Comparison of Genomic and Proteomic Biomarkers in CTCs And Biopsies from HRPC Patients

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molecular profiling & diagnostics

Clinical Application: Verification of the method of using CTCs as a surrogate marker for the primary tumor

Key Words: tumor biopsies, CTCs, protein expression, FISH, surrogate marker, comparison study

Background: The link between circulating tumor cells (CTCs) and the primary tumor has always been of interest in the field of oncology. The ability to use CTCs as a sort of "liquid biopsy" to assess the biological status of a patient's cancer is one that holds great value. This study examines a set of both genomic and proteomic biomarkers in hormone-refractory prostate cancer patients (HRPC), attempting to verify through biomarker comparison in CTCs and tissue (both archival and fresh biopsy) the idea of using CTCs as a surrogate for the primary tumor.

Methods: Three tubes of blood were drawn from a total of twenty HRPC patients with available archival tissue. Metastatic site biopsies were taken from these patients as well. Two tubes of blood were processed on the CellSearch® platform, using the Profile Kit. These cells were then fixed, placed on slides, and stained for CTC phenotypic markers (DAPI, cytokeratin (CK), CD45) and the biomarkers of interest. The slides were analyzed using Laser Scanning Cytometry (LSC); CTCs were identified as DAPI+/CK+/CD45- cells, and the expression levels of the biomarkers was reported in these cells. The archival and biopsy slides for each patient were stained and analyzed in the same manner as the CTCs. All three tissue types were then hybridized and analyzed for the FISH markers of interest. The third tube of blood was enriched for EpCAM+ cells using the Miltenyi AutoMACS® platform. RNA was extracted from the enriched cells, reverse transcribed to obtain cDNA, and preamplified before qRT-PCR was performed.

Results:

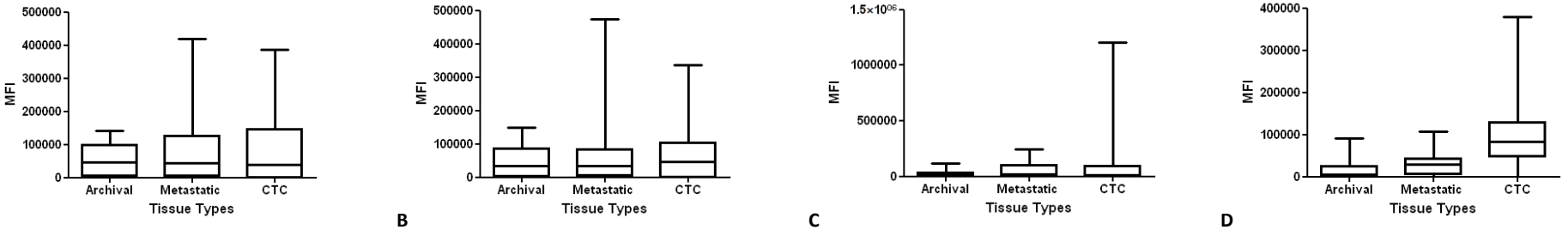

Figure 1. Biomarker Expression Levels in Different Tissues from All Patients. Results from the protein expression analysis across archival tissue, metastatic tissue, and CTCs are shown here. The Mean Fluorescent Intensity (MFI) of a single biomarker is plotted on each graph; biomarkers A, B, and C indicate a similar staining pattern across all three tissue types. Additional analysis indicated that biomarker A showed a statistically significant correlation between the expression levels in metastatic tissues and CTCs, while biomarker B showed a statistically significant correlation between both expression levels in archival and metastatic tissues and metastatic tissues and CTCs. Biomarker D exhibited a statistically significant correlation in archival and metastatic tissues.

Higher expression of biomarker C was observed in the CTCs of some patients compared to archival and metastatic tissues, perhaps indicating reactivation of marker-dependent pathways in the CTCs of these patients. This marker was also analyzed by FISH; a high degree of concordance was found between the archival and metastatic tissues, but not with CTCs, illustrating further that some change is occurring in the CTCs to modify the expression patterns of this marker. Another marker (not shown) was also examined by FISH and PCR, but minimal concordance was found (3/11 patients between archival and metastatic tissues showed deletion of abnormal phenotype by FISH; only 1 patient exhibited this deletion in all tissue types).

Impact: Correlation was found between the expression of specific biomarkers in archival tissue, metastatic tissue, and CTCs in HRPC, lending validity to the idea of using CTCs as a surrogate marker for the study of the primary tumor. Further cases should be completed in order to more fully explore the depth of this correlation.

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