Assessment of HER-2 Status by fluorescence in situ hybridization (FISH) in circulating tumor cells (CTCs) isolated from metastatic gastric cancer patients

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Abstract

Human epidermal growth factor receptor 2 (HER-2) is involved in the pathogenesis and poor outcomes of gastric cancer. Targeted drugs inhibiting HER-2 pathway such as trastuzumab showed benefit in patients with HER2-positive metastatic gastric cancer. Reliable evaluation of HER-2 status is a useful and significant tool for treatment selection in gastric cancer patients. However, tumor biopsy in patients with recurrent and/or metastatic disease is not always possible. Here, we suggest isolation and culturing of circulating tumor cells (CTCs) as an alternative to tumor tissue biopsy. Ten milliliters of blood samples were collected in ACDA tubes from 34 patients with metastatic gastric cancer. The blood samples were divided into two parts: one for immunofluorescent staining and other for culturing. Both samples were processed by size-base filtration using CTC isolation kit. CTCs (≥2) were detected in 12 of 35 patients (34.3%, range 2-11). CTC culturing was successful in 30 of 35 cases (85.7%). Cultured CTCs were analyzed for HER-2 amplification by fluorescence in situ hybridization (FISH), and compared to HER-2 status on the primary tumor tissues. The overall concordance of HER2 status was 67% between cultured CTCs and primary tumor tissues. These results suggest that the isolation and culture of CTCs can be a substitute method for tumor tissue biopsy, and may provide clinical applications, including serial blood samplings for the personalized cancer therapy based on their genomic information.

Introduction

Human epidermal growth factor receptor 2 (HER2), also known as CerbB-2 and ERBB2, is a proto-oncogene located on chromosome 17q21 that encodes a trans-membrane protein with tyrosine kinase activity, a member of the HER receptor family and is involved in signal transduction pathways, leading to cell growth and differentiation[1].

Amplification of the HER2 gene and overexpression of its product were first discovered in breast cancer and are significantly associated with worse outcomes[2]. In gastric and gastroesophageal cancer, the frequency of HER2 overexpression varies widely in the literature; studies have yielded inconsistent findings regarding its prognostic relevance[3]. With the recent introduction of trastuzumab for the treatment of patients with advanced gastric cancer, the clinical demand for HER2 assessment is rapidly increasing. However, HER2 testing in gastric cancer differs from testing in breast cancer because of inherent differences in tumor biology, intratumoral heterogeneity of HER2 expression and incomplete membrane staining that are commonly observed in gastric tumors[4].

Circulating tumor cells (CTCs) shed from primary tumor tissue, are circulating in bloodstream and causing metastasis [5]. CTCs have similar molecular characteristics of primary tumor tissue [6], therefore it is possible to monitor drug sensitivity and resistance, and predict prognosis of therapy through liquid biopsy using CTCs.

Here, we isolated live CTCs from gastric cancer patients, and successfully cultured to expand sufficient amount for FISH analysis of HER-2 status.

Reference

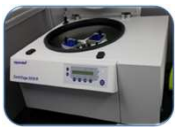
[1] Akiyama T et al. Science (1986) 232:1644–1646.
[2] Slamon DJ, et al. Science (1987) 235:177–182.
[3] He C et al. World J Gastroenterol (2013) 19:2171–2178.
[4] Abrahão-Machado LF et al. World J Gastroenterol (2013) 19:6438–6446.
[5] Cristofanilli M et al N Engl J Med (2004) 351:781-791.
[6] Cohen SJ et al. J Clin Oncol (2008) 26:3213-3221.

Materials and Methods

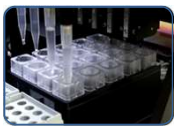
◆ Blood collection and CTC enrichment process

Blood samples (15 ml) from gastric cancer patient were collected and processed within 4 hrs. Cells enriched from 5 ml of blood were immunostained for identification of CTCs. Remaining cells were used for CTC culturing.

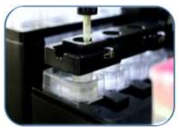
Blood collection



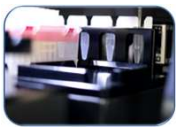
Pre-processing



Size-based gravity flow filtration



Retrieval of CTCs



◆ Immunofluorescence analysis

Cells on slides were then blocked with 1% BSA in PBS for 30 min, and incubated with primary antibodies followed by secondary antibody incubation. Primary antibodies were mouse anti-EpCAM (Cell Signaling Technology) and rabbit anti-CD45 (Santa Cruz). EpCAM signals were amplified with Tyramide Signal Amplification System (Life Technologies) according to the manufacturer's protocol. Secondary antibody for CD45 was goat anti-rabbit Alexa 594 (Invitrogen). The slides were mounted with Fluoroshield with DAPI (ImmunoBioScience).

◆Culture of CTCs

Enriched CTCs were collected and cultured in growth medium at 37°C, 5% CO2. After 16-18 days of culturing, cells were fixed in 4% paraformaldehyde for FISH analysis.

◆HER-2 Fluorescent in site hybridization

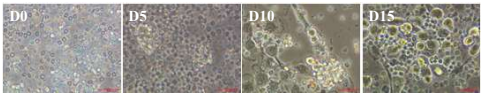
HER-2 status was assessed by fluorescence in situ hybridization (PathVysion HER2/neu kit, Abbott molecular, Des Plaines, Illinois) In determining the HER2/neu status by FISH, the following cutoffs were used: (1) amplified, if the HER2/neu:CEP17 ratio was greater than 2.2 or the HER2/neu was greater than 6 copies/nucleus; (2) equivocal, if the HER2/neu:CEP17 ratio was between 1.8 and 2.2; and (3) negative, if the HER2/neu:CEP17 ratio was less than 1.8. All cases were assessed by expert pathologists.

Results

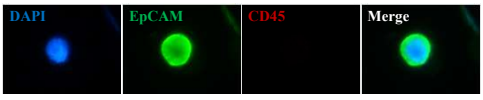
◆HER-2 Fluorescent in site hybridization

Variables		Total (n=35)	%
Age	Median (min-max)	59 (30-86)	
Sex	Male	23	65.7
	Female	12	34.3
Stage (AJCC7)	I	5	14.3
	II	8	22.8
	III	8	22.8
	IV	14	40.0
Cell Type	AWD/AMD	12	34.3
	APD/SRC	22	62.8
	Mucinous	1	2.9

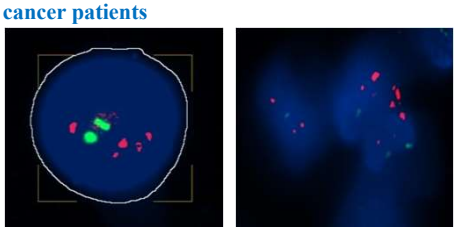
◆ Representative images of CTC culture at day 0, 5, 10, and 15 (X400).



◆Immunofluorescent staining of CTC for EpCAM (green), CD45 (red), and nuclei (blue) (X400).



◆ Representative FISH images for the detection of HER-2 amplification in CTC from gastric cancer patients



Conclusion

In our study, we successfully cultured CTCs in 30 of 35 cases (85.7%). Cultured CTCs were analyzed for HER-2 amplification by fluorescence in situ hybridization (FISH), and compared to HER-2 status on the primary tumor tissues. The overall concordance of HER2 status was 67% between cultured CTCs and primary tumor tissues. These results suggest that the isolation and culture of CTCs can be a substitute method for tumor tissue biopsy, and may provide clinical applications, including serial blood samplings for the personalized cancer therapy based on their genomic information.

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