High-Throughput Imaging and High Content Analysis of Disease Relevant lncRNAs Examined by RNA Fluorescence in situ hybridization (RNA FISH)

Hans E. Johansson1, Arturo V. Orjalo, Jr.1, Sally R. Coassin1, Jerry L. Ruth1, Fabio Stossi2, Michael A. Mancini2

1: Biosearch Technologies, Inc., Novato, CA; 2: Baylor College of Medicine, Houston, TX

ABSTRACT

The list of known lncRNAs has increased dramatically in recent years. Novel lncRNAs are being discovered at an unprecedented rate and their functions are still largely unknown. Hybridization-based technologies have demonstrated to be powerful tools for the discovery of novel lncRNAs. By combining high-throughput imaging techniques with RNA in situ hybridization (RNA FISH) we have successfully identified a set of novel lncRNAs in colorectal cancer cell lines that were not previously reported in the literature. We have developed a classification method based upon a set of intensity quantification of RNA particles at the single cell level and determined their localization and pattern (texture). As specific lncRNAs (e.g. MALAT1) we have validated an imaging-based platform to perform RNA FISH in both manual (coverslip) and automated (384 well) formats that allows quantification of gene expression and patterns during the cell cycle since large number of cells can be analyzed simultaneously. We are now using this strategy to detect and study the expression of novel lncRNAs that could be useful both in the basic science and in the clinical setting with possible uses both in diagnostics and prognostics.

METHODS

For each target, a mix of multiple 20-mer oligonucleotides, each labeled with a single Quasar® 570 or 670 fluorophore was designed* and synthesized. All probe sets contained ≥ 36 oligonucleotides each. Hybridizations were carried out on coverslips or slides for 4 to 16 hours at 37 °C in 100 µl hybridization solution (10% dextran fluorophore) with variable X-chromosome karyotype.

In situ oligonucleotides for use in RNA Fluorescence in situ hybridization (RNA FISH). In contrast to traditional cell-disruptive RNA analyses (e.g. northern blotting, qPCR, and microarrays) that yield average and molecule fluorescence in situ hybridization (smRNA FISH) now bring such central aspects of cellular mRNA metabolism into view. Streamlining phenomena in the dark. Examples are differential stability of mRNA expression from different promoters, and inhibition of expression in trans. For each target, a mix of multiple 20-mer oligonucleotides, each labeled with a single Quasar® 570 or 670 fluorophore was designed* and synthesized. All probe sets contained ≥ 36 oligonucleotides each. Hybridizations were carried out on coverslips or slides for 4 to 16 hours at 37 °C in 100 µl hybridization solution (10% dextran fluorophore) with variable X-chromosome karyotype.

In the list of known lncRNAs has increased dramatically in recent years. Novel lncRNAs are being discovered at an unprecedented rate and their functions are still largely unknown. Hybridization-based technologies have demonstrated to be powerful tools for the discovery of novel lncRNAs. By combining high-throughput imaging techniques with RNA in situ hybridization (RNA FISH) we have successfully identified a set of novel lncRNAs in colorectal cancer cell lines that were not previously reported in the literature. We have developed a classification method based upon a set of intensity quantification of RNA particles at the single cell level and determined their localization and pattern (texture). As specific lncRNAs (e.g. MALAT1) we have validated an imaging-based platform to perform RNA FISH in both manual (coverslip) and automated (384 well) formats that allows quantification of gene expression and patterns during the cell cycle since large number of cells can be analyzed simultaneously. We are now using this strategy to detect and study the expression of novel lncRNAs that could be useful both in the basic science and in the clinical setting with possible uses both in diagnostics and prognostics.

REFERENCES


REFERENCES


Nuclear RNA LANDMARKS

Figure 5. Detection of In lncRNAs with Stellaris FISH probes.

Four prototypical In lncRNA FISH probe sets were used to demonstrate unique subnuclear localization patterns known to be associated with nuclear domains. The pre-mRNA containing SNHG11 locates to Cajal bodies (where its snRNPs are processed), STAG1 localizes to nucleolus (ribosomal synthesis), MALAT1 concentrates to RNA splicing speckles (mRNA splicing), and NEAT1 accumulates in paraspeckles (transcription factor regulation). Probe sets directly labeled with Quasar® 570 dyes were used in HeLa cell, and manually carried out in a 384 well, glass bottom plate (Greiner). Nuclei are labeled with the DNA specific dye DAPI (blue). Imaging was performed with a multiphoton capable DeltaVision deconvolution microscope using a 40x/1.0NA air lens.

ACKNOWLEDGMENTS

We thank Ron Cook for expert advice.

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

Figure 7. Quantitative analysis of subnuclear lncRNA patterns in primary endometrial cell lines. Stellaris FISH probes were used to label the InRNA MALAT1 in cell lines derived from two patients with different endometrioid ovarian adenocarcinomas (left panel) and from a primary ovarian adenocarcinoma (right panel). The staining was used to identify nuclear boundaries (green outline). RNA FISH staining is punctate in both cell lines, it exhibits a more complex nuclear-staining in the 3D smear cells. This is reflected in two feature patterns, including one that captures the linearity of structure (correlation, C) and one that captures the homogeneity of pattern (homogeneity, H). Similar analysis for NEAT1 (C, D, E, F, G) shows that its correlation is statistically different between the two cell lines while its homogeneity is not, highlighting that different measures can define different types of pattern changes.

 sclAIrNES

REFERENCES


