—Preliminary Note—

Cell growth regulation of scirrhous gastric carcinoma

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In scirrhous carcinoma, cancer cell infiltration is demonstrated in abundant fibrous stroma¹⁾. The fibrous stromal formation may be induced by infiltrating cancer cells and mesenchymal cells, and fibroblasts may contribute to the production of extracellular connective tissue. Tumor cell and mesenchymal cell may synthesize various types of collagen and glycosaminoglycan²⁾. However the mechanism of intercellular influences between cancer cells and mesenchymal cells is not clearly understood. This study was undertaken to clarify the interactions between cancer cells and mesenchymal cells in scirrhous carcinoma of the human stomach.

The MA IV cell line was established from a signet ring cell carcinoma of the human stomach^{3,4)}, and was compared with the biological features of the MKN 28 cell line which was derived from a differentiated adenocarcinoma of the stomach⁵⁾. These cells were maintained in RPMI medium with 10% fetal bovine serum. Human fibroblasts were derived from the subcutaneous tissue of a healthily 3-year-old male and maintained in MEM medium with 10% fetal bovine serum.

MA IV and MKN 28 cells were cultured for one week. The media were removed and filtrated with a 0.22 μ m filter unit, Millex-GV. Human skin fibroblasts were plated at 2.5×10^5 cells per well and cultured with 50% MEM and 50% tumor cell conditioned media. The number of cells was counted by culture counter on the 1st, 2nd, 6th, 10th and 12th day. As a control, the fibroblasts were cultured in tumor cell free media.

The number of fibroblasts decreased in the MA IV, MKN 28 and control conditioned media on the first day and gradually increased from the second day. A 30% increase in the number of fibroblast was noted in MA IV conditioned medium compared with the other conditioned media on the 10th day ($p < 0.01$) (Fig. 1-1).

The MA IV cell line was cultured for 14 day in a type IV collagen solution (Cell matrix, Nitta Gelatine Co., Ltd., Osaka) containing Ham's F10, reconstitution buffer (2.2g NaHCO_3 and 4.77g HEPES in 100ml of 0.05N NaOH) and 20% fetal bovine serum. The cells were then pulsely labelled with 100 μ g bromodeoxyuridine (BrdU) (Becton Dickinson, Co., Ltd., USA). After 30 minutes of incubation, the labelled cells were fixed in 70% ethanol for 30 minutes and incubated in 4N HCl for 20 minutes. Subsequently 1:200 anti-BrdU (Becton Dickinson, Co., Ltd., USA) as special antibody, and 1:45 peroxidase conjugated anti mouse-IgG as secondary antibody were used. The BrdU positive cells were counted under a light microscope, and the results were estimated by t-test.

Growth appearance of fibroblast in the conditioned media of MA IV and MKN 28

Fig. 1-1 Fibroblast proliferation in the conditioned medium of MA IV compared with control and MKN 28 conditioned media ($p < 0.01$)

Growth curve of MA IV in collagen matrix type IV

Fig. 1-2 MA IV cell proliferation in collagen matrix type IV compared with control medium

Fig. 2-1 The cell density is much more prominent in collagen matrix(B) compared with control medium(A) on the third day ($\times 51.2$)

Fig. 2-2 Immunohistochemical BrdU uptakes are noted in dense nuclei (arrows)
A: control, B: collagen matrix ($\times 400$)

The number of MA IV cells increased from the first day in collagen matrix containing type IV ($p < 0.01$) (**Fig. 1-2**). The cell density increased much more in collagen matrix than in the control media on the third day (**Fig. 2-1**). BrdU was localized in the nuclei of the immature cells which contained a scant number of mucous granules. The average percentage of the BrdU uptake cells was 21.2% in collagen matrix (± 1.2 standard error) compared with 14.9% (± 0.3 standard error) in the control media on the 3rd, 7th, 9th and 14th day (**Fig. 2-2**).

We observed that the proliferation of fibroblasts was promoted in the conditioned medium of cancer cells. This result suggests that cancer cells may regulate the fibroblast growth and secrete a substance which may stimulate the in vitro proliferation of interstitial connective tissue. The present results indicate that MA IV cells proliferate and BrdU positive cells increase in the presence of collagen type IV. Type IV collagen is allegedly stated as specific for basement membranes which are extracellular structures underlying most epithelial cells and surrounding muscle, nerve, fat and smooth muscle cells⁷⁾. Type IV collagen which is produced by cancer cells may promote cancer cell and fibroblast growth. Tumor cells and mesenchymal cells such as fibroblast may contribute to basal lamina formation in cancer cells. Cell growth is promoted by the basal lamina formation. Type IV collagen production increase depends on the carcinoma and stroma cell proliferation²⁾.

The development of scirrhous carcinoma may be mediated by intercellular interactions between stroma cells and malignant cells. Not only tumor cells but also mesenchymal cells are important factors in determining tumor cell proliferation. These results suggest that the proliferation and the differentiation of gastric cancer cells correlate with fibroblast growth and the production of extracellular matrix such as type IV collagen.

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