

Role of genetic detection in peritoneal washes with gastric carcinoma: The past, present and future

Hyun-Dong Chae

Hyun-Dong Chae, Department of Surgery, School of Medicine, Catholic University of Daegu, Daegu 705-718, South Korea

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Correspondence to: Hyun-Dong Chae, MD, PhD, Department of Surgery, School of Medicine, Catholic University of Daegu, 3056-6, Daemyung-4-Dong, Namgu, Daegu 705-718, South Korea. hdchae@cu.ac.kr
 Telephone: +82-53-6504429
 Fax: +82-53-6247185

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Abstract

The most frequent cause of treatment failure following surgery for gastric cancer is peritoneal dissemination, mainly caused by the seeding of free cancer cells from the primary gastric cancer, which is the most common

type of spread. Unfortunately, there is no standard modality of intraperitoneal free cancer cells detection to predict peritoneal metastasis until now. We reviewed English literature in PubMed was done using the MeSH terms for gastric cancer, peritoneal wash, and reverse transcriptase polymerase chain reaction. All the articles were reviewed and core information was tabulated for reference. After a comprehensive review of all articles, the data was evaluated by clinical implication and predictive value of each marker for peritoneal recurrence. There are still many limitations to overcome before the genetic diagnosis for free cancer cells detection can be considered as routine assay. To make it a reliable diagnostic tool for detecting free cancer cells, the process and method of genetic detection with peritoneal washes should be standardized, and the development of simple diagnostic devices and easily available kits are necessary. Herein, we reviewed the past, present and future perspectives of the peritoneal lavage for the detection of intraperitoneal free cancer cells in patients with gastric cancer.

Key words: Gastric cancer; Peritoneal metastasis; Free cancer cells; Reverse transcriptase polymerase chain reaction; Genetic detection

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Core tip: The most common cause of treatment failure after gastric cancer surgery is peritoneal metastasis, mainly caused by free cancer cells from primary cancer. Genetic detection using reverse transcriptase polymerase chain reaction analysis has been used for the detection of free cancer cells. The process and method of genetic detection with peritoneal washes should be standardized, and the development of simple diagnostic devices and easily available kits are necessary in the future. In this article, we summarize the current evidence of genetic detection in peritoneal washes from gastric cancer patient.

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INTRODUCTION

Although the incidence of gastric cancer has been declining in western countries, gastric cancer is still the fourth most common cancer and the second leading cause of cancer deaths worldwide^[1]. Peritoneal dissemination of gastric cancer, which is caused by free cancer cell seeding from the primary tumor, is the most common cause of treatment failure after surgery. In general, the frequency of peritoneal dissemination is increased with depth of invasion of the gastric wall^[2]. Intraperitoneal free cancer cells detected in peritoneal washes from gastric cancer patients have been reported to be significant and independent prognostic factor for recurrence and survival after surgery.

To predict peritoneal metastasis, several methods have been used for many studies, which are conventional cytology^[3,4], ThinPrep^[5], and molecular markers^[4,6-8]. Conventional cytology had been regarded as the gold standard for detecting cancer cells in peritoneal washes. However, the usefulness of conventional cytology for prediction of peritoneal metastasis has controversy because of its low sensitivity^[3,4,7,9].

Recently, reverse transcriptase polymerase chain reaction (RT-PCR) analysis has been used for the genetic detection of free cancer cells^[10-12]. Several target genes have been used, which are carcinoembryonic antigen (CEA), heparanase, matrix metalloproteinase-7 (MMP-7), cytokeratin 20 (CK-20), telomerase, and melanoma-associated gene (*MAGE*)^[2,13]. The sensitivity of RT-PCR is higher than that of conventional cytology^[14-16]. Several studies reported that the results of RT-PCR analysis from peritoneal washes correlate strongly with peritoneal recurrence and prognosis after curative surgery in patients with advanced gastric cancer^[6,14-20].

In this reason, we reviewed the present status and future perspectives of peritoneal lavage for the detection of intraperitoneal free cancer cells in patients with gastric cancer (Figure 1).

DETECTION OF INTRAPERITONEAL FREE CANCER CELL WITH GASTRIC CARCINOMA

The past

Conventional cytology from the peritoneal washes had been widely used to detect free cancer cells and to predict peritoneal metastasis^[3,9], based on several studies reporting a correlation between peritoneal recurrence and presence of intraperitoneal free cancer cells^[21-24].

In patients with serosal involvement, 50% of patients develop peritoneal recurrence even if curative resection is performed^[25,26]. Bando *et al*^[3] reported that 24% of positive cytology from 1297 gastric cancer patients. This result presented higher positive rate than other studies, probably because many patients with advanced stage of gastric cancer were included in the study (296 patients had peritoneal metastasis). Ribeiro *et al*^[27] reported much higher incidence of positive cytology (41%) from their study from 49 patients with advanced gastric cancer including metastatic disease. Other studies that evaluated patients with gastric cancer underwent curative resection showed approximately 5% of positive rate from peritoneal washes cytology^[3,8-10,25,27,28].

In many past studies, intraperitoneal free cancer cells detected in peritoneal washing cytology have been demonstrated as significant and independent prognostic factor, influencing both recurrence free survival and overall survival of patients with gastric cancer. Therefore, peritoneal wash cytology is recommended in the Japanese Classification of Gastric Carcinoma from 1998^[29]. Recently, the American Joint Committee on Cancer tumor node metastasis staging system classified the positive peritoneal cytology in gastric cancer as metastatic disease (M1) in the 7th edition^[1].

However, the conventional cytology is often criticized for its relatively low sensitivity to detect intraperitoneal free cancer cells and to predict peritoneal metastasis^[3,4,7,9]. Furthermore, previous studies reported that the conventional cytology in patient without any macroscopic peritoneal metastasis (P0) after curative resection had very low sensitivity (5%-15%)^[3,4,7]. Although obtaining peritoneal cytology has been advocated by Japanese and Dutch investigators^[30,31], this is not a uniform practice in other Western centers. Immunocytochemical methods that used a panel of monoclonal antibodies, directed to gastric cancer-associated antigens had improved detection of peritoneal cytology by providing more sensitive and may have a higher specificity than conventional cytology^[32].

The present

General principles: The high sensitivity of RT-PCR analysis has made it possible to detect micrometastasis on the cancer tissue specific messenger RNA (mRNA) expression in peripheral vein, bone marrow, lymph nodes, and peritoneal cavity^[19,33-35]. Although RT-PCR analysis of peritoneal washes is a more sensitive than conventional cytology, the result variations in RT-PCR between different laboratories have been reported. Therefore standardization and quality control of the process of RT-PCR is very important.

For the detection of gastric cancer micrometastases in peritoneal washes, RT-PCR technique has been used from the twenty-first century^[10-12]. The primer sequences, which are used in previous studies, for RT-PCR of peritoneal lavage in patients with gastric cancer are summarized in Table 1. The sensitivity of RT-PCR is higher than that of conventional cytology^[10-12]. Based on several studies, There is strong correlation between

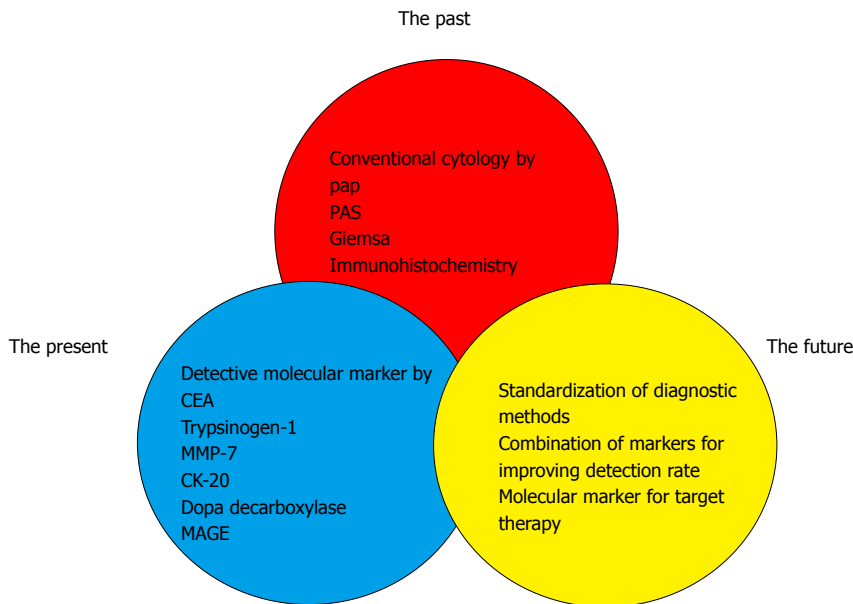


Figure 1 Detection of free cancer cell in peritoneal washes of gastric cancer patients: The past, present and future. CEA: Carcinoembryonic antigen; MMP-7: Matrix metalloproteinase-7; CK-20: Cytokeratin-20; MAGE: Melanoma associated gene.

the results of RT-PCR analysis from peritoneal washes and prognosis, including peritoneal recurrence rate, after curative resection in patients with advanced gastric cancer^[6,14,16-20] (Table 2).

Although many investigators have demonstrated that molecular techniques using RT-PCR may serve as a useful method for the detection of free cancer cells, there are still several problems. These are time-consuming, expensive, relatively laborious compared with conventional cytology, and the accuracy is widely variable between laboratories and the methods of processing the peritoneal washes is not yet standardized. Current experimental studies which aim to identify a rapid, accurate, and cost-effective detection method are proposed. The transcription-reverse transcription concerted reaction system and the LightCycler system have shown promise in intraperitoneal free cancer cell detection.

Molecular markers: (1) CEA: CEA is the most commonly studied tumor marker and is predominantly used clinically in patients with gastrointestinal cancer. Structurally, it is a glycoprotein with a molecular weight of 200 kd and is a component of the glycocalyx, located on the luminal side of the cell membrane of normal epithelial intestinal cells. Although its exact function is unknown, CEA has been shown to be involved in cell adhesion and is able to inhibit apoptosis induced by loss of anchorage to the extracellular membrane.

In 1997, Nakanishi *et al*^[16] had proposed the first study that more sensitive detection of free cancer cells could be achieved through amplification of CEA mRNA by means of the RT-PCR, and reported a high sensitivity of the CEA RT-PCR assay for the detection of peritoneal micrometastasis and a 20% improvement in the positive rate, as compared with that of cytology alone. CEA is presented in all of gastric cancer cell lines examined irrespective of the differentiation degree,

and it is absent in blood and mesothelium. That is indicating the specificity of CEA RT-PCR for detection of carcinoma cells in peritoneal lavage fluid.

Since this report, many investigators have been used CEA as the target gene of RT-PCR for the patients with gastric cancer. From 2001, many other studies have reported that quantitative CEA mRNA detection using RT-PCR is an accurate method for predicting the risk of peritoneal recurrence in patients with gastric cancer^[36-42].

Many studies reported that the detection of CEA mRNA using RT-PCR of peritoneal washes was the most reliable prognostic marker for recurrence and peritoneal carcinomatosis in patient with gastric cancer, and more sensitive than conventional cytology^[8,17]. Kodera *et al*^[43] found that the use of RT PCR for CEA mRNA resulted in a detection rate of free cancer cells of 28% (41 of 148), with a 14% higher detection rate than for peritoneal wash cytology. However, the sensitivity of the CEA RT-PCR assay for the detection of peritoneal micrometastasis still remains low. Furthermore, false-positive results, caused by expression of CEA in non-cancer cells like mesothelial cells and lymphocytes, is remained as main problem of this technique^[20].

(2) Trypsinogen-1: Trypsin is a member of the serine protease family composed of three trypsinogen genes (*trypsinogen 1, 2 and 4*) with a potential role in cancer invasion^[44,45]. These family members are sharing same nucleotide structures above 90% with each other.

Trypsin has potent proteolytic activity, and its inappropriate activation may lead to peritoneal dissemination of infiltrative gastric cancer by defoliation of cancer cells from the surface of advanced primary tumor, attachment and invasion to extracellular matrix under the mesothelium^[46].

(3) MMP-7: Recently, MMPs are gaining attention and attracted by many investigators who are interested in new biomarkers for cancer spread and metastasis^[47].

Table 1 Primer sequences used for reverse transcriptase polymerase chain reaction

Gene	Sequence of primer pair (5'→3')	Tm (°C)	Amplicon length (bp)
CEA	F: TCTGGAAGTCTCTCTCTCTCAGCTGG R: TGTAGCTGTGCAAAATGCTTTAAGGAAGAAGC	69	160
CK-20	F: GGTGCGGACTACAGTGCATATTACA R: CCTCAGCAGCCAGTTTAGCATTATC	72	121
Trypsinogen	F: ACCACCATGAATCCACTCCTG R: GCTTTAGCTATTGGCAGCTAT	62	
MMP-7	F: ATGTTAACTCCCGCGTCATA R: CAGCATACAGGAAGTTAATCC	72	418
DDC	F: AAGCACAGCCATCAGGATTCA R: TGGACATGCTTGCGGATATAAG	70	
L3PP	F: GATGCTGTGTGTTTGTAT GTTGAC R: CTTGACTGTGCTGATCACATT	95	
MAGE A1-6	F: CTGAAGGAGAAGATCTGCC R: CTCCAGGTAGTTTCTCTGCAC	60	855

Tm: Melting temperature; F: Forward; R: Reverse; CEA: Carcinoembryonic antigen; CK-20: Cytokeratin-20; MMP-7: Matrix metalloproteinase-7; DDC: Dopa decarboxylase; L3PP: L-3 phosphoserine phosphatase heparanase; MAGE: Melanoma associated gene.

Table 2 Reports on molecular marker with peritoneal washes in gastric cancer

Ref.	Year	Method	Molecular marker	Outcome
Nakanishi <i>et al</i> ^[17]	1997	RT-PCR	CEA	
Fujimura <i>et al</i> ^[46]	1998	RT-PCR	Trypsinogen	
Kodera <i>et al</i> ^[20]	2002	RT-PCR	CEA	Peritoneal recurrence, survival
Nakanishi <i>et al</i> ^[17]	2000	Q-RT-PCR	CEA	Peritoneal recurrence, survival
Yonemura <i>et al</i> ^[6]	2001	RT-PCR	MMP-7	
Sugita <i>et al</i> ^[14]	2003	Q-RT-PCR	CEA and CK-20	Peritoneal recurrence, survival
Sakakura <i>et al</i> ^[62]	2002	Q-RT-PCR	Dopa decarboxylase	
Shimomura <i>et al</i> ^[66]	2004	Q-RT-PCR	L3-PP	
Kodera <i>et al</i> ^[7]	2006	RT-PCR	CEA	Overall survival
Jeon <i>et al</i> ^[13]	2010	RT-PCR	MAGE	Overall survival
Jeon <i>et al</i> ^[71]	2014	RT-PCR	MAGE and CEA	3-yr survival

CEA: Carcinoembryonic antigen; CK-20: Cytokeratin-20; MMP-7: Matrix metalloproteinase-7; L3-PP: L-3 phosphoserine phosphatase heparanase; MAGE: Melanoma associated gene.

MMPs have key role in degrading extracellular matrix by proteolytic activity, as well as regulating other enzymes, chemokines and cell receptors^[48,49]. MMP-7, also called Matrilysin, is a distinct family member in MMPs family because its highest proteolytic activity for wide range of molecules and pivotal role in activating other MMPs for cell degradation^[50-53]. Another specific characteristic of matrilysin in contrast to other MMPs is that it is mainly expressed by tumor cells and not by stromal cells^[54-56].

Yonemura *et al*^[6] reported that MMP-7 mRNA was not expressed by fibroblasts, peripheral blood, mesothelial cells, normal gastric mucosa, or the peritoneal lavage fluid of patients with benign disease. In contrast, it was expressed by all of the examined specimens of peritoneal dissemination in gastric cancer. By combining cytology and MMP-7 RT-PCR, the sensitivity rate for the prediction of peritoneal dissemination was improved to 11% over the prediction by routine cytology.

(4) CK-20: The keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells. Recently, many investigators identified CK-20 as

one of the potential cancer-related biomarkers used for detecting peritoneal free cancer cells in gastric cancer^[57].

CEA had been reported that there are strong positivity of mRNA expression in differentiated gastric carcinoma, but relatively weak or undetectable in poorly differentiated type^[57]. Thus, CK-20 has been the use as option for multiple marker assay with CEA for the detection of nodal metastasis^[58]. Also for the peritoneal recurrence, many studies reported the usefulness of multi-marker assay, employing both CEA and CK-20 RT-PCR^[14,15,59,60].

(5) Dopa decarboxylase (DDC): DDC is an enzyme for the metabolism of dopamine, and also has a role for the synthesis of important neurotransmitters including serotonin. Although DCC is investigated as cancer-related marker with its high expression in lung cancer and neuroblastoma^[61], the role of this marker in gastric cancer is still unclear.

Sakakura *et al*^[62] have analyzed in their studies for the global gene expression of a gastric cancer cell line, and approximately 21168 genes were established from the tumor and metastatic site of gastric cancer patients.

Among them, they found that 24 genes were upregulated and 17 genes downregulated, and DCC was one of these upregulated genes. DCC-specific RT-PCR is potentially a novel marker for peritoneal dissemination of gastric cancer and reliable and efficient for the prediction of peritoneal recurrence of gastric cancer.

(6) L-3 phosphoserine phosphatase heparanase (L3-PP): L3PP is one of the up-regulated genes and hundreds of protein phosphatases are known. It is an intermediary enzyme in both amino acid biosynthesis and gluconeogenesis, and is an enzyme that acts specifically in the synthesis of serine from 3-phosphoserine^[63,64]. It has been reported that activity of the enzyme L3-PP increases when cell multiplication and frequency of mitosis increases^[65].

Shimomura *et al*^[66] had reported that L3-PP over-expression in gastric cancer cells from peritoneal metastasis by cDNA microarray as well as RT-PCR suggest that overexpression of L3-PP is probably involved in peritoneal metastasis of gastric cancers. When they had used combination RT-PCR of CEA and L3-PP to reduce false-negative result of CEA mRNA, the sensitivity of peritoneal dissemination was improved from 71.4% to 85.7%.

(7) Melanoma associated gene (*MAGE*): *MAGE* has been shown to be a cancer-specific marker that suppresses apoptosis and plays an important role in carcinogenesis^[67]. *MAGE* gene expression in RT-PCR of gastric cancer is relatively higher than that of other markers^[68,69]. Although the rate of expression is different according to their subtype, expression of *MAGE-4*, *MAGE-6*, *MAGE-8*, *MAGE-9*, *MAGE-10*, and *MAGE-12* genes was very high as 82% in gastric cancer tissue^[70]. Furthermore, previous studies reported that *MAGE* showed no expression in normal gastric tissue^[69,70]. In this reason, *MAGE* has been attracted for the new target gene for predicting prognosis of gastric cancer, and expected as a marker for target therapy because of its specific expression^[69,70].

Jeon *et al*^[71] had reported, in their trial on the comparison of the 2 markers (CEA and *MAGE*) after long-term follow-up, that *MAGE* RT-PCR showed better specificity and more significant associations with peritoneal recurrence than CEA RT-PCR, and *MAGE* RT-PCR results was proved to be the most important prognostic factor for recurrence in patients with gastric cancer after curative resection.

The future

Genetic detection in peritoneal washes with gastric carcinoma can be performed only at university hospitals and large volume cancer centers. Furthermore, there is no standard for processing of peritoneal fluid and the method of genetic detection. To make it a reliable diagnostic tool for detecting free cancer cells, the process and method of genetic detection with peritoneal washes should be standardized, and the development of simple diagnostic devices and easily available kits are necessary.

Also, effective modality of treatment for the patient with a positive peritoneal molecular diagnosis should be needed. If the molecular marker for peritoneal free cancer cell is used not only for the diagnosis but also for the therapeutic modality with the development of target therapy, it might be one of useful method for the treatment of advanced gastric cancer and the prevention of peritoneal recurrence.

CONCLUSION

The methods of detecting intraperitoneal free cancer cells represent are still area of evolution. In past, conventional cytology was regarded as the only method for detecting peritoneal free cancer cells from gastric cancer. However low sensitivity of this method had been criticized, and many studies about various method with peritoneal washes were performed for better prediction of peritoneal metastasis. In present, genetic detection using RT-PCR analysis has been used for improving the detection rate. It has been suggested that these tools have better sensitivity in detecting intraperitoneal free cancer cells with better correlation to peritoneal recurrence. But it can be performed only at university hospitals and large volume cancer centers. Furthermore, there is no standard for processing of peritoneal fluid and the method of genetic detection and effective modality of treatment for the patient with a positive peritoneal molecular diagnosis. In near future, standardization of diagnostic methods, combination of markers for improving detection rate, and development of molecular marker for target therapy could provide us with being relevant in clinical decision-making, detection methods need to be accurate, reliable, cost-effective and effective modality of treatment for the patient with a positive peritoneal diagnosis.

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