日本財団補助金による

1999 年度日中医学協力事業報告書

－中国人研究者・医療技術者招聘助成－

財団法人　日　中　医　学　協　会

理事長　中　島　章　　殿

12 年　2 月　21 日

研究室で撮影した本人のスナップ写真、及び発表論文等のコピーを添付

1．招へい責任者　覚道　健一

所属機関　和歌山県立医科大学第二病理学教室　職名　教授

所在地　〒641-0012　和歌山市紀三井寺811-1　電話　073-441-0635

招へい研究者氏名　汪　筱娟

所属機関　和歌山県立医科大学第二病理学教室　職名　研究生

研究テーマ　消化管間質腫瘍107例症例におけるｂｃｌ－2，ｐ53免疫組織化学的検討

2．日本滞在日程

７月	７月９日来日した。第二病理教室にて研究活動を始めた。同時に県国際交流課主催の日本語教室で週１回学習を始める。
８月	病理業務を研修した。
９月	１６日に行われる大学院医学研究科受験し（語学試験と面接），合格した。
１０月	ウエスタンブロット，ノーザンブロットの分子医学的技術を研修した。
１１月	ＰＣＲ－ＳＳＣＰ法の技術研修を行った。
１２月	人消化管間質腫瘍におけるｂｃｌ－２，ｐ５３免疫組織化学的検討をした。
１月	研究結果のとりまとめと，データの解析，文献考察を行い，現在まで論文化へ努力中である。同時にＤＮＡシークエンス解析を研修した。
２月	２月１８日和医大第二病理学訪中団の通訳として同行した。
４月より	和歌山県立医科大学大学院医学研究科入学予定。医学振興会より奨学金をうけることが内定した。

3．研究報告

別紙書式を参考に、報告本文4000字以上で報告して下さい（枚数自由・ワープロ使用）

タイトル・要旨等は日本語で、KEY WORDS以下は日本語或いは英語で記入して下さい。

研究成果の発表予定がある場合は発表原稿・抄録集等を添付して下さい。

論文発表に当っては、***日中医学協会助成事業－日本財団補助金による***旨を明記して下さい。

学会名称　　第89日本病理学会総会

学会テーマ　　消化管間質腫瘍107症例におけるｂｃｌ－２，ｐ５３免疫組織化学的検討

日本側主催代表者氏名・所属・役職

北村幸彦　　大阪大学医学部　　教授

中国側主催代表者氏名・所属・役職

無

学会報告

報告者氏名・所属・役職

汪　筱娟　　和歌山県立医科大学第二病理学教室　研究生

消化管間質腫瘍107症例におけるbcl-2、ｐ53免疫組織化学的検討

汪　筱娟、森　一郎、宇都宮洋才、中村美砂、中村靖司、覚道健一
和歌山県立医科大学第二病理学教室

消化管の非上皮腫瘍中で間質腫瘍（GIST）は頻度が高く、その悪性度判定として腫瘍細胞密度、腫瘍径、周辺浸潤、核分裂数などがある。このうち核分裂数は最も重要な指標とされている。

ｐ53はその変異が大多数の癌において認められる癌抑制遺伝子である。一方、bcl-2は細胞周期器に関わるタンパク質である。一部の腫瘍ではbcl-2陽性およびｐ53陰性の間に相関があり、予後規定因子としての重要性が報告されている。

今回我々は免疫組織化学的手法を用いて消化管間質腫瘍107症例におけるbcl-2およびｐ53発現を検討した。Bcl-2陽性症例は84.1％であり、良性と高分化に有意に陽性例が多かった($p<0.01$)。ｐ53陽性症例は49％であり、低分化に有意に多かった($p<0.01$)。しかし、Bcl-2とｐ53発現の間には負の相関がなかった。同時bcl-2とp53発現は核分裂数、腫瘍細胞密度、腫瘍径、周辺浸潤との間にも相関の傾向が認められた。

以上よりBcl-2、ｐ53発現は消化管間質腫瘍においても悪性指標として有用であると考えられる。

キーワード：　消化管，間質腫瘍，bcl-2，p53，免疫組織化学

＊日中医学協会助成事業―日本財団補助金による。

bcl-2 & p53 Expression and Relation to Histopathological Features in 107 Cases of the Gastrointestinal Stromal Tumor

Xiaojuan Wang, M.D., Ichiro Mori, M.D., Ph.D., Wutsunomiya , M.D., Ph.D., Misa Nakamura, M.D., Ph.,D., Yasushi Nakamura, M.D., Ph.D, Kakudo Kenichi, M.D., Ph.D., F.I.A.C.

Summary

Aim We examined bcl-2 and p53 expression in 107cases of human gastrointestinal stromal tumor (GIST) to study their relation to histopathological features by immunohistochemical technique(LSAB).

Method Labelled streptavidin-biotin(LSAB) method.
Primary antibody: Ncl-bcl-2(Novocastra Laboratories Ltd. Batch:111701, United Kingdom); Ncl-p53-D07(Novocastra Laboratories Ltd. Batch:753, United Kingdom)

Results bcl-2 positive rate was 84.1% (90/107) and significantly expressed in benign (85.7%, 36/42, $p<0.01$) and well differentiated GIST (94.9%, 37/39, $p<0.01$). P53 positive rate was 49% and significantly expressed in poorly differentiated GIST (88.5%, 23/26). There are tendentious relationship between p53 expression and mitosis number($r=0.35$), cellularity($r=0.36$), peripheral infiltration($r=0.34$) although no significant statistically, as well as relationship between bcl-2 negative and maximal tumor size($r= -0.16$), peripheral infiltration ($r=-0.23$). But no inverse correlation was identified between bcl-2 expression and p53 expression.

Conclusion Our results suggest that immunohistochemical p53 overexpression and bcl-2 negative expression might be an important parameter in predicting malignancy. Mitosis might be a helpful criteria in malignancy diagnosis.

Keywords: gastrointestinal stromal tumor, bcl-2, p53, immunohistochemical technique

bcl-2 & p53 Expression and Relation to Histopathological Features in 107 Cases of the Gastrointestinal Stromal Tumor

Xiaojuan Wang, M.D., Ichiro Mori, M.D., Ph.D., Utsunomiya , M.D., Ph.D., Misa Nakamura, M.D., Ph.,D., Yasushi Nakamura, M.D., Ph.D, Kakudo Kenichi, M.D., Ph.D., F.I.A.C.

Introduction

Gastrointestinal stromal tumors (GIST) are the most common non-epithelial tumors of the gastrointestinal tract. Its malignant diagnosis and grade mainly depending on mitostic number, and its malignant potential is correlated with the large tumor size, high cellularity, necrosis and peripheral infiltration. Sometimes it is very difficult to make a distinction between benign and malignancy.

It is known that overexpression of tumor-suppressor gene p53 suggesting a loss of p53 function which is implicated in the pathogenesis of many tumors and correlates with a poorly differentiation of tumor. bcl-2 is involved in cell cycle regulation and apoptosis. In some cancers bcl-2 expression correlates with negative p53 staining and its prognostic significance has already been reported in number of malignancies. Gastrointestinal stromal tumors, however, have rarely been examined.

The aim of this study was to examine bcl-2 and p53 expression in 107 cases human gastrointestinal stromal tumor (GIST) to study their relation to histopathological features by immunohistochemical technique(LSAB).

Material and Method

Hundred and seven cases of GIST were selected from the Department of Pathology , the Affilicated Hospital of Shandong Medical University, Jinan, Shandong, China, in the 30-year period from 1969 to 1999. All of these surgical specimens were routinely fixed in formalin and embedded in paraffin. The cases occurred between the age of 6 days and 73-year-old, with the mean of 49.3, and the tumor ranged in size from 0.5 to 30cm. The male to female ratio was 63:44. Twenty-five cases located in the esophagus, 38 in the stomach, 33 in the small intestine and eleven in the

large intestine. The original diagnoses included leiomyoma, leiomyoblastoma, leiomyosarcoma and GIST.

Gross features including tumor size were checked in surgical and pathological records. Microscopically, these cases were devided into three groups depending on mitostic number (10 high power fields in one group, observed 5 groups at the fields of cellularity in each case, then obtained a mean.). Group I as benign, no mitosis is seen, ie.0/10HPF; Group II as well differentiated malignancy, with mitosis 1-4/10HPF and Group III as poorly differentiated malignancy, with mitosis>5/10HPF. Simutaneously, 1)microscopic arrangement pattern of tumor cells; leiomyoma type, schwannoma type, leiomyoblastoma type and mixed type; 2)cellularity; low, intermediate and high; 3)peripheral infiltration; 4) necrosis and 5)the presence of hemorrhge were also noted in every case.

Immunohistochemical studies of bcl-2 and p53 protein were detected by standard Labelled Streptavidin –Biotin (LSAB) method with microwave epitope retrieval (5 x 3min, in 10mmol/L citrate buffer, pH6.0). Sections were 3 μm cut from paraffin blocks, placed onto silane-coated slides, and dried at 60 C for minutes. The sections were dewaxed in Xylene , rehydrated through graded alcohols and treated with 3% hydrogen peroxide for 10 minutes to inactivate endogenous peroxidase activity. They were then incubated with the primary antibody of Ncl-bcl-2 (Novocastra Laboratories Ltd. Batch: 111701, United Kingdom) and Ncl-p53-D07 (Novocastra Laboratories Ltd. Batch: 753, United Kingdom) for over night at 4C. The dilution of antibody was 1: 400 and 1: 300, respectively for bcl-2 and p53. Sections were incubated with biotinylated secondary antibody, bollowed by peroxidase-conjugated streptavidin by use of the Universal DAKO LSAB kit (Dako Corp.) at 10 minutes for each step. Staining was visualized by use of 3-amino-9ethyl-carbazole; the sections were washed in water and counterstained with methlgreen. A stomach carcinoma with documented p53 mutation was used as a positive control and a follicular lymphoma was used as a positive control for bcl-2. Negative controls were performed by substituting normal sheep serum for primary antibody.

The results were evaluated in the following criteria: negative (-) for bcl-2 and p53, with no tumor cell stained; positive (+) for bcl-2 (tumor cell stained) and positive (+)~(++) for p53 (less than 50% of tumor cells

stained for+, and more than 50% of tumor cells stained for++).

Statistical methods: Chi-squared test and relative analysis. $P<0.05$ was considered to represent statistical significance.

Results

Gross and microscopic features

The gross, microscopic features and immunohistochemical studied of the 107 cases GIST were summarized in Table*.

The age of case ranged from 6 days to 73-year-old, with the mean of 49.3, and the mean of 43.5 for benign , the mean of 52.9 for malignancy. The size of tumor was measured in maximal diameter, occurring between 0.5cm and 30cm, with the mean of 6.56cm and a mean of 3.97cm for benign and a mean of 8.41cm for malignancy. Of the 107, the male were 63 cases and the female were 44 cases. Twenty-five cases located in esophagus, 38 in stomach, 33 in small intestine and eleven in large intestine.

Microscopically, 42 cases were in Group I, Group II were 39 cases and 26 cases in Group III. Cellularity were 7 intermediate and 35 low in benign, 20 cases low, 29 intermediate , 16 high in malignancy. No periphral infiltration in Group I, but 35 cases (53%) were seen with a periphral infiltration in Group II and group III. Necrosis was observed 45 cases(69%) in Group II and Group III , but only 4 cases(9%) in Group I, hemorrhge was noted 44 cases(67%) in Group II and III and only 5 cases(16%) in Group I.

Immunohistochemical expression

bcl-2 positive rate was 84.1% (90/107) in total and significantly expressed in benign (85.7% , 36/42, $p<0.01$) and well-differentiated GIST (94.9%, 37/39, $p<0.01$), expression in poorly-differentiated is 65% (17/26), but no significant difference between benign and well differentiated. P53 positive rate was 49% in total and significantly expressed in poorly differentiated GIST (88.5%, 23/26, $p<0.01$). The expression of p53 between well-differentiated (53.8%, 21/39) and benign group (19%, 8/42) also has a significant difference ($p<0.01$), as well as the expression of p53 between well and poorly-differentiation. Positive(++) of p53 was seen only in malignancy(18/65) but no significant different expression between well

and poorly differentiated. There are tendentious relationship between p53 expression and mitosis number(r=0.35), cellularity(r=0.36), peripheral infiltration (r=0.34) although no significant statistically, as well as the relationship between bcl-2 negative and maximal tumor size(r=-0.16), and peripheral infiltration (r=-0.23). But no inverse correlation was identified between bcl-2 expression and p53 expression.

Table* Gross, microscopical features and immunohistochemical studied

	Total (n=107)	Group I(benign) (n=42)	Group II(well-) (n=39)	Group III(poorly-) (n=26)
Mean age	49	43	54	52
Female: male	63:44	24:18	26:13	13:13
Location, E:S:SI:LI	25:38:33:11	24:9:4:5	1:20:16:2	0:9:13:4
Mean volume	6.56	3.97	7.34	9.84
Arrange, Lei:Sch:Blas:Mix	85:22:0:0	27:6:4:5	23:13:2:1	5:13:4:4
Cellularity, I, II, III	56:36:15	35:7:0	14:16:9	7:13:6
Mitosis (10HPF)	0~28	0	1~4	
>5~28				
Hemorrhage	49	5	29	15
Necrosis	49	4	31	14
bcl-2 (+)	90	36	37	17
p53	52	8	21 (4++)	23 (11++)

E: esophagus, S: stomach, SI: small intestine, LI: large intestine

Lei: leiomyoma type, Sch: schwannoma type, Blas: blastoleiomyoma, Mix: mixed type

HPF: high power field

Discussion

Gastrointestinal stromal tumors are the most common mesenchymal tumors which traditionally have been designated as smooth muscle tumors. Its myogenic differentiation was unable to demonstrate in most series of research, although with increasing analytic tools. Furthermore, the biological behavior of GIST is difficult to predict.

bcl-2 is one of the many proteins that regulate programmed cell death and is overexpressed in B-cell lymphomas. It is encoded in the 18q21 region that is frequently involved in the t(14:18)(q32:q21) in follicular

lymphomas. The p53 tumor suppressor gene encodes a 53KD nuclear protein involved in the regulation of cell growth. Mutations in the p53 gene are among the most common genetic abnormalities in human cancers and more than 95 per cent occur in exons 5-8. In contrast to non-stabilized wild-type protein, which has a short half-life(5-20min.), mutated p53 protein becomes stabilized and can be detected by immunohistochemical techniques. Mutation and overexpression of p53 have been reported in leiomyosarcoma of soft tissue and uterus[1-7], as well as the expression of bcl-2[8-10] and the correlation of p53 negative expression with bcl-2 expression[11]. However, rare study has been examined in the gastrointestinal stromal tumor[12-14]. The aim of this study was to evaluate bcl-2 and p53 as additional prognostic markers, as well as the correlation with the differentiation, maximal tumor sizes, cellularity, periphrel infiltration, hemorrhge and necrosis and the correlation of p53 negative expression with bcl-2 expression.

This study has revealed the presence of a bcl-2 expression in benign and well-differentiated GIST than in poorly differentiated GIST, the same as the other reports. The bcl-2 over-expression in benign and well differentiated suggests that it might play an important role in preventing apoptosis among benign and well-differentiated GIST. Although bcl-2 itself does not stimulate cell growth, an inhibition of apoptosis by bcl-2 may provide a survival advantage to the cells of benign and well-differentiated GIST. In the study no significant difference between benign and well differentiated. Since cellular proliferation and apoptosis form a complex mechanism, another pathway could also be implicated in the regulation of the cell and cell growth in benign and well-differentiated GIST.

Mutation of the P53 gene occurs in many human cancers and p53 overexpression has been demonstrated by immunohistochemistry in a variety of tumor types. Positive staining for p53 has been correlated with tumor differentiation and with a poor prognosis in a series of reports. The present study showed that p53 positive rate was significantly expressed in poorly differentiated GIST. The expression of p53 between well-differentiated and benign group also has a significant difference, as well as the expression of p53 between well and poorly differentiation. Moreover, positive (++) of p53 was seen only in malignancy (18/65), but no significant difference between well and poorly differentiated. As the

other reports, the result suggests that p53 gene might play an important role in formation and differentiation of gastrointestinal stromal tumors.

In the study inverse correlation between bcl-2 and p53 expression was not revealed, although an inverse correlation of expression has been reported in a number of human malignancies previously. The finding suggests that expression of bcl-2 in GIST might have not been induced by mutant p53.

Pathologically and clinically, it is important to predict the biological nature of GIST. So far the importance of the mitotic index has especially emphasized due to its objectivity. The large tumor size, high cellularity, tumor hemorrhge and tumor necrosis and have been reported as indicator in detecting malignant potential of GIST. In our materials there are tendentious relationship between p53 expression and mitosis number, cellularity, peripheral infiltration although no significant statistically, as well as the relationship between bcl-2 negative expression and maximal tumor size, peripheral infiltration.

In conclusion, the present study showed that bcl-2 is frequently expressed in benign and well-differentiated GIST and p53 is frequently expressed in malignant GIST both in well and poorly differentiated GIST, especially in the condition of strong p53 positive, a malignant potential might be considered. But no correlation between bcl-2 negative expression with p53 positive expression. On the other hand, mitosis was a helpful criteria to distinguish benign and malignant GIST, as well as to grade well -differentiated and poorly-differentiated GIST.

REFERENCES

1. Lopes JM, Silva P, Seixas M, et al. Microsatellite instability is not associated with degree of malignancy and p53 expression of gastrointestinal stromal tumours. Histopathology 1998 Dec; 33 (6): 579-81.
2. Chang MS, Choe G, Kim WH, et al. Small intestinal stromal tumors: a clinicopathologic study of 31 tumors. Pathol Int 1998 May; 48(5): 341-7.
3. Michael DJ, Maura AF, James AR, et al. p53 immunoreactivity and mutation of the p53 gene in smooth muscle tumours of the uterine corpus. J Pathol 1995; 177:65-70.
4. Expression of steroid receptors, Ki-67, and p53 in uterine leiomyosarcomas. Int J Gynecol Pathol 1999 Jan; 18(1):20-8.
5. Meye A, Bache M, Hinze R, et al. Molecular characterization and liposomal transfection of a p53-mutated cell line established from a poorly differentiated leiomyosarcoma. Int J Oncol 1998 Aug; 13 (2): 241-8.
6. Hall KL, Teneriello MG, Taylor RR, et al. Analysis of Ki-ras, p53, and MDM2 genes in uterine leiomyomas and leiomyosarcomas. Gynecol Oncol 1997 May; 65(2): 330-5.
7. Yoo J, Lee HK, Kang CS, et al. p53 gene mutations and p53 protein expression in human soft tissue sarcomas. Arch Pathol Lab Med 1997 Apr; 121(4): 395-9.
8. Miettinen M, Sarloma-Rikala M, Lovatich AJ. Cell-type- and tumour-type-related patterns of bcl-2 reactivity in mesenchymal cells and soft tissue tumours. Virchows Arch 1998 Sep; 433(3): 255-60.
9. Suster S, Fisher C, Moran CA. Expression of bcl-2 oncoprotein in benign and malignant spindle cell tumors of soft tissue, skin, serosal surfaces, and gastrointestinal tract. Am J Surg Pathol 1998 Jul; 22(7): 863-72.
10. Chilosi M, Facchettti F, Dei Tos AP, et al. bcl-2 expression in pleural and extrapleural solitary fibrous tumours. J Pathol 1997 Apr; 181(4):

362-7.
11. Y-L Zhai, T Nikaido, T Toki, et al. Prognostic significance of bcl-2 expression in leiomyosarcoma of the uterus. Br J Cancer 1999; 80 (10), 1658-1664.
12. Kenichi Tazawa, Kazuhiro Tsukada, Hiroyasu Makuuchi, et al. An immunohistochemical and clinicopathological study of gastrointestinal stromal tumours. Pathol Int 1999; 49:786-798.
13. Hillemanns M, Pasold S, Bottcher K, et al. Prognostic factors of gastrointestinal stromal tumors of the stomach. Verh Dtsch Ges Pathol 1998; 82:261-6.
14. Uma N.M.Rao, Sydney DF, Merka WJ, et al. Comparative immunohistochemical and molecular analysis of uterine and extrauterine leiomyosarcomas. Mod Pathol 1999; 12(11): 1001-1009.