Tumorigenicity, Motility and Liver Metastasis of Human Gastric Carcinoma Lines with High Metastatic Potential in the Liver of Nude Mice

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ABSTRACT

To analyze the human gastric carcinoma metastasis to the liver, 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 five times, we were able to obtain a cell line, designated AZ-H5c, with high metastatic potential in nude mice. It was observed that animals had liver metastasis in 10 of 12 (83%) cases injected with AZ-H5c, whereas only 14% with parental AZ521. The growth activity *in vivo* of AZ-H5c cells is much more rapid than that of AZ521 cells, but its growth activity *in vitro* is slower. The mortile activity *in vitro* of AZ-H5c is stronger than that of AZ521. These results suggest that our model can provide a new approach to basic and clinical studies of cancer metastasis.

Key words: Metastasis, Human gastric carcinoma, Motile activity, Growth activity, Nude mice

INTRODUCTION

Liver metastasis is very often observed in human gastric carcinoma, which is one of the most frequent causes of cancer death. The establishment of relevant animal metastatic models of these tumors is highly important in the search for the development of new therapeutics of gastric carcinoma. Many attempts to demonstrate metastatic activity in the liver from human cancers has been carried

out by using intrasplenic injections of cancer cells(1, 2, 3) or by using intact tissue orthotopic implant techniques(4, 5, 6, 7). However, animal models suitable for the investigation of gastric tumor metasasis to the liver have not been reported. Recently, we reported the establishment and characterization of a human gastric carcinoma line with high metastatic potential in the liver of nude mice, AZ-H3c, by using intrasplenic injections of gastric cancer cells(7). In this paper, we newly established a line, designated as AZ-H5c, with more highly metastatic potential in the liver than AZ-H3c. By comparing AZ-H5c with parental AZ521, we were also able to analyze several features such as the motility and tumorigenicity of the cell.

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 16-18 g were used.

Cell lines

The human gastric carcinoma line AZ521 was obtained from the Japanese Cancer Reseach Resouces Bank (Tokyo). The procedure for the establishment of the human gastric carcinoma cell line with high metastatic capability was previously described(8). Briefly, a human gastric carcinoma cell line, AZ521 (5×10^6), was injected into the spleens of nude mice. After five weeks, mice were sacrificed and the livers with 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 AZ-H1c. The same procedure was repeated using AZ-H1c cells, and AZ-H2c, AZ-H3c, AZ-H4c were established. AZ-H5c was established upon the fifth cycle of selection. Each resultant cell line at *in vitro* passage 3-7 was used for the experiments.

Evaluation of metastatic potential of cell lines

As previously reported(8), AZ521, AZ-H1c, AZ-H2c, AZ-H3c, AZ-H4c and AZ-H5c cells (5×10^6/0.1 m*l* in PBS) were injected into the spleens of nude mice using a 26-guage needle. The mice were sacrificed approximately 5 weeks after the injection, and autopsies were performed. The ability of cells to produce metastasis in nude mice was evaluated.

In vivo growth assay

1×10^7 cultured AZ521 and AZ-H5c cells (passage-5) were inoculated sub-

cutaneously into six 6-week-old nude mice. The resulting tumors were measured with calipers and their volume was estimated by using the following formula; $V=L\times W\times H/2$ (V, volume; L, length; W, width; H, height).

In vitro growth assay

AZ521 and AZ-H5c cells were plated on 24 well plates (Corning, New York, USA) on day 0 (1×10^4 cells/well). The culture supernatants were exchanged with new ones once every other day. On day 1, 2, 3 and 4, cells were detached from culture dishes by 5-min treatment with 0.25% trypsin at 37 ℃. The number of viable tumor cells was determined by the trypan blue exclusion test. The experiments were performed in duplicate.

In vitro motility assay

Transwell cell culture chambers (Costar Corp., Cambridge, USA) were used for the motility assay. AZ521 and AZ-H5c cells (1×10^5) were suspended in serum-free DMEM, and added to the upper chamber. The lower chamber contained DME supplemented with 10% fetal calf serum and human cellular fibronectin (12.5μg/m*l*, as a chemoattractant). Cells were incubated for 6 hours at 37 ℃ in a CO_2 incubator. At the end of the incubation, cells on the upper surface of the filter were completely removed by wiping with a cotton swab. Cells were fixed in methanol and stained with Giemsa solution. Cells that invaded the lower surface of the filter were counted under a light microscope at a magnification of $\times100$. Each assay was done in triplicate.

RESULTS

Pathohistology

Fig. 1 shows microscopic views of the subcutaneous tumor of AZ521 and AZ-H5c, and the metastatic liver tumor after intrasplenic injection of AZ-H5c. The pathohistology of these foci was a poorly differentiated adenocarcinoma and the same appearance between parental AZ521 and AZ-H5c tumors was essentially recognized.

Liver metastasis of lines

To evaluate the potential of cell lines to metastasize to the liver, we employed a greater number of mice (N=65). As shown in Fig. 2, AZ-H5c is the highest metastatic line. There was a difference between AZ521 and AZ-H5c lines. It was also observed that all cell lines showed metastasis to mesenteric lymphnodes. However, none of the lines had pulmonary metastasis (data not shown).

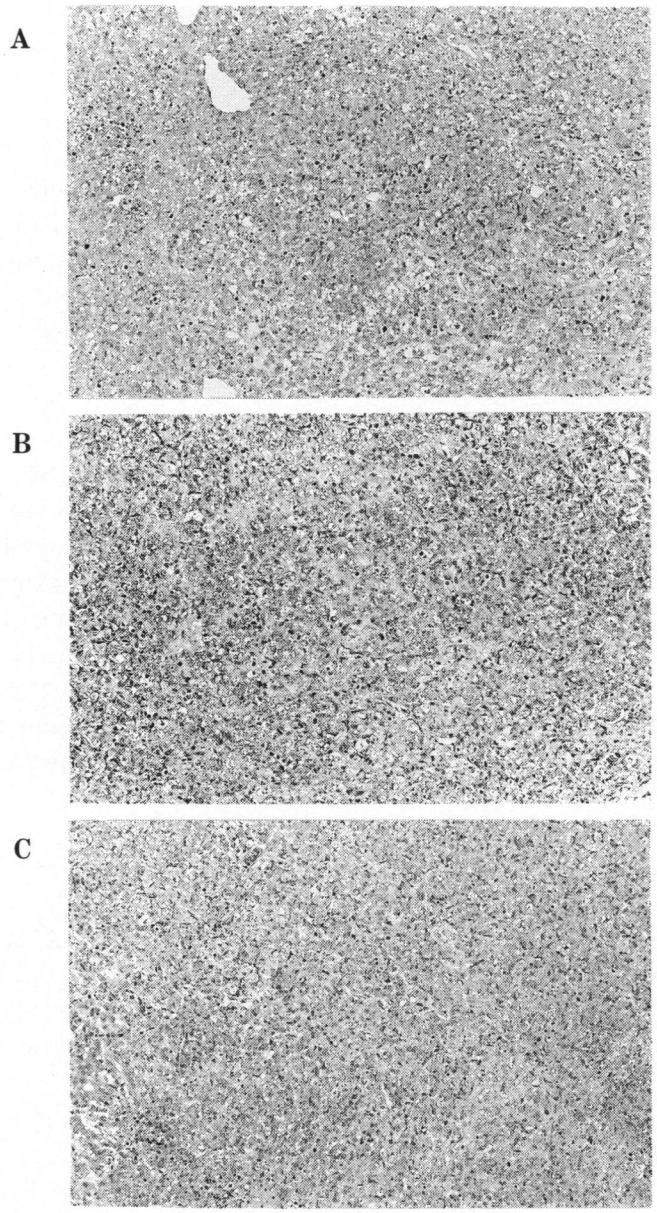

Fig. 1 Microscopic views of the subcutaneous tumor of AZ521 and AZ-H5c, and the metastatic liver tumor after intrasplenic injection of AZ-H5c. A, subcutaneous tumors of AZ521; B, subcutaneous tumors of AZ-H5c; C, the metastatic liver tumors after intrasplenic injection with AZ-H5c. Tissues were fixed, embedded, sectioned, and stained by H&E using standard procedures. (×100).

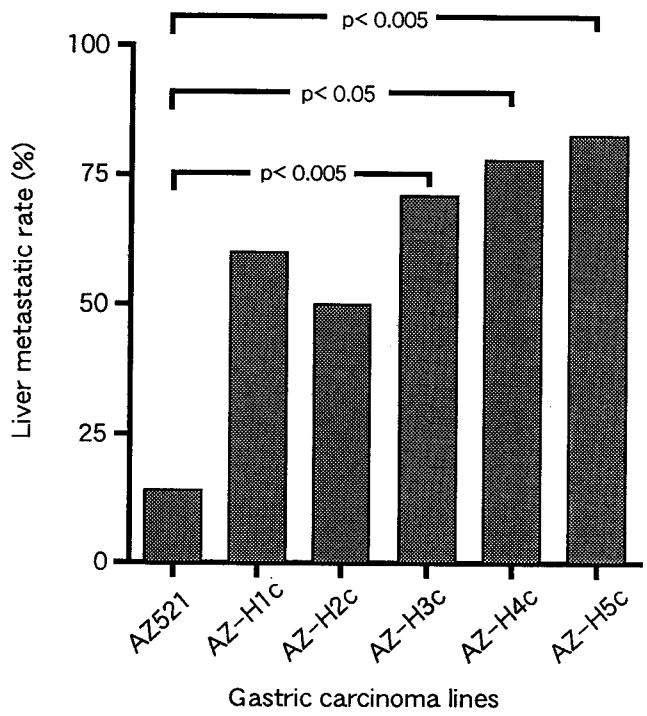

Fig. 2 Liver metastatic rates after intrasplenic injection of gastric cacinoma lines, AZ521, AZ-H1c, AZ-H2c, AZ-H3c, AZ-H4c and AZ-H5c.

Growth activity in vivo

Tumor weights of AZ521 and AZ-H5c, that were inoculated subcutaneously into nude mice, were 18,686±5,243 mg and 761±225 mg, respectively. As shown in Fig. 3, the growth activity *in vivo* of AZ-H5c acts more rapidly than that of AZ521.

Growth activity in vitro

Cell number of AZ521 and AZ-H5c, that were cultured on plate after 96 hours, were 100,000±4,000/m*l* and 57,500±7,500/m*l*, respectively. As shown in Fig. 4, the growth activity *in vitro* of AZ-H5c is slower than that of AZ521.

Motile activity in vitro

Cell number of AZ521 and AZ-H5c cells, that were invaded to the lower surface of the filter, were 0.833±0.792/field and 118.000±11.994/field, respectively. As shown in Fig. 5, the motile activity *in vitro* of AZ-H5c is stronger than that of AZ521.

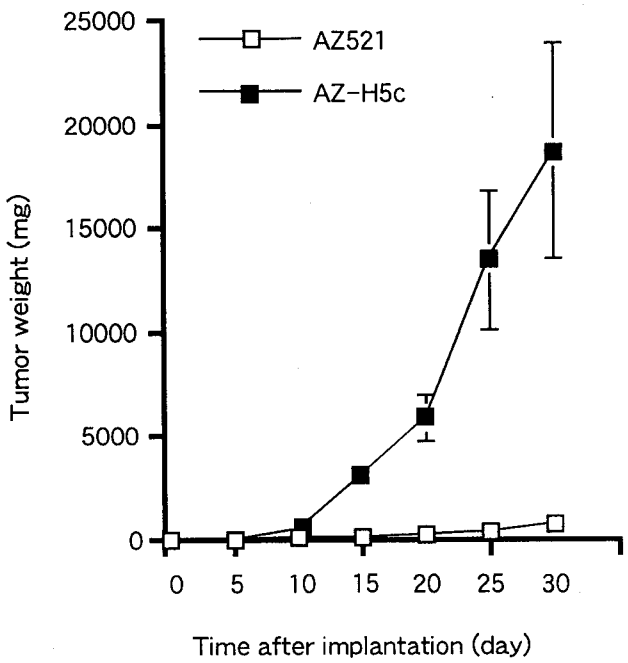

Fig. 3 *In vivo* growth activities of AZ521 and AZ-H5c. AZ-H5c cells were much more rapidly grown as compared with AZ521. Bars represent mean ± standard error.

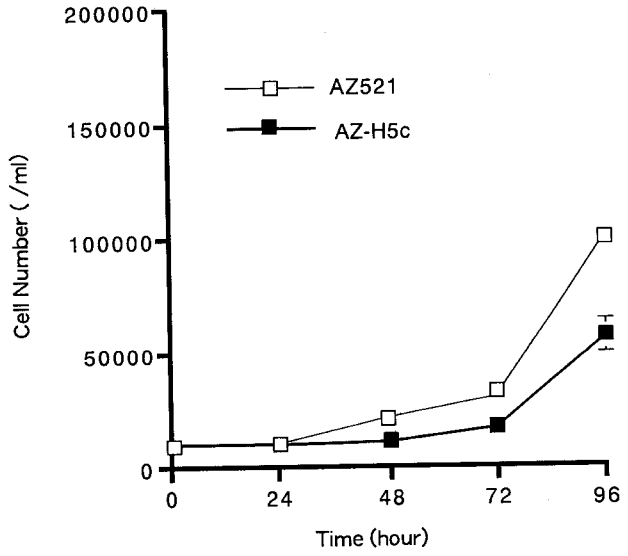

Fig. 4 *In vitro* growth activities of AZ521 and AZ-H5c. Bars represent mean ± standard error.

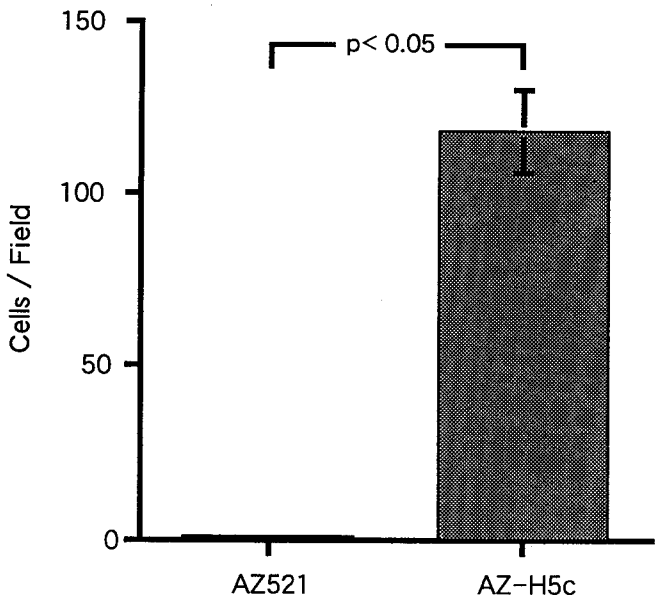

Fig. 5 *In vitro* motile activities of AZ521 and AZ-H5c. Bars represent mean ± standard error.

DISCUSSION

In the present study, we established a new line of human gastric carcinoma with high metastatic potential. Namely, metastatic tumors in nude mice intrasplenically injected with parental AZ521 cells were harvested, and the cell line, AZ-H1c, was established *in vitro*. Subsequently, an additional two cycles of the selection procedure yielded another cell line designated AZ-H3c with a high metastatic capability in the livers of nude mice. A further two cycles of the selection procedure yielded AZ-H5c, with an even higher metastatic capability than AZ-H3c. These are the first *in vitro* lines of such human gastric carcinomas.

The inhibition of metastasis is one of the major goals of cancer therapy. There are several mechanisms that confer a high liver metastatic capacity to cells. It is well known that hematogenous metastasis occurs as a consequence of 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 movement, (v) arrest in the capillary beds of a target organ, (vi) extravasation toward the organ parenchyma, and (vii) tumor cell growth(9).

Therefore, a mechanism that affects each of these steps can influence the metastatic potential of tumor cells. Since our model starts with an intrasplenic injection, it does not exactly represent 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 the AZ-H5c cells injected to the subcutis of nude mice grew much more rapidly than the AZ521 cells. At present, it is not known whether the differential growth ability in the subcutis reflects back to the primary lesion or within the metastatic lesion(10, 11, 12). On the other hand, the growth activity *in vitro* of AZ-H5c is slower than that of parental AZ521. The discrepancy between *in vivo* and *in vitro* findings probably reflects enviromental elements such as tumor growth factors. The motile activity *in vitro* of AZ-H5c is stronger than that of parental AZ521, but its invasiveness is rather weak, similar to that of the parental AZ521 (data not shown).

In conclusion, we present newly established 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 a low metastatic parental line demonstrated that at least the difference in the growth potential is likely responsible for the acquired high metastatic capacity. While further studies are necessary to confirm the characterizations of AZ521 and AZ-H5c lines. Therefore, our experimental model can further provide insights into the metastasis research for human gastric carcinoma.

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