

The expression and clinical significance of CD44v in human gastric cancers

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INTRODUCTION

CD44 was originally implicated as a "homing" receptor directing the migration of recirculating lymphocytes^[1]. CD44 expression has been confirmed not only in lymphocytes but also in a wide variety of epithelial tissues. It is considered as an important cell adhesion molecule for cell to cell interactions^[2]. Molecular cloning and analysis of the genetic structure have revealed that the CD44 gene has at least 19 exons, of which 12 can be alternatively spliced to make up a wide variety of CD44 splice variants which have been found in various types of human malignancies and have been considered as a marker in tumor progression and metastasis^[3]. We analysed the expression of aberrant CD44 transcripts with RT-PCR method in gastric carcinomas, and discussed the clinical significance of metastasis-related CD44v gene.

MATERIALS AND METHODS

Materials

Fresh tissue specimens including normal gastric mucosa, gastric tumor and mucosa adjacent to tumor were tissues taken from 20 patients with gastric cancers who underwent surgical treatment in the Department of General Surgery in our hospital from November 1997 to February 1998. The diagnosis was confirmed before operation, by endoscopic and pathological examinations. The normal mucosae were taken from mucosae 10 cm away from carcinomas, and the adjacent tissues 2 cm away from the carcinomas. The specimens were derived in 30 minutes after surgical resection, and were frozen in liquid nitrogen prior to store below -70 °C.

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There were 15 males and 5 females, aged 30-79 years, with an average of 58. Pathological examination showed two types of tumors according to *Laureu's* classification division of Finland: intestinal type (well-differentiated) and diffuse type (poorly-differentiated). All the gastric mucosae, gastric cancers and their adjacent tissues expressed CD44s: 16 of 20 gastric cancer cases including 14 cases of diffuse type and 2 cases of well-differentiated type had positive expression of CD44v; 5 of 10 adjacent tissues had positive expression of CD44v. In contrast, there was no positive expression of CD44v in all 10 normal gastric mucosae.

RT-PCR method

Primers sp1 and sp2 were taken from the cDNA sequence described by Matsumura^[4]. sp1: 5'GACACATATTGCTTCAATGCTTCAGC3', sp2: GATGCCAAGATGATCAGCCATTCTGGAAT3', pointing to part of exons of standard CD44s, the amplified product was CD44s; primer p1 and p2 were taken from No.1 and No.5 exons in human cDNA sequence described by Hofmann^[5], p1: 5'GACAGACACCTCAGTTTTTCTGGA3', p2: 5'TTCCTTCGTGTGTGGGTAATGAGA3', pointing to the abnormal splice variants of CD44 gene containing v7 exon, the amplified product was CD44v. The two couples of primers were both synthesized by SAGON Co, Canada, kits and TaqDNA polymerase were purchased from PE Co. USA.

Total RNA extraction was prepared according to AGPC single-step method^[6]. RT-PCR: model RNA 3 µL, down primers (sp1, p1) 2 µL (25 pmol) and 2 µL AMV-RTase were put into 20 µL reaction system to synthesize cDNA. Putting 10 µL cDNA into a reaction tube, the primers sp2, p2 and 2 µL TaqDNA polymerase were added, then the amplification happened in 50 µL reaction system. The conditions of PCR reaction was 94 °C for 50", 52 °C for 50" and 72 °C for 1'33" undergoing 35 cycles. For negative controls, template cDNA was not added to the reaction mix. The amplified product was electrophoresed in a 2% agarose gel, observed and photographed with ultraviolet radiography.

Statistical analysis

Data were analyzed for Fisher exact examination by

running statistical software SPSS for Win95. *P* value was set at 0.05 for statistical significance.

RESULTS

Amplified with sp1 and sp2 as primers, the CD44s specific 482 bp product was obtained from all the gastric cancers, the adjacent tissues and normal gastric mucosae (Figure 1).

With p1, p2 as primers, 10 normal gastric mucosae showed no RT-PCR fragment; 5 of 10 adjacent tissues showed specifically the 740 bp CD44v product of RT-PCR fragment, these 5 cases with positive CD44v expression were diffuse-type gastric cancers with distant lymph node metastasis; 16 of 20 gastric cancers expressed specific 740 bp band, of which, 14 of 15 were diffuse cancers, only 2 of 5 cases were well-differentiated cancers; 15 of 16 cases with lymph node metastasis had CD44v expression, only 1 case had CD44v expression in all 4 cases without lymph node metastasis. All the cases with CD44v expression in adjacent tissues were diffuse-type gastric cancer with distant lymph node metastasis (Figure 2).

The relationship between the biological behaviors of gastric cancer and CD44v mRNA expression is presented in Table 1.

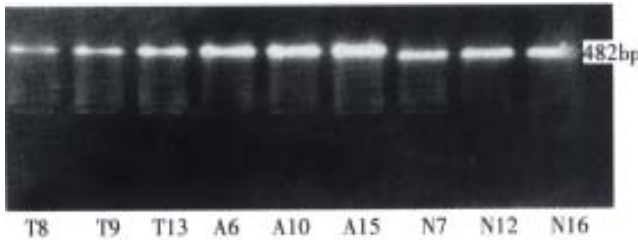


Figure 1 Electrophoretic pattern of RT-PCR product for CD44s in gastric tissues.



Figure 2 Electrophoretic pattern of RT-PCR product for CD44v in gastric tissues.

Table 1 Relationship between CD44v expression and histological classification of gastric cancer, lymph node metastasis, clinical pathology and tumor size

	<i>n</i>	CD44v mRNA		<i>P</i> value
		+	-	
Histological types				
Diffuse type	15	14	1	<i>P</i> <0.05
Well-differentiated type	5	2	3	
Lymph node metastasis				
Positive	16	15	1	<i>P</i> <0.05
Negative	4	1	3	
Tumor size				
>5 cm	13	12	1	<i>P</i> >0.05
<5 cm	7	4	3	
Clinicopathology				
Ulcerative type	13	12	1	<i>P</i> >0.05
Infiltrative type	7	4	3	
Stage				
I-II	7	4	3	<i>P</i> >0.05
III-IV	13	12	1	

DISCUSSION

As an adhesive molecule, CD44 exists on the surface of cells. The NH₂-terminal function area of CD44 can join the hyaluronate in the basement membrane to extracellular matrix, thus to regulate the movement and function of cells. By this mechanism, neoplastic cells can adhere itself to the extracellular matrix and basement membrane of the host cell, resulting in invasion and metastasis of malignancy. On the other hand, the degraded products of hyaluronic acid can motivate the growth of local vessels providing the basis for invasion and metastasis. This varied CD44v is a promising marker in diagnosing gastric cancer clinically^[4,7].

Yokozaki^[8]'s research suggests that the rate of abnormal expression of CD44 gene whether in well or poorly-differentiated gastric cancer may approach to 100%. In gastric cancer cells, CD44 gene generates abnormal splice variant CD44v during transcription, whereas in normal gastric mucosa cells, CD44 gene only expresses CD44s protein. In metastatic cells of gastric cancer the abnormal expression of CD44v is strengthened more than those in primary cancer cells. Therefore, CD44 gene's abnormal expression may not only serve as a good marker for identifying gastric cancer in normal mucosa, but also predict cancer metastasis.

We have got similar result to Yokozaki's. The expression rate of CD44v in gastric tissue is up to 80% with difference between two types of gastric cancer, but not high in the adjacent tissue and normal mucosa. It shows that the variant expression of CD44v is related to the phenotype of gastric malignancy, and may serve as a useful indicator in tumor metastasis, and have potential significance in diagnosing gastric cancer.

The formation and metastasis of gastric cancer are related to the abnormal expression of onco-gene (c-erb2, c-myc, ras) and suppressor onco-gene (p53). CD44's abnormal expression may be an earlier event than that of ras, p53 and other genes, and may be related to the activation of ras gene. The mechanism in promoting the formation of cancer cell and metastasis may probably be related to the function of CD44. The cancer cell with CD44v expression may get lymphocyte's guise so as to escape the recognition and execution from human immune system and easily invades lymph node, resulting in metastasis^[9].

Our results show that 16 of 20 gastric cancer specimens and 5 of 10 adjacent tissue specimens had positive CD44v expression, and none of the normal mucosa had CD44v expression (Chi-square $\chi^2 = 17.160$, $P < 0.05$). There was no statistical significance as comparing the former two with the normal mucosa. The CD44v expression rates in two types of gastric cancer were different significantly ($P < 0.05$), the rates were also markedly different ($P < 0.05$) in cases with or without lymph node metastasis. Expression of CD44v mRNA was related to the genesis and prognosis of gastric cancer, and is one of the biological markers indicating metastasis and poor prognosis. However the expression of CD44v had nothing to do with clinical stage or size of the cancer. Considering the limited data available, further investigations are needed.

No specific strand was discovered in the 20 cases of normal gastric mucosal tissues, Northern blot-hybridization method has also been used for CD44 variant gene expression product in different tumors, *i.e.* gastric cancer, lung adenocarcinoma, colon cancer and esophagus cancer tissues.

However, the method is less sensitive than RT-PCR method, it is also time-consuming and needs large quantities of RNA and tissues. RT-PCR technique can be used to detect CD44 gene's variant expression product rapidly with a tiny amount of tissues in batches, so it is highly valuable in clinical practice. False negative result is possible if only RT-PCR is used for detecting CD44, thus further researches are needed to improve the detecting sensitivity, when RT-PCR with Southern blot-hybridization technique or semiquantitative density technique are used in combination.

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