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Direct detection of circulating tumor cells (CTCs) in blood using digital cast PCR for early detection of lung cancer

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Enumeration and molecular characterization of circulating tumor cells (CTCs) in human blood holds great potential in cancer diagnosis, survival prognosis, and treatment guidance. However, current methods require extensive process to isolate rare CTCs in blood. We reported a new approach for direct CTC analysis in blood without cell sorting by using digital sample enrichment and reverse transcription competitive allele-specific TaqMan qPCR (RT-qCastPCR) for rare mutations and RT-qPCR for cancer cell-specific marker genes. CastPCR is capable of detecting 1 mutant in 1,000,000 wild-type molecules. Blood samples from lung cancer patients or normal individuals with spiked-in known lung cancer cell lines were partitioned in aliquots of 5 - 50 μL onto 96-well plates, such that each well contained either one cancer cell or none in the presence of 50 – 500 thousand normal white blood cells. Genetic mutations and panel of cancer-specific markers including CK19 and CEA for CTC identification and enumeration were determined by using both castPCR and RT-qPCR assays. The sample partition process resulted in a digital enrichment of 20 – 200 folds (the relative ratio of CTC to normal cells) in a CTC-positive well. Digital castPCR accurately identified known mutation and CK19 in spiked-in samples of ~10 - 60 cells per mL whole blood by two cell lines, but there was no positive well in the absence of spiked-in cells. Furthermore, cell type specific markers (CK19) and known EGFR mutations were identified in the same sample wells, indicating that identified mutation was specifically derived from cancer cells. In five blood samples from lung cancer patients of stage I - IV, EGFR mutation (p.L858R) was detected in all samples. CTC numbers in 3 early- stage lung cancers (I and II) were 11 - 32 cells/mL blood. In contrast, > 96 CTCs/mL were detected in stage IV patients. In conclusion, our data suggest that combination of digital sample enrichment with castPCR and RT-qPCR could be used to directly enumerate CTCs and detect cancer mutations in whole blood samples of lung cancer patients.