Detection of discordant HER2 status by FISH in circulating tumor cells and disseminated tumor cells in early stage breast cancer using a microfluidic-based cell enrichment and extraction platform (OncoCEE™)

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Abstract

Background: Evaluation of HER2 in circulating (CTCs) and disseminated (DTC) tumor cells may aid therapy in breast cancer. We report here the discordance in HER2 status in CTCs and DTCs in early stage breast cancer by fluorescence in situ hybridization (FISH) using a microfluidic cell platform (OncoCEE™)

Methods: Blood (10ml) and BM (1-2ml) from patients with Stage T1 and T2 breast cancer was collected in OncoCEE-Sure™ collection tubes. Mononuclear cells were recovered using a Percoll density gradient method, incubated with a mixture of 10 primary capture antibodies (Abs), and introduced into streptavidin coated OncoCEE™ microchannels for tumor cell capture. For blood samples, captured cells were stained with anti cytokeratin (CK) and CD45 Abs for CTC enumeration followed by FISH using probes specific to centromere 17 and HER2. For BM samples, captured cells were subjected to HER2 FISH analysis. The ratio of HER2:CEP17>2.0 in any CK+/ CD45- and CK-/CD45- cell was regarded as positive for HER2 .

Results: Blood and BM from 68 patients (68 Blood, 54 BM; 54 matched blood and BM) with stage T1N0 (41), T1N1 (6), T2N0 (11), T2N1(2), T2N2 (1), T2N3 (2) with HER2+ (n=7) and HER2- (n=61) breast cancers were studied. The 7 patients with HER2 + primary tumor had HER2+ DTCs in 3/7 (43 %) and HER2 + CTCs in 1/6 (17 %) patients. HER2 + DTCs and HER2 + CTCs occurred in 10/47 (21%) and in 4/57 (7%) patients with HER2- primary breast tumors. The discordance of HER2 status was observed in 14% in CTCs and in 22% in DTCs.

Conclusion: 1. The cell enrichment and extraction microfluidic platform (OncoCEE™) provides a sensitive approach for evaluation of HER2 in CTCs and DTCs. 2. CTCs and DTCs acquired HER2 gene amplification in 21% and 7% of patients with HER2 negative early stage primary breast cancer. 3. CTCs and DTCs lost HER2 gene amplified status in 57% and 83% of patients with HER2 positive early stage primary breast cancer. 4. The clinical significance of alterations in HER2 status among CTCs and DTCs in early stage breast cancer needs further investigation.

Introduction

The occurrence of circulating tumor cells (CTCs) in blood and disseminated tumor cells (DTCs) in bone marrow(BM) of patients with early and advanced breast cancer is well recognized. These tumor cells most likely play an important role in the complicated process of metastasis. Enumeration and characterization of these cells may increase early detection, improve design of personalized therapies, aid in the monitoring of treatment efficacy, enhance prognostic accuracy and advance our understanding of the biology of metastatic disease. Detection of CTCs and DTCs can be challenging because of their extreme rarity in peripheral blood or BM. The majority of techniques currently utilized for the detection of CTCs and DTCs do not allow detailed phenotypic and genotypic characterization of these cells. HER2/neu is the most commonly evaluated target in breast cancer for determining eligibility of patients for treatment with humanized monoclonal antibody trastuzumab. Evaluation of HER2 gene amplification in CTCs and DTCs might provide useful information for monitoring response to trastuzumab therapy and for consideration of this therapy in patients with HER2 negative primary tumor with however HER2 positive CTCs and/or DTCs. In this study we report the discordance in HER2 status in CTCs and DTCs in early stage breast cancer as detected by fluorescence in situ hybridization (FISH) using a microfluidic cell enrichment and extraction platform (OncoCEE™).

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 - SureTM. Biosept) 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 samples using a Percoll density gradient method in Leucosep tubes. The recovered cells were 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 400 G for 10 minutes following washings with PBS/ Casein/ Arginine/ EDTA. The resulting 1mL cell pellet was then introduced into the cell enrichment and extraction (CEE) microchannels. Each CEE microchannel was attached to a syringe pump. The resuspended cells (300µL) were introduced to the CEE microchannels at a volumetric flow rate of 18 µL/ min. The cells in the CEE microchannels were then subjected to immunofluoresecent staining using AlexaFluor – 488 tagged mixture of cytokeratin antibodies directed against CK 7,17,18,19, and AlexaFluor - 594 tagged CD45 antibody. The microchannels were examined under the microscope and cells with the phenotype CK +/ CD45 -/ DAPI + or CK - / CD 45 - / DAPI + were localized, enumerated and their precise location were recorded so as to allow re-localization of the same cells following FISH analysis. Following enumeration of the CTCs, the CEE microchannels were processed for multi-color FISH using two direct labeled probes (Abbot Molecular) specific to the centromeres of chromosomes (CEP 17- Spectrum Green) and the locus specific HER2 probe (Spectrum orange). The scoring of the signals was performed on both CK+ and CK- CTCs that were enumerated and localized in the microchannels prior to FISH testing. The ratio of HER2: CEP 17 >2.0 in any CD 45 negative CK+ or CK- cell was regarded as positive for HER2 gene amplification.

Results

- Peripheral blood and BM from 68 patients staged as T1N0 in 41, T1N1 in 6, T2N0 in 11, T2N1 in 2, T2N2 in 1 and T2N3 in 2 patients were studied.
- The primary invasive mammary tumor was positive for HER2 gene amplification in 7 patients and negative in 61 patients.
- HER2 gene amplified CTCs were detected by FISH performed on the intact CTCs in the microchannels in 1 out 6 (17%) HER2 + primary tumor and in 4 out of 57 (7%) in HER2 primary tumors.
- HER2 gene amplified DTCs were detected in 3/7 (43%) HER2+ primary tumors and in 10/47 (21%) of HER2 primary tumors.
- Discordance of HER2 status as evaluated by FISH of intact CTCs and DTCs in the microchannels was observed in 14%, in CTCs and 22%, in DTCs.
- Discordant HER2 status was contributed more often by DTCs in comparison to CTCs in patients with early stage breast cancer.

Results

MICROFLUIDIC CHANNELS

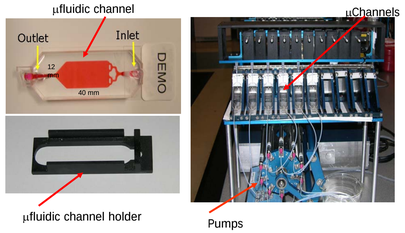

Fig 1: Illustration of the microfluidic platform (Biocept, Inc. San Diego) used in the study.

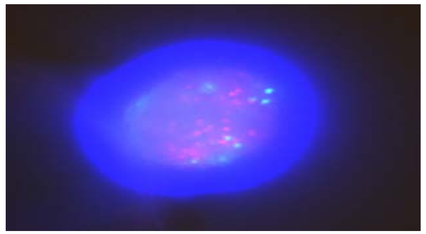

Fig 2: Illustration of a CK + and CD45- CTC. Note the numerous orange signals indicating increased copy numbers of HER2. The primary tumor was negative for HER2 gene amplification

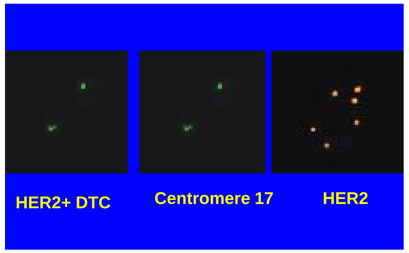

Fig 3: Illustration of a disseminated tumor cell (DTC) in bone marrow showing increased copy numbers of HER2 as evidenced by increased orange signals. The primary tumor was negative for HER2 gene amplification.

TABLE 1 :- HER2 gene amplification in circulating tumor cells and disseminated tumor cells in HER2 positive primary breast tumors.

TUMOR STAGE	GENOMIC TYPE	HER2+ CTCs	HER2+ DTCs
T1N0	Luminal B	+	+
T1N0	Luminal B	–	–
T1N1	HER2 +	–	–
T1N0	Luminal B	–	–
T2N0	Luminal B	–	–
T1N1	Luminal B	–	+
T2N1	Luminal B	–	+

TABLE 2: HER2 gene amplification in circulating tumor cells in blood and disseminated tumor cells in bone marrow in HER2 negative primary breast tumors.

TUMOR STAGE	GENOMIC TYPE	HER2+ CTCs	HER2+ DTCs
T1N0	Luminal A	+	–
T1N0	Luminal A	+	–
T1T0	Luminal A	+	–
T1N0	Luminal A	+	–
T1N0	Luminal A	–	+
T1N0	Luminal A	–	+
T2 N0	Luminal A	–	+
T1N0	Triple negative	–	+
T1N0	Luminal A	–	+
T1N0	Luminal A	–	+
T1N0	Luminal A	–	+
T1N0	Luminal A	ND	+
T1N1	Luminal A	ND	+
T1N0	Luminal	–	+

Conclusions

1. The cell enrichment and extraction microfluidic platform (OncoCEE™) provides a sensitive approach for evaluation of HER2 in CTCs and DTCs.
2. CTCs and DTCs acquired HER2 gene amplification in 21% and 7% of patients with HER2 negative early stage primary breast cancer.
3. CTCs and DTCs lost HER2 gene amplified status in 57% and 83% of patients with HER2 positive early stage primary breast cancer.
4. The clinical significance of alterations in HER2 status among CTCs and DTCs in early stage breast cancer needs further investigation.

Thanks to Ms. Nettie Glaze for her administrative support