Detection of Long Noncoding RNA in Cancer by Fluorescence *In Situ* Hybridization

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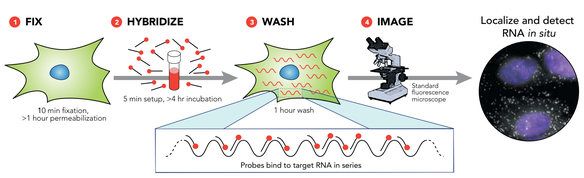
HYPOTHESIS

Long noncoding RNAs (lncRNAs) cannot be detected by immunohistochemistry, as no protein product is produced. Fluorescence *in situ* hybridization (FISH) using labeled DNA probes against chromosomal targets is also not useful, as transcription is not detected or quantified. The use of fluorescent synthetic oligonucleotide probes directed against the transcribed lncRNA should allow both the detection and quantification of the targets. To demonstrate this, we designed and tested fluorescent oligonucleotide probe sets against six lncRNAs (MALAT1, NEAT1, XIST, HOTAIR, H19, and PCA3) implicated in cancer.

METHODS



For each target, a mix of multiple 20mer oligonucleotides, each with a single Quasar® 570 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 sulfate,10% formamide in 2X SSC). Samples were then washed, nuclear stained with DAPI, and imaged.

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

RESULTS

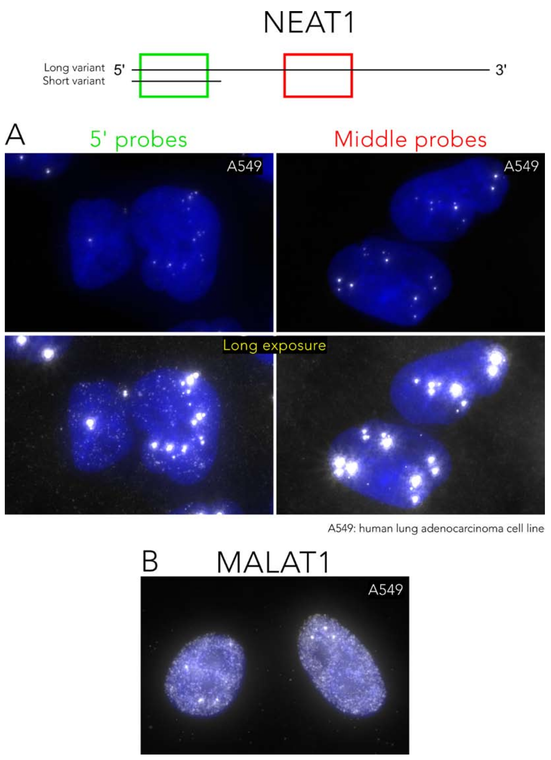


Figure 1. NEAT1 and MALAT1 RNA FISH

(A) NEAT1 RNA distribution of its transcript variants in A549 cells. The short variant can be observed outside of nuclear paraspeckles, as seen in the long exposure.
(B) MALAT1 RNA distribution in nuclear speckles in A549 cells.

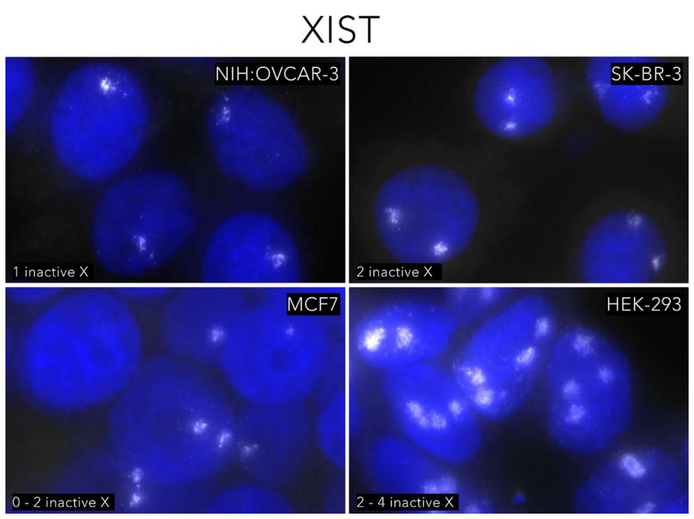


Figure 2. XIST RNA distribution in various human cell lines

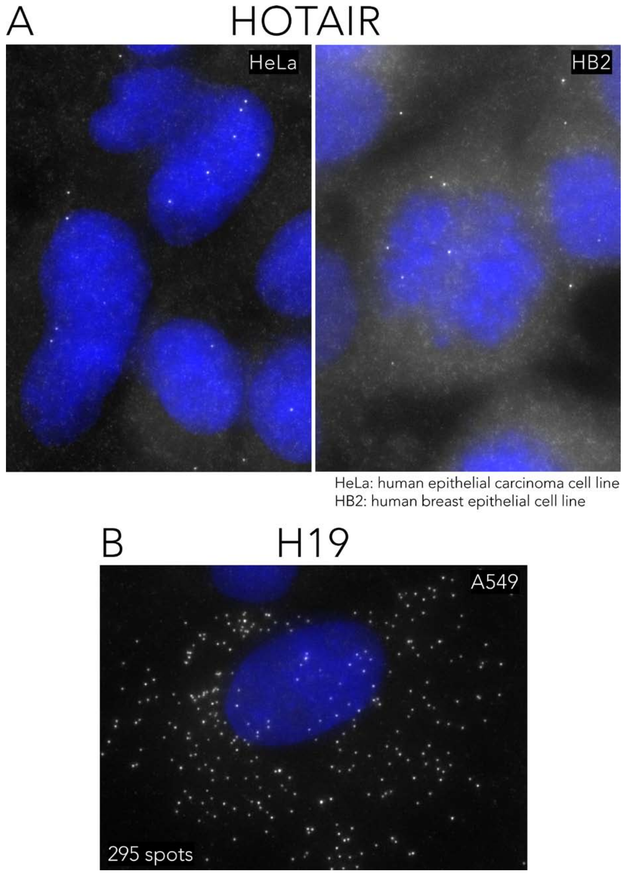


Figure 3. HOTAIR and H19 RNA FISH

(A) HOTAIR RNA distribution in HeLa cells and HB2 cells.
(B) H19 RNA distribution and quantification in a single A549 cell.

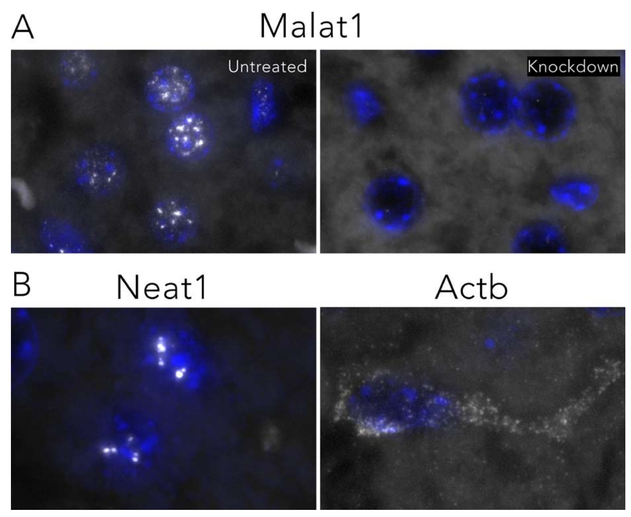


Figure 4. Malat1 and Neat1 RNA FISH in FFPE mouse liver tissue

(A) Malat1 RNA probed in liver tissue knocked down for Malat1.
(B) Neat1 and beta-actin RNA distribution in liver tissue.

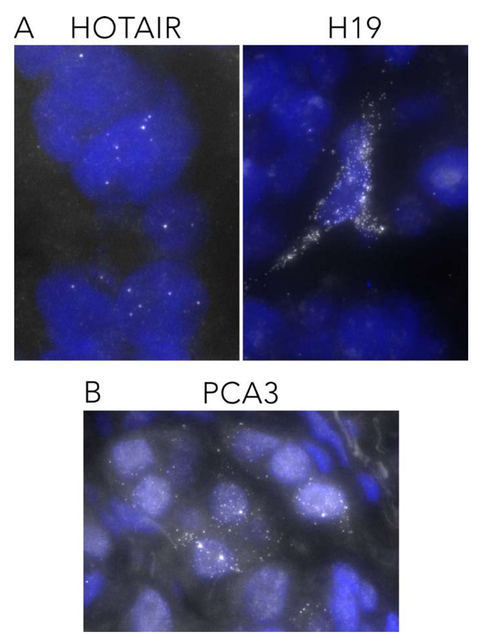


Figure 5. HOTAIR, H19, and PCA3 RNA FISH in human tissue

(A) HOTAIR and H19 RNA distribution in frozen ovarian tissue.
(B) PCA3 RNA distribution in FFPE prostate tissue (collected with appropriate permissions).

CONCLUSIONS

Long noncoding RNAs are easily detected by fluorescence *in situ* hybridization using a mixture of oligonucleotide probes, each labeled with a single fluorophore. Single molecule detection allows localization and quantification of the lncRNAs in question.

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