

Gastrin Gastrin/CCK-B

mRNA

가 ,¹ ,²
 .¹ .² .²

The Expression of Gastrin and Gastrin/CCK-B Receptor mRNA in Cancer and Normal Tissue of Large Intestine

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Purpose : Gastrin, a peptide hormone produced by th cells of the gastric antrum plays a major role in r acid secretion in the stomach, and acts as a trophic in the gastrointestinal tract. The relationship betwe and the development of colorectal cancer remains co versial. To study its possible role in developme nt o eration of colorectal cancer, we evaluated the exp of gastrin and gastrin/CCK-Breceptor mRNA in cancer normal tissue from colorectal cancer patients. We a viewed clinical records to evaluate the correlations gastrin receptor expression and clinical characte colorectal cancer.

Methods : Reverse transcription-polymerase chain re (RT-PCR) was used to evaluate mRNA expression for gast and gastrin/CCK-B receptor in 26 surgical specime colorectal cancer.

Results : The mRNA expression of gastrin was detecte 24 out of 26 cancer specimens and 9 out of 26 norm colon specimens (p<0.05). The mRNA expression gastrin/ CCK-B receptor was detected in 18 out of cancer specimens and 17 out of 26 normal colon specim (p>0.05). There was no significant correlation b gastrin receptor expression and clinical characte colorectal cancer.

Conclusions : The gastrin gene products might be important than gastrin/CCK-B receptor in developne or proliferation of colorectal cancer, which supp hypothesis that gastrin gene products play a role lification of colorectal cancer as an autocrine facto
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Key Words : Gastrin, Gastrin/CCK-B receptor, Colorectal cancer
가 , 가 ,

2
가
4
가
가
gastrin (tro-
phic factor)
3
gastrin
mouse rat
gastrin
, gastrin antisense gastrin
4
gastrin 가가 ,⁵ Thor-
burn⁶
gastrin 가
Zollinger-Ellison syndrome
gastrin

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crine 가 .^{8,9} auto-gas-

trin CCK-A gastrin/CCK-B 가

gastrin CCK 1000 CCK-A inner primer DNA PCR

CCK gastrin/CCK-B gastrin 1, 2 PCR

.¹⁰ gastrin gastrin/CCK-B 가 RNA primer Table

가 gastrin/CCK-B mRNA

gastrin gastrin mRNA

factor gastrin .¹¹ autocrine

gastrin gastrin/CCK-B mRNA

1) 1997 9 1998 9 가

26 -80°C

2) (1) **Gastrin** **Gastrin/CCK-B** **GAPDH**

: (polymerase chain reaction-PCR)

Biosystems 381A DNA (Foster City, CA)

oligonucleotide purification cartridge (OPC[®]) oligonucleotide-pak column, Applied Biosystems, Inc)

primer intron genomic DNA가

cDNA PCR

2 PCR

inner primer DNA

(2) **Total RNA** : RNA

ribonuclease (RNase)

180°C가 dry oven 8

diethylpyrocabonate (DEPC) 0.1% Total RNA

100 mg Polytron tissue homogenizer RNA lysis solution (4.48 M guanidine thiocyanate, 28 mM sodium citrate, 0.56% sarcosyl) 500 ul, 50 ul 2 M sodium acetate/pH4, 500 ul water-saturated phenol 200 ul

49 : 1 chloroform/isoamylalcohol 가

15 4°C

15,000 rpm 20 tube

absolute ethanol 2 volume 가 20

-20°C 1

4°C 15,000 rpm 20

Table 1. Gastrin and GAPDH gene primer

Gastrin gene primer	
GAS5	5'-ATG CAG CGA CTA TGT GTG TAT G-3'
GAS3	5'-GAA GTC CAT CCA TCC ATA GGC-3'
Gas5IN	5'-TAT GTG CTG ATC TTT GCA CTG-3'
Gas3IN	5'-CAT GGT CCC TGC TTC TTG GA-3'
Glyceride aldehyde phosphodiesterase (GAPDH)	
Gpd5s	5'-GTG GAC ATT GTT GCC ATC AAC GAC CCC-3'
Gpd3as	5'-GCC CCA GCC TTC TCC ATG GTG GT-3'

Table 2. Gastrin/CCK-B receptor gene primer

HGR5s	5'-CAT CTG CCG ACC ACT GCA GGC-3'
HGR5INs	5'-ATT GTA GCC ACG TGG CTG CTG TCC-3'
HGR3as	5'-CAC GTA GCA GCC ATC GCT GTC-3'
HGR4as	5'-ACG CCC GTT CTG GTG AAC AGC-3'
HGRP (probe)	5'-GTG GTC CAG CCC CAC-3'

3 : RNA
 75% ethanol 1 ml 가 10 tube
 5 ethanol heat-
 ing block RNA RNase-free water
 260 nm

(3) (Reverse transcription reaction):

total RNA 2 ug MMLV (mal-
 rony murine leukemia virus)-RTase 200 unit/ul, random
 primer 1 ug, dNTPs 200 uM, 1x reverse transcriptase
 buffer, RNase inhibitor 40 unit/ul 가 가
 20 ul 41°C
 95°C 5
 cDNA

(4) PCR: PCR 1 PCR

10x buffer 2 ul, dNTP 0.2 mM, primer sense
 antisense primer가 10 pmole, Taq. polymerase
 1.25 unit, cDNA 20 ul 5 ul
 20 ul
 . 2 PCR 1 PCR 1 ul 1
 PCR PCR

PCR9600 (Perkin Elmer Cetus, version 1.0)
 95°C 5 , 94°C 1 , 55°C
 1 , 72°C 1 35 72°C 10
 . 2 -PCR filter가
 pipette tip 1 -PCR
 100 1 5 ul
 . 1 PCR
 filter tip clean bench
 pipette UV cross linker

GAPDH (glyceride aldehyde phospho-
 diesterase) total RNA
 cDNA 20 ul 2 ul 25

(5) : gel 2 3% Nu-

Sieve GTG[®] agarose (FMC, Rockland, ME. USA) 1x
 TBE buffer (10.8 g Tris-base, 5.5 g Boric acid, 4 ml
 0.5 M EDTA (pH8.0)/1000 ml)
 heating block 0.5 ug/ml
 Ethidium bromide 가
 gel 1x TBE buffer
 gel loading buffer (6x sample load-

Gastrin Gastrin/CCK-B mRNA 3
 ing buffer; 0.25% Bromophenol (BPB), 0.25% Xylene
 cyanol FF, 40% Sucrose or 30% Glycerol)
 well size marker
 (phix 174/HaeIII)

3)

SAS windows system
 V6.12 (SAS Institute Inc. Cary, NC. USA) Chi- square,
 Mantel-Haenszel Chi-square test p < 0.05

1)

	58.8	38	77
		13	
Dukes	B가 14	(53.8%),	C가 12
(46.2%)	A		12
(46.2%),	14	(53.8%)	

2) Gastrin Gastrin/CCK-B mRNA

Gastrin RT-PCR 235 bp
 (Fig. 1). Gastrin/CCK-B RT-PCR
 가 (S)
 129 bp, (L) 144 bp
 (Fig. 2). GAPDH
 RT- PCR 270 bp
 Gastrin mRNA 26
 9 (34.6%), 24 (92.3%)가
 (P < 0.05), gastrin/CCK-B
 26 17 (65.4%),
 18 (69.2%)가
 (P >

0.05)(Table 3).

3)

	Gastrin/CCK-B	mRNA	가
	12	8 (66.7%)	
	가	14	10
	(71.4.7%)		

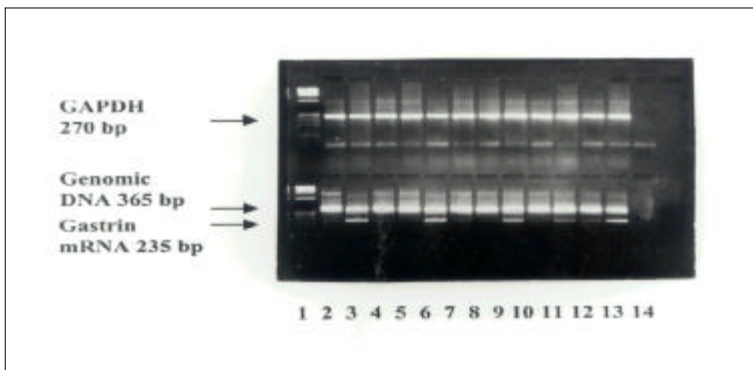


Fig. 1. Detection of human gastrin mRNA by RT-PCR. Lane 1; Size marker (X174/HaeIII). Lane 2, 4, 6, 8, 10, 12; normal tissue. Lane 3, 5, 7, 9, 11, 13; tumor tissue. Lane 14; negative control.

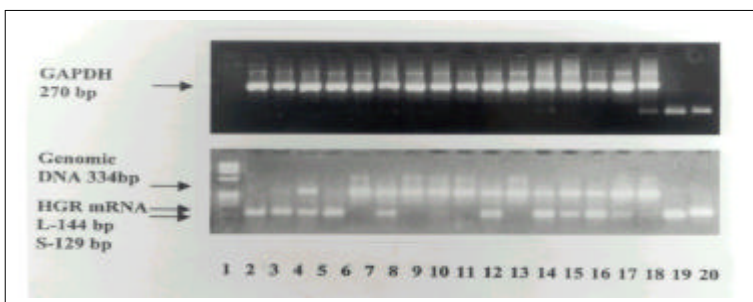


Fig. 2. Amplification of human gastrin/CCK-B receptor mRNA by RT-nested PCR. (HGR; human gastrin receptor). Lane 1; Size marker (X174/HaeIII). Lane 2, 4, 6, 8, 10, 12, 14, 16; normal samples. Lane 3, 5, 7, 9, 11, 13, 15, 17; tumor samples. Lane 19; HGR short form (S). Lane 20; HGR long form (L).

Table 3. mRNA expression rate of gastrin and gastrin/CCK-B receptor in colorectal cancer and normal colorectal tissue

	Gastrin*	Gastrin/CCK-B receptor
	No. of expressed/ Total (%)	No. of expressed/ Total (%)
Cancer	24/26 (92.3%)	18/26 (69.2%)
Normal	9/26 (34.6%)	17/26 (65.4%)

*P < 0.05; † No. = number.

(P > 0.05).
 Gastrin/CCK-B mRNA 12
 8 (66.7%)
 14
 10 (71.4%)
 (P > 0.05).
4) Gastrin/CCK-B mRNA
 Gastrin/CCK-B mRNA 9
 4 (44.4%)
 17
 12 (70.6%)

(P < 0.05).
 gastrin mRNA
 . Biagini ¹²
 gastrin mRNA 30
 26 (86.7%)
 10
 1
 . Monges
¹³
 7
 gastrin mRNA가 4
 gastrin mRNA가
 gastrin mRNA가
 gastrin
 가
 autocrine factor
 . Imdahl ¹⁴
 gastrin mRNA
 56
 ribonuclease protection assay (RPA)
 48 (85.7%)
 . Finley ¹⁵ RT-PCR
 10
 10
 gastrin mRNA가

gastrin PCR

Matsushima¹⁶ gastrin/CCK-B 가 10 2
 8 1 (12.5%),
 (20%) 가 Northern blot nested PCR 가 .
 Imdal¹⁴ RPA gas-
 trin/CCK-B 가 57 6 (10.5%)

가 RT-PCR
 Biagni¹² gastrin/CCK-B 10 8
 (80%), 30 23 (76.7%)
 가 RT-PCR
 CCK-B 92%,
 57%, 65%, 100%
 17

gatrin/CCK-B 가

gatrin/CCK-B 17
 gatrin/CCK-B

Gly-gastrin 가
 Gly-gastrin 가
 gastrin
 Gastrin progastrin
 Imdahl¹⁴ gastrin gastrin
 T3 gastrin/CCK-B mRNA 가 mRNA
 gastrin/CCK-B mRNA
 exon amino acid (short form) RT-PCR
 (long form) 가 가
 20 gastrin/CCK-B 129 bp 144 bp 129 bp
 144 bp 가
 G
 21

gas-
 trin/CCK-B gastrin
 gastrin autocrine 가

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gastrin posttranslational processing progastrin
 Gly-gastrin
 18 gastrin Gly-gastrin
 19

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