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Rapid communication

Establishment of a human cancer cell line with high potential for peritoneal dissemination

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Introduction

Peritoneal dissemination is one of the most important prognostic factors for gastrointestinal cancers. Even if cancer cells are exposed to the serosal surface of the gastrointestinal tract or are recognized cytologically in ascitic fluid at operation, some develop peritoneal dissemination but some do not. It seems that certain characteristics of cancer cells affect the development of peritoneal dissemination. However, few studies of the mechanism of peritoneal dissemination and the characteristics of cells which develop peritoneal dissemination have been undertaken.[1–5] It is useful to establish and analyze cells with high potential for peritoneal dissemination in order to study those mechanisms and characteristics. We report herein the establishment of a human cell line with high potential for peritoneal dissemination.

Materials and methods

Three gastric cancer cell lines (MKN-7, MKN-45, and AKT-GC-F) and five colon cancer cell lines (Colo201, Colo205, LS174T, SW1116, and AKT-CC-K) were used in this study. AKT-GC-F and AKT-CC-K cells were established in our laboratory. All cell lines were of human origin and were maintained in our laboratory under usual conditions. Each cell suspension including 1×10^5 cancer cells was injected into the peritoneal cavities of severe combined immunodeficiency (SCID) mice ($n = 3$). Four weeks after injection, the mice were killed to detect cancer nodules in the abdominal cavity. One detected cancer nodule was selected, cut into pieces $2 \times 2 \times 2$ mm in size, and then inoculated in the mesentery of new SCID mice ($n = 4$). Four weeks later, the presence or absence of peritoneal dissemination originating from the nodule inoculated in the mesentery was observed. Peritoneal nodules were confirmed as cancer dissemination by both histologic examination and cancer cell growth in vitro.

The peritoneal dissemination nodule, if detected, was excised, cut into $2 \times 2 \times 2$ mm pieces and again inoculated in the mesentery of new SCID mice ($n = 4$). The dissemination nodule then was serially inoculated in new SCID mice ($n = 4$). The metastatic potential of peritoneal dissemination was evaluated at four weeks after inoculation by two parameters: the increased number of dissemination nodules and the increased ratio of weight of all dissemination nodules to that of the inoculated nodule.

Results

Although cancer cell lines, except MKN-7, could grow in the mesentery, peritoneal dissemination from the inoculated nodule was observed in only two gastric cancer cell lines (MKN-45 and AKT-GC-F).

The dissemination nodules from MKN-45 and AKT-GC-F cells were serially inoculated in the mesentery. The number and the weight ratio of dissemination nodules from AKT-GC-F cells were unchanged after serial inoculation. However, those from MKN-45 cells increased serially as shown in Table 1. MKN-45 cells from each generation could grow in vitro and could develop peritoneal dissemination after a one-month incubation in vitro.

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Table 1. Number and weight ratio of dissemination nodules of MKN-45 cells in each generation at 4 weeks after inoculation

Peritoneal dissemination nodule	Generation				
	Parent (n = 3)	1st (n = 4)	2nd (n = 4)	3rd (n = 4)	4th (n = 4)
Number	2 ± 0.5	2 ± 1.5	6 ± 2.3	9 ± 5.0	27 ± 6.5
Weight of dissemination nodule (mg)	81 ± 24	99 ± 4	139 ± 18	212 ± 7	370 ± 31
Weight of inoculated nodule (mg)	908 ± 214	863 ± 34	1337 ± 69	1000 ± 13	1293 ± 87
Weight ratio (mean)	0.09	0.11	0.10	0.21	0.29

mean ± SD

Discussion

The peritoneal dissemination mechanism is thought to be as follows: cancer cells 1) invade the serosa of the original organ and are exposed to the abdominal cavity; 2) detach themselves from the primary tumor; 3) are transferred to other sites in the peritoneum; 4) attach to the peritoneum; 5) invade the peritoneum; and 6) grow there. Previous models for peritoneal dissemination used either injection of cell suspension[1,2] or implantation of a tumor to the outer surface of the stomach or colon.[5,6] They do not, however, include all steps of the peritoneal dissemination mechanism. Our method of cell selection for peritoneal dissemination includes all steps of the mechanism and is assumed to be suitable for studying the characteristics of cells with potential for peritoneal dissemination.

Using our selection method, it was revealed that six of eight cancer cell lines did not have the potential for peritoneal dissemination. Colo201 and Colo205 cell lines which were established from the ascitic fluid of patients with colon cancer[7] did not develop peritoneal dissemination. The explanation may be that cancer cells lose the disseminating potential after maintenance in vitro for an extended period, or that even cancer cells in ascitic fluid cannot develop peritoneal dissemination when they have no potential for attachment and/or invasion to the peritoneum. Cancer cells in ascitic fluid at operation do not always develop peritoneal dissemination clinically, and treating cancer cells with some antibody or receptor ligands suppressed the development of peritoneal dissemination experimentally.[1,2] These findings suggest that cancer cells need certain characteristics to develop peritoneal dissemination.

We were able to establish a cell line with high potential for peritoneal dissemination. The difference in cell adhesion and invasion of the peritoneum, and the expression of adhesion molecules, matrix metalloproteinase and carbohydrates between cells with and without high potential for peritoneal dissemination, will be elucidated using adhesion and invasion assay, flow cytometry, and immunohistochemistory.

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