432l Detection of Disseminated Tumor Cells in Bone Marrow of Breast Cancer Patients

Xiaodong Tong, Kristie Melnik, Stephan Braun, Maciej Zborowski, and Jeffrey Chalmers

Both recent and historical studies have shown that the presence of disseminated tumor cells (DTCs) in bone marrow of patients with small, potentially curative, resected tumors is an indicator of relapse at distant sites from the initial tumor (Braun et al. NEJM 342:525-533; 2000). Thus, detection of occult metastatic tumor cells in bone marrow of breast cancer patients is of considerable importance and desired as an important potential indicator of prognosis and diagnosis in oncology. However, currently used and reported methods of detection are labor intensive and inaccurate; i.e. visual, microscopic observation of on the order of 10E7 total cells with a typical frequency of occurrence of 1 cancer cell per 10E6 of nucleated cells.

To become a routine, clinical diagnostic methodology, an accurate, rapid and reproducible technology for detection of these cancer cells is needed. We are developing a process with three sequential separation steps to enrich for the disseminated tumor cells in the bone marrow of breast cancer patients. These steps include: removal of erythrocytes using density gradient centrifuge or lysis buffer, flow-through magnetic cell separation to remove the normally occurring leukocytes using CD13, CD33 and CD45 as the targets for paramagnetic bead-conjugated antibodies, and immunocytochemistry staining with cytokeratin and Hoechst 33342 to stain the disseminated tumor cells.

Under the optimization conditions, this enrichment procedure routinely yields a 1.5-2 log depletion based on the nucleated cells. Cytokeratin positive cells were found in the bone marrow specimens of 11 of the 19 patients with breast cancer. To further study the relationship between the presence of occult cytokeratin-positive cells in bone marrow with the potential of tumor relapse, it is proposed to analyze the recovered tumor cells by real-time RT PCR and micro-array.