Detection of HER2 status of circulating tumor cells and disseminated tumor cells using a microfluidic platform (cell enrichment and extraction technology [CEE™]).

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Abstract

Background: Evaluation of HER2 status of circulating tumor cells (CTCs) and disseminated tumor cells (DTCs) can provide valuable information to make therapeutic decisions. We report here the utility of a microfluidic platform for evaluation of HER2 gene amplification in CTCs and DTCs by fluorescent in situ hybridization (FISH).

Methods: Blood (10ml) and BM (1–2ml) were collected from patients with operable breast cancer. Mononuclear cells were recovered, incubated with a mixture of 10 primary capture antibodies (Abs), introduced into CEE™ microchannels (Biocept, Inc, San Diego), stained with fluorescent cytokeratin (CK) and CD45 Abs and then processed for FISH using probes specific to centromere 8 (spectrum aqua), 17 (spectrum green) and HER2 (spectrum orange). The ratio of HER2:CEP1 >2.2 in any CD45 negative cell (CK+ or CK-) was regarded as positive for HER2 gene amplification.

Results: Blood and BM from 50 patients with T1N0(26),T1N1(5),T2N0(6),T2N1 (3),T2N3(3),T3N0 (2),T3N1(1),T4N1(2),T4N3(2) with 7 HER2+ and 43 HER2- invasive tumors were studied. HER2+ CTCs were noted in 4/49 (8.0 %) patients with HER2+ (1) and HER2- (3) tumors. HER2+ DTCs were noted in 14/50 (28%) patients with tumors that were HER2+ in 2 and HER2- in 12 patients. Six of the 7 patients with HER2 amplified tumors did not show any HER2 amplified CTCs and 5 of the same patients did not contain HER2+ DTCs. Overall, HER2 gene amplified CTCs and DTCs were noted in blood and BM simultaneously in only 2 patients and in either the blood (2) or BM (12) in the remaining patients.

Conclusions:
1. The cell enrichment and extraction microfluidic technology (CEE™) provides a sensitive platform for evaluation of HER2 by FISH in intact CTCs and DTCs.
2. HER+ CTCs and DTCs were encountered in HER2+ as well as in HER2- primary tumors.
3. Discordant HER2 status was contributed mainly by HER2+ DTCs occurring in HER2 -primary breast tumor.
4. The clinical implications of evaluating HER2 in CTCs and DTCs in patients with invasive breast cancers need further investigation.

Introduction

The presence of circulating tumor cells (CTCs) in peripheral blood and disseminated tumor cells (DTCs) in bone marrow (BM) constitutes a central event in the metastatic cascade in patients with breast cancer. The rarity of these cells in comparison to the surrounding hematopoietic cells makes their detection very challenging. Enumeration and characterization of these cells can be useful for early detection, monitoring therapy, ascertaining prognosis and in advancing our understanding of the biology of metastatic disease. The majority of techniques currently utilized for the detection of CTCs and DTCs do not allow detailed phenotypic and genotypic characterization of the cells: HER2/neu is the most commonly evaluated target in breast tumors for determining eligibility of patients for treatment with the humanized monoclonal antibody trastuzumab. Evaluations of HER2 status of CTCs and DTCs can provide valuable information for monitoring response to trastuzumab therapy and for consideration of trastuzumab therapy in patients with HER2- primary tumor demonstrating HER2+ CTCs and /or DTCs. Microfluidic platforms (CTC-Chip) have been reported that allow efficient and selective isolation of CTCs in peripheral blood. In this study we report the utility of a novel microfluidic platform utilizing cell enrichment and extraction technology (CEE™, Biocept, Inc. San Diego) for evaluation of HER2 gene amplification in CTCs and DTCs by fluorescent in situ hybridization (FISH).

Methods

Matched specimens of peripheral blood (10ml) and BM (1-2ml) were collected from patients with operable breast cancer in a prospective institution review board-approved protocol into 10 mL vacutainer tubes containing 1.5 mL acid-citrate-dextrose solution (ACD solution A vacutainers; BD, Franklin Lakes, NJ). Anti-clumping reagent (Cell-Sure™, Biocept, San Diego) was injected into the vacutainer tubes within 60 minutes, stored at room temperature and processed within 24 hours of collection. Mononuclear cells were recovered from the peripheral blood and BM samples using a Percoll density gradient method in Leucosep tubes (Greiner bio-one, Monroe, NC). The recovered cells are incubated with Fc blocker (100 µg/mL) and a capture antibody cocktail adjusted to a concentration of 1µg/mL for 30 minutes at room temperature. After centrifugation, secondary antibody was added to the cell pellet, incubated for 30 minutes at room temperature and centrifuged three times at 400G for 10 minutes following washings with PBS/Casein/ EDTA. The resulting 1mL cell pellet was then introduced into the cell enrichment and extraction (CEE™) microchannels. Each CEE™ microchannel is attached to a syringe pump. The resuspended cells (800µL) are passed through the microchannels at a volumetric flow rate of 18 µL/min. The cells in the microchannels were then subjected to immunofluorescent staining using an AlexaFluor–488-tagged mixture of cytokeratin antibodies directed against CK 7, 17, 18, 19, pan-cytokeratin and AlexaFluor-594-tagged CD45 antibody. The microchannels were examined under the microscope and cells with the phenotype CK+/CD45-/DAPI+ or CK-/CD45-/DAPI+ were localized, enumerated and their precise location recorded so as to allow re-localization of the same cells following FISH analysis. After enumeration of the CTCs, the microchannels were processed for multi-color FISH using three direct labeled probes (Abbot Molecular) specific to the centromeres of chromosomes 8 (CEP 8-Spectrum Aqua) and 17 (CEP 17-Spectrum Green) and the locus-specific HER2 probe (Spectrum Orange). Scoring of the signals was performed on both CK+ and CK- CTCs that were enumerated and localized in the microchannels prior to FISH testing. A ratio of HER2:CEP17>2.2 in any CD45 negative CK+ or CK- cell was regarded as positive for HER2 gene amplification. Sections of the primary tumor were fixed in buffered formalin, routinely processed, embedded in paraffin wax and cut at 5µ thickness. Unstained sections were used for performing interphase FISH for HER2 gene amplification using the PathVysion DNA probe kit (Abbott Lab). A ratio of HER2:CEP17 of ≥2.2 (counting of at least 60 tumor nuclei) was regarded as positive for HER2 gene amplification.

Results

- Peripheral blood and BM from 50 patients staged as T1N0 in 26, T1N1 in 5, T2N0 in 6, T2N1 and T2N3 in 3, T3N0 in 2, T3N1 in 1 and T4N1 and T4N3 in 2 were studied.
- The primary invasive breast tumor tissue was positive for HER2 gene amplification by FISH in 7 patients (14%).
- HER2 gene amplified CTCs were detected in 4/49 (8.0%) patients, with underlying HER2+ tumor in one and HER2- tumor in 3 patients.
- HER2 gene amplified DTCs were detected in 14/50 (28%) patients.
- The primary tumor was HER2+ in 2 and HER2- in 12 patients, respectively.
- Six of the 7 patients with HER2 amplified primary tumor did not show any HER2 amplified CTCs and 5 of the same patients did not show any HER2+ DTCs.
- Overall, HER2 gene amplified CTCs and DTCs were noted in blood and BM simultaneously in only 2 patients.
- Discordant HER2 status was contributed mainly by HER2+ DTCs occurring in HER2- primary breast tumors (24%; 12 of 50 cases)

Results

MICROFLUIDIC CHANNELS

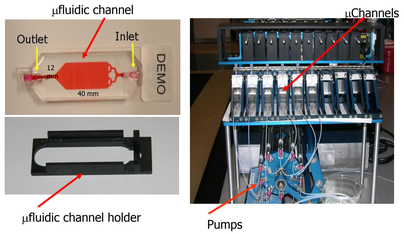

Fig 1: Illustration of the CEE™ microfluidic platform (Biocept, Inc. San Diego) used in the study.

HER2 GENE AMPLIFIED CYTOKERATIN-POSITIVE CIRCULATING TUMOR CELL IN BLOOD

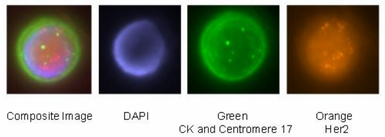

Fig 2: Illustration of a CK+ and CD45- CTC, by fluorescence in situ hybridization (FISH). Note the numerous orange signals in the CTC indicating HER2 gene amplification. The primary tumor was negative for HER2 gene amplification.

HER2 GENE AMPLIFIED CYTOKERATIN-NEGATIVE DISSEMINATED TUMOR CELL IN BONE MARROW

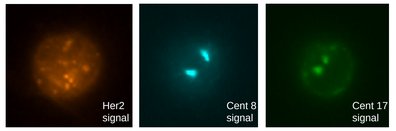

Fig 3: Illustration of HER2 gene amplification in cytokeratin-negative DTC in bone marrow when the primary tumor was negative for HER2 gene amplification.

Results

TABLE 1: Detection of HER2+ circulating tumor cells (CTCs), HER2+ disseminated tumor cells (DTCs), genomic type and HER2 gene amplification status of the primary breast tumor from patients with different stage breast cancer

TUMOR STAGE	GENOMIC TYPE	HER2 STATUS PRIMARY TUMOR	HER2+ CTCs	HER2+ DTCs
T1NO	LUMINAL A	–	+	+
T1NO	LUMINAL A	–	+	–
T3NO	LUMINAL A	–	+	+
TINO	LUMINAL A	–	–	+
TIN1	LUMINAL A	–	–	+
T2NO	LUMINAL A	–	–	+
T1NO	TRIPLE NEGATIVE	–	–	+
T1N1	LUMINAL A	–	–	+
T1N0	LUMINAL B	+	+	–
TINO	LUMINAL A	–	–	+
T4N3	HER2+	+	–	+
TIN1	LUMINAL A	–	–	+
TIN1	LUNIMAL A	–	ND	+
TIN1	TRIPLE NEGATIVE	–	–	+
T2NO	LUMINAL B	+	–	+
TIN1	LUMINAL A	–	–	+

* ND = not done

TABLE 2: Status of HER2 gene amplification by FISH in circulating tumor cells (CTCs) and disseminated tumor cells (DTCs) in HER2-positive primary breast tumors.

TUMOR STAGE	GENOMIC TYPE	HER2+ CTC	HER2+ DTC
TI NO	LUMINAL B	+	–
TI NO	LUMINAL B	–	–
TI NO	LUMINAL B	–	–
TI NO	LUMINAL B	–	–
T2 NO	LUMINAL B	–	+
T2 N1	LUMINAL B	–	+
T4 N3	HER2+	–	–

Conclusions

1. The cell enrichment and extraction (CEE™) microfluidic technology provides a sensitive platform for evaluation of HER2 by FISH in intact CTCs and DTCs.
2. HER2+ CTCs and DTCs were encountered in patients with HER2+ as well as in HER2- primary tumors.
3. Discordant HER2 status was contributed mainly by HER2+ DTCs occurring in HER2- primary breast tumors.
4. The clinical implications of evaluating HER2 in CTCs and DTCs in patients with invasive breast cancers needs further investigation.

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