



삼차원 조건적 역분화 배양법을 이용한 순환종양세포의 검출

Detection of Circulating Tumor Cells Using Three-dimensional and Conditionally Reprogrammed Culture Methods

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Background: Circulating tumor cells (CTCs) are rare in the circulation of patients with tumors and are very difficult to detect. In this study, we cultured CTCs in a three-dimensionally reprogrammed condition using the peripheral blood of patients with cancer.

Methods: We evaluated the sensitivity of a three-dimensional (3D) conditionally reprogrammed cell (CRC) culture method using SNU-484 gastric cancer cells. From April 2017 to January 2018, blood was collected from 30 patients diagnosed with colorectal or breast cancer at the Daegu Catholic University Medical Center. After lysis of the red blood cells, the blood samples were centrifuged to isolate the mononuclear cells. We used Matrigel to create 3D conditions and reprogrammed the isolated cells using a Rho-kinase inhibitor and J2 feeder cells. After 30 days of culture, the cells were collected and RNA was extracted and amplified for the expression of *GAPD*, *hTERT*, and *MAGE A1-6* using real-time PCR.

Results: The SNU-484 cells were cultured from three cells using the 3D CRC culture method. The cultured blood samples showed some cells or few colonies. The positive rates of *hTERT* and *MAGE A1-6* mRNA were 50% and 30%, respectively. The positive rates of both mRNAs were 60% and 20%, respectively. *MAGE* and both mRNAs showed significantly increased odds ratios at the tumor, nodes, and metastases (TNM), N, and M stages in all subjects.

Conclusions: As 60% of CTCs were detected in the blood of 30 patients with cancer, the 3D CRC culture method was very effective for CTC culture.

Key Words: Neoplastic cells, Circulating, Breast cancer, Colorectal cancer, Culture

INTRODUCTION

Circulating tumor cells (CTCs) were first reported by Thomas Ashworth in 1869 [1] as a small number of tumor cells found in

the blood of patients with tumors. Several studies have shown that CTCs play an important role in tumor metastasis through epithelial-mesenchymal transition [2] or tumor stem cells [3], and the analysis of molecular biological features by detecting CTCs is important for the diagnosis and prognosis of various tumor diseases [4]. These advantages have led to the concept of ‘liquid biopsy’ [5] to diagnose tumors by detecting CTCs, plasma-free DNA, and exosomes in the blood.

CTCs are present in trace amounts in the blood [6], are physically and immunologically removed from the circulation [7], and have a short half-life of 1–2.4 hours [8]; therefore, it is not easy to detect CTCs in the blood. To overcome these limitations, many separation methods have been developed based on the characteristics of CTCs. Biophysical separation methods utilize the size of CTCs [9] and the difference in the rate of movement in the elec-

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tric field [10]. Immunological separation methods use monoclonal antibodies against epithelial cell adhesion molecule (EpCAM) [11] or tumor cell-specific antigens [12].

There have been many attempts to cultivate isolated CTCs [13, 14], but the isolation process is difficult and shows a low positive rate of 10–20%. To increase the success rate of CTC culture, we have attempted to culture tumor cells in the blood using the conditionally reprogrammed cell (CRC) culture method [15, 16]. CRC culture is a method in which normal and tumor cells of the epithelial lineage are immortalized when the feeder cells and the Rho-kinase inhibitor (Y-27632) are used together to activate intracellular telomerase and regulate the cell cycle by suppressing the p16/Rb pathway [17]. Unlike cell immortalization by viral oncogenic mRNA transduction, the reprogrammed cell population maintains a normal karyotype and the original phenotype [18]. The CTC culture-positive rates obtained in a previous study using the CRC culture method were 33.3% for breast cancer and 16.1% for colorectal cancer [15, 16].

To further increase the CTC detection rate, this study intends to detect CTCs using a three-dimensional (3D) CRC culture technique using Matrigel. Matrigel is a reconstructed basement membrane matrix from mouse Engelbreth-Holm-Swarm (EHS) sarcoma [19], used for 3D culture. It contains components, such as collagen and laminin, as well as various growth factors secreted from the extracellular matrix [19], which promote the growth of tumor cells and stimulate the invasion of tumor cells in vitro [20].

MATERIALS AND METHODS

1. Sensitivity of 3D CRC culture method

To evaluate the sensitivity of the 3D CRC culture method, we used SNU-484 gastric cancer cells obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea). A six-well plate was coated with 400 μ L of Matrigel (Corning, Bedford, MA, USA) and the SNU-484 cells were inoculated at concentrations of 1, 3, 6, 13, 25, and 100 cells and then incubated for 4 weeks with the conditioned F media [17]. After measuring the number of 100 SNU-484 cells using a cell counter EVE (NanoEnTek, Seoul, Korea), the number of cells was adjusted by a 2-fold serial dilution. After 4 weeks, we confirmed the culture of SNU-484 cells by observing colony formation under a microscope. This experiment was repeated four times.

2. Patients and samples

From April 2017 to January 2018, using cell-free DNA BCT bottles (Streck, Omaha, NE, USA), 6 mL of blood samples were collected from patients (with informed consent) diagnosed with colorectal or breast cancer at the Daegu Catholic University Medical Center. Patients with anemia were excluded (males and females with hemoglobin less than 10 g/dL and 8 g/dL, respectively), and a total of 30 samples were drawn. Blood sampling was performed in the proximal vein of the tumor during surgery in patients who underwent tumor removal surgery or in the peripheral vein in the unoperated group. In addition, information such as sex, age, diagnosis, and cancer stage was collected from the electronic medical records of the hospital. Cancer staging was assessed according to the 7th edition of the American Joint Committee on Cancer Staging Manual. This study was approved by the institutional review board of Daegu Catholic University Medical Center (CR-17-051-R).

3. 3D CTC culture method

1) Mononuclear cell collection

The venous blood collected from the patients was centrifuged at $300 \times g$ for 20 minutes at room temperature (22 to 24°C) to remove plasma components. Then, 6 mL of RBC lysis buffer (Roche Diagnostics GmbH, Mannheim, Germany) was added to remove the red blood cells, and the mixture was rolled at room temperature for 15 minutes. The mixture was centrifuged at $350 \times g$ for 10 minutes at room temperature, the supernatant was removed, and the remaining cell layer was used for culturing.

2) Cell lines

Swiss 3T3 mouse fibroblasts (J2 strain, obtained from the Georgetown University Medical Center) were used as feeder cells. The SNU-484 cell line was purchased from KCLB and used as a positive control in mRNA real-time PCR.

3) Preparation of conditioned F media

F media from DMEM media supplemented with fetal bovine serum, glutamine, penicillin/streptomycin, Ham's F12 nutrient mixture, hydrocortisone, epidermal growth factor, insulin, cholera toxin, fungizone, and gentamycin (all from Thermo Scientific, Waltham, MA, USA) was used for culturing J2 fibroblasts. The cultured J2 cells were separated from the culture dish using trypsin-

EDTA, irradiated with 30 Gy of gamma rays (Gammacell 3000 Elan; MDS Nordion, Ottawa, Canada), and then plated again in 30 mL of F medium. The F medium with irradiated J2 cells was collected 3 days later. Three volumes of the obtained supernatant were mixed with one volume of fresh F medium, and 5 μ mol/L Y-27632 (ENZO Life Sciences, Farmingdale, NY, USA) was added to prepare conditioned F medium for culturing the CTCs.

4) 3D CTC culture using conditioned F media

For the 3D culture, 500 μ L of Matrigel Matrix was coated on a 35 mm Petri dish (Sarstedt, Nümbrecht, Germany) according to the manufacturer's instructions (Thin Gel). The coated dish was inoculated with a mononuclear component containing isolated CTCs and cultured in 3 mL of conditioned F medium for 1 month at 37°C in a 5% CO₂ environment. During the incubation period, cell growth was observed using a phase-contrast inverted microscope (Eclipse Ti-U; Nikon, Tokyo, Japan) and photographed. After the culture was completed, the cells were separated from the culture dish using 10 units of Dispase (Corning) and then used for detection.

4. CTC detection with real-time PCR

Cells isolated from Matrigel were centrifuged at 1,000 \times g for 5 minutes to obtain cell pellets, and RNA was extracted using TRIzol reagent (Invitrogen; Thermo Scientific). The extracted RNA was reverse transcribed using the ImProm-II Reverse Transcription System (Promega Corporation, Madison, WI, USA) to obtain cDNA. Melanoma-associated antigen (*MAGE*) A1-6, human telomerase reverse transcriptase (*hTERT*), and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) mRNA were de-

tected using real-time PCR and a LightCycler 480 (Roche Diagnostics GmbH). The *hTERT* and *MAGE* A1-6 mRNAs were amplified using real-time nested PCR and a LightCycler 480 SYBR Green I Master (Roche Diagnostics GmbH), but the cycles of PCR were different. We interpreted the PCR products as positive results using fluorescent signals and melting temperature analysis. The primers used for each polymerization reaction and amplification process are listed in Table 1.

5. Statistical analysis

The results were analyzed using SPSS Statistics 20 (International Business Machines Co., Armonk, NY, USA), and Fisher's exact test and logistic regression analysis were used to compare the positive rates of *hTERT* and *MAGE* A1-6 real-time PCR between cancer stages.

RESULTS

1. Sensitivity of 3D CRC culture method

The SNU-484 cell line was cultured when 13 or more cells were added; 75% with six cells, 50% with three cells, and no culture with one cell (Table 2). The SNU-484 cells were cultured from three cells using the 3D CRC culture method.

2. CTC culture with 3D CRC culture

Within 1 week of culture, all specimens showed cell aggregates; and after 4 weeks, all specimens showed some cells, cell aggregates, or small colonies under a microscope. Fig. 1A, B show a colony of well grown colorectal and breast cancers, respectively, imaged using phase-contrast microscopy. Colorectal tumor cells

Table 1. Primer sequence for each mRNA, product base pair, and PCR process

mRNA	Primer sequence	Product (bp)	PCR process
<i>GAPDH</i>	OF 5'-TCG GAG TCA ACG GAT TTG GTC GTA-3'	320	95°C 5 s, 59°C 5 s, 72°C 18 s, 33 cycles
	OR 5'-CAA ATG AGC CCC AGC CTT CTC CA-3'		
<i>hTERT</i>	OF 5'-CGG GCT GCT CCT GCG TTT GGT G-3'	311	95°C 5 s, 68°C 5 s, 72°C 16 s, 25 cycles
	OR 5'-AGC CGC GGT TGA AGG TGA GAC TGG-3'	152	95°C 5 s, 66°C 5 s, 72°C 10 s, 25 cycles
	IF 5'-TCA CCT CAC CCA CGC GAA AAC CTT-3'		
	IR 5'-CGT GGG CCG GCA TCT GAA CAA A-3'		
<i>MAGE</i> A1-6	OF 5'-CTG AAG GAG AAG ATC TGC C-3'	465	95°C 5 s, 60°C 5 s, 72°C 25 s, 30 cycles
	OR 5'-CCA GCA TTT CTG CCT TTG TGA-3'	262	95°C 5 s, 62°C 5 s, 72°C 12 s, 25 cycles
	IF 5'-AAG GAG AAG ATC TGC CAG TG-3'		
	IR 5'-GAG GCT CCC TGA GGA CTC T-3'		

Abbreviations: *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *hTERT*, human telomerase reverse transcriptase; *MAGE*, melanoma-associated antigen; OF, outer forward; OR, outer reverse; IF, inner forward; IR, inner reverse.

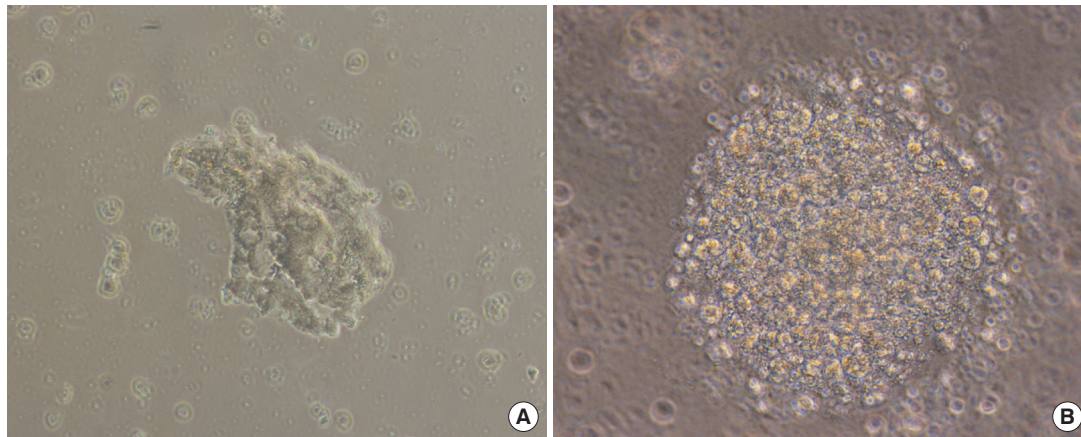


Fig. 1. (A) Cultured cells from a patient with colorectal cancer, captured on day 18 of culture, at 300× magnification. (B) Cultured cells from a patient with breast cancer, captured on day 15 of culture, at 300× magnification. Colorectal tumor cells formed large undifferentiated aggregates while breast tumor cells formed large spheres.

Table 2. Sensitivity of three-dimensional CRC culture method

Cell line	Cell count	Matrigel (μL)	Number	Positive rates (%)
SNU-484	100	400	4	100
SNU-484	25	400	4	100
SNU-484	13	400	4	100
SNU-484	6	400	4	75
SNU-484	3	400	4	50
SNU-484	1	400	4	0

formed large undifferentiated aggregates, whereas breast tumor cells formed large spheres

3. Real-time PCR of CTC culture

Table 3 shows the tumor staging and PCR results for patients with breast cancer. Using RNA extracted from the cultured cells, *GAPDH* mRNA was positive in all patients, *bTERT* mRNA in 25% (2/8), and *MAGE* A1-6 mRNA in 12.5% (1/8). None of the mRNAs were positive for both the mRNAs, and three (37.5%) were positive for either mRNA. Most patients with breast cancer were in cancer stage I or lower.

Table 4 shows the tumor staging and PCR results for the CRC tumor group. *GAPDH* mRNA was positive in all patients, *bTERT* mRNA in 59.1% (13/22), and *MAGE* A1-6 mRNA in 36.4% (8/22). Six (27.3%) were positive for both the mRNAs, and 15 (68.2%) were positive for either mRNA. In patients with colorectal cancer, patients with stage II or lower showed significant differences in both real-time PCR positive rates compared to patients with stage III or higher ($P < 0.05$).

The total positive rates in the patients with breast and colorectal

Table 3. Demographic information and PCR results of patients with breast cancer

Sex	Age	Stage	T	N	M	<i>hTERT</i>	<i>MAGE</i>	<i>hTERT</i> and <i>MAGE</i>	<i>hTERT</i> or <i>MAGE</i>
F	78	0	0	0	0	-	-	-	-
F	55	0	0	0	0	-	-	-	-
F	45	Ia	1	0	0	-	-	-	-
F	50	Ia	1	0	0	-	-	-	-
F	42	Ia	1	0	0	-	-	-	-
F	53	Ia	1	0	0	+	-	-	+
F	50	IIa	1	1	0	+	-	-	+
F	50	IIIc	1	3	0	-	+	-	+
Positive rate (N=8)						25%	12.50%	0	37.50%

GAPDH results are omitted.

Abbreviations: *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *hTERT*, human telomerase reverse transcriptase; *MAGE*, melanoma-associated antigen.

cancers were as follows: *bTERT* mRNA in 50% (15/30) and *MAGE* A1-6 mRNA in 30% (9/30). Six (20%) were positive for both the mRNAs, and 18 (60%) were positive for either mRNA.

4. Odds ratio of the cancer stage of patients with PCR results

Significant associations were found between the cancer stage of patients and the PCR results of the cultured cells. Both the mRNAs (*MAGE* and *bTERT*) showed significantly increased odds ratios at the TNM, N, and M stages in all subjects. In patients with breast cancer, analysis was not possible due to the small number of subjects; and in patients with colorectal cancer, these were significant at the M stage ($P < 0.05$, Table 5).

Table 4. Demographic information and PCR results of patients with colorectal cancer

Sex	Age	TNM Stage	T	N	M	<i>hTERT</i>	<i>MAGE</i>	<i>hTERT</i> and <i>MAGE</i>	<i>hTERT</i> or <i>MAGE</i>
M	56	I	1	0	0	-	-	-	-
M	72	I	2	0	0	+	-	-	+
F	88	IIa	3	0	0	-	-	-	-
M	62	IIa	3	0	0	-	-	-	-
M	55	IIa	3	0	0	+	-	-	+
F	48	IIa	3	0	0	+	-	-	+
M	79	IIa	3	0	0	+	-	-	+
M	75	IIa	3	0	0	+	-	-	+
M	77	IIa	3	0	0	-	+	-	+
M	80	IIIa	3	1	0	+	+	+	+
F	50	IIIb	2	2	0	-	-	-	-
M	59	IIIb	3	1	0	-	-	-	-
F	61	IIIb	3	1	0	+	-	-	+
M	64	IIIb	3	1	0	+	-	-	+
M	68	IIIb	3	2	0	-	-	-	-
F	78	IIIb	3	2	0	-	+	-	+
F	87	IIIb	4	1	0	+	+	+	+
M	72	IVa	3	1	1	-	-	-	-
M	36	IVa	3	2	1	+	+	+	+
M	80	IVa	3	2	1	+	+	+	+
M	32	IVa	4	1	1	+	+	+	+
M	64	IVa	4	2	1	+	+	+	+
Positive rates for stages I, II (N=9)						55.60%	11.10%	0	66.70%
Positive rates for stages III, IV (N=13)						61.50%	53.80%	46.2%*	69.20%
Total positive rate						59.10%	36.40%	27.30%	68.20%

*Significant difference between the two subgroups ($P < 0.05$).Abbreviations: *hTERT*, human telomerase reverse transcriptase; *MAGE*, melanoma-associated antigen.

DISCUSSION

Detection of CTCs in the blood has been investigated using various methods based on antibodies, transcripts, or proteins [21, 22]. Recently, many researchers have utilized cell-free DNA (cfDNA) instead of CTCs [23-25]. In some solid tumors, cfDNA has produced promising results [26]. However, this approach is limited because cfDNA yields the same information regarding mutations and epigenetic modifications as the tumor tissue without providing any information about protein and RNA transcription profiles of the tumor phenotype [27]. As CTCs can provide all information about tumor cells, if the low culture-positive rate can be increased, the CTC culture method would be the most suitable method for studying tumor progression and metastasis.

To efficiently separate CTCs, we used the CD45 depletion method [28], MetaCell [29], and RBC lysis buffer. In our experience, the CD45 depletion method required a lot of time, and the MetaCell method did not separate CTCs well depending on the sample, therefore, we used the RBC lysis method. For the successive culture of CTCs, blood collection tubes and prompt processing of blood specimens are important. Rodríguez-Lee et al. [30] showed that overall cell retention was optimal in cfDNA tubes at 24 hours. Therefore, we collected blood specimens with Streck cfDNA tubes and processed the CRC culture within 24 hours. To determine whether the cultured cells were cancer cells, we as-

Table 5. Odds ratio of the cancer stage of patients with PCR positive results

PCR	TNM stage (> III vs. < II)		T stage (> 3 vs. < 2)		N stage (> 1 vs. 0)		M stage (> 1 vs. 0)	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Total (N=30)								
<i>hTERT</i>	2.25 (0.52-9.7)	0.277	1.33 (0.3-5.91)	0.705	2.25 (0.52-9.7)	0.277	5.09 (0.5-52.29)	0.171
<i>MAGE</i>	7.56 (1.5-38.15)	0.014*	0.9 (0.37-2.21)	0.819	7.56 (1.5-38.15)	0.014*	16 (1.45-176.5)	0.024*
Both	5.44 (1.14-25.95)	0.033*	0.46 (0.18-1.21)	0.117	5.44 (1.14-25.95)	0.033*	46 (3.33-634.9)	0.004*
Either	2.98 (0.68-13.17)	0.149	2.6 (0.56-2.02)	0.221	2.98 (0.68-13.17)	0.149	3.14 (0.31-32.28)	0.335
BRC (N=8)								
<i>hTERT</i>	5 (0.15-66.59)	0.368	NA		5 (0.15-66.59)	0.368	NA	
<i>MAGE</i>	NA		NA		NA		NA	
Both	NA		NA		NA		NA	
Either	NA		NA		NA		NA	
CRC (N=22)								
<i>hTERT</i>	1.28 (0.23-7.19)	0.779	1.28 (0.23-7.19)	0.779	1.28 (0.23-7.19)	0.779	3.56 (0.33-38.78)	0.298
<i>MAGE</i>	9.33 (0.89-97.62)	0.062	1.6 (0.52-4.89)	0.410	9.33 (0.89-97.62)	0.062	13 (1.11-152.4)	0.041*
Both	4.59 (0.76-27.62)	0.096	0.86 (0.29-2.55)	0.782	4.59 (0.76-27.62)	0.096	30 (2.14-421.1)	0.012*
Either	1.12 (0.18-6.93)	0.899	2.67 (0.42-6.83)	0.297	1.12 (0.18-6.93)	0.899	2.18 (0.2-24.21)	0.525

*Significant correlation. $P < 0.05$.Abbreviations: BRC, breast cancer; CRC, colorectal cancer; *hTERT*, human telomerase reverse transcriptase; *MAGE*, melanoma-associated antigen; OR, odds ratio; CI, confidence interval; NA, not applicable.

sayed for *MAGE* and *bTERT* mRNA. In our previous study [15, 16, 28], these two genes were also used to identify tumor cells in CTC cultures. Although *bTERT* mRNA might be expressed in the cells of the CRC culture [17], its expression could differentiate human cancer cells from mouse fibroblast J2 cells due to its positive and negative expression, respectively.

The sensitivity of the CRC culture method was also evaluated. As the cell concentration was measured using the dilution method, the number of cells used for the sensitivity measurement may not be accurate. To overcome this limitation, the sensitivity evaluation was repeated four times. In a previous study [16], the minimum number of SNU-484 cells capable of forming a colony was six. Liu et al. [17] reported a minimum number of four (primary keratinocytes) and two (cervical cancer cells). At present, the minimum number is three. However, in Liu's study, there was no description of the number of repetitions and how to measure the number of injected cells, therefore, direct comparison of results may be difficult. Our previous studies used a three-dimensional (3D) CRC technique, whereas this study used a 3D CRC technique. These results suggest that 3D CRC culture enhanced the ability to detect CTCs.

With 3D CRC culture, cell aggregates or colonies were observed in all 30 cultured specimens, but not all the cultured cells were CTCs when PCR results were considered. As shown in Table 3, the positive rate of CTC in patients with breast cancer was 37.5%. In a study by Zhang et al. [13], in which CTCs were first cultured in vitro, three out of 15 (20%) patients with invasive breast cancer were positive for culture. In a study by Yu et al. [14], six metastatic cancers out of 36 breast cancer samples (16.7%) were cultured; however, in a previous study by the author [15] using the 2D CRC culture method showed a positive rate of 33.3%, indicating a positive rate similar to that in this study. As shown in Table 4, the positive rate of CTCs in patients with colorectal cancer was 68.2%. To detect CTCs in patients with colorectal cancer, a detection method using surface antigen or cfDNA was mainly used rather than culture [31-34]. The CTC detection rate varied from 18.9-91.3% [31-34], and was proportional to the cancer stage, and the DNA detection method showed a higher positivity than the surface antigen detection method. In a study by Cayrefourcq [31] in patients with metastatic colorectal cancer, the positive rates of surface antigen detection and culture were 70.4% and 2.8%, respectively. Our study [16] using a 2D CRC culture showed a posi-

tive rate of 16.1%. Therefore, the 2D CRC culture method is very useful for detecting CTCs in patients with CRC. Compared to the 2D culture method, the 3D CRC culture method showed a similar CTC detection rate in breast cancer patients but was very high in colorectal cancer patients. The proportion of patients with breast and colorectal cancers with stage 1 or less was 75.0% and 17.9%, respectively, which might explain why the effect of the 3D CRC culture method is not prominent in patients with breast cancer with many early stage cancers.

In the association study between cancer stage and real-time PCR results, the results of *MAGE* and both real-time PCR showed increased odds ratios at the TNM, N, and M stages. In previous studies on CRC culture [15, 16], *bTERT* showed higher sensitivity than *MAGE* despite *MAGE* known to be expressed specifically in tumor cells.

A limitation of this study is that the number of patients with colorectal and breast cancers was not large. The number of patients with colorectal cancer was not sufficient for each stage, therefore, they were grouped into two groups for statistical analysis. The number of patients with breast cancer was particularly small; therefore, statistical analysis was not performed.

The CTC culture method has not been utilized in the clinical field because of its low positive rate; however, this study showed new hopes for 3D CTC culture by showing a CTC detection rate of 68.2% in patients with colorectal cancer. In future studies, we will confirm the sensitivity of the 3D CRC culture method by supplementing the number of cases and analyzing the characteristics of the tumor from the cultured cells in more detail.

요 약

배경: 혈중종양세포는 종양환자의 혈액에서 소량으로 존재하여 검출이 매우 어렵다. 본 연구에서는 종양환자에서 말초혈액을 이용하여 혈중종양세포를 3차원 재분화 조건하에서 배양하였다.

방법: SNU-484 위암세포주를 사용하여 3차원 재분화 배양법의 민감도를 확인하였다. 2017년 4월부터 2018년 1월까지 대구가톨릭 의료원에서 대장암 및 유방암으로 진단받은 30명의 혈액을 배양하였다. 혈액내 적혈구를 용혈시킨 후 원심 하여 단핵구 세포층을 분리하였다. Matrigel을 사용하여 3차원 배양조건을, Rho-kinase 억제제와 J2 세포주를 이용하여 재분화 조건을 각각 형성하였다. 배양 30일 후 배양된 세포를 수거하여 RNA를 추출하였고 *GAPD*, *bTERT* 및 *MAGE* A1-6 유전자를 실시간 중합효소연쇄반응으로

증폭하였다.

결과: SNU-344 세포주를 사용한 3차원 재분화 배양법으로 3개 세포부터 배양되었다. 말초혈액을 배양하여 세포 응집이나 소수의 세포 집락을 형성하였다. 배양된 세포들의 *bTERT* 및 *MAGE A1-6* 유전자 양성률은 각각 50% 및 30%였다. 두가지 유전자중 한 개 유전자 및 동시양성률은 각각 60% 및 20%였다. *MAGE* 양성 및 동시양성은 환자들의 TNM 병기, N 병기 및 M 병기에 따라 의미 있는 교차비 증가를 나타내었다.

결론: 30명의 종양환자의 말초혈액에서 60%의 혈중종양세포 검출률을 나타내어 3차원적 재분화 배양법은 혈중종양세포 검출에 매우 유용하였다.

Conflicts of Interest

None declared.

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