고신대학교 의과대학 학술지 제23권 제1호
Kosin Medical Journal
Vol. 23. No. 1, pp. 26~30, 2008

대장암 환자에서 Survivin mRNA의 발현과 간 전이와의 연관성

최종순, 장희경

고신대학교 의과대학 가정의학교실, 병리학교실

The Expression of Survivin mRNA in Patients with Colorectal Carcinomas to The Liver Metastasis

Jong-Soon Choi[1], Hee-Kyung Chang[2]

Department of Family Medicine[1] and Pathology[2], Kosin University College of Medicine, Busan, Korea

Abstract

Background : Survivin is a novel member of the inhibitors of apoptosis proteins (IAP) gene family. Although survivin expression has been reported in colon cancer, the results are still controversial and its clinical significanceincluding chemoprevention remains unclear. Some reports described thegene as a potential target in anticancer or chemopreventive therapy. To facilitate understanding of survivin in the colon cancer, the clinicopathological significance was investigated with the expression of survivin.

Methods : Surgical specimen were obtained from a total 37 cases of consecutive patients with various stages of colorectal carcinoma who had undergone a resection. To determine survivin mRNA in colorectal carcinomas and adjacent normal colorectal tissue samples, total RNA was isolated from each of the samples after lysis in quanidinium isothiocyanate and phenol extraction and reverse-transcription polymerase chain reaction was done. The PCR products were confirmed by DNA sequencing after subcloning.

Results : The positive rate of survivin in cancer tissue was 94.6%(35/37) and that of non-neoplastic colorectal tissue was 51.4%(19/37). Survivin expression tended to increase in cancer tissue, but has statistically marginal significance(0.05<p=0.065< 0.1). The correlation of survivin expressions to age, gender, invasion depth, lymph node metastasis, vessel and perineural invasions, and liver metastasis was not found.

Conclusions : The expression of survivin appears not to be eligible to differentiate malignant from benign lesions in colon, even though it has marginal significance(0.05<p=0.065< 0.1). The role of survivin-associated apoptosis may be unnoticeable, in the light of insignificant correlation of gene expression to the other prognostic factors in colon cancer.

Key words : Colorectal cancer, SurvivinmRNA, liver metastasis

Introduction

Survivin located in chromosome 17q25 is a new member of the inhibitor of apoptosis (IAP) family and is expressed predominantly in fetal tissue but is also found in many common human cancers.[1)] Survivin inhibits the processing of caspase-3 and caspase-7, terminal effectors of apoptosis, and also inhibits the induction of apoptosis by Fas, Bax and caspases.[2)]The clinical significance of survivin expression and correlation with the biological aggressiveness of cancer remain unclear. There are some reports that survivin expression correlated with poor survival among patients with various tumors, including neuroblastoma[3)], nonsmall-cell lung cancer[4)], breast carcinoma[5)], esophageal cancer[6)], gastric carcinoma.[7)] Therefore, survivin expression is thought to be an important prognostic marker in cancers. In addition to that, there was a report that survivin can be a good candidate for transcription targeting of cancer gene therapy in a wide variety of tumors.[8)] Recently, Gianani[9)] reported the opposite data to previous study of which survivin expressed in all normal, hyperplastic polyps, adenomatous polyps, and adenocarcinoma cases by

교신저자 : 최 종 순
주소 : 602-702 부산시 서구 암남동 34
고신대학교 의과대학 가정의학과
TEL : 051-990-8332
E-mail : fmcjs@naver.com

immunohistochemistry and concluded the expression of survivin is not specific marker of adenocarcinoma of colon. However, the survivin expression in colorectal cancer are still controversial and its clinical significance remains unclear.

To clarify their clinical implication and relationship to apoptosis in colorectal cancer, survivin expression in normal and cancer tissues from patients with colorectal cancers were evaluated with analysis of correlation to clinical and pathological characteristics of the patients.

Materials and Methods

Patients and clinical samples

Surgical specimen were obtained from a total 37 cases of consecutive patients with various stages of colorectal carcinoma who had undergone a resection(Table 1). Fresh-frozen primary colorectal carcinoma samples and non-neoplastic normally looking colorectal mucosal tissues more than 2cm from the tumor mass were subjected to mRNA extraction. Their pathologies were confirmed by frozen section, before mRNA extraction.

Table 1. Clinicopathological parameters of 37 patients with colorectal cancers

Characteristics	No. of patients(n=37)
Mean Age(yrs±SD)	59.22 (±18.635)
Gender	
Male	19 (55.9%)
Female	18 (48.6%)
Depth of tumor invasion(pT)	
I	1 (2.7%)
2	6 (16.2%)
3	14 (37.8%)
4	16 (43.2%)
Lymph node metastasis	
Negative	23(62.2%)
Positive	14(37.8%)
Vessel invasion	
Negative	26(70.3%)
Positive	11(29.7%)
Perineural invasion	
Negative	28(75.7%)
Positive	9(24.3%)
Liver metastasis	
Negative	33(89.2%)
Positive	4(10.8%)

RNA isolation

Fresh-frozen colorectal cancer and corresponding non-neoplastic colorectal tissue blocks were cut into 4μm sections using cryostat microtome at -20°C. The first and last sections were immediately stained with methylene blue and examined under a microscope to confirm histologically normal tissues without tumor cell infiltration and tumor tissue consisting at least 80% of tumor cells. Total RNA was isolated from each of the 38 samples after lysis in quanidinium isothiocyanate and phenol extraction using the commercial kit(Trizol, Invitrogen Laboratories, San Diego, U.S.A.).

Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

c-DNA was synthesized from 4 μg of total RNA in a 25-μL reaction mixture containing 6 μL of 5X reverse transcriptase reaction buffer, oligo(dT)(100pmole/uL) 1μL, 10mM dNTP 4μL, 40 unit/μL of RNAsin, 0.5μuL(200units/L) of Moloney leukemia virus reverse transcriptase(MMLV RTase). The mixture was incubated at 4 °C for 60 min., heated to 94 °C for 3 min., and then chilled on ice. To test the cDNA integrity, GADPH was amplified in each samples.

Nested PCR for detection of Survivin mRNA

To ensure that RNA was not degraded, a PCR assay with primers specific for beta-actin cDNA was carried out in each cases under the following cycling conditions: 25 cycles, 30 sec. at 94 °C, 30 sec. at 60 °C, and 30 sec. at 72 °C with pre-denaturation for 2 min at 94 °C and post-extension at 72 °C. A 10 μL, aliquot of each reaction mixture was size-fractionated on 1% agarose gel and visualized with ethium bromide staining. c-DNA was synthesized by random priming using less than 1 μg total RNA as template. The primer pairs used for survivin gene amplification was forward primer 5'-GCA TGG GTG CCC CGA CGT TG-3' (corresponding to position 48-67 of the survivin mRNA [Genbank accession NM 001168]) and reverse primer 5'-GCT CCG GCC AGA GGC CTC AA-3' (position 475-494) which produced a PCR product of 431 bp. As an internal standard, a fragment of human G3PDH

was amplified by PCR using forward primer 5'-ACC ACA GTC CAT GCC ATC AC-3' and reverse primer 5'-TCC ACC ACC CTG TTG CTG TA-3' which gave a PCR product of 450bp. The PCR amplification was performed in a final volume of 20 μL which comprised 1 μL of the reverse transcriptase reaction mix, 3 pmol each of 5' and 3' primers for the survivin or G3PDH genes, 160 μM of deoxynucleotide triphosphate, 1 mM MgSO4 and 0.3 units of Taq DNA polymerase in reaction buffer. PCR was performed with an initial denaturation step at 94°C for 2 min. followed by 30 cycles of 20 seconds at 94°C for denaturation, 30 sec. at 68°C for annealing and 45 sec. at 72°C for extension. The final extension step was prolonged to 5 min. at 72°C. PCR products were separated on 2% agarose gels by electrophoresis.

Direct DNA Sequencing of PCR products

Wizard plus SV minipreps kit was used for the template DNA preparation for sequencing after subcloning of RT-PCR products. Automatic DNA sequencer(ABI sequencer 3700, Macrozen, Seoul, Korea) was used for sequencing. The sequence data were analyzed by the NCBI(NIH,USA) Blast search program.

Statistical Analysis

The statistical analysis was preformed using SPSS(version 11, Stanford, USA). The significance level was set at $p<0.05$.

Results

The expected survivin product of 450 bp was amplified(Fig.1). Thirty-five out of 37 cancer samples (94.6%) and 19 out of 37 normal control tissues (51.4%) had detectable levels of survivin mRNA. Survivin expression tended to increase in cancer tissue, but it has statistically marginal significance(0.05<p=0.065< 0.1).The each correlation of survivin expressions to age, gender, invasion depth, lymph node metastasis, vessel and perineural invasions, and liver metastasis was not demonstrated(Table 2).

Table 2. The relationship of positively detected cases by PCR with Survivin primers with clinicopathologic parameters in 37 cases of colorectal carcinomas

Characteristics	No. of patients (n=37)	Numbers of cases Survivin**		in tumor	in non-tumor
		Positive	Negative	Positive	Negative
Gene expressions		35(94.6%)	2(5.4%)	19(51.4%)	18(48.6%)
Age(yrs)					
Mean	59.22	59.37	56.50	59.10	56.21
Gender					
Male	19	18	1	9	8
Female	18	17	1	10	10
Depth of tumor invasion(pT)					
I	1	1	0	0	1
II	6	5	1	2	4
III	14	13	0	7	6
IV	16	15	0	10	6
Lymph node metastasis					
Negative	23	21	2	10	13
Positive	14	14	0	9	5
Vessel invasion					
Negative	26	24	2	12	14
Positive	11	11	0	7	4
Perineural invasion					
Negative	28	26	2	14	14
Positive	9	9	0	5	4
Liver metastasis					
Negative	33	31	2	17	16
Positive	4	4	0	2	2

P = 0.065

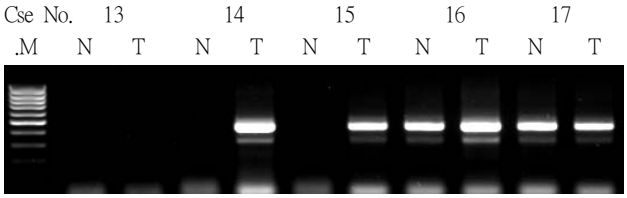

Fig. 1. Electrophoretic analysis of nested RT-PCR amplification products with survivin from non-neoplastic(N) colorectal mucosa and cancer tissues(T). The expected survivin product of 431 bp was amplified. The product of GAPDH of 450bp was amplified.

Discussion

Apoptosis and various apoptosis regulatory molecules have an important role in the accumulation of cells.[10] Survivin, a new member of the IAP family, is expressed in many cancers and inhibits apoptosis. It has been reported that survivin blocks downstream part of both apoptotic pathways by directly inhibiting the terminal effector

caspase-3 and caspase-7, and interfering with caspase-9 activity/processing.[11] In addition, survivin counteracts pro-apoptotic stimuli induced by interleukin-3, Fas, Bax, tumor necrosis factor, anticancer drugs and X-irradiation.[2,11] As previously described survivin expression is inversely correlated with apoptosis during tumorigenesis, and positively correlated with proliferation and angiogenesis with the inhibition of apoptosis by survivin, predicting poor prognosis and shorter survival in human cancers.[11] In this study mRNA expression of survivin in normal and cancer tissues of the colon was studied. It was found that 94.6% of cancer samples and 51.4% of normal colon tissue had detectable levels of survivin mRNA on RT-PCR. Recent publications have demonstrated survivin expression in all the most common human cancers.[11] However, it has been reported that survivin expression is not detected in normal adult tissues with immunohistochemical survivin expression being absent in normal tissues.[5,11] Otherwiase, Gianani reported survivin expressed in all normal, hyperplastic polyps, adenomatous polyps, and adenocarcinoma cases by immunohistochemistry and concluded the expression of survivin is not a specific marker of adenocarcinoma of colon.[9] In this study 51.4% of normal colon tissues exhibited detectable survivin expression by RT-PCR. Using the more sensitive RT-PCR method, survivin expression has been detected in many kinds of normal tissues although the levels of survivin expression in normal tissues were lower than paired malignant tissues in every report.[4,6,13] Though the role of survivin in cancer tissues is being steadily elucidated, some reports demonstrate that survivin has a crucial role during malignant transformation.[12] Ultimately the significance of survivin expression in normal tissues, particularly the correlation with malignant potential, remains unclear. Some previous works have suggested that survivin expression may have a role in cell proliferation to be useful for chemoprevention of colon cancer.[11-14] Therefore, further studies are required to address the role of survivin in normal tissue such as whether survivin in normal tissue relates to tumorigenesis and whether patients with positive survivin expression in some normal tissues have an increased potential risk for the development of cancer. The further studies for anti-tumor or chemopreventive therapy using survivin will be needed.

Conclusions

Although survivin expression showed in about 50% of non-neoplastic mucosal tissues, this study demonstrates an increased level of survivin expression in almost of all colon cancers.

The facts that the survivin-positive non-neoplastic colon mucosa show inflammatory and regenerative changesshow us the possibility which recurrent chronic inflammation can be the precancerous lesion. Therefore, these results suggest that as many previous reports have described, survivin might have an important role in tumor development and progression. Further study about the relationship of survivin with other cancer gene expressions in view point of prognostic insight including liver metastasis in patients with colon cancer will be needed.

References

1) Ambrosini G, Adida C, Altieri DC : A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 3:917-923, 1997
2) Tamm I, Wang Y, Sausville E, Scudiero D A, Vigna N, Oltersdorf T et al : IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res 58:5315-5322, 1998
3) Islam A, Kageyama H, Takada N, Kawamoto T, Takayasu H, Isogai E et al : High expression of Survivin, mapped to 17q25, is significantly associated with poor prognostic factors and promotes cell survival in human neuroblastoma. Oncogene 19:617-624, 2000
4) Monzo M, Rosell R, Felip E, Astudillo J, Sanchez J, Maestre J et al : A novel anti-apoptosis gene: re-expression of survivin messenger RNA as a prognosis marker in non-small-cell lung cancers. J Clin Oncol 17:2100-2106, 1999
5) Tanaka K, Iwamoto S, Gon G, Nohara T, Iwamoto M, Tanigawa N : Expression of survivin and its relationship to loss of apoptosis in breast carcinomas. Clin Cancer Res 6:127-132, 2000
6) Kato J, Kuwabara Y, Mitani M, Shinoda N, Sato A, Toyama T et al : Expression of survivin in esophageal cancer: correlation with the prognosis and response to chemotherapy. Int J Cancer 95:92-98, 2001
7) Okada E, Murai Y, Matsui K, Isizawa S, Cheng C, Masuda M et al : Survivin expression in tumor cell nuclei is predictive of a favorable prognosis in gastric cancer patients. Cancer Lett

163:109-115, 2001
8) Zhu ZB, Makhija SK, Lu B, Wang M, Kaliberova L, Liu B, Rivera AA, Nettelbeck DM, Mahasreshti PJ, Leath CA, Barker S, Yamaoto M, Li L, Alvarez RD, Curiel DT : Transcriptional targeting of tumors with a novel tumor-specific survivin promoter. Cancer Gene ther 11:256-262, 2004
9) Gianani R, Jarboe E, Orlicky D, Frost M, Bobak J, Lehner R, Shroyer. Expression of survivin in normal, hyperplastic. And neoplastic colonic mucosa. Hum Pathol 32:119-25, 2001
10) Krajewska M, Krajewski S, Epstein J I, Shabaik A, Sauvageot J, Song K et al : Immunohistochemical analysis of bcl-2, bax, bcl-X, and mcl-1 expression in prostate cancers. Am J Pathol 148:1567-1572, 1996
11) Yamamoto T, Tanigawa N : The role of survivin as a new target of diagnosis and treatment in human cancer. Med Electron Microsc 34:207-213, 2001
12) Sui L, Dong Y, Ohno M, Watanabe Y, Sugimoto K, Tokuda M : Survivin expression and its correlation with cell proliferation and prognosis in epithelial ovarian tumors. Int J Oncol 21:315-320, 2002
13) Ito T, Shiraki K, Sugimoto K, Yamanaka T, Fujikawa K, Ito M et al : Survivin promotes cell proliferation in human hepatocellular carcinoma. Hepatology 31:1080-1086, 2000
14) Zhang T, fields JZ, Ehrlich SM, Boman BM : The chemopreventive agent silindac attenuates expression of the antiapoptotic protein surviving in colorectal carcinoma cells. J Pharmacol Exp Ther 308:434-437, 2004

국문요약

연구배경 및 목적

Survivin은 새로이 고사(아포프토시스)-억제 유전자군에 추가된 유전자이다. Survivin 발현이 대장암에서 보고는 되고 있으나, 상이한 결과들로 논란이 많으며, 임상적 의의에 대해서도 불명확하다. 더욱이 이 유전자는 암 치료나 예방적 항암치료의 표적으로의 가능성이 제기되고 있다. 대장암 survivin 유전자의 발현을 조사하고 임상 및 예후 인자들과의 관련성을 분석하여 대장암의 발생과 임상 측면에서이들 유전자의 역할을 이해하고 예방과 치료에 이용할 수 있는 지를 조사하고자 하였다.

대상 및 방법

다양한 병기의 37예의 신선한 대장암 수술 조직을 대상으로 하였다. 암 조직과 주위 비종양성 조직에서 전체 mRNA를 분리하여 역전사-연쇄중합효소반응으로 survivin mRNA를 검출하였다. PCR 산물을 확인하기 위하여 아클로닝후에 DNA 염기 분석을 시행하였다.

결 과

대장에서 Survivin의 발현율은 대장암 조직에서는 94.6%였고, 비종양성 조직에서는 51.4%으로, 대장암 조직에서 survivin의 발현이 높았으나, 비종양성 조직에서의 경계성 영역의 유의한 차이가 인정되었다 ($0.05<p=0.065< 0.1$). 연령, 성별, 침윤 깊이, 림프절 전이, 혈관과 신경 침범, 간으로의 전이 등의 임상 및 병리학적 인자들과 관련성은 없었다.

결 론

survivin은 대장암에서 암특이적으로 발현되지 않았다 ($0.05<p=0.065< 0.1$). 대장암의 치료나 chemoprevention에서 SSX(synovial sarcoma on X chromosome gene)와 survivin의 이용은 가능하나 제한적일 것으로 생각된다. SSX와 survivin 발현의 상관성이 인정되지 않으므로, 대장암에서 SSX은 survivin-관련 항아포토시스 경로와는 무관할 것으로 생각된다.

중심단어 : 대장암, survivin mRNA