Visualizing KRAS point mutations in non-small cell lung cancer tumors using the BaseScope in situ hybridization assay

Genomics

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About 25% of non-small cell lung cancer (NSCLC) patients bear one or more KRAS mutations in their tumors, which is correlated with poor prognosis. Precise identification of somatic mutations in tumors is important for developing targeted therapies. Sequencing technologies allow for mutation-profiling but they do not permit direct visualization and association of genetic alterations with cellular morphology.

To address this need we developed a specialized RNA in situ hybridization (ISH) method known as BaseScope. The BaseScope assay has a unique signal amplification system that allows for highly sensitive and specific detection of single nucleotide point mutations in tissues. BaseScope probes specific for KRAS G12C, G12A, G12V, G12S and wild type KRAS were designed and expression of each point mutation was assessed in a NSCLC tumor microarray with 48 tumor cores with known KRAS mutation status as determined by DNA sequencing. RNA quality and background signal threshold for each tumor core were determined using PPIB (positive) and dapB (negative) control probes.

Using the sequencing data as the gold standard, the BaseScope assay demonstrated 83-100% sensitivity and 97-100% specificity for various KRAS mutations [Table 1]. For KRAS G12C, the assay correctly identified all 6 sequencing-positive cores and identified the rest as negatives. For KRAS G12V, the assay detected 5 of 6 mutated cores with 100% specificity. Interestingly, for KRAS G12S and KRAS G12A mutations, the BaseScope assay demonstrated 100% sensitivity and 97% specificity. Furthermore, it was observed that 100% of the KRAS-mutated tumors showed expression for both wild type and mutant KRAS alleles within these NSCLC tumors.

In summary, we demonstrate the development of an RNA ISH assay for point mutations detection with morphological context in FFPE tissues. This assay has the unique ability to identify very small subclones whose frequency within the tumor might fall below the detection limit of sequencing.