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

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Abstract

Background: Evaluation of HER2 status of circulating tumor cells (CTCs) and disseminated tumor cells (DTCs) can provide invaluable 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 CEE™ microchannel (Biocept, Inc, San Diego), stained with fluorescent centromere (C:K) and CEP 17 and then processed for FISH using probes specific to centromere 8 (lilac/slate), 17 (green) and HER2 (spectra red) on a Leica A60R fluorescent microscope. Any HER2-positive CTCs (e.g., in BM) were regarded as positive for HER2 gene amplification.

Results: Blood and BM were collected from 30 patients with T2NO, T1NO, T1DNO and T2DNO in Tumor Stage II, and HER2 was assessed. HER2 CT Cs were detected in 3 of 4 (75%) patients with HER2+ and 3 of 27 (11%) patients with HER2- in the blood. In BM, 4 of 5 (80%) patients with tumors that were HER2+ and 2 of 13 (15%) patients with HER2- were noted. The HER2+ patients did not show any HER2- amplified CTCs and vice versa. The number of patients that did not contain HER2- DTCs was lower (75%). Overall, HER2 amplified CTCs and DTCs were noted in blood and BM simultaneously in only 2 patients (10%).

Conclusions: The cell enrichment and extraction microfluidic technology (CETM™) provides a sensitive platform for evaluation of HER2 by FISH in intact CTCs and DTCs. HER2 CTCs and DTCs were detected in BM and blood as well as in HER2 primary tumors.

1. Discrepancy HER2 status was most commonly encountered in patients in whom HER2+ DTCs were observed in BM as HER2- primary tumors.

2. 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 of these cells can be useful for early detection, monitoring therapy, ascertaining prognosis and in advancing our understanding of the biology of breast cancer. The majority of techniques currently used for the detection of CTCs and DTCs do not allow detailed phenotypic and genotypic characterization of the cells. HER2 is the most commonly evaluated target in breast cancers for the prediction of patients with the humoral monoclonal antibody trastuzumab. Evaluation of HER2 status in CTCs and DTCs could provide valuable information to guide post-surgical adjuvant therapy and for consideration of trastuzumab therapy in patients with HER2-positive primary tumors demonstrating circulating HER2-positive CTCs in BM and/or DTCs. Microfluidic technology is a rapid technique that allows high efficiency and selective isolation of CTCs in peripheral blood. In this study we report the utility of a novel microfluidic technology platform (CETM™) (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 and processed for FISH using probes specific to centromere 8 (lilac/slate), 17 (green) and HER2 (spectra red) on a Leica A60R fluorescent microscope. Any HER2-positive CTCs (e.g., in BM) were regarded as positive for HER2 gene amplification.

Results

| Blood and BM were collected from 30 patients with T2NO, T1NO, T1DNO and T2DNO in Tumor Stage II, and HER2 was assessed. HER2 CT Cs were detected in 3 of 4 (75%) patients with HER2+ and 3 of 27 (11%) patients with HER2- in the blood. In BM, 4 of 5 (80%) patients with tumors that were HER2+ and 2 of 13 (15%) patients with HER2- were noted. The HER2+ patients did not show any HER2- amplified CTCs and vice versa. The number of patients that did not contain HER2- DTCs was lower (75%). Overall, HER2 amplified CTCs and DTCs were noted in blood and BM simultaneously in only 2 patients (10%). |

Conclusions

1. Discrepancy HER2 status was most commonly encountered in patients in whom HER2+ DTCs were observed in BM as HER2- primary tumors.

2. The clinical implications of evaluating HER2 in CTCs and DTCs in patients with invasive breast cancers need further investigation.

Microfluidic Channels

**Microfluidic Channels**

**Peripheral blood and BM from 50 patients staged as T1NO, T1DNO, T2NO, T2DNO, T3NO, T4N0, T4N1 and T4N2 in BM were studied.**

The primary invasive breast tumor was positive for HER2 gene amplification by FISH in 7 patients (14%).

HER2 gene amplified CTCs were detected in 4/49 (8.1%) patients, with underlying HER2+ tumors in one and HER2+ tumors in 3 patients.

HER2 gene amplified DTCs were detected in 4/50 (8.0%) patients.

The primary invasive tumor was HER2+ in 2 and HER2- in 58 patients.

Six of the 7 patients with HER2+ primary tumors did not show any HER2 amplified CTCs and 5 of the same patients did not show any HER2 amplified DTCs.

Discordant HER2 status was contributed mainly by HER2- DTCs occurring in HER2+ primary breast tumors (24%; 12 of 50 cases).

**Conclusions**

1. The cell enrichment and extraction (CETM™) microfluidic technology provides a sensitive platform for evaluation of HER2 by FISH in intact CTCs and DTCs.

2. HER2+ DTCs and CTCs were encountered in patients with HER2+ as well as HER2- primary tumors.

3. Discrepancy 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.


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

<table>
<thead>
<tr>
<th>TUMOR STAGE</th>
<th>GENOMIC TYPE</th>
<th>HER2 STATUS</th>
<th>PRIMARY TUMOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNM</td>
<td>HER+</td>
<td>HER+ DTCs</td>
<td></td>
</tr>
<tr>
<td>HER2+</td>
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**Table 2**: Status of HER2 gene amplification by FISH in circulating tumor cells (CTCs) and disseminated tumor cells (DTCs) in HER2-positive primary breast tumor.

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**Fig 1**: Illustration of the CETM™ microfluidic platform (Biocept, Inc. San Diego) in use.

**Fig 2**: Illustration of a CTC and CEP-17/CDC-45 by fluorescent in situ hybridization (FISH). Note the numerous orange signals in the CTC indicating HER2 amplification. The primary tumor was negative for HER2 gene amplification. HER2 GENE AMPLIFIED CYTOKERATIN-POSITIVE CIRCULATING TUMOR CELL IN BLOOD

**Fig 3**: Illustration of a HER2+ CTC in blood (Abeco, Inc. San Diego). Detection of HER2 status in circulating tumor cells and disseminated tumor cells using a microfluidic platform (cell enrichment and extraction technology [CETM™]).