Sequencing the chemokine receptor CXCR4 in individual circulating tumor cells (CTCs) of patients with breast cancer (BrCa).

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Abstract Disclosures

Abstract:

Background: Recent efforts of deep sequencing patients tumors have confirmed that clonal populations causing distant metastases are represented within the primary carcinoma. However from large numbers of tumor cells the frequency of mutations in individual clones cannot be inferred. Endocytic recycling of CXCR4, reportedly overexpressed in at least 23 different cancer types, is important for cancer cell motility. The C-terminus of CXCR4 has been reported to contain important motifs for the regulation of receptor trafficking. In this study we aim to establish the methodology for identifying possible mutations from the C-terminus of CXCR4 gene in individual CTCs.

Methods: Blood samples from BrCa patients were collected in CellSave tubes and processed with Veridex CellSearch Epithelial Cell Kit. After enumeration, samples of 6 positive patients (2 locally advanced and 4 metastatic, range 13-296 CTC/7.5 ml), were removed from Veridex cartridge and loaded on DEPArray, Silicon Biosystems, a fluorescence and morphology image-based cell sorting system, enabling up to 20 recoveries of single or multiple cells. Recovered cells were then amplified with Amp1/1 Whole Genome Amplification (WGA), Silicon Biosystems. An aliquot was used for gene-specific PCR and sequencing by capillary electrophoresis. The overall method was first validated, in blind, spiking KRAS-mutated cells in healthy donor blood samples. Results: On 5/6 (83%) single tumor cells recovered from DEPArray in spiking experiment, KRAS gene was successfully amplified: the blinded mutation was correctly identified and 100% purity was confirmed by DNA fingerprinting. In a duplicate spiking sample sequenced without single-cell sorting and WGA no mutation was detected. In patients, CXCR4 C-terminus sequence was obtained (range 1-9 sequences per patients) for 34/62 (55%) single and 6/11 (55%) multiple CTCs (range 2-8).

Conclusions: Mutation sequencing on multiple individual CTCs is possible following single-cell sorting with the method proposed. We succeeded in sequencing the C-terminus of CXCR4 gene from single EpCAM+ CTCs. As the study is still ongoing, these results are preliminary due to the small cohort of patients.

Associated Presentation(s):