Estrogen receptor and progesterone receptor immunocytochemistry staining in circulating tumor cells as compared to primary tumor or metastatic biopsy

Julie Ann Mayer, Tony J. Pircher, Tam Pham, Karina L. Wong, and Farideh Z. Bischoff

Biocept Inc., San Diego California

ABSTRACT

INTRODUCTION: Hormone receptor (Estrogen receptor (ER) and progesterone receptor (PR)) status in all breast cancer patients is recommended by immunohistochemistry (IHC) and is considered standard practice for selection of treatment options. However, the analytical sensitivity of IHC in detecting low levels of ER/PR is often poor and likely due to methodological variation. Because biopsy is not often feasible in all patients presenting with recurrent and/or metastatic breast disease, circulating tumor cells (CTCs) offer an attractive alternative source of tumor tissue for determining ER/PR status and can be monitored more readily to enable a more effective course of treatment..

METHODS: Twenty ml of peripheral blood was collected prospectively from 14 patients diagnosed with late stage metastatic/recurrent breast cancer. CTCs were isolated using the microfluidic OncoCEE™ platform. A cocktail of antibodies was utilized for CTC capture and detection with an expanded anti-cytokeratin (CK) cocktail mixture and anti-CD45. ER/PR protein expression was assessed by immunocytochemistry (ICC) on the cells captured within the microchannels and compared to IHC performed on the primary tumor or metastatic biopsy.

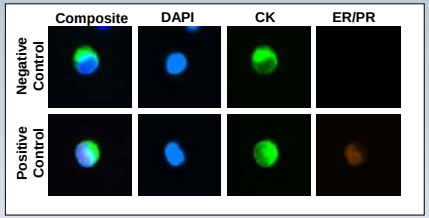
RESULTS: CK+/CD45-/DAPI+ cells were located and assessed for ER/PR immunocytochemistry. Among the 14 cases a high concordance (12/14; 86%) in ER/PR status between primary tumor/metastatic biopsy and CTCs was observed. Two cases were found to be discordant where one was positive by IHC and negative on the CTCs and the other was negative on by IHC on the primary tumor/metastatic biopsy and positive on the CTCs. However, both cases that were discordant had low numbers of CTCs detected.

CONCLUSIONS: There is significant heterogeneity between ER/PR protein expression in CTCs and primary tumor/metastatic biopsy and this status may change over time due to therapy. ER/PR ICC on CTCs from peripheral blood using the OncoCEE™ platform is shown to be feasible with high concordance (86%) in ER/PR status between primary tumor/metastatic biopsy (by IHC) and CTCs (by ICC). The significance of heterogeneity at the ER/PR protein level in CTCs ascertaining to the prognosis and predictive response to anti-estrogen therapy needs further evaluation in larger prospective clinical trials.

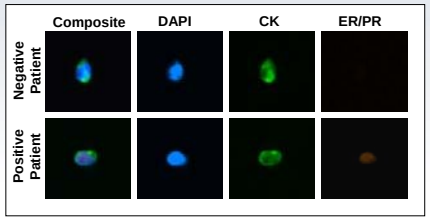
BACKGROUND

Estrogen receptor alpha (ER) is a widely accepted biomarker and great detail has been studied regarding the protein and RNA levels. It is estimated that up to 75% of breast cancers rely on estrogen receptor signaling for their means of growth and targeting this pathway has clear clinical efficacy[1]. Several treatments exist for estrogen receptor positive breast cancer patients that can alter estrogen receptor signaling. Selective estrogen receptor modulators (SERMs) such as tamoxifen act as a receptor antagonist, aromatase inhibitors, downregulation of estrogen receptor itself by antiestrogens such as fulvestrant, and even more drastic measures such as ovarian ablation[1]. More recently a number of studies have demonstrated amplification at the ESR1 locus, although the frequency of the amplification continues to be extensively debated[2-4].

ER/PR ICC ON SPIKED TUMOR CELLS



ER/PR ICC ON PATIENT CTCs



RESULTS

Biocept ID	Total CK+ CTCs	ER/PR+ CTCs	Biocept's ER/PR ICC Status	Tissue ER/PR IHC Status
11-18070	2	0	neg	neg
11-18028	39	0	neg	neg
11-18030	~3100	0	neg	neg
11-18050	1	0	neg	neg
11-18197	1	0	neg	neg
11-18245	2	0	pos	neg
11-18100	1	0	neg	pos
11-18049	16	6	pos	pos
11-18125	12	10	pos	pos
11-18185	54	25	pos	pos
11-18199	24	14	pos	pos
11-18226	140	8	pos	pos
11-18338	73	29	pos	pos
11-18345	3	1	pos	pos

Data is shown in Table 1. Among the 14 cases a high concordance (12/14; 86%) in ER/PR status between primary tumor/metastatic biopsy and CTCs was observed. Discordant patient samples are highlighted in blue.

DISCUSSION

The CEE™ technology provides a sensitive platform for enhanced capture, detection and molecular characterization (ER/PR) of both CK+ and CK- CTCs.

This platform allows for evaluation of ER/PR status by ICC in intact CTCs within the microchannels .

Sensitivity and accuracy levels of this test need to be tested on a larger patient population.

Though some discordance between tumor and CTCs is expected given variation in tumor heterogeneity, biopsy size, and robustness of the technical assay (especially for IHC), a blood-based CTC assay may offer more reliable testing given the advantages of simple repeat testing and the use of larger blood volumes, which may help to ensure informative results.

METHODS AND MATERIALS

Laboratory Information and Patients Patients with advanced stage breast cancer were enrolled from January 2011 to June 2011. Peripheral blood was collected under appropriate third party institution review board approved protocols (ConversantBIO & BioOptions).

Cell Separation Leucosep tubes (Greiner bio-one, Monroe, NC) utilizing a Percoll density gradient method were used to recover the peripheral blood mononuclear cell fraction (PBMC). Fc blocker (100ug/mL) and a capture antibody cocktail (1µg/mL of each antibody in the cocktail) were added for 30 minutes at room temperature to the recovered PBMC fraction. Following a wash and centrifugation, biotinylated secondary antibody was added for 30 minutes at room temperature. Following washing, the final pellets were subsequently applied to the CEE™ microchannels.

CTC Enrichment and Detection CEE™ microchannels are manufactured at Biocept, Inc. (San Diego, CA). The cell fraction is run through the microchannel and the captured cells stained with a mixture of anti-cytokeratin antibodies labeled with AlexaFluor-488. Cells were simultaneously stained with anti-CD45 labeled with AlexaFluor-594. ER/PR ICC was performed using anti-ER and anti-PR monoclonal rabbit antibodies and secondary anti-Rabbit AlexaFluor-546. The microchannels undergo microscopic analysis for enumeration of CK+/CD45-/DAPI+ (criteria for CTC identification), CK-/CD45+/DAPI+ (criteria for background WBCs) and all CK+ cells are assessed for ER/PR ICC.

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