Genetic profiling of circulating tumor cells from the cerebrospinal fluid of breast cancer patients with leptomeningeal metastasis

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Abstract

Background: The standard to diagnose leptomeningeal metastasis (LM) is to identify malignant cells in the cerebrospinal fluid (CSF). Using conventional cytology its sensitivity remains low with approximately 50% and 85% for the first and the second lumbar puncture. Once diagnosed with LMs, most patients are treated with untargeted therapies with limited effectiveness and significant side effects.

Objective: We aimed to developed a workflow using Sievewell 370 K microwell chips to enable molecular analysis of tumor cells from the CSF (CSF-CTCs).

Methods: Individual cells were separated on the chip, identified with cytokeratin antibodies, and isolated via micromanipulation. 15 CSF samples from 9 patients with suspected LM were analyzed on the chips and by conventional cytology. The isolated cells were characterized by low pass whole genome and panel sequencing and compared to extracranial tissue biopsies.

Results: On-chip immunostaining achieved less than 5% cell loss and over 95% single cell isolation for the MCF7 breast cancer cell line. For 10 of the 15 CSF samples, concordant results were obtained by analysis on the microwell chips and conventional cytology: 1 sample was tumor cell-negative, 9 samples were tumor cell-positive. In the other 5 samples, tumor cells were detected by analysis on the microwell chip with either inconclusive (3 samples) or negative (2 samples) results obtained by cytological analysis. With the microwell chips, 5 to 10,000 (mean 1,218; median 116) CSF-CTCs were found per mL CSF. The detection of chromosomal aberrations confirmed the malignant origin of detected tumor cells, including those from samples that were tumor cell-negative in conventional cytology. A high level of clonality was observed among the tumor cells in the CSF of each patient, yet these CSF tumor cells often exhibited distinct differences compared to the corresponding extracranial metastases or primary tumors. Panel sequencing revealed private mutations of CSF-CTCs in resistance related genes and tumor driver genes.

Conclusion: The microwell-based CSF-CTC detection appears superior to conventional cytology. Private mutations could impact drug susceptibility and therapy resistance. Therefore, the CSF-based liquid biopsy should be considered for genetic profiling of LMs, potentially improving diagnosis, treatment monitoring, and targeted therapy selection.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.