Application Note

Serum miRNA and lncRNA detection as a potential biomarker of lung cancer

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Lung cancer is one of the leading causes of mortality globally, and the second most common cancer among both men and women in the United States. Although it has been extensively studied for years, there is still a great deal unknown that relates to its detection for clinical diagnosis, prognosis and mechanism.

The discovery of cancer biomarkers, specific molecules that help distinguish between normal and malignant conditions, may potentially be developed into a more effective diagnostic tool for lung cancer. To explore the possibility of using serum miRNAs and lncRNAs as lung cancer biomarkers, we used the miScript® PCR System and the human RT² lncRNA Cancer PathwayFinder PCR Array to evaluate cancer-related miRNA and lncRNA levels, respectively, in non-small cell lung cancer (NSCLC) patient serum samples.

Introduction

Early diagnosis of lung cancer is still the key factor related to prognosis after treatment. Detection of lung cancer through traditional methods is limited in its effectiveness for early detection. Currently, the only recommended screening test for lung cancer is low-dose computed tomography, which is time-consuming and costly. A minimally invasive approach could be used to accelerate earlier detection of lung cancer.

miRNAs and lncRNAs contribute to the post-transcriptional regulation of mRNAs in all cell types for both healthy and diseased cells. Various miRNAs and lncRNAs have also been identified as either oncogenes or tumor suppressors. As both miRNAs and lncRNAs exhibit stable expression in circulation, their potential as tumor biomarkers is promising. To unlock potential miRNA and lncRNA signatures in circulation, high-performance tools capable of detecting low copy numbers and rare transcripts are imperative. For miRNA expression profiling, the miScript PCR System offers a suite of specialized products designed to overcome common challenges associated with circulating miRNA quantification. Similarly, the human RT² lncRNA PCR Arrays offer a leading solution for circulating lncRNA quantification. These technologies enable cutting-edge discoveries that identify minimally invasive biomarker signatures for the early detection of lung cancer.

Materials and methods

Serum from NSCLC patients and healthy donors (Bioreclamation, NY, and Asterand, Detroit, MI) were analyzed. The control group comprised total RNA from healthy donor serum samples. Group 1 comprised total RNA from NSCLC cancer patient serum samples. Total RNA from 200 µl serum sample was purified with the miRNeasy Serum/Plasma Kit.

For miRNA quantification, the miScript II RT Kit was used for cDNA synthesis. The Human Serum/Plasma miScript miRNA PCR Array was used for miRNA detection with the miScript SYBR® Green PCR Kit. For lncRNA detection, the RT² PreAMP cDNA Kit and RT² Human lncRNA Cancer PathwayFinder PreAMP Primer Mix were used for cDNA synthesis and target lncRNA preamplification. The Human RT² lncRNA Cancer PathwayFinder PCR Array was used for lncRNA detection with the RT² SYBR Green qPCR Mastermix. For both miRNA and lncRNA, real-time PCR was carried out on an Applied Biosystems® 7900HT Sequence Detection System. Data was analyzed using the GeneGlobe Data Analysis Center.

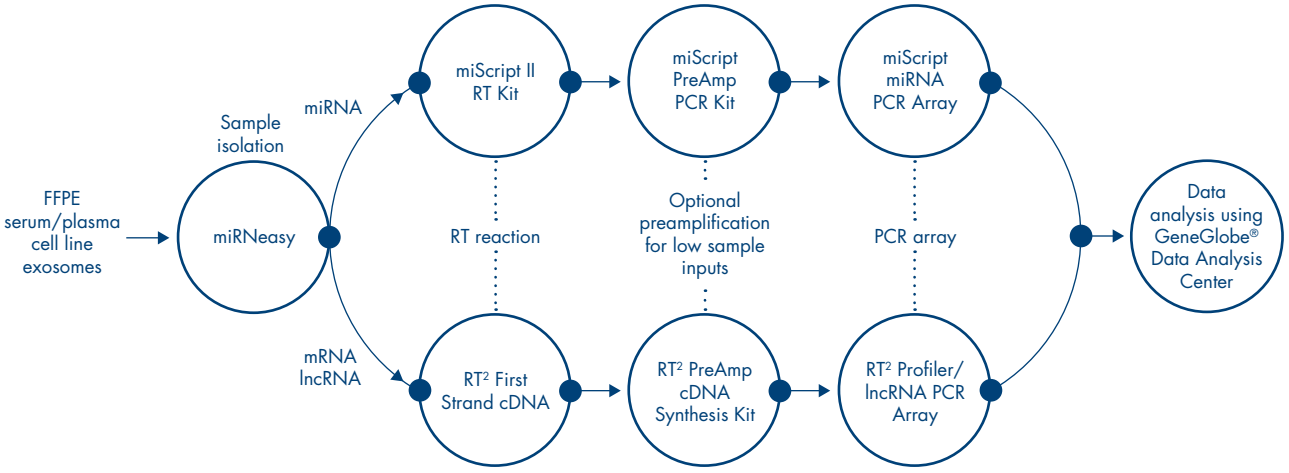

Figure 1. Total RNA Discovery workflow. miScript and RT² systems can be used to simultaneously quantify miRNA and lncRNA, respectively.

Results

Regarding miRNA quantification, the Human Serum/Plasma miScript miRNA PCR Array was used to profile the expression of the 372 most abundantly expressed miRNAs in serum or plasma. When ± 3-fold was used as a cutoff for expression regulation, 65 miRNAs exhibited differential expression. Upon applying a p-value of 0.05, the increased expression of 12 miRNAs and the decreased expression of one miRNA were determined to be significant (Figure 2). Differentially expressed miRNAs include hsa-miR-29a-3p, hsa-miR-29c-3p, hsa-miR-140-3p, hsa-miR-143-3p, hsa-miR-145-5p and hsa-miR-338-3p that have been previously associated with lung cancer.

For lncRNA detection, the Human RT² lncRNA Cancer PathwayFinder PCR Array enabled the detection of signal from 41 cancer-related lncRNAs in NSCLC patient serum samples – demonstrating that the RT² lncRNA Cancer PathwayFinder PCR Array and PreAMP Kit simplify lncRNA detection in serum. In contrast to healthy donor serum control samples, there was a trend for increased levels of lncRNA in cancer patient serum samples, including PVT1 and RMRP which were significantly upregulated (77-fold and 24-fold, $p<0.01$, $Cq<25$ in cancer samples) (Figure 3).

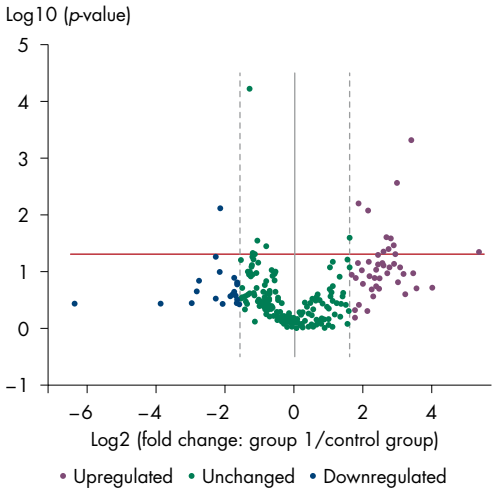

Figure 2. Differential detection of miRNA expression using the miScript PCR System. Volcano plot of miRNA expression changes in NSCLC patient serum samples, compared with healthy donor serum samples. Y: p-value; X: log 2 (fold change).

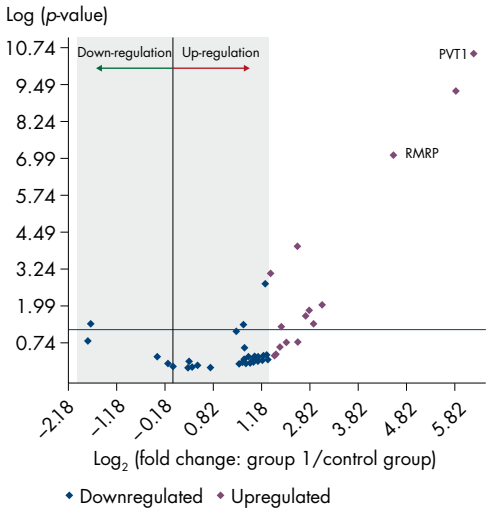

Figure 3. Successful detection of lncRNAs in serum samples with preamplification. Volcano plot of lncRNA gene expression changes in NSCLC patient serum samples, compared with healthy donor serum samples. Y: p-value; X: log 2 (fold change).

Conclusions

- miScript and RT² lncRNA PCR systems enable robust quantification of serum miRNAs and lncRNAs.
- Preamplification can enable quantification of lncRNAs, which are typically expressed at low levels in serum.
- miScript miRNA PCR Arrays and RT² lncRNA PCR Arrays can help identify differentially expressed miRNAs and lncRNAs in cancer serum samples compared with normal controls, thus, providing the opportunity to identify novel biomarkers.
- Ingenuity® Pathway Analysis can be used to model, analyze and understand the complex biological system underlying the experimental findings.

Ordering Information

Product	Contents	Cat. no.
miRNeasy	Isolation of total RNA (>18 nt) from cells, serum/plasma, tissue and FFPE	Varies
RT² lncRNA PCR Arrays	For profiling lncRNAs by pathway or disease, available in 96-well, 384-well and Rotor-Disc® 100 formats for almost all qPCR instruments	330721
RT² lncRNA PCR Array Modification	Add up to four genes to an RT² lncRNA PCR Array	330711
Custom RT² lncRNA PCR Array	Build your own RT² lncRNA PCR Array for RNAseq and microarray verification	330731
RT² RNA QC PCR Arrays	Arrays for quality-control analysis prior to experiments using RT² lncRNA PCR Arrays; available in 96-well, 384-well and Rotor-Disc 100 formats	330291
RT² lncRNA qPCR Assay (200)	For 200 x 25 µl reactions: laboratory-verified SYBR Green qPCR assay	330701
miScript miRNome	For SYBR Green-based, real-time PCR analysis of miRNA assays in 96-well, 384-well or Rotor-Disc formats	331222
miScript miFinder	For SYBR Green-based, real-time PCR profiling of miRNAs using the miScript PCR System	331221
Custom miScript miRNA PCR Array	For SYBR Green-based, real-time PCR profiling of custom miRNA panels using the miScript PCR System	331231

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

See how you can benefit from Total RNA Discovery at **www.qiagen.com/RNADiscovery**.