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2) 第 32 回日本外科系連合学会学術集会（2007, 06. 2321, 東京）

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村上英嗣、緒方　裕、赤木由人、石橋生哉、森眞二郎、牛島正貴、福嶋敬愛、小篠洋之、白水和雄：大腸癌肝転移に対する外科治療と成績

H．知的財産権の出願・登録状況（予定を含む。）

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

厚生労働科学研究費補助金（がん臨床研究事業）

分担研究報告書

大腸癌肝転移の肝切除後の再発形式についての検討

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研究要旨

大腸癌肝転移に対する肝切除後の予後向上のためには、肝切除後の再発形式の実態を把握し、それに対する対策を立てることは重要である。肝切除後は残肝再発および肝外再発を高頻度に認め、特に同時性肝転移では、肝切除時に微小肝転移が存在する可能性が高く、肝切除後の残肝再発予防を目的とした予防的肝動注療法は有効であった。肝切除後の再発形式を考慮した補助化学療法を選択することは重要であると考えられた。また、肝外再発は、同時性肝転移、異時性肝転移いずれも高頻度に認められたが、異時性肝転移でやや高率であった。　以上より、同時性肝転移では、肝動注療法と全身化学療法の併用が、異時性肝転移では、全身化学療法を肝切除後の補助療法として選択することは効率的な方法であると考えられた。

A．研究目的

大腸癌肝転移の肝切除後の再発形式を明らかにすることで、肝切除後の補助化学療法の選択をどのようにするか明らかにすることを目的とする。

B．研究方法

1990年から2000年までに東京都立駒込病院外科で行われた大腸癌肝転移213例（同時性：116例、異時性：97例）を対象に、肝切除後の再発形式を肝動注療法の実施の有無別に比較検討した。

（倫理面への配慮）

本研究では、個人情報が問題となることはないが、個人情報保護については十分に配慮して研究を行った。

C．研究結果

肝動注施行群と非施行群の残肝再発率は同時性肝転移で、34.7%と66.7%に対し、異時性肝転移では37.1%と47.7%で、肝動注群で有意に（$p<0.05$）残肝再発の予防効果があった。一方、肝外無再発率は、同時性肝転移で、53.1%と48.9%、異時性肝転移で71.4%と62.8%で、肝外再発は異時性肝転移例でやや多い傾向があった。

D．考察

肝切除後の残肝再発は、同時性肝転移群でやや多く、同時性肝転移では微小肝転移に対

する予防処置を行うことが必要であり、残肝
再発予防を目的-00-

E．結論

同時性肝転移と異時性肝転移では、肝切除の再発頻度が異なり、同時性肝転移では残肝再発に、異時性肝転移では肝外再発に重点を置いた補助化学療法を選択すべき である。

F．健康危険情報

なし

G．研究発表

別紙

（発表誌名巻号・頁・発行年等も記入）

H．知的財産権の出願・登録状況

なし

Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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Ⅳ. 研究成果の刊行物・別刷

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Intraoperative Quantitative Detection of CEA mRNA in the Peritoneal Lavage of Gastric Cancer Patients with Transcription Reverse-transcription Concerted (TRC) Method. A Comparative Study with Real-time Quantitative RT-PCR

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Abstract. *Background: Real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) for detection of carcinoembryonic antigen (CEA) mRNA in the peritoneal lavage of gastric cancer patients is now recognized as a useful method for the prediction of peritoneal recurrence after curative surgery. One problem with this method is that it is time-consuming and difficult to perform an intraoperative diagnosis, which is essential for intraperitoneal adjuvant chemotherapy. Patients and Methods: In order to overcome these problems, we introduced a transcription-reverse transcription concerted reaction (TRC), which is a non-PCR-based, isothermal mRNA amplification method, as an ultra-rapid diagnostic method, and compared its diagnostic power with qRT-PCR for peritoneal washes from 112 gastric cancer patients. Results: TRC measurement could be completed within 1.0-1.5 h and showed the same detection sensitivity ranging from 10^2 to 10^6 copies for standard CEA mRNA as qRT-PCR. The CEA mRNA copy number, as determined by TRC, was well correlated with the depth of tumor invasion (pT category), similar to the result obtained using qRT-PCR. With CEA mRNA copy numbers of 100 as a TRC cut-off value, the resultant sensitivity and specificity of TRC (85% and 100%, respectively) were higher than for cytology (62%, 100%) and comparable to qRT-PCR (92%, 100%). Conclusion: TRC has a diagnostic power almost equivalent to qRT-PCR but with the advantage of ultra-rapid detection. TRC would therefore be available for intraoperative sensitive diagnosis of occult tumor cells in the peritoneal cavity of gastric cancer patients.*

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Key Words: Gastric cancer, intraoperative diagnosis, TRC method, quantitative RT-PCR, peritoneal metastasis, peritoneal washes cytology.

The prognosis for advanced gastric cancer has not improved drastically despite recent advances in multimodal treatment strategy. Peritoneal carcinomatosis is the most frequent pattern of recurrence after curative surgery, and therefore the most important prognostic factor (1-3). We previously demonstrated from animal models of peritoneal micrometastasis that only early onset of chemotherapy targeting micrometastasis could effectively eliminate peritoneal metastasis and improve survival of mice remarkably, or effect a complete cure in some animals (4, 5). Therefore, development of a sensitive detection method for a small number of cancer free cells or micrometastases in the peritoneal cavity is essential for the prevention of peritoneal relapse and for establishing individualized therapy for high-risk populations of gastric cancer patients who may benefit from adjuvant chemotherapy after macroscopically complete surgical resection.

Cytological examination of the peritoneal washes sampled for the prediction of peritoneal recurrence is already an established prognostic factor (6, 7). However, conventional cytology lacks sensitivity and peritoneal recurrence has been predicted in only 50% of patients by this modality (8-10). This is because of the difficulty of detecting a small number of tumor cells at the micrometastasis level in the peritoneal washes. Qualitative and quantitative RT-PCR (qRT-PCR) with carcinoembryonic antigen (CEA) as a genetic marker is recognized to be the most reliable method to allow such sensitive detection (11-13). Cumulative evidence from many retrospective studies, as well as a prospective study, indicate

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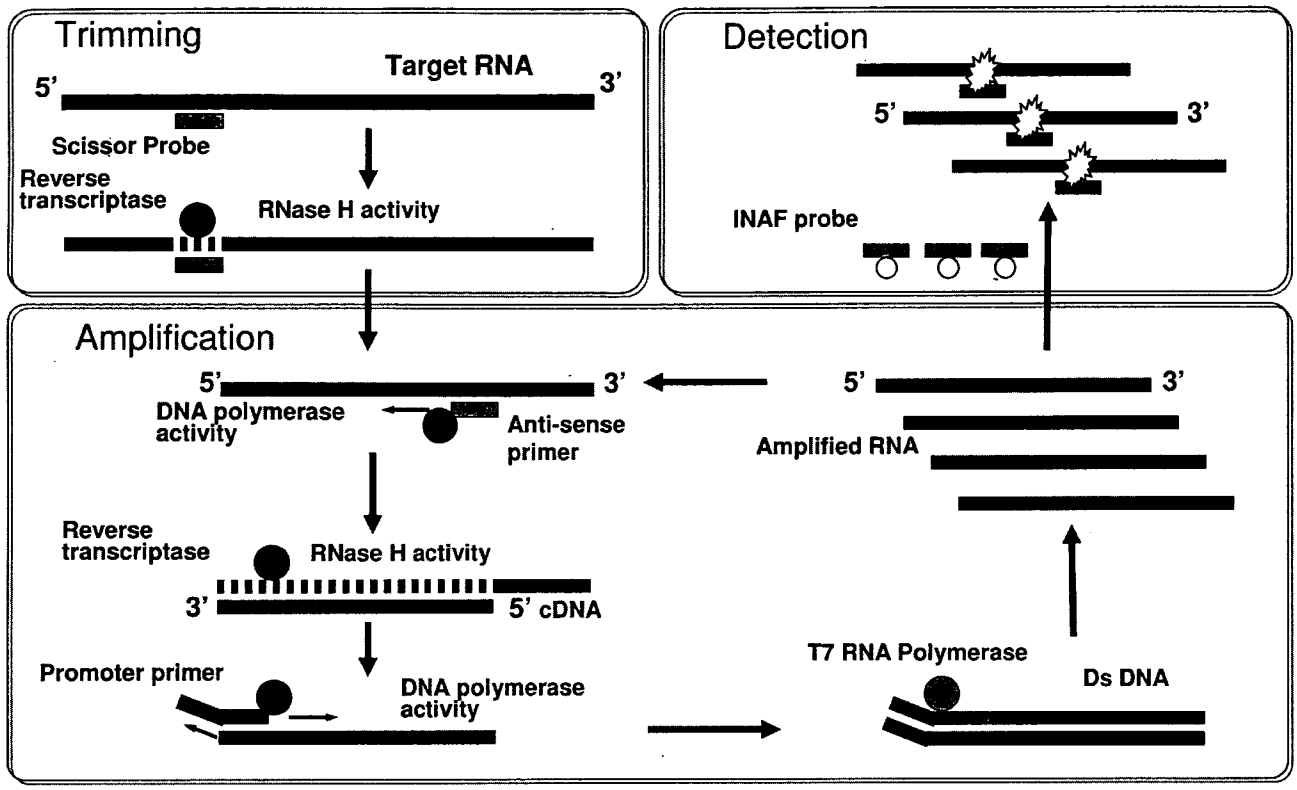

Figure 1. *Schematic representation of the principles of the TRC reaction for detection of CEA mRNA. It consists of a sequence of steps including trimming of mRNA with scissor probe and RNaseH activity of RT, complementary DNA (cDNA) synthesis with RT, double-stranded DNA (dsDNA) synthesis by DNA polymerase activity of RT and subsequent transcription (mRNA amplification) of promoter-bearing dsDNA with T7 RNA polymerase. Detection of amplified CEA mRNA is achieved with a CEA-specific intercalation activating fluorescence (INAF) hybridization probe. RT: Reverse transcriptase.*

reaction mixture simultaneously (excitation wavelength, 470 nm and emission wavelength, 520 nm).

Each run consisted of patient samples with unknown CEA mRNA concentrations (up to 12 samples), a negative control without a template and CEA mRNA standards. Standard mRNA containing the near full-length CEA mRNA was synthesized *via in vitro* transcription of promoter bearing double-stranded DNA as a template with SP6 RNA polymerase. Two external CEA mRNA standards (low copy standard: $1x10^2$ copies and high copy standard: $1x10^6$ copies) were used for making a calibration curve. Quantitation of CEA mRNA in each sample was performed automatically by reference to this standard curve constructed each time using the TRCR-160 software. With this software, the samples calculated below 10 copies are displayed <10, because the dynamic range for the detection of CEA mRNA was from 10^2 to $1x10^6$ copies.

Real-time quantitative RT-PCR. cDNA was synthesized from total RNA using random hexanucleotide primers (Pharmacia, Biotech, Uppsala, Sweden) and SuperScript II RNase H-reverse transcriptase (Invitrogen, Carsbad, CA, USA) according to the manufacturer's instructions. The resultant first-strand cDNA was stored at -80°C until analysis.

Single-step real-time RT-PCR for CEA mRNA was performed using CEA-specific oligonucleotide primers and two fluorescent hybridization probes on the LightCycler instrument (Roche Diagnostics, Mannheim, Germany) as described elsewhere (13). To quantify and demonstrate the integrity of the isolated RNA, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was also analyzed with real-time RT-PCR using the appropriate primers and

Table I. *Oligonucleotide sequence of promoter primer, antisense primer, scissor probe and INAF probe for TRC method used in this study*

Promoter primer (1508-1530)[a] 23 mer
5'-*AAT TCT AAT ACG ACT CAC TAT AGG GAG A*CC AAC ATC ACT GAG AAG AAC AGC-3'
Antisense primer (1668-1687) 20 mer
5'-GTT CAC AGG TGA AGG CCA CA-3'
Scissor probe (1490-1513) 24 mer
5'-TGT TGG AGA TAA AGA GCT CTT GTG-3'
INAF probe (1582-1601) 20 mer
5'-ACT GTG ATT GTC TTG ACT GT-3'

[a]Numbers in parentheses indicate corresponding position of the target genome sequences (GeneBank Accession No. M29540); sequence of the promoter primers in italics is the T7 RNA polymerase binding sequence.

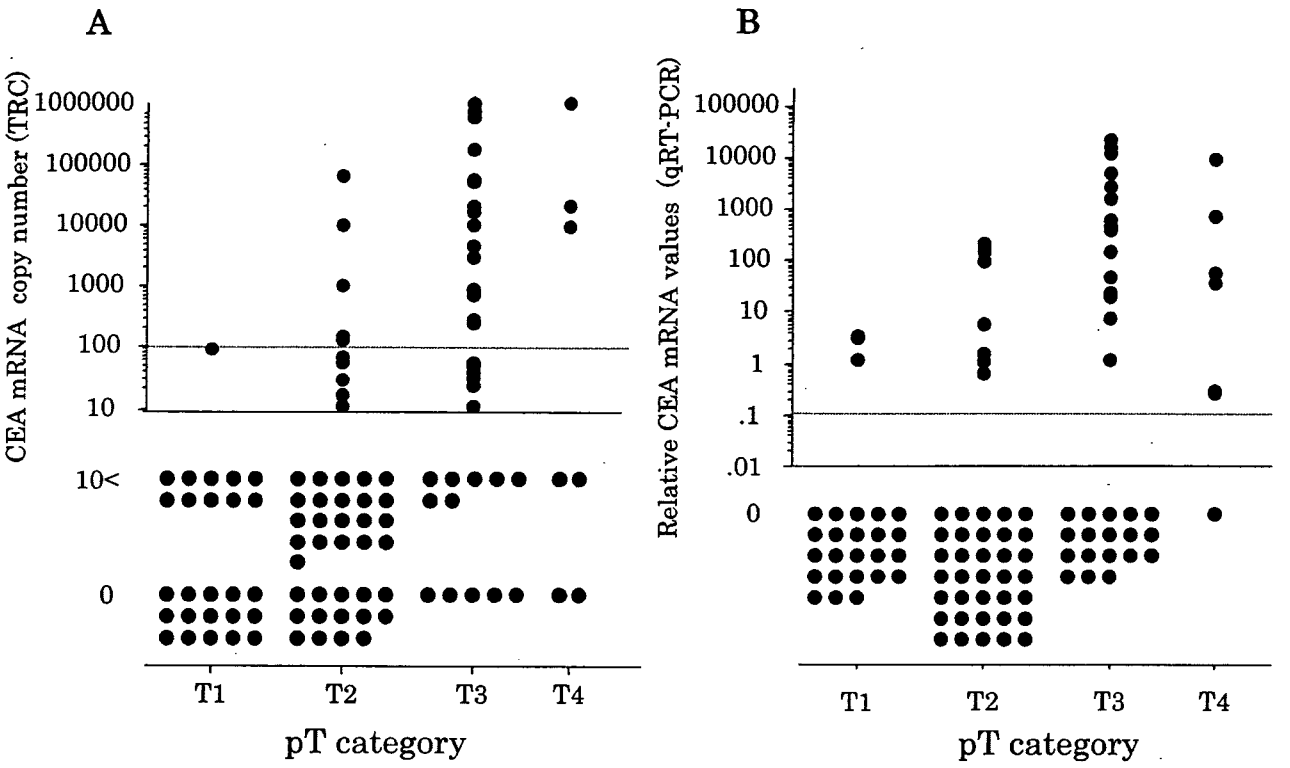

Figure 3. *Comparison of CEA mRNA expression of peritoneal washes from gastric cancer patients measured with TRC and qRT-PCR according to the depth of invasion (pT category). (A) CEA mRNA copies quantitated with TRC. (B) Relative CEA mRNA values assessed with qRT-PCR. In the TRC method, samples with CEA mRNA less than 10 copies are represented as 10< en bloc, not as real values determined by the exploration of the curve. Definition of pT category is as follows: T1, mucosal to submucosal invasion; T2, muscularis propria to subserosal invasion; T3, serosal invasion and T4, invasion to adjacent tissues.*

$1x10^2$ to $1x10^6$ copies of CEA mRNA, indicating the proof of quantification of sample with unknown messages by this TRC method.

Comparison of detection sensitivity and time between TRC and qRT-PCR. Standard CEA mRNA ($1x10^6$ copies) and cDNA synthesized from this mRNA preparation with reverse transcriptase were serially diluted (1x10-$1x10^6$ copies) and then subjected to measurement with TRC and qRT-PCR, respectively. Figure 2C shows the log-linear correlation of CEA mRNA values measured by both TRC and qRT-PCR ranging from $1x10^2$-$1x10^6$ copy, indicating almost the equivalent detection sensitivity and dynamic range of both methods. Total assay time for qRT-PCR, including pretreatment of peritoneal washes (10 min), RNA extraction and cDNA synthesis (90 min), along with amplification and subsequent real-time data analysis (70 min), was approximately 3 hours, whereas the entire reaction time with TRC, including pretreatment of peritoneal washes (10 min), RNA extraction (30-50 min), amplification and subsequent real-time data analysis (20 min), was only 1.0-1.5 hours, less than half the period required for qRT-PCR.

CEA mRNA level in peritoneal washes according to depth of tumor invasion (pT category). The average CEA mRNA copy numbers of the peritoneal washes as measured by TRC (T1: 7.2, T2: 1,700, T3: 105,027 and T4: 147,051) were well correlated with the depth of tumor invasion (Figure 3A), similar to the average CEA mRNA values (T1: 0.29, T2: 14.4, T3: 1,755 and T4: 1,368) as determined using qRT-PCR (Figure 3B). Median CEA mRNA copies as assessed using TRC and qRT-PCR were (T1: 0, T2: 10, T3: 51 and T4: 10) and (T1, T2, T3: 0 and T4: 37.18), respectively. The mean relative CEA mRNA value of peritoneal washes as measured with TRC in the 9 mucosa-confined gastric cancer patients, which were considered clinically benign in terms of peritoneal metastasis and therefore as negative controls in this study, was 11.9±28.5 (SD). No CEA mRNA was detected using TRC in the peripheral blood leukocytes from 10 healthy volunteers or primary human cultured mesothelial cells, the 2 major cellular constituents in peritoneal washes. CEA mRNA values of the patients with synchronous peritoneal metastasis with TRC ranged from 10 to 1,000,000.

Cut-off value of the TRC method. In the present study, the cut-off value of CEA mRNA copies with the TRC method