1009–1018 (1993).
18) Kawaguchi, S. and Uede, T. Distribution of integrins and their matrix ligands in osteogenic sarcomas. *J. Orthop. Res.,* **11,** 386–395 (1993).
19) Feldman, L. E., Shin, K. C., Natale, R. B. and Todd, R. F., III β1 Integrin expression on human small cell lung cancer cells. *Cancer Res.,* **51,** 1065–1070 (1991).
20) Kanai, Y., Shimoyama, Y., Oda, T. and Hirohashi, S. Abnormalities of cell adhesion molecules in multistage carcinogenesis. *Gann Monogr. Cancer Res.,* **42,** 127–137 (1994).
21) Takeichi, M. Cadherins in cancer: implications for invasion and metastasis. *Curr. Opin. Cell. Biol.,* **5,** 806–811 (1993).
22) Springer, T. A. Adhesion receptors of the immune system. *Nature,* **346,** 425–434 (1990).
23) Ikeda, Y., Ezaki, M., Hayashi, I., Yasuda, D., Nakayama, K. and Kono, A. Establishment and characterization of human pancreatic cancer cell lines in tissue culture and in nude mice. *Jpn. J. Cancer Res.,* **81,** 987–993 (1990).
24) Morgan, R. T., Woods, L. K., Moore, G. E., Quinn, L. A., McGravan, L. and Gordon, S. G. Human cell line (COLO 357) of metastatic pancreatic adenocarcinoma. *Int. J. Cancer,* **25,** 591–598 (1981).
25) Morikawa, K., Walker, S. M., Jessup, M. and Fidler, I. J. *In vivo* selection of highly metastatic cells from surgical specimens of different primary human colon carcinomas implanted into nude mice. *Cancer Res.,* **48,** 1943–1948 (1988).
26) Giavazzi, R., Campbell, D. E., Jessup, J. M., Cleary, K. and Fidler, I. J. Metastatic behavior of tumor cells isolated from primary and metastatic human colorectal carcinomas implanted into different sites in nude mice. *Cancer Res.,* **46,** 1928–1933 (1986).
27) Furukawa, T., Kubota, T., Watanabe, M., Kitajima, M. and Hoffman, R. M. A novel "patient-like" treatment model of human pancreatic cancer constructed using orthotopic transplantation of histologically intact human tumor tissue in nude mice. *Cancer Res.,* **53,** 3070–3072 (1993).
28) Tan, M. H. and Chu, T. M. Characterization of the tumorigenic and metastatic properties of a human pancreatic tumor line (AsPC-1) implanted orthotopically into nude mice. *Tumor Biol.,* **6,** 89–98 (1985).
29) Marincola, F., Taylor-Edwards, C., Drucker, B. and Holder, W. D., Jr. Orthotopic and heterotopic xenotransplantation of human pancreatic cancer in nude mice. *Curr. Surg.,* **44,** 294–297 (1987).
30) Kawaguchi, S., Kikuchi, K., Ishii, S., Takada, Y., Kobayashi, S. and Uede, T. VLA-4 molecules on tumor cells initiate an adhesive interaction with VCAM-1 molecules on endothelial cell surface. *Jpn. J. Cancer Res.,* **83,** 1304–1316 (1992).
31) Fidler, I. J. Critical factors in the biology of human cancer metastasis: Twenty-eighth G.H.A. Clowes Memorial Award Lecture. *Cancer Res.,* **50,** 6130–6138 (1990).
32) Fu, X., Bestermen, J. M., Monosov, A. and Hoffman, R. M. Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. *Proc. Natl. Acad. Sci. USA,* **88,** 9345–9349 (1991).
33) Fu, X., Herrera, H. and Hoffman, R. M. Orthotopic growth and metastasis of human prostate carcinoma in nude mice after transplantation of histologically intact tissue. *Int. J. Cancer,* **52,** 987–990 (1992).
34) Wang, X., Fu, X. and Hoffman, R. M. A new patient-like metastatic model of human lung cancer constructed orthotopically with intact tissue via thoracotomy in immunodeficient mice. *Int. J. Cancer,* **52,** 992–995 (1992).
35) Ruoslahti, E. and Gianotti, F. G. Integrins and tumor cell dissemination. *Cancer Res. (Cold Spring Harbor),* **1,** 119–126 (1989).
36) Hart, I. R. and Saini, A. Biology of tumor metastasis. *Lancet,* **339,** 1453–1457 (1992).
37) Zetter, B. R. Adhesion molecules in tumor metastasis. *Semin. Cancer Biol.,* **4,** 219–229 (1993).
38) Juliano, R. L. and Varner, J. A. Adhesion molecules in cancer: the role of integrins. *Curr. Opin. Cell. Biol.,* **5,** 812–818 (1993).
39) Schwartz, M. A. Signaling by integrins: implications for tumorigenesis. *Cancer Res.,* **53,** 1503–1506 (1993).
40) Humphries, M. J., Olden, K. and Yamada, K. M. A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells. *Science,* **233,** 467–470 (1986).
41) Zutter, M. M., Mazoujian, G. and Santoro, S. A. Decreased expression of integrin adhesive protein receptors in adenocarcinoma of the breast. *Am. J. Pathol.,* **137,** 863–870 (1990).
42) Gui, G. P. H., Wells, C. A., Browne, P. D., Yeomans, P., Jordan, S., Puddefoot, J. R., Vinson, G. P. and Carpenter, R. Integrin expression in primary breast cancer and its relation to axillary nodal status. *Surgery,* **117,** 102–108 (1995).
43) Friedrechs, K., Ruiz, P., Franke, F., Gille, I., Terpe, H.-J. and Imhof, B. High expression level of α6 integrin in human breast carcinoma is correlated with reduced survival. *Cancer Res.,* **55,** 901–906 (1995).
44) Qian, F., Vaux, D. L. and Weissman, I. L. Expression of the integrin $\alpha 4\beta 1$ on melanoma cells can inhibit the invasive stage of metastasis formation. *Cell,* **77,** 335–347

(1994).
45) Giancotti, F. G. and Rouslahti, E. Elevated levels of the alpha 5 beta 1 fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. *Cell,* **60,** 849–859 (1990).
46) Hemler, M. E., Sanchez-Madrid, F., Flotte, T. J., Krensky, A. M., Burakoff, S. J., Bhan, A. K., Springer, T. A. and Strominger, J. L. Glycoproteins of 210,000 and 130,000 m.w. on activated T cells: cell distribution and antigenic relation to components on resting cells and T cell lines. *J. Immunol.,* **132,** 3011–3018 (1984).
47) Bergelson, J. M., St., John, N., Kawaguchi, S., Pasqualini, R., Berdichevsky, F., Hemer, M. E. and Finberg, R. W. The I domain is essential for echovirus 1 interaction with VLA-2. *Cell Adhes. Commun.,* **2,** 455–464 (1994).
48) Weitzman, J. B., Pasqualini, R., Yakada, Y. and Hemler, M. E. The function and distinctive regulation of the integrin VLA-3 in cell adhesion, spreading, and homotypic cell aggregation. *J. Biol. Chem.,* **268,** 8651–8657 (1993).
49) Hemler, M. E., Haung, C., Takada, Y., Schwarz, L., Strominger, J. L. and Clabby, M. L. Characterization of the cell surface heterodimer VLA-4 and related peptides. *J. Biol. Chem.,* **262,** 11478–11485 (1987).
50) Lee, R. T., Berdichevski, F., Chen, G. C. and Hemler, M. E. Integrin-mediated collagen gel matrix reorganization by cultured human vascular smooth muscle cells. *Circ. Res.,* **75,** 209–214 (1995).
51) Hemler, M. E., Ware, C. F. and Strominger, J. L. Characterization of a novel differentiation antigen complex recognized by a monoclonal antibody (A-1A5): unique activation-specific molecular forms on stimulated T cells. *J. Immunol.,* **131,** 334–340 (1983).
52) Kageshita, T., Yoshii, A., Kimura, T., Kuriya, N., Ono, T., Tsujisaki, M., Imai, K. and Ferrone, S. Clinical relevance of ICAM-1 expression in primary lesions and serum of patients with malignant melanoma. *Cancer Res.,* **53,** 4927–4932 (1993).