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Advancements in minimal residual disease detection: a practical approach using single-cell droplet PCR for comprehensive monitoring in hematological malignancy

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Abstract: The identification of chromosomal abnormalities accompanied by copy number alterations is important for understanding tumor characteristics. Testing methodologies for copy number abnormality have limited sensitivity, resulting in their use only for the sample provided at the time of diagnosis or recurrence of malignancy, but not for the monitoring of minimal residual disease (MRD) during and after therapy. We developped the "DimShift" technology which enable to measure the copy number of target gene/chromosome in each cell, which is given by the single cell droplet PCR. Qualitative result of DimShift given by peripheral blood was perfectly concordant with that of bone marrow. These findings and performances are promising to be the new methodology for MRD detection in malignant diseases utilizing bone marrow as well as peripheral blood.

Keywords: chromosomal abnormalities, droplet digital PCR, minimal residual disease, myelodysplastic syndrome

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Chromosomal abnormalities are frequently observed in hematological disorders. For example, myelodysplastic syndrome (MDS) is frequently accompanied by chromosomal aneuploidies, as in chromosomes 5−, 7−, 8+, 17−, or 20−.[1–3] These chromosomal aneuploidies are worth identifying and monitoring since they provide information regarding disease status or indicate poor prognosis.[4,5] Cytogenetic methods, such as fluorescence *in situ* hybridization or G-banding, are widely used to detect chromosomal copy numbers.[6] However, because they are low-throughput methods that require numerous steps and may not be welcomed in routine laboratory workflows. Therefore, in this study, we developed a novel high-throughput and sensitive assay for aneuploidy detection using droplet polymerase chain reaction (PCR). A PCR-based method would facilitate more rapid and more frequent monitoring of chromosomal status. Generally speaking, PCR is not suitable for counting just one or two copies, although this technique works as a powerful tool for detecting a novel genetic marker, such as BCR-ABL,[7] which is frequently present in cells of patients with chronic myelogenous leukemia and acute lymphoblastic leukemia, but not in cells from healthy patients. In a general real-time PCR, the information of the template molecules is retained during the exponential amplification phase and is reflected as the difference in Ct values. However, the sensitivity is not high enough to determine the ratio of abnormal cells if a sample is a mixture of normal karyotype and aneuploid cells. In digital PCR, where DNA is amplified in a nanoliter-sized compartment, the reaction is efficient enough for the amplification of only one molecule of DNA.[8] At the endpoint, however, the high efficiency makes it impossible to preserve information regarding the number of template molecules. The results only provide information as to whether or not the DNA of interest was amplified, that is, whether the target DNA was present or absent. We speculated that information on the number of template molecules might be retained before the reaction reaches its

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