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GROWTH RESPONSES OF HUMAN GASTRIC CANCER CELLS TO HORMONES AND FACTORS

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ABSTRACT

Various hormones and factors were added to human gastric cancer cells growing in vitro in a serum-free medium and growth rates were examined. The proliferation effect of transferrin was common to cell lines derived from differentiated carcinoma, MKN28, and another cell line derived from undifferentiated carcinoma, MKN45. Insulin was efficient only in the MKN28 cell line, and hydrocortisone was efficient only in the MKN45 cell line. Although serum-free media supplemented with transferrin, insulin or hydrocortisone failed to show significant effects on growth, they were helpful to maintain the subsistence of cells.

INTRODUCTION

Many substances have been known to stimulate the growth of non-neoplastic or neoplastic cells. Growth of some cancers, especially of endocrine-responsive tissue, for example, breast, endometrium and prostate, is dependent on the same hormones that control the growth of the normal tissue. The growth of epithelial cells of gastrointestinal tracts also is influenced by various gastrointestinal hormones.[11] Moreover, various kinds of growth factors have been discovered up to now.[4]

However, there is little information about which substances control the growth of human stomach cancers. This is one of the most important issues when we consider the large number of gastric cancers in Japan. In the present study, we tested the effect of

various substances, including hormones and growth factors, on the growth of two human gastric cancer cell lines which derived from different histological types.

MATERIALS AND METHODS

Cells and Culture Two cell lines, MKN28 and MKN45, used throughout this study were derived from human gastric cancers. The MKN28 cell line was derived from a metastatic tumor of the lymph node of a 70-year-old Japanese woman who had a differentiated adenocarcinoma of the stomach.

The MKN45 cell line was derived from a metastatic tumor of the liver of a 62-year-old Japanese woman who had an undifferentiated adenocarcinoma of the stomach. Their properties in vitro are described in detail elsewhere.[7)13)14)] The cultures were fed with RPMI1640 medium (Nissui Seiyaku, Tokyo, Japan) supplemented with 10% fetal bovine serum (M. A. Bioproducts, Walkersville, Md. USA) and 200μg/ml of kanamycin sulfate (Meiji Seika, Tokyo, Japan). The cells were grown on plastic tissue culture dishes in an atmosphere of 5% CO_2 at 100% humidity.

Experimental Media Serum-free RPMI1640 medium contained substances which are shown in Table 1. Human transferrin (99% iron free), epidermal growth factor (EGF) from mouse submaxillary glands, hydrocortisone, bovine pancreatic insulin, a mixture of bovine and porcine pancreatic glucagon, ß-estradiol 3-benzoate (estradiol), estriol 3-benzoate (estriol), 17α-hydroxyprogesterone (progesterone), testosterone propionate (testosterone), and prostaglandin E_2 and $F_2\alpha$, were purchased from Sigma (St. Louis, MO. USA). Amogastrin (gastrin) was obtained from Nippon Kayaku (Tokyo, Japan).

Experimental Procedure For each experiment, exponentially growing cells were trypsinized, and 5×10^4 cells suspended in 1.5ml of the serum-supplemented medium were plated into 35mm plastic tissue culture dishes. The serum-supplemented medium was refreshed 24hr after the seeding. On the 3rd day, various concentrations of hormones and factors dissolved in 1.5ml serum-free medium were added to the dishes. The effects were estimated on the 2nd day after the addition. Cell number was determined by the trypan blue dye-exclusion test, experimental cell counts were obtained by duplicate plates in one test, and the average numbers were calculated in two or three tests.

Ultrastructural Examination For transmission electron microscopy, the cell pellets were fixed in 2.5% glutaraldehyde buffered at pH7.4 with 0.1m phosphate for 30min, postfixed in 1% osmium tetraoxide for 1hr, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi HS-9 electron microscope.

RESULTS

Transferrin increased the relative cell number in both cell lines (MKN28; 144% at 1μg/ml, MKN45; 165% at 1μg/ml). EGF had no effect on the cell number of either cell line at any concentration tested (0.1-10μg/ml). Hydrocortisone was efficient in the

MKN45 cell line (159% at 1μg/ml), and insulin was efficient in the MKN28 cell line (151% at 1μg/ml). Glucagon and gastrin did not significantly increase the cell number in either cell line (Table 2).

Sex hormones and prostaglandins also had no effect (Table 3).

Table 1. Substances used for cell growth study

Substance	Concentration
Transferrin	0.1~ 10μg/ml
EGF	0.1~ 10μg/ml
Hydrocortisone	1 ~100μg/ml
Insulin	0.1~ 10μg/ml
Glucagon	1 ~100μg/ml
Gastrin	1 ~100μg/ml
Estradiol	0.1~ 10μg/ml
Estriol	0.01~ 1μg/ml
Progesterone	0.1~ 10μg/ml
Testosterone	1 ~100μg/ml
Prostaglandin E_2	1 ~100μg/ml
Prostaglandin $F_2\alpha$	1 ~100μg/ml

Table 2. Responses of gastric cancer cells to factors and adrenal and gastrointestinal-pancreatic hormones

		% of growth	
Addition	Concentration	MKN28	MKN45
None (control)	—	100	100
Serum	5%	174	172
	10%	176	188
Transferrin	1μg/ml	144	165
	10μg/ml	135	130
EGF	1ng/ml	110	92
	10ng/ml	98	83
Hydrocortisone	1μg/ml	89	159
	10μg/ml	79	128
Insulin	0.1μg/ml	126	54
	1μg/ml	151	64
	10μg/ml	131	47
Glucagon	10μg/ml	84	123
	20μg/ml	73	130
	50μg/ml	89	104
	100μg/ml	95	122
Gastrin	10μg/ml	107	93
	20μg/ml	93	81
	50μg/ml	60	63
	100μg/ml	35	54

We were unable to obtain multiple effects by using a mixture of hormones and factors. Insulin prevented the effects of the blends (Table 4). The cells grown in serum -free medium showed degenerative features characterized by dilated mitochondria (Fig. 1). Serum-free medium supplemented with the mixture preserved cells without degeneration (Fig. 2).

Table 3. Responses of gastric cancer cells to sex hormones and prostaglandins

Addition	Concentration	% of growth MKN28	MKN45
None (control)	—	100	100
Estradiol	0.1μg/ml	81	120
	1μg/ml	80	134
	10μg/ml	77	109
Estriol	0.01μg/ml	114	97
	0.1μg/ml	107	92
	1μg/ml	130	108
Progesterone	1μg/ml	78	56
	10μg/ml	88	55
Testosterone	10μg/ml	85	94
	100μg/ml	74	63
Prostaglandin E_2	1ng/ml	98	94
	10ng/ml	105	86
	100ng/ml	98	88
Prostaglandin $F_2\alpha$	1ng/ml	105	110
	10ng/ml	97	100
	100ng/ml	116	81

Table 4. Comparison of the growth of MKN28 and MKN45 cells in serum-free media supplemented with factors and hormones

Addition	% of growth MKN28	MKN45
None (control)	100	100
Serum (10%)	176	188
Serum (10%)+Insulin (1μg/ml)	175	172
Transferrin (1μg/ml) +Insulin (1μg/ml)	139	ND[a]
Transferrin (1μg/ml) +Hydrocortisone (1μg/ml)	ND	135
Transferrin (1μg/ml)+EGF (1ng/ml) +Insulin (1μg/ml)	140	ND
Transferrin (1μg/ml)+EGF (1ng/ml) +Hydrocortisone (1μg/ml)	ND	138
Transferrin (1μg/ml)+EGF (1ng/ml) +Insulin (1μg/ml)+Hydrocortisone (1μg/ml)	144	87

a) ND: not done

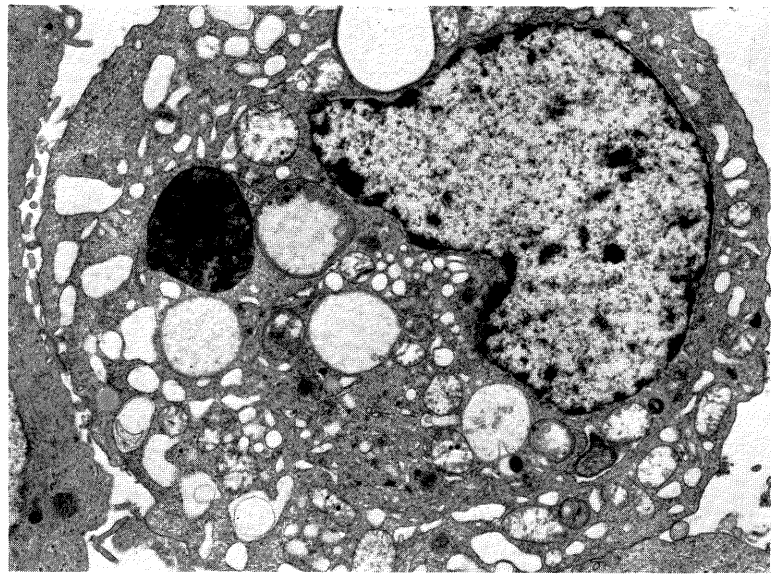

Fig. 1. Numerous swollen cells are seen in the serum-free medium. They have degenerative features characterized especially by dilated mitochondria. MKN45 cells. ×6490.

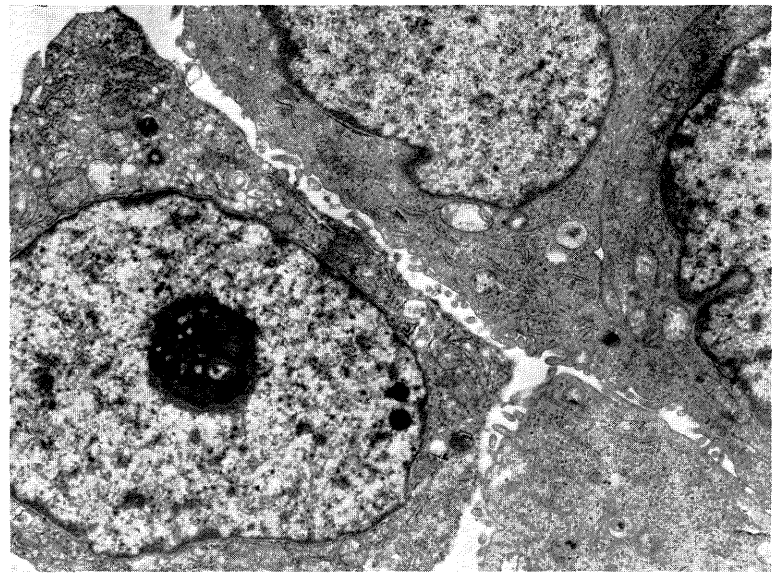

Fig. 2. There are a little or no degenerative features in the cells cultivated in the serum-free medium supplemented with factors and hormones (Transferrin: 1μg/ml, EGF: 1ng/ml, Hydrocortisone: 1μg/ml). These features are not different from those of cells cultivated in the serum supplemented medium. MKN45 cells. ×6490.

Discussion

In the present study, one factor and two hormones were found to have significant growth effects (a growth increase of over 140%) on two human gastric cancer cell lines cultivated in a serum-free medium.

The effect of transferrin was common to both the MKN28 cell line derived from a differentiated carcinoma and the MKN45 cell line derived from an undifferentiated carcinoma. Many investigators have reported that transferrin is essential for the growth of various types of non-neoplastic and neoplastic cells in vitro.[1] Probably, the transferrin effect is common to many cells irrespective of the organ.

Although EGF has been found to have a growth effect on various kinds of cells,[2] it was not found in our cell lines. According to Hirata[6], the MKN28 cell line and the MKN45 cell line have EGF-receptors, but those of the MKN28 cell line ($Kd=3.0\times10^{-10}$ M, Maximal binding sites per cell=3100) and the MKN45 cell line ($Kd=14.7\times10^{-10}$M, Maximal binding sites per cell=1200) are considerably less in number than those of other cells easily responsive to EGF, such as the $HeLaS_3$ cell line ($Kd=1.5\times10^{-10}$M, Maximal binding sites per cell=22500).

The effect of hydrocortisone was explained by the inhibition of prostaglandin E biosynthesis wich resulted in stimulation of cell proliferation.[18] According to this theory, the effect ought to be found in both cell lines. The difference in the effect of hydrocortisone between the two cell lines was probably due to the difference in number of the receptor. Insulin induced growth only in the MKN 28 cell in the present study. Insulin has been shown to give a great stimulation of growth in the human colon cancer cell line HC84S,[15] and differentiated carcinomas of the stomach are thought to arise from intestinal metaplasia.[16][17] Thus, insulin may have an affinity to the intestinal type mucosal cell. On the other hand, insulin seemed to have a suppressing effect rather than a promoting effect in the MKN45 cell line. MKN45 cells are said to have the ability to differentiate into mucous neck cells.[14] In addition insulin may have a suppressive effect on the mucus neck cell type cells. Gastrin, meanwhile, has trophic effects on the gastrointestinal tract mucosa,[5][10] and along with glucagon, has been found to have a significant growth effect on rat stomach cancer cells.[12] Our two cell lines did not significantly react to either hormone. This may be due to the loss of the receptors.

On the experimental carcinogenesis of gastric cancer in rats, research has shown that the incidence in males is much higher than in females, and that female hormones have suppressive effects on the carcinogenesis.[3] In our study, sex hormones did not have a significant influence on the growth of two cell lines. As a matter of fact, we were not able to detect estrogen and progesterone receptors in either cell line.

Some investigators have shown that prostglandin $F_2\alpha$ has a stimulatory effect in the growth of cultured mouse fibroblasts.[9] On the other hand, prostaglandin E is believed to have a suppresive effect.[19] These prostaglandins also did not have an influence on the

growth of our gastric cancer cells. This may also be due to the loss of receptors.

Growth of a few human cancer cells have been successfully controlled without decreased growth rates in serum-free media supplemented with hormones.[8)15)] Although our hormone-supplemented media failed to preserve the growth rates, electronmicroscopic examinations showed that these media delayed the degeneration of cells. Our data suggest that these factors and essential for the maintenance of cell subsistence.

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