Cancer panel analysis of cultured circulating tumor cells and primary tumor tissue from breast cancer patients

Eunjoo Hwang[1], Ji-hyun Uh[1], Hye Seon Lee[1], Cham Han Lee[1], Soo Jeong Lee[1] , Sei Hyun Ahn[2], Byung Ho Son[2], Jong Won Lee[2], Jong Han Yu[2], Nak-Jung Kwon[3], Woo Chung Lee[3], Kap-Seok Yang[3], Sung Ho Choi[1], Myoung Shin Kim[1], Jinseon Lee[1], Byung Hee Jeon[1]

[1]Cytogen Inc., Seoul, Korea,
[2] Department of Surgery, College of Medicine, University of Ulsan and Asan Medical Center, Seoul Korea
[3] Macrogen Inc, Seoul, Korea

PURPOSE

The isolation and culture of CTCs can be applied as a substitute method for tumor tissue biopsy, and may provide many clinical applications, including genomic analysis of tumor and personalized cancer therapy according to the genomic information. This method could provide better understanding of tumor metastasis and noninvasive monitoring the disease progression.

INTRODUCTION

Circulating tumor cells (CTCs) shed from primary tumor tissue, are circulating in bloodstream and causing metastasis (1). CTCs have similar molecular characteristics of primary tumor tissue (2), therefore it is possible to monitor drug sensitivity and resistance, and predict prognosis of therapy through liquid biopsy using CTCs.

Breast cancer mortality ranks the fifth in all kind of cancers, and the first in female cancers. Although effective therapies on the basis of hormone receptors and Her2 expressions showed high survival rate, recurrence and metastasis are unavoidable. Furthermore, recurrent tumors and metastases have different genetic characteristics, thus different therapies than initial treatments are needed (3). CTCs from breast cancer patients can be an indicator for detection of recurrence and metastasis (4), predictor for survival rate (5), and standard for therapy decision (6). Here, we isolated live and intact CTCs on the basis of size difference, successfully cultured to expand sufficient amount for genomic analysis.

MATERIALS AND METHODS

Blood collection and CTC enrichment process

10ml of blood from 6 breast cancer patient were collected and processed within 4 hrs. CTCs from blood were enriched using Smart Biopsy cell isolator (Cat#CIS020, Cytogen, Inc.) with CTC culture kit (Cat# CIKC10, Cytogen, Inc.).

Blood | **Density gradient centrifugation** | **Cell isolator** | **Recovery of filtered CTCs from HDM chip**

Culture of CTCs

Enriched CTCs were collected and cultured in growth medium (MSCGM, Lonza) at 37°C, 5% CO2. After 16-18 days of culturing, cells were fixed in 4% paraformaldehyde for immunofluorescent staining and the remaining cell pellets were kept at -80°C until cancer gene panel analysis.

Immunofluorescence analysis

Cells on slide were blocked with 1% BSA in PBS for 30 min, and incubated with mouse anti-EpCAM
antibody (Cell Signaling Technology). EpCAM signals were amplified with Tyramide Signal
Amplification System (Life Technologies) according to the manufacturer's protocol.

Ion AmpliSeq Cancer Panel (ICP) analysis

Genomic DNA of CTCs was extracted, amplified, and screened for mutations in 50 genes (2,800 COSMIC mutations) using the Ion AmpliSeq Comprehensive Cancer Panel.

RESULTS

Clinical Information of Patients

Patient ID	Age	AJCC/TMN stage[a]	No of cultured cells	
			No. of total cells	No. of EpCAM+ (%)
AMC-15-01	47	IIA	4.0 x 10^5	34.92
AMC-15-02	38	IIA	5.0 x 10^5	53.74
AMC-15-03	43	IIA	5.0 x 10^5	53.76
AMC-15-04	51	IIB	5.2 x 10^5	41.20
AMC-15-05	37	IIIC	8.3 x 10^5	86.54
AMC-15-06	46	IIB	4.5x 10^5	86.14

Table 1. Clinical information of breast cancer patient and IF staining analysis of EpCAM+ cell

Six patients with breast cancer from the ASAN Medical Center, Seoul, Korea were included in this study. The median age was 44 years (range, 37–47 years). Cancer stages were evaluated using the Tumor, Node, and Metastasis (TMN) system based on the recommendations of the 7th American Joint Committee on Cancer (AJCC)/Tumor, Node, and Metastasis (TMN).

Characterization of CTC features

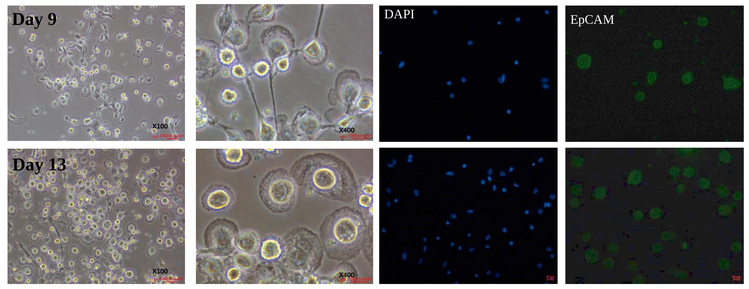

Figure1. Representative microscopic images and immunofluorescent staining for nuclei (blue) and EpCAM (green) pictures (X200) of cultured CTCs at day 9 and day 13

During the early phase of culture (until day 9), cells were grown either attached or suspended as single cells (Fig.1A, B). Cells were expanded during the rest of the culture process until they reached a population of 4 × 105 to 8 × 105 cells (Fig. 1C, Table 1) and the attached cells showed cell membrane ruffling (Fig. 1D). These observations suggested the selective expansion of epithelial cells and improved cell motility.

RESULTS

Cancer Gene Panel Analysis of cultured CTCs

Patient ID	Gene ID	Mutations type	A.A. mutation[a]	Cosmic number
AMC-15-01	*PDGFRA*	SNP[b]	N659K	COSM22414
	MET	SNP	Unknown	COSM710
	PTEN	INS[c]	N323fs*2	COSM23626
	PTEN	INS	T321fs*3	COSM4994
	PTEN	INS	N323fs*2	COSM4990
AMC-15-02	*PDGFRA*	SNP	V824V	COSM22413
	HRAS	SNP	H27H	COSM249860
	SMARCB1	SNP	Unknown	COSM1090
AMC-15-03	*PDGFRA*	SNP	V824V	COSM22413
	HRAS	SNP	H27H	COSM249860
	SMARCB1	SNP	Unknown	COSM1090
AMC-15-04	N/A[d]			
AMC-15-05	*CDKN2A*	SNP	H66R	COSM14253
AMC-15-06	*MLH1*	SNP	V384D	COSM26085
	MET	SNP	Unknown	COSM710
	HRAS	SNP	H27H	COSM249860

[a]AA mutation: amino acid mutation [b]SNP: single nucleotide polymorphism
[c]INS: insertion [d]N/A: Not applicable

We detected mutations in PDGFRA, MET, PTEN, HRAS, SMARCB1, CDKN2A, and MLH1 from genomic analysis of cultured CTCs. Mutations in these genes have been reported in breast tumor tissues

Comparison of COSMIC mutations in Primary tumor tissue and cultured CTCs

	Gene ID	Mutation type	A.A Mutation[a]	Cosmic number
Primary tissue	*NOTCH1*	DEL[b]	V1578delV	COSM13047
	HRAS	SNP[c]	H27H	COSM249860
	TP53	SNP	H193Y	COSM10672
CTC	*PDGFRA*	SNP	V824V	COSM22413
	HRAS	SNP	H27H	COSM249860
	SMARCB1	SNP	Unknown	COSM1090

We confirmed the same mutation of HRAS in both cultured CTCs and primary tumor tissues. This suggested that the cultured CTCs maintained genomic profiles similar to those of primary tumor tissues

REFERENCES

1. Cristofanilli M et al. J Clin Oncol 23:1420–1430, 2005.
2. Antonio M et al. PLoS One, 9:e103883, 2014.
3. Suzuki M et al. Mol Oncol 1:172–180, 2007.
4. Mario G et al. Breast Cancer Res 16:440, 2014.
5. Brigitte R et al. J Natl Cancer Inst 106: doi: 10.1093/jnci/dju066, 2014.
6. François CB et al. Cancer Metastasis Rev 32:179–188, 2013.

CONCLUSION

Here we isolated and cultured CTCs with high purity and obtained sufficient numbers of CTCs for genomic analysis. We also confirmed that cultured CTCs maintain genomic profiles similar to those of primary tumor tissues through cancer gene panel analysis. These results suggest that the use of cultured CTCs is an appropriate technology for breast cancer treatment and can be an applicable tool of serial liquid biopsy in the treatment