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-
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 × 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 × 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 (Gammasell 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 µmol/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 medium

For the 3D culture, 500 µL 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 × 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 detected 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

| mRNA    | Primer sequence                                      | Product (bp) | PCR process |
|---------|-------------------------------------------------------|--------------|-------------|
| GAPDH   | OF 5’-TCG GAG TCA AGC GAT TIG 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 CTC TCT CA-3’              |              |             |
| hTERT   | OF 5’-CGG GCT GCT CCT GCG TTT GTT G-3’               | 311          | 95°C 5 s, 68°C 5 s, 72°C 16 s, 25 cycles |
|         | OR 5’-AGC GCC GGT TGA AGG TGA GAC TGG-3’             |              |             |
|         | IF 5’-TCA CCT CAC CCA GGC GAA AAG CCT T3’           | 152          | 95°C 5 s, 66°C 5 s, 72°C 10 s, 25 cycles |
|         | IR 5’-CGT GCG GCG GCA TCT GAA CAA A-3’              |              |             |
| MAGE 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 TTI CCT TIG CCT TGA-3’                |              |             |
|         | IF 5’-AAG GAG AAG ATC TGC CAG T-3’                 | 262          | 95°C 5 s, 62°C 5 s, 72°C 12 s, 25 cycles |
|         | IR 5’-GAG GCC GCC GCC CAA CCA-3’                   |              |             |

Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; hTERT, human telomerase reverse transcriptase; MAGE, melanoma-associated antigen; OF, outer forward; OR, outer reverse; IR, inner reverse; IF, inner forward; IR, inner reverse.
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, hTERT 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, hTERT 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 cancers were as follows: hTERT 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 hTERT) 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).
### 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 4. Demographic information and PCR results of patients with colorectal cancer**

| Sex | Age | TNM Stage | T | N | M | hTERT | MAGE | MAGE or hTERT |
|-----|-----|-----------|---|---|---|-------|-------|---------------|
| 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  | IIa       | 3 | 1 | 0 | +     | +     | +             |
| F   | 50  | IIb       | 2 | 2 | 0 | -     | -     | -             |
| M   | 59  | IIb       | 3 | 1 | 0 | -     | -     | -             |
| F   | 61  | IIb       | 3 | 1 | 0 | +     | -     | -             |
| M   | 64  | IIb       | 3 | 1 | 0 | +     | -     | -             |
| M   | 68  | IIb       | 3 | 2 | 0 | +     | -     | -             |
| F   | 78  | IIb       | 3 | 2 | 0 | +     | -     | -             |
| F   | 87  | IIb       | 4 | 1 | 0 | +     | +     | +             |
| M   | 72  | Iva       | 3 | 1 | 1 | -     | -     | -             |
| M   | 36  | Iva       | 3 | 2 | 1 | +     | +     | +             |
| M   | 80  | Iva       | 3 | 2 | 1 | +     | +     | +             |
| M   | 52  | 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.

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

| PCR | T/M 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)** | 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 |
| hTERT | 7.56 (1.5-38.15) | 0.014* | 0.9 (0.3-2.21) | 0.819 | 7.56 (1.5-38.15) | 0.014* | 16 (1.45-176.5) | 0.024* |
| MAGE | 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* |
| Both | 2.98 (0.68-13.17) | 0.149 | 2.6 (0.56-20.2) | 0.221 | 2.98 (0.68-13.17) | 0.149 | 3.14 (0.31-32.28) | 0.335 |
| **BRC (N=8)** | 5 (0.15-66.59) | 0.368 | NA | NA | 5 (0.15-66.59) | 0.368 | NA | NA |
| hTERT | NA | NA | NA | NA | NA | NA | NA | NA |
| MAGE | NA | NA | NA | NA | NA | NA | NA | NA |
| Both | NA | NA | NA | NA | NA | NA | NA | NA |
| Either | NA | NA | NA | NA | NA | NA | NA | NA |
| **CRC (N=22)** | 1.28 (0.23-7.19) | 0.779 | 1.28 (0.23-7.19) | 0.779 | 12.8 (0.23-7.19) | 0.779 | 3.56 (0.33-38.78) | 0.298 |
| hTERT | 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* |
| MAGE | 4.59 (0.76-27.2) | 0.096 | 0.86 (0.29-2.55) | 0.782 | 4.59 (0.76-27.2) | 0.096 | 30 (2.14-421.1) | 0.012* |
| Both | 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 positive 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 179%, 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 발초혈액을 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. 혈액에서 적혈구들을 용열 시킨 후 원심하여 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 세포 양성이 50% 및 30%였다. 두가지 유전자 중 하나의 유전자 양성은 각각 60% 및 20%였다. MAGE 양성 및 동시 양성은 각각 50% 및 60%였다.

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

Conflicts of Interest

None declared.

Acknowledgment

This study was supported by the Industry Academic Cooperation Foundation of the Daegu Catholic University (201712).

REFERENCES

1. Ashworth TR. A case of cancer in which cells similar to those in the tumours were seen in the blood after death. Aust Med J 1869;14:146-7.
2. Pecot CV, Bischoff FZ, Mayer JA, Wong KL, Pham T, Bottsford-Miller J, et al. A novel platform for detection of CK+ and CK- CTCs. Cancer Discov 2011;1:580-6.
3. Marrinucci D, Bethel K, Kolatkar A, Luttgen MS, Malchiodi M, Baehring F, et al. Fluid biopsy in patients with metastatic prostate, pancreatic and breast cancers. Phys Biol 2012;9:016003.
4. Haber DA and Velculescu VE. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. Cancer Discov 2014;4:650-61.
5. Azim HA, Jr., Rothé F, Aura CM, Bavington M, Maetens M, Rouas G, et al. Circulating tumor cells and response to neoadjuvant paclitaxel and HER2-targeted therapy: a sub-study from the NeoALTTO phase III trial. Breast 2013;22:1060-5.
6. Miller MC, Doyle GV, Terstappen LW. Significance of circulating tumor cells detected by the CellSearch System in patients with metastatic breast colorectal and prostate cancer. J Oncol 2010;2010:617421.
7. Kang Y and Pantel K. Tumor cell dissemination: emerging biological insights from animal models and cancer patients. Cancer Cell 2013;23:573-81.
8. Meng S, Tripathy D, Frenkel EP, Shete S, Naftalis EZ, Huth JF, et al. Circulating tumor cells in patients with breast cancer dormancy. Clin Cancer Res 2004;10:8152-62.
9. Dong Y, Skelley AM, Merdek KD, Sprott KM, Jiang C, Pierceall WE, et al. Microfluidics and circulating tumor cells. J Mol Diagn 2013;15:149-57.
10. Gupta V, Jafferji I, Garza M, Melnikova VO, Hasegawa DK, Pethig R, et al. ApoStream™, a new dielectrophoretic device for antibody independent isolation and recovery of viable cancer cells from blood. Biomicrofluidics 2012;6:24133.
11. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature 2007;450:1235-9.
12. Gleghorn JP, Pratt ED, Denning D, Liu H, Bander NH, Tagawa ST, et al. Capture of circulating tumor cells from whole blood of prostate cancer patients using geometrically enhanced differential immune-capture (GEDI) and a prostate-specific antibody. Lab Chip 2010;10:27-9.
13. Zhang L, Ridgway LD, Wetzel MD, Ngo J, Yin W, Kumar D, et al. The identification and characterization of breast cancer CTCs competent for brain metastasis. Sci Transl Med 2013;5:180ra48.
14. Yu M, Bardia A, Aceto N, Bersani F, Makkad MW, Donaldson MC, et al. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. Science 2014;345:216-20.
15. Jeong YJ, Park SH, Jeon CH. Detection of circulating tumor cells in patients with breast cancer using the conditionally reprogrammed cell culture method and reverse transcription PCR of hTERT and MAGE A1. Oncol Lett 2020;20:78.
16. Yang CS, Kim IH, Chae HD, Kim DD, Jeon CH. Detection of circulating tumor cells in patients with breast cancer using the conditionally reprogrammed cell culture method and reverse transcription PCR of hTERT and MAGE A1. Oncol Lett 2020;20:78.
17. Liu X, Ory V, Chapman S, Yuan H, Albanese C, Kallakury B, et al. ROCK inhibitor and feeder cells induce the conditionally reprogramming of epithelial cells. Am J Pathol 2012;180:599-607.
18. Suprynowicz FA, Upadhyay G, Krawczyk E, Kramer SC, Hebert JD, Liu X, et al. Conditionally reprogrammed cells represent a stem-like state of adult epithelial cells. Proc Natl Acad Sci USA 2012;109:20035-40.
19. Benton G, Kleinman HK, George J, Amaouотова I. Multiple uses of basement membrane-like matrix (BME/Matrigel) in vitro and in vivo.
20. Liu X and Wu X. Utilizing matrigel transwell invasion assay to detect and enumerate circulating tumor cells. Methods Mol Biol 2017;1634:277-82.
21. Ferreira MM, Ramani VC, Jeffrey SS. Circulating tumor cell technologies. Mol Oncol 2016;10:374-94.
22. Ilie M, Szaler-Grusman E, Hofman V, Long-Mira E, Suttmann R, Darbonne W, et al. Expression of MET in circulating tumor cells correlates with expression in tumor tissue from advanced-stage lung cancer patients. Oncotarget 2017;8:26112-21.
23. Masuda T, Hayashi N, Iguchi T, Ito S, Eguchi H, Mimori K. Clinical and biological significance of circulating tumor cells in cancer. Mol Oncol 2016;10:408-17.
24. Szilágyi M, Pös O, Márton É, Buglyó G, Solész B, Keserű J, et al. Circulating cell-free nucleic acids: Main characteristics and clinical application. Int J Mol Sci 2020;21:6827.
25. Stewart CM and Tsui DWY. Circulating cell-free DNA for non-invasive cancer management. Cancer Genet 2018;228-9:169-79.
26. Jiang T, Ren S, Zhou C. Role of circulating-tumor DNA analysis in nonsmall cell lung cancer. Lung Cancer 2015;90:128-34.
27. Brock G, Castellanos-Rizaldos E, Hu L, Coticchia C, Skog J. Liquid biopsy for cancer screening, patient stratification and monitoring. Transl Cancer Res 2015;4:280-90.
28. Kim DD, Yang CS, Chae HD, Kwak SG, Jeon CH. Melanoma antigen-encoding gene family member A1-6 and hTERT in the detection of circulating tumor cells following CD45- depletion and RNA extraction. Oncol Lett 2017;14:837-43.
29. Bobek V, Gurlich R, Eliasova P, Kolostova K. Circulating tumor cells in pancreatic cancer patients: Enrichment and cultivation. World J Gastroenterol 2014;20:17163-70.
30. Rodríguez-Lee M, Kolatkar A, McCormick M, Dago AD, Kendall J, Carlsson NA, et al. Effect of blood collection tube type and time to processing on the enumeration and high-content characterization of circulating tumor cells using the high-definition single-cell assay. Arch Pathol Lab Med 2018;142:198-207.
31. Cayrefourcq L, Mazard T, Joosse S, Solassol J, Ramos J, Assenat E, et al. Establishment and characterization of a cell line from human circulating colon cancer cells. Cancer Res 2015;75:892-901.
32. Kajit JT, Kunkel M, Dickier DT, Joude J, Allen JE, Das A, et al. Circulating tumor cell levels are elevated in colorectal cancer patients with high tumor burden in the liver. Cancer Biol Ther 2015;16:690-8.
33. Takeda K, Yamada T, Takahashi G, Iwai T, Ueda K, Kuriyama S, et al. Analysis of colorectal cancer-related mutations by liquid biopsy: Utility of circulating cell-free DNA and circulating tumor cells. Cancer Sci 2019;110:3497-509.
34. Bidard FC, Kiavue N, Ychou M, Cabel L, Stern MH, Madic J, et al. Circulating tumor cells and circulating tumor DNA detection in potentially resectable metastatic colorectal cancer: a prospective ancillary study to the unicancer prodige-14 trial. Cells 2019;8:516.