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Role of Orthotopic Fibroblasts in the Development of Scirrhous Gastric Carcinoma

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We examined the interaction between scirrhous gastric cancer cells and organ-specific fibroblasts *in vivo* and *in vitro*. Co-inoculation of scirrhous gastric cancer cells with gastric fibroblasts into nude mice specifically increased tumorigenicity, compared with that of gastric cancer cells alone. Furthermore, the histologic findings of the xenograft produced by co-inoculation with gastric fibroblasts was similar to that of human scirrhous gastric carcinoma. Conditioned medium from gastric fibroblasts significantly stimulated the growth of gastric cancer cells. These findings suggest that the growth of scirrhous gastric cancer cells was affected by orthotopic fibroblasts.

Key words: Scirrhous gastric carcinoma — Orthotopic fibroblast — Growth factor

The prognosis of gastric cancer has improved, but that of scirrhous gastric cancer has not improved significantly because its biologic behavior is poorly understood. Human scirrhous gastric carcinoma (diffusely infiltrating carcinoma or Borrmann's type IV carcinoma) is characterized by extensive carcinoma cell infiltration and proliferation with fibrosis in the stroma.[1] The typical histologic findings of scirrhous gastric carcinoma suggest that the development of scirrhous gastric carcinoma may be controlled by an intercellular relationship between the cancer cells and the stroma cells. Several studies have been published recently on the effect of fibroblasts on the growth of cancer cells.[2–10] However, there have been no reports about the effect of gastric fibroblasts on the growth of scirrhous gastric cancer cells or on the histologic behavior of xenografts. Therefore, we examined the interaction between scirrhous gastric cancer cells and fibroblasts.

We used three cell lines in this study; OCUM-2M, NF-8, HS-27F. The gastric scirrhous cancer cell line OCUM-2M was derived from a primary gastric tumor taken at total gastrectomy from a 49-year-old Japanese female with scirrhous gastric cancer on October 13, 1992. The primary culture was initiated as follows: the primary tumor was excised under aseptic conditions, and minced with forceps and scissors. The tumor pieces were cultivated in Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal calf serum (GIBCO, Grand Island, NY), 1% glutamine, 0.5% pyruvate, penicillin (100 IU/ml) and streptomycin (100 mg/ml), and incubated in humidified incubators at 37°C in an atmosphere of 5% CO_2 in air. The cancer cells and fibroblasts initially grew in a monolayer. After about 3 weeks, floating cancer cells began to grow gradually. About 5 weeks later, the floating cancer cells were collected and transferred to another culture dish. Serial passages then were carried out every 4–7 days. The floating cancer cells were designated OCUM-2M. The gastric fibroblast cell line NF-8 was derived from the gastric tissue surrounding the tumor. NF-8 and OCUM-2M were derived from the same patient. The ectopic fibroblast cell line HS-27F was derived from the foreskin of a newborn baby. The fibroblasts were used before the 8th passage in culture.

Fibroblast conditioned medium was prepared as follows. Approximately 5×10^5 fibroblasts were seeded into 100 mm plastic dishes with 10 ml of DMEM containing 10% fetal calf serum (FCS), and incubated at 37°C for 3 days. The conditioned medium was centrifuged at 1,000*g* for 5 min, and stored at −20°C until use.

We examined the *in vivo* effect of the co-inoculation of tumor cells with fibroblasts. The injection procedure was as follows. Subconfluent gastric cancer cells (OCUM-2M) and fibroblasts (NF-8 or HS-27F) were released by trypsin/EDTA (GIBCO) treatment and counted. Suitable numbers of tumor cells and fibroblasts were resuspended in 0.2 ml of DMEM and inoculated subcutaneously into four-week-old female BALB/c nu/nu nude mice. The tumor growth was followed for six weeks. The histologic features of the xenografts were examined by hematoxylin-eosin staining (H-E).

The effect of the fibroblasts on DNA synthesis in scirrhous gastric cancer cells was determined by measuring [^{3}H]thymidine incorporation. A 90 μl portion of the tumor cell suspension (1×10^5 cells/ml) was added to 90 μl of conditioned medium in each well of 96-well flat-bottomed microtiter plates (Falcon), and incubated at 37°C for 24 h. As a control, 90 μl of DMEM containing 10% FCS was used. DNA synthesis was assayed after incubation with [^{3}H]thymidine (1 μCi/well; RCC Amersham 10 μCi/mmol) for 24 h at 37°C. The cells

then were rinsed and collected on a membrane filter, and the radioactivity was counted in a liquid scintillation counter.

The effect of co-culture with OCUM-2M and NF-8 on cell growth was examined by using the double chamber method. The OCUM-2M cells (4×10^4) were seeded in the upper chamber on a membrane with a pore size of 3 μm (Falcon) in each well, and NF-8 cells (7×10^4) were seeded under the membrane in the same well. As a control, 4×10^4 OCUM-2M or 7×10^4 NF-8 were seeded in wells separately. The numbers of cells were counted at 72 h after the seeding. Statistical analysis was performed using Student's t test.

The co-inoculation of scirrhous gastric cancer cells with gastric fibroblasts into nude mice remarkably increased the tumorigenicity, compared with that of gastric cancer cells alone. The inoculation of 5×10^6 OCUM-2M with 2×10^6 NF-8 resulted in tumor formation in all treated mice (4/4). However, the inoculation of 5×10^6 OCUM-2M alone and the co-inoculation of 5×10^6 OCUM-2M with 2×10^6 HS-27F both resulted in poor tumor formation (1/4) (Table I). Furthermore, the tumor size observed after the co-inoculation of OCUM-2M and NF-8 was much larger than that observed after the inoculation of OCUM-2M alone (Fig. 1). The histo-

Table I. Effect of Co-inoculation of Fibroblasts on Tumor Formation in Nude Mice

Cell line	No. of cancer cells injected		
	1×10^5	1×10^6	5×10^6
OCUM-2M alone	0/4[a)]	1/5	1/4
OCUM-2M with HS-27F	0/3	1/5	1/4
OCUM-2M with NF-8	0/4	4/5	4/4

a) Number of mice bearing a tumor/total number of mice.

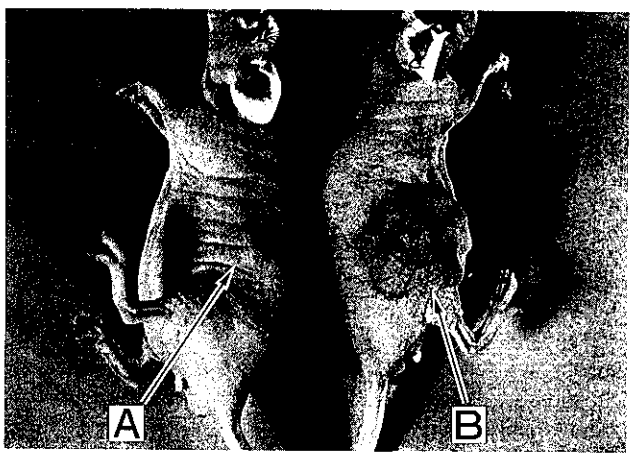

Fig. 1. Effect of gastric fibroblasts on xenograft growth in nude mice. The tumor size achieved by the co-inoculation of OCUM-2M and NF-8 (B) was much larger than that achieved by the inoculation of OCUM-2M alone (A). These tumors were observed at 4 weeks after the inoculation.

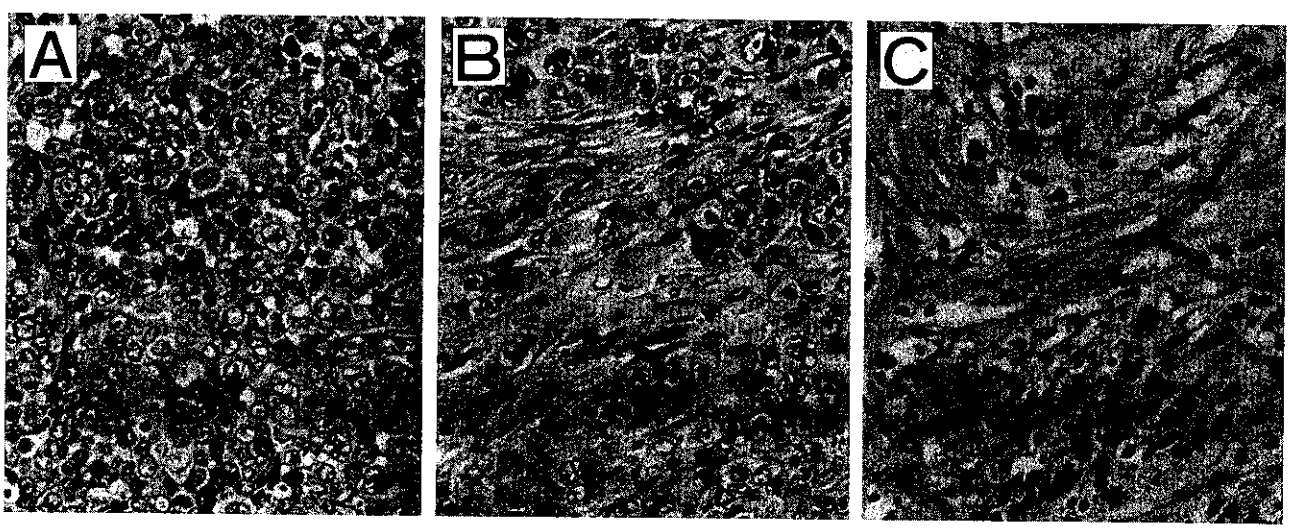

Fig. 2. The histologic findings of the xenografted tumor and the primary tumor (H-E, ×400). The xenografted tumor of OCUM-2M alone shows poorly differentiated adenocarcinoma with poor fibrosis (A). The xenografted tumor of OCUM-2M and NF-8 shows infiltrative growth with a scirrhous pattern (B), which is similar to the histologic findings of the primary scirrhous gastric carcinoma (C) from which OCUM-2M was derived.

logic findings of the xenografts produced by co-inoculation with NF-8 were similar to those of human scirrhous carcinoma with extensive fibrosis, whereas poor fibrosis was observed in the xenografts produced by the inoculation of OCUM-2M alone (Fig. 2). There was no significant difference between the findings obtained by co-inoculation of OCUM-2M with HS-27F and those obtained by inoculation of OCUM-2M alone. The DNA synthesis of the tumor cells following the addition of conditioned medium from NF-8 was significantly increased to 136% of the control value. On the other hand, conditioned medium from HS-27F did not stimulate DNA synthesis of tumor cells (Fig. 3). The growth of OCUM-2M and NF-8 was increased significantly by co-culture compared to single culture (Fig. 4).

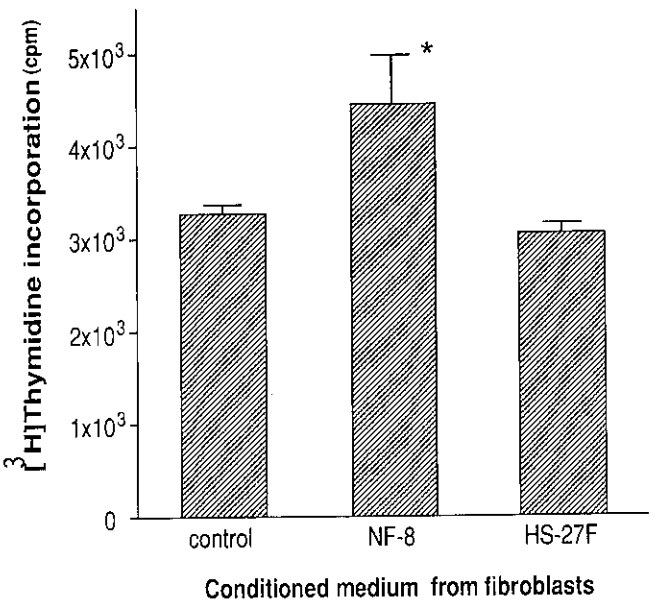

Fig. 3. The effect of conditioned medium from fibroblasts on DNA synthesis of OCUM-2M. The results are shown as the means of 4 samples, and the bars correspond to the standard deviations. The significance of differences was determined by using Student's t test. *, $P<0.01$ compared to control.

In conclusion, the histologic findings of the xenograft produced by co-inoculation of scirrhous gastric cancer cell with gastric fibroblasts in nude mice were similar to those of human scirrhous gastric cancer. In addition, the tumorigenicity of co-inoculation of scirrhous gastric cancer cells with gastric fibroblasts in nude mice was increased considerably compared to that of inoculation of scirrhous gastric cancer cells alone. These findings demonstrate that the presence of the gastric fibroblast line NF-8 can promote the growth of the human scirrhous gastric cancer cell line OCUM-2M, while that of fibroblasts from skin can not. Our findings also suggest that there is a close relationship between cancer cells and orthotopic fibroblasts in the development of scirrhous gastric cancer, and that gastric fibroblasts produce a paracrine factor(s) which may play an important role in the progression of scirrhous gastric cancer.

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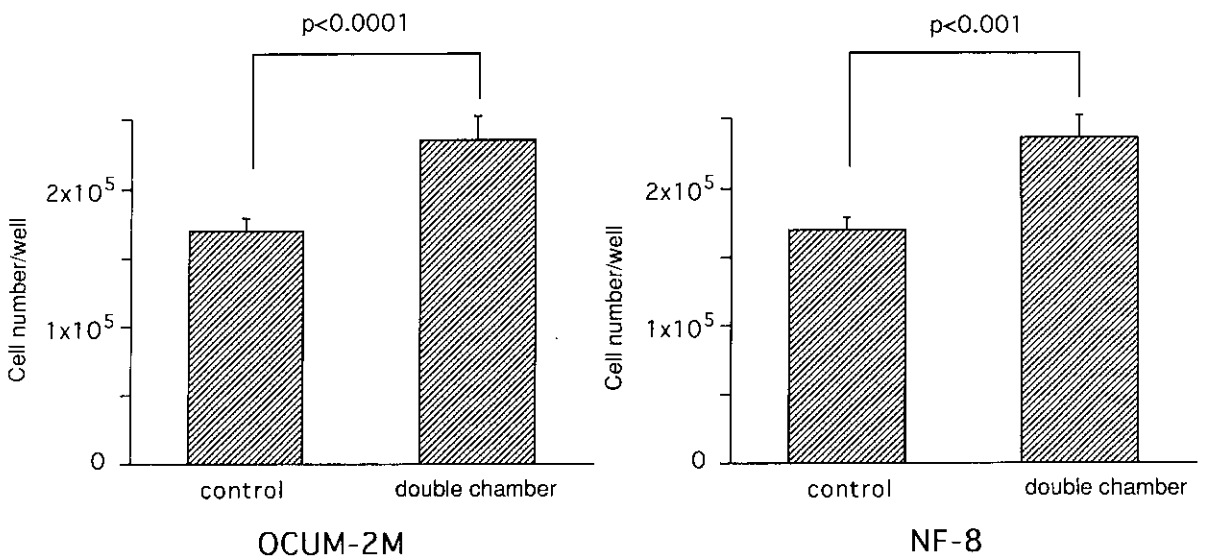

Fig. 4. The effect of co-culture with OCUM-2M and NF-8 by using a double chamber method. The growth of OCUM-2M and NF-8 was increased significantly by co-culture. The results are shown as the means of 4 samples, and the bars correspond to the standard deviations. The significance of differences was determined by using Student's t test.

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