EML4-ALK pre-mRNA and mature mRNA fusion detection using RNA fluorescence in situ hybridization (RNA FISH)

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ABSTRACT

Gene fusions that activate otherwise silent signaling enzymes, such as the anaplastic lymphoma receptor tyrosine kinase (ALK), are responsible for a significant number of lung and other cancers. Several drugs that target ALK have found success in the treatment of patients with ALK-gene fusions. RNA fluorescence in situ hybridization (FISH) is used for molecular diagnostics for ALK fusions, detects both functionally active and silent (non-transcribed or -translated) fusions.

We have applied Stellaris® RNA FISH in two approaches to detect EML4-ALK fusion RNAs. In Stellaris RNA FISH pools of directly fluorescently labeled 20-mer oligonucleotides with similar Tm are used for specific and sensitive detection and subcellular localization of the target RNAs.

Chromosomal 2 translocations (EML4-ALK) fuse the promoter and 5′ half of EML4 (active in normal lung tissue) and the 3′ and kinase encoding half of the otherwise silent ALK gene. To detect both mature cytoplasmic mRNA and nuclear pre-mRNA from both wild type and fused EML4 and ALK genes, eight RNA FISH probe sets labeled with four different fluorophores were designed against the different 5′ and 3′ segments. We chose the non-small cell lung cancer (NSCLC) adenocarcinoma H2228 cell line that carries one wild type and two mutant chr: 2 with two different inversions (presumed active), for testing.

Quadruplex RNA FISH was carried out on fixed cells with probe sets against each of the different 5′ and 3′ segments of EML4 and ALK mRNAs. Imaging revealed spectrally distinct co-localized signals consistent with wild type cytoplasmic EML4 mRNAs, and with EML4-ALK fusion mRNAs. However, no signal was detected for the ALK 3′ probe set, consistent with the absence of wild type ALK mRNA. Additionally, larger nuclear foci were detected with the EML4 (5′ and 3′) and the ALK (only exon probe sets, indicating three active chromosome 2 loci. Active transcription was confirmed for the wild type EML4 gene with probe sets targeting the first and last intron. Signals from the combination of EML4 (first intron) and ALK (both introns) were also found to co-localize on one chromosome, thus verifying the active transcription of one fusion gene. In contrast, at the second mutant chr 2 locus, only the EML4 (first intron) probe set gave a signal, indicating a non-productive ALK-fusion.

When EML4-ALK probe sets were applied to circulating tumor cells derived from patients with NSCLC, mutant cells were clearly distinguished from wild type cells. Similarly, EML4-ALK fusion mRNAs could be detected in NSCLC tissue samples.

In summary, we have successfully developed and applied Stellaris RNA FISH probe sets to detect mature cytoplasmic and immature nuclear EML4-ALK fusion mRNA. RNA FISH thus provides a useful tool to identify gene fusion genes in cultured tumor cells, circulating tumor cells and in tissue.

CONCLUSIONS AND REFERENCES

Stellaris FISH is a powerful method that enables detection, localization, and quantification of RNA at the single cell level. Single molecule RNA FISH (smFISH) provides an accurate method to capture the stochastic behavior of genes, providing further insight into cell-to-cell gene expression variation. By using spectrally distinct fluorescent labels, Stellaris FISH can distinguish different RNA variants from one or multiple genes. Furthermore, because most post-transcriptional processing, including pre-mRNA splicing, occurs co-transcriptionally, RNA FISH targeting the location of introns can be used as a proxy for the encoding gene. Thus, the Stellaris FISH method can serve as a functional proxy for DNA FISH.