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

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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 alternative source of tumor tissue for determining ER/PR status and can be monitored more readily to enable a more effective course of treatment. 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 ovariectomy. Most recently a number of studies have demonstrated amplification at the ER/PR loci, however the frequency of amplification continues to be extensively debated.1

METHODS: Twenty ml of peripheral blood was collected prospectively from 34 patients diagnosed with late stage metastatic/recurrent breast cancer. CTCs were isolated using the microfluidic OncocCEETM platform. A cocktail of antibodies was utilized for CTC capture, and detection was accomplished 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: In a prospective study CK+CD45-/CD45+ cells were detected in 22 of 34 (65%) patients with late stage breast cancer and assessed for ER/PR immunocytochemistry. Among the 22 CK+ CTCs cases a concordance of 75% (16/22) was observed in ER/PR status between primary tumor and CTCs and was observed and 90% (19/22) concordance was obtained when compared to the metastatic biopsy. Overall a high concordance of 86% (19/22) was achieved. Five cases were discordant based on primary tissue alone. Two of these cases are concordant when compared to the metastatic biopsy. Overall three cases were found to be discordant: all three were positive by IHC on the primary tumor/micrometastatic breast and negative on the CTCs and important to note that all three discordant cases had relatively low numbers of CTCs detected.

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

REFERENCES

METHODS AND MATERIALS

Laboratory Information and Patients Patients with advanced stage breast cancer were enrolled from January 2011 to April 2012. Peripheral blood was collected in heparinized tube and sent to Biocept for immediate processing and IHC analysis. All samples were processed and analyzed in accordance with approved protocols (Columbia University Medical Center, AdapBio & 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 (100µg/mL) and a capture antibody cocktail (4µg/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. After washing, the final pellets were subsequently applied to the CEETM microchannels.

CTC Enrichment and Detection CEEETM 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 antibodies and secondary anti-Rabbit AlexaFluor-546. The microchannels undergo microscopic analysis for enumeration of CK+CD45+/CD45+ (CTC identification). CK+CD45+/CD45- (background WBCs) and all CK+ cells are assessed for ER/PR ICC.

RESULTS


data is shown in Table 1. Among the 22 CK+ CTC cases a concordance of 75% (16/22) in ER/PR status between primary tumor and CTCs was observed and a 90% (19/22) concordance was obtained when compared to the metastatic biopsy. Overall a high concordance of 86% (19/22) was achieved. Discordant patient samples are highlighted in blue.

CONCLUSION

The CEEETM technology provides a sensitive platform for enhanced capture, detection and molecular characterization (ER/PR) in intact CTCs within the microchannels . Sensitivity and accuracy levels of this test need to be tested on a larger patient population.

SUMMARY

Though some discordance between tumor and CTCs is expected given variation in tumor heterogeneity, biopsy size, and robustness of the patient population, a high concordance of 86% (19/22) was achieved. Discordant patient samples are highlighted in blue.

REFERENCES