



Thymidylate Synthase mRNA

5 - FU

,¹ ,²

1 . 2 . 2 . 2 .

Relationship between Expression of Thymidylate Synthase mRNA and Resistance of Colon and Gastric Cancer Cells to 5-FU

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Purpose: Thymidylate synthase (TS) is an important target for chemotherapeutic drugs such as 5-fluorouracil (5-FU). Over-expression of TS has been linked to chemotherapy resistance, but their relationship was not completely understood. We compared the expression level of TS with resistance of colon and gastric cancer cell lines to 5-FU.

Methods: Expression of TS mRNA was determined by RT-PCR assay in 9 colon and 10 gastric cancer cell lines. Cytotoxicity of 5-FU was determined by MTT assay. Apoptosis was determined using propidium iodide staining by flow cytometry.

Results: All cancer cell lines tested showed differential levels of TS mRNA expression. Colon cancer cell Colo320 (the highest expression of TS) was more resistant to 5-FU than SNU-C1 (the lowest expression of TS) was. Flow cytometry also showed that 5-FU induced apoptosis less in Colo320 than SNU-C1. But in gastric cancer cells SNU-1 (the highest expression of TS) was not resistant to 5-FU than SNU-16 (the lowest expression of TS) was.

Conclusions: The high level of expression of TS was correlated with resistance of colon cancer cells to 5-FU, but not in gastric cancer cells. Thus, TS may be differently involved in the resistance of gastric and colon cancer cells to 5-FU, which may depend on the origin of cancer cells and status of apoptosis related genes. *J Korean Soc Coloproctol* 2003;19:67-73

Key Words: 5-fluorouracil, Thymidylate synthase, Resistance, Gastric cancer cells, Colon cancer cells

5-Fluorouracil (5-FU) , , 가
 . 5-FU 가
 . (1) 5-FU
 fluorodeoxyuridine monophosphate (FdUMP)가
 thymine (key enzyme) thymidy-
 late synthase (TS) DNA
 , (2) 5-FU가 RNA (incorporation) RNA
 , (3) 5-FU가 DNA
 DNA¹⁻⁹
 5-FU가 TS 가 , DNA
 RNA
 가 .¹⁰ 5-FU TS
 , TS DNA
 dUMP (methylation) dTMP
 . dTMP dTDP
 dTTP가 . dTTP DNA
 dTTP
 DNA 가
 . TS가 dUMP dTMP
 methyl donor
 (CH₂-FH₂, methylene tetrahydrofolate) . FdUMP
 가 TS (methylene tetrahydrofolate)
 (ternary complex)가 TS
 dUMP
 5-FU leucovorin (folinic acid)
 TS FdUMP 5-FU

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5-FU 15~20%
 15% 5-FU
 가 5-FU (resistance)
 , 가
 10,11
 TS TS 5-FU
 ,
 .¹² TS
 , TS
 TS
 가 5-FU가
 TS
 , 5-FU
 TS
 가

1)

(SNU-C1, SNU-C4, SNU-C5, COLO 320HSR, LoVo, DLD-1, HT-29, HCT-8, HCT-116) (SNU-1,-5, -16, -216, -484, -601, -638, -668, -719) (South Korea) 10% fetal bovine serum (Sigma, USA) RPMI 1640 (Giboco BRL) 37°C, 5% CO₂

2) Transcript

(1) RNA : Acid guanidinium thiocyanate-phenol-chloroform¹³ 9 RNA 10 1×10⁷ 가 PBS (4 M guanidinium thiocyanate, 25 mM sodium citrate pH 7.0, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol) 1 ml polypropylene tube 1 ml 2 M sodium acetate (pH 4.0) 0.1 ml, phenol 1 ml, chloroform-isoamylalcohol (24:1) 0.3 ml 15 3,000 rpm 20 Corex^R 가 isopropanol -20°C 1

10,000 g 20 RNA iso-
 -20°C 1
 10,000 g 15 RNA RNA
 75% ethanol ,
 DEPC water RNA
 (10D=40µg/ml) 260 nm (DU^R 650 spectrophotometer), RNA A260/A280 , RNA RNA 5µg 28S 18S band
 180°C 8 Tris, SDS DEPC 0.1%가

(2)

- : First strand cDNA 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 10 mM DTT (Promega), 1 U/µl RNasin (Promega), 1 mM each dNTP, oligo (dT)₂₀ 100 ng MMLV reverse transcriptase (Promega) 200 U가 20µl RNA 1µg . PCR 1xPCR (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1 mM MgCl₂, 100µg/ml gelatin, 0.05% triton X-100) 25 ng RNA cDNA, primer 20 pmole, 50µM dNTP taq DNA polymerase (Perkin Elmer) 2.5 unit 가 25µl (GeneAmp PCR system 2400, Perkin Elmer). 5µCi [α -³²P]dCTP 가 primer PCR Table 1, 2 . PCR 25µl TS 10µl β -actin 5µl 7% polyacrylamide gel gel X-ray autoradiography

3)

5-FU RT-PCR 가 TS 가 5-FU MTT (dimethyl thiazole-diphenyltetrazolium-bromide)¹⁶ MTT formazan for mazan microplate reader

Table 1. Primer sequences used for PCR

Gene	S&AS [†]	Primer sequences	Sequence region [‡]	of PCR	Cycle	Length Reference [§] products
TS	S	5'-GGGCAGATCCAACACATCCTC-3'	208 ~ 28	1111	22	Takeishi et al ¹⁴
	AS	5'-AAGACAAAGCCACCCCAAGTTAGA-3'	1291 ~ 318			
β -actin*	S	5'-GACTATGACTTAGTTGCGTTA-3'	1912 ~ 332	501	17	Nakajimalijima et al ¹⁵
	AS	(5'-GCCTTCATACATCTCAAGTTG-3')	2412 ~ 392			

* β -actin, PCR control (house keeping gene), [†] S = Sense; AS = antisense, [‡] The oligonucleotide primers constructed for PCR correspond to the sense and antisense based within these reported sequences, [§]References for primer sequences

Table 2. PCR condition of TS and β -actin

Gene	Hot start	Denaturation	Annealing	Extension
TS	94°C, 12 min	94°C, 30sec	66°C, 30 sec	72°C, 1 min
β -actin		94°C, 30sec	53°C, 30 sec	72°C, 1 min

IC₅₀

50%

4)

(FACSCalibur™, Becton-Dickinson, Boston, MA)

5-FU

(PBS; phosphate buffered saline), 80% cold ethanol

30 ethanol

0.1% Triton X-100, 0.1 mM EDTA, 0.05 mg/ml RNase A (50 units/mg), 50 μ g/ml propidium iodide (PI)

7† 18 sub-G₁ DNA

17

5)

± Student's t-test

P < 0.05

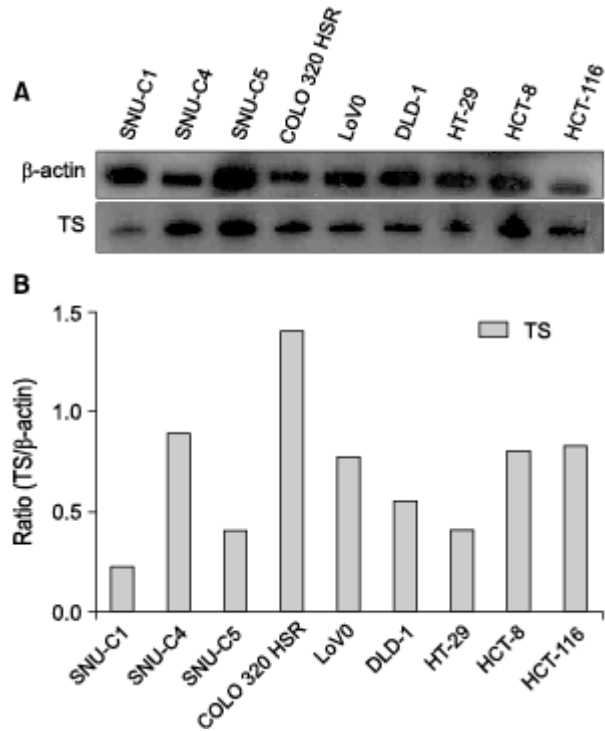


Fig. 1. Expression of TS mRNA in colon cancer cell lines. β -actin was used as a control for RNA (A). Expression level of TS mRNA was represented as the ratio of TS mRNA and β -actin (B).

1) Thymidylate synthase

TS mRNA RT-PCR
 9 10
 TS가
 TS mRNA/ β -actin (T/A)
 Colo320
 TS mRNA가 가 (T/A=1.41)
 SNU-C1 가 (T/A=0.22) (Fig. 1).
 SNU-1 가 (T/A=1.10)
 SNU-16 가 (T/A=0.28)
 (Fig. 2).

2) TS 5-FU

TS mRNA 5-FU
 TS mRNA가 가
 Colo320 가 SNU-
 C1 MTT assay TS mRNA가
 Colo320 SNU-C1
 5-FU (Fig.)

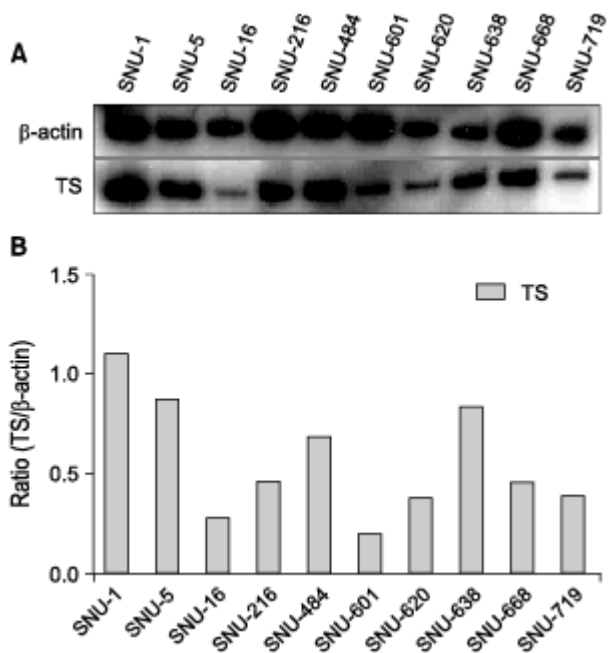
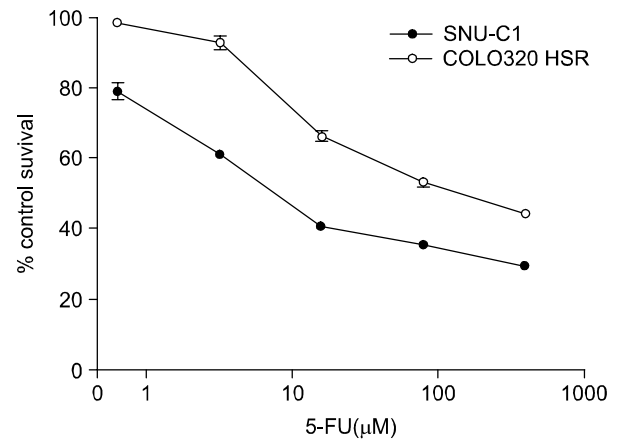


Fig. 2. Expression of TS mRNA in gastric cancer cell lines. β -actin was used as a control for RNA (A). Expression level of TS mRNA was represented as the ratio of TS mRNA and β -actin (B).

3). TS mRNA 가
 SNU-1 TS mRNA 가
 SNU-16 TS mRNA 가
 mRNA 가 TS
 (Fig. 4).

3) TS 5-FU apoptosis
 Colo320 SNU-C1 5-FU 4
 apoptosis
 5-FU Colo320 apoptosis
 가 SNU-C1 (Fig. 5).

TS DNA



5-FH	
IC ₅₀ (μ l)	
SNC-C1	COLO320
7.59	140.78

Fig. 3. Cytotoxic effects in the colon cancer cells to 5-FU with the lowest and highest expression of TS. The experiments were carried out in triplicate. The 50% inhibitory concentration (IC₅₀) for a particular agent was defined as the drug concentration which results in a 50% reduction in cell number as compared to the untreated control. Bars, SE; The all means were significantly different from the control at the level of p value 0.05 when using the Student's t-test.

가 Colo320가 TS 가 SNU-C1
 5-FU . SNU-C1
 p53 , Colo320 p53
 5-FU
 . p53 TS
 SNU-C1 SNU-C4
 TS
 SNU-C4
 5-FU TS가
 가 . 가
 9 TS mRNA
 5-FU (IC₅₀)
 (r) 0.636
 가
 (P value: 0.066). 5-FU
 가 TS thymidine 5-FU
 thymidine DNA apoptosis가
 Fas가 Fas Ligand propidium
 apoptosis가 24-27
 iodide 5-FU apoptosis TS
 MTT Colo320 TS SNU-C1
 apoptosis가 .
 Terashima 28 TS
 가 5-FU TS 5-FU
 dihydropyrimidine dehydrogenase (DPD)
 가 가 . Choi 29 5-FU
 doxorubicin
 TS 가
 가 .
 5-FU TS
 5-FU TS
 가 , Grem 23
 가 (60) 가 5-FU
 5-FU TS
 가 DPD
 , 가 (doubling time)
 p53 가

가 5-
 fluorouracil thymidylate synthase
 1) TS mRNA
 2) 5-FU TS
 mRNA 가 ,
 3) 5-FU apoptosis
 TS apoptosis
 TS apoptosis

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