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Detection and validation of novel RNA cancer biomarkers by single molecule RNA fluorescence *in situ* hybridization (smRNA FISH)

#3399

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ABSTRACT

RNA biomarkers are discovered and validated through advanced transcriptome analysis at a fast pace. These markers that allow the interrogation of the active genes hold promise for more precise staging, prognosis, and treatment of cancer. Long RNA markers comprise pre-mRNAs found in the cell nucleus, long non-coding RNAs (lncRNAs) that may be found in both the nucleus and the cytoplasm, as well as mature and mostly cytoplasmic mRNAs. Importantly, comparative studies in mouse have demonstrated that transcript levels are more strongly correlated with clinical traits than the corresponding protein levels. Because lncRNA and mRNA biomarkers have the potential to contribute to the development of targeted cancer therapies, there is an obvious need for a robust and dependable methodology to reliably detect and validate these RNA targets. RNA fluorescence *in situ* hybridization (RNA FISH) provides a powerful means to detect specific RNAs in single cells, while still maintaining tissue morphology. Significant advances in RNA FISH technology, such as the hybridization of multiple, fluorescently labeled 20-mer oligonucleotides to the RNA target, allow for the detection of single RNAs and yields information on the RNA's distinct spatial distribution within tissues and even within cells. In this study, we investigate emerging RNA biomarkers in prostate cancer cell lines and tissue utilizing RNA FISH. We also examine established breast cancer biomarkers such as human epidermal growth factor receptor 2 (HER2 / ERBB2), estrogen receptor α (ER α / ESR1), and progesterone receptor (PR / PGR) in breast cancer tumors and cell lines. The results and tools presented here will contribute to advancing the current capabilities of the detection and treatment of specific cancers, as well as in the continued discovery and development of cancer drug candidates.

METHOD

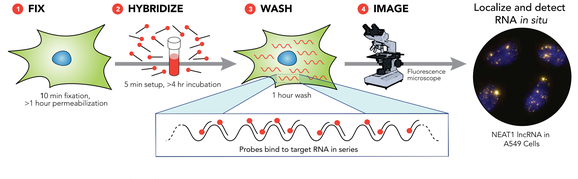

Figure 1. Schematic of Stellaris® RNA FISH assay
For each target, a mix of multiple 20-mer oligonucleotides, each labeled with a single Quasar® 570, Quasar 670, or CAL Fluor® Red 610 fluorophore was designed* and synthesized. All probe sets contained at least 32 oligos. Adherent cells were grown on #1 cover glass and subsequently fixed and permeabilized. Hybridizations were carried out for 4 to 16 hours at 37 °C in 50 µl hybridization solution (10% dextran sulfate, 10% formamide in 2X SSC). Samples were then washed, DAPI stained, and imaged.

*Stellaris probe designer and protocols: www.biosearchtech.com/stellaris

Microscope specifications used for image acquisition:
Nikon Eclipse Ti
Andor Clara CCD camera
Prior Lumen 200 Illumination System
CFI Plan Apo VC 60X Oil

RNA FISH IN ADHERENT CELL LINES

MALAT1 is a predictive RNA biomarker for metastasis development in lung cancer.

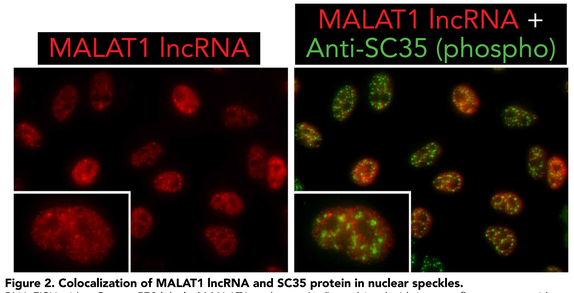

Figure 2. Colocalization of MALAT1 lncRNA and SC35 protein in nuclear speckles.
RNA FISH with a Quasar 570 labeled MALAT1 probe set (red) combined with immunofluorescence with an anti-p-SC35 antibody (green) reveals co-localization in the human lung adenocarcinoma cell line, A549.

Tumor expression of ER and/or PR, as observed in 70% of invasive breast cancers, is responsive to endocrine therapy such as tamoxifen. Overexpression of Her2, as observed in 15-20% of invasive breast cancers, is associated with a diminished prognosis (e.g., high risk of recurrence). However, a regimen of directed therapies that target Her2 has proven beneficial.

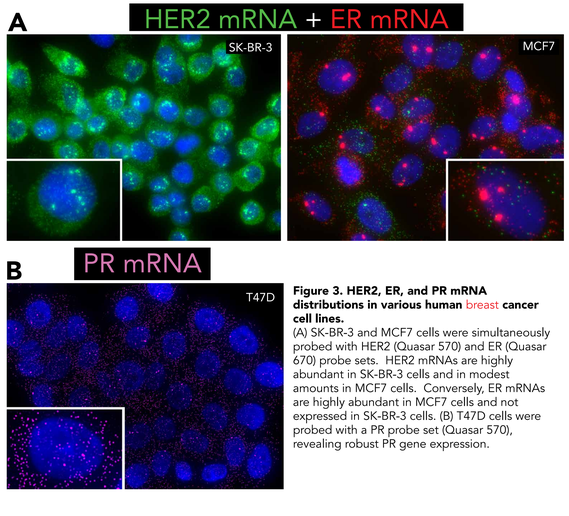

Figure 3. HER2, ER, and PR mRNA distributions in various human breast cancer cell lines.
(A) SK-BR-3 and MCF7 cells were simultaneously probed with HER2 (Quasar 570) and ER (Quasar 670) probe sets. HER2 mRNAs are highly abundant in SK-BR-3 cells and in modest amounts in MCF7 cells. Conversely, ER mRNAs are highly abundant in MCF7 cells and not expressed in SK-BR-3 cells. (B) T47D cells were probed with a PR probe set (Quasar 570), revealing robust PR gene expression.

RNA FISH IN ADHERENT CELL LINES (CONTD)

TDRD1 is upregulated as a direct ERG target gene and is strongly associated with ERG overexpression in primary prostate cancer.

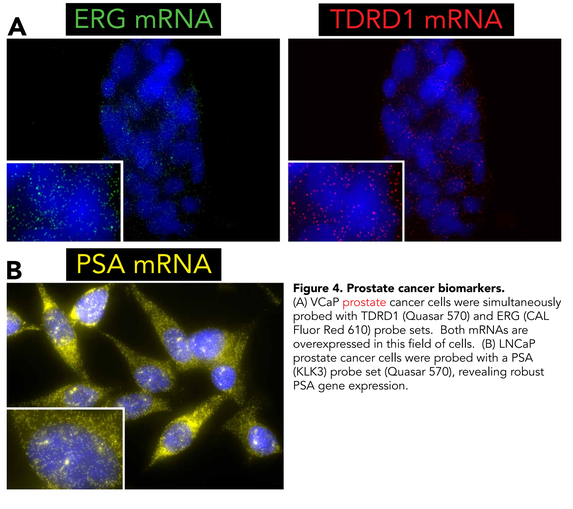

Figure 4. Prostate cancer biomarkers.
(A) VCaP prostate cancer cells were simultaneously probed with TDRD1 (Quasar 570) and ERG (CAL Fluor Red 610) probe sets. Both mRNAs are overexpressed in this field of cells. (B) LNCaP prostate cancer cells were probed with a PSA (KLK3) probe set (Quasar 570), revealing robust PSA gene expression.

RNA FISH IN FFPE TISSUE

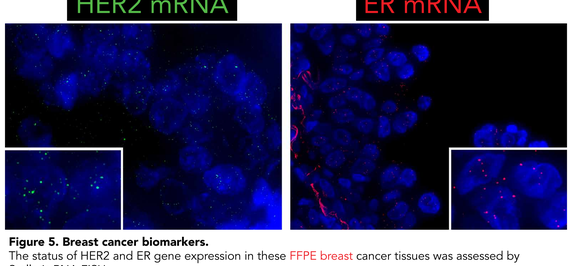

Figure 5. Breast cancer biomarkers.
The status of HER2 and ER gene expression in these FFPE breast cancer tissues was assessed by Stellaris RNA FISH.

RNA FISH IN FFPE TISSUE (CONTD)

Overexpression of PCA3 lncRNA and ERG are specific prostate biomarkers.

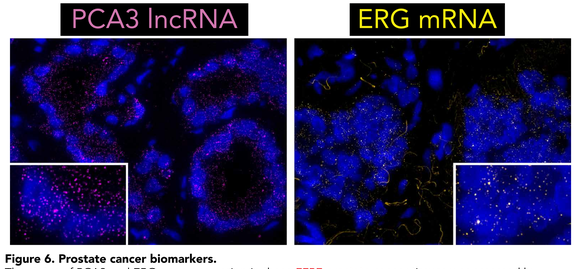

Figure 6. Prostate cancer biomarkers.
The status of PCA3 and ERG gene expression in these FFPE prostate cancer tissues was assessed by Stellaris RNA FISH.

CONCLUSIONS AND REFERENCES

Stellaris FISH is an RNA detection method that enables detection, localization, and quantification of RNA biomarkers (both mRNA and lncRNA) at the single cell level. The amplification and overexpression of genes in cell lines and tissue can be robustly assessed with this technology, allowing the Stellaris method to serve as a proxy for immunofluorescence and DNA FISH.

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