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Abstract

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HUMAN CELL LINE (HGC-27) DERIVED FROM THE METASTATIC LYMPH NODE OF GASTRIC CANCER

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Abstract. A cell line (HGC-27) was established by culture of the metastatic lymph node from a gastric cancer patient diagnosed histologically as undifferentiated carcinoma. HGC-27 cells were polygonal or short spindle-shaped and adhered to glass surfaces as a monolayer. The cells were probably derived from gastric cancer cells, as their origin from mesenchymal tissues can be excluded morphologically and enzyme-histochemically. Enzyme activities were generally negative or low, except for adenosine triphosphatase, lactic dehydrogenase and leucine aminopeptidase. These scanty findings might reflect the undifferentiated character of the original tumor cells. The cloning efficiency was 5.3% in liquid medium and 1.0% in soft agar. The doubling time was about 17 hr. Chromosomal analysis revealed a mode of 109 and 110 chromosomes.

The necessity for establishing human cancer cell lines has recently become more important in etiological, immunological and therapeutic studies of cancers. In spite of extensive efforts made over the years to establish cultures of human cancers only limited success has been achieved so far (2, 3). In Japan where gastric cancer is the most frequently encountered malignant disease, the establishment of gastric cancer cell lines is especially important. However, gastric cancer cell lines are not easily available in spite of some reports of successful establishment (1, 5, 6). The present authors have tried to cultivate gastric cancer cells *in vitro* from 13 cases of primary lesion, 7 cases of metastatic lymph nodes and 4 case samples of ascitic fluid. Successful culture was established in only one permanent cell line (HGC-27) derived from a metastatic lymph node. This report describes the culture history and some biological characteristics of the HGC-27 cells.

The lymph node with the metastasis was resected from a patient with gastric cancer on November 9, 1973, and used for tissue culture. In the primary lesion of the stomach, cancer cells proliferated intensively and infiltrated throughout the wall and were histologically well-differentiated tubular adenocarcinoma in the mucosa and undifferentiated carcinoma in other areas (Fig. 1).



Fig. 1

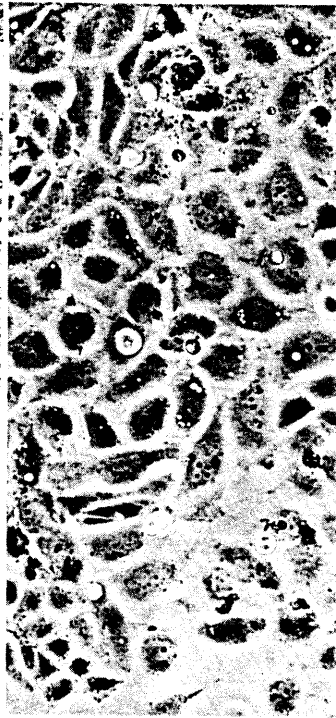


Fig. 2

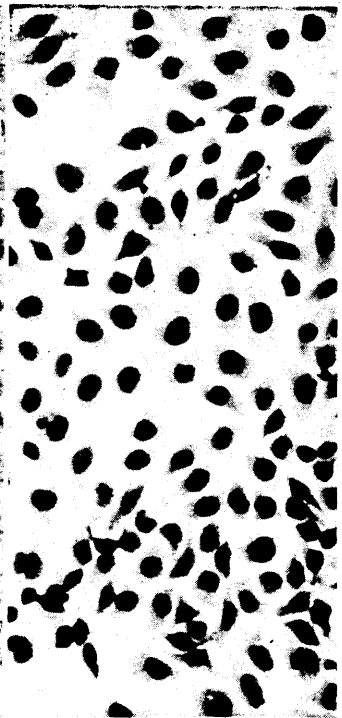


Fig. 3

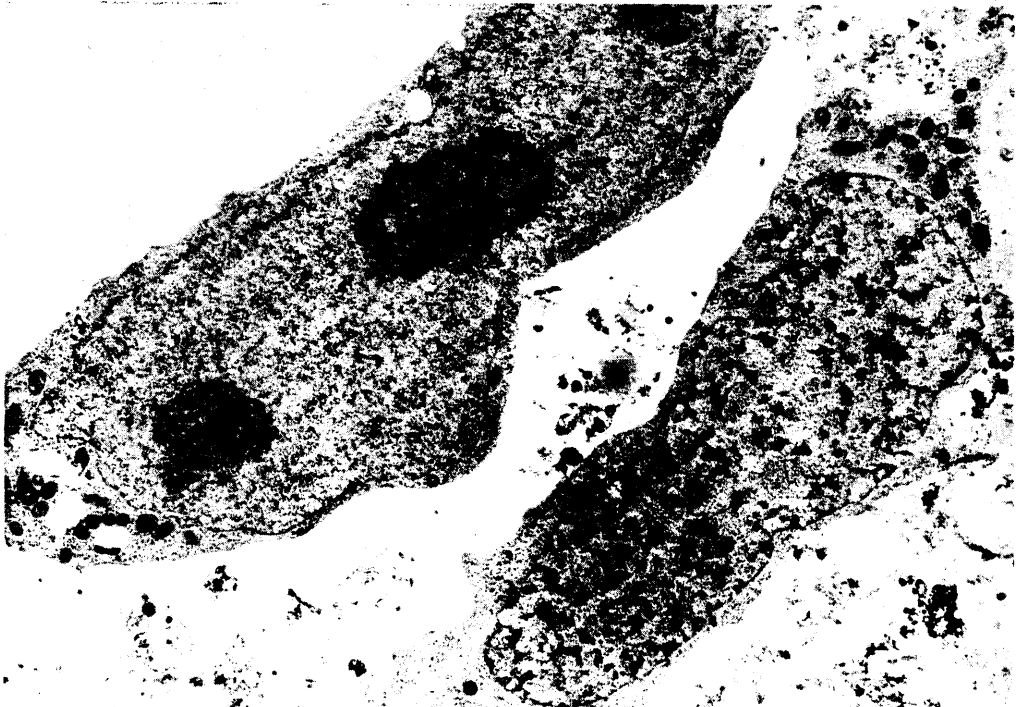


Fig. 4

The lymph node used in culture was completely replaced by undifferentiated cancer cells.

The lymph node was finely minced by scissors and pipetted. The tissue was so medullary and soft that the cancer cells were easily liberated and dispersed without trypsin digestion. The dispersed cells were suspended in Ham's F-12 medium, pH 7.2, supplemented with 20% fetal calf serum (FCS) and incubated at 37°C in 5% CO₂ and 95% air. Polygonal epithelioid cells were predominant and proliferated well, adhering mostly to the glass surface from the beginning of cultivation (Fig. 2). The medium was changed every 2-3 days and the first subcultivation was performed at 46 days. Subcultures were performed by trypsin digestion every 2 weeks up to 5 months and every 4-7 days at 1:10 ratio after 5 months. The medium was changed to RPMI-1640 supplemented with 10% FCS at 3 months after initiation of culture. HGC-27 cell line has now been maintained in culture for over 2 years and has been subcultured more than 120 times.

In cultures, polygonal epithelioid cells were predominant and showed a slight atypism and pleomorphism. The cells proliferated as a monolayer, showing areas of slight pile-up in dense culture (Fig. 3). In sparse culture, some cells were elongated, and assumed short spindle-shapes, resembling fibroblasts. Epithelioid cells, however, might assume such forms depending on the culture conditions, as the spindle-shaped cells were observed together with epithelioid cells after several clonings. Ultrastructurally, the cytoplasm contained abundant free ribosomes mostly taking the form of polysomes and well-developed mitochondria. Endoplasmic reticulum and Golgi apparatus were poorly developed. The nucleus had dotted heterochromatins distributing diffusely, showing a tigroid-like appearance. The nucleoli were either well-defined or ill-defined. Intracytoplasmic canaliculi were occasionally seen, but its significance was obscure. Desmosomes and secretory granules were not observed (Fig. 4). Morphologically, the HGC-27 cells lacked distinctive adenocarcinoma cell characteristics. The usual histochemical investigations for mucin, such as Alcian blue-PAS

Fig. 1. Histological section of primary carcinoma of the stomach showing well-differentiated tubular adenocarcinoma in the mucosa and undifferentiated carcinoma in other areas. H-E stain. $\times 72$.

Fig. 2. Growth of polygonal epithelioid tumor cells showing a pavement-like pattern in an early passage. Phase contrast. $\times 170$.

Fig. 3. HGC-27 cells in the 121st passage. Polygonal or slightly elongated epithelioid cells showing monolayered growth. Giemsa stain. $\times 170$.

Fig. 4. Electron micrograph of HGC-27 cells in the 34th passage. Note numerous free ribosomes, scattered mitochondria and scanty endoplasmic reticulum. Nuclei have irregular profiles and are composed of euchromatin or diffusely scattered heterochromatin and dense nucleoli. $\times 7,400$.

double staining and mucicarmine staining, were negative. The results of the enzyme-histochemical investigations are summarized in Table 1. Moderate to

TABLE 1. ENZYME ACTIVITIES IN HGC-27 CELLS

Enzyme	Activities
Alkaline phosphatase	- ~ ±
Acid phosphatase	- ~ ±
Adenosine triphosphatase	++
Glucose-6-phosphatase	-
Non-specific esterase	- ~ ±
β-Glucuronidase	- ~ ±
Leucine aminopeptidase	+ ~ ++ ~ +++
Lactic dehydrogenase	+ ~ ++ ~ +++
Succinic dehydrogenase	- ~ +
Glucose-6-phosphate dehydrogenase	±
Phosphorylase	-

-, negative; ±, trace; +, weak; ++, moderate; and +++, intense.

high activities of adenosine triphosphatase (ATPase), leucine aminopeptidase (LAP) and lactic dehydrogenase (LDH) and weak succinic dehydrogenase (SDH) activity were detected, although other enzymes were absent or showed only trace activities. These results differed from the data of the enzyme-histochemical studies for gastric cancer *in vivo* (4). Immunofluorescent antibody technique using fluorescein isothiocyanate-conjugated anti-human immunoglobulin rabbit serum (Behringwerke) revealed no detectable surface or intracytoplasmic immunoglobulins. Phagocytoses of Indian ink and iron chondroitin sulfate were not observed under the physiological conditions. Aggregates were not formed in gyratory cultures. Cloning efficiency at the 22nd passage was 5.3% in liquid medium and 1.0% in soft agar medium. The doubling time estimated from the growth curve was approximately 17 hrs (passage 21). Chromosome counts of passage 11 cells ranged from 73 to 119 with a mode of 109 and 110 chromosomes being 28%. The cells were heterotransplanted into mice treated with antithymocyte serum (ATS) subcutaneously or into the cheek pouches of hamsters treated with ATS or cortisone; tumor growth was not found.

It is essential to ascertain that the HGC-27 cells were derived from gastric cancer cells. Their discrimination from lympho-reticular cells, endothelial cells

and fibroblasts is particularly important, as the HGC-27 cells were cultivated from the lymph node. In the cultural process, HGC-27 cells showed a marked initial proliferation of epithelioid cells and were established without a lag phase. The morphology of the established cells was identical to the cells in the early period of culture. Poor development of rough-surface endoplasmic reticulum and Golgi apparatus and the lack of collagen production probably exclude the possibility of their being mature fibroblasts. The probability of these cells being macrophages or reticulum cells may be excluded because of the absence of both phagocytosis and lysosomal enzymes activity, such as β -glucuronidase and acid phosphatase. The lymphoid cell possibility may also be excluded as the HGC-27 cells did not produce immunoglobulins and proliferated as a monolayer adhering to the glass surface. These findings strongly suggest that the HGC-27 cells were derived from gastric cancer cells, although they did not have the morphological and enzyme-histochemical characteristics of glandular epithelial cells. The reason for HGC-27 cells lacking distinctive characteristics may be that the original tumor was undifferentiated carcinoma without mucin production and tubular formation. However, the possibility that the HGC-27 cells were derived from undifferentiated mesenchymal cells cannot be entirely excluded. The reasons for the failure of heterotransplantation are obscure and remain to be solved. Transplantation into nude mice should be examined.

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