Serum lncRNA detection as a potential biomarker of lung cancer

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Lung cancer is the leading cause of cancer mortality, and the second most common cancer among both men and women in the United States. Although it has been studied extensively for many years, there is still a great amount of knowledge to be uncovered with respect to its clinical diagnosis, prognosis and mechanism. The discovery of cancer biomarkers, specific molecules that help distinguish between normal and cancerous conditions, may potentially be used to develop more effective diagnostic tools for lung cancer. To explore the possibility of using serum lncRNAs as lung cancer biomarkers, we used the Human RT² lncRNA Cancer PathwayFinder PCR Array to evaluate cancer-related long noncoding RNA (lncRNA) levels in Non Small Cell Lung Cancer (NSCLC) patient serum samples.

Introduction

Early diagnosis of lung cancer is still the key factor for mortality rate control. Currently, the only recommended screening test for lung cancer is low-dose computed tomography, which is time consuming and costly. A blood-based screening method would be more convenient. lncRNAs have been linked to the development of cancer and thus their potential as tumor biomarkers in blood-based tests seems promising. Researchers are increasingly aware of the important role played by lncRNAs in tumorigenesis, and a variety of lncRNAs have been identified as oncogene or tumor suppressors. Although the biomarker potential of lncRNAs has been discussed vigorously, the lack of a tool for screening serum/plasma lncRNAs has limited studies so far. Better lncRNA profiling and confirmation of stable lncRNA biomarkers will improve the early diagnostic efforts of lung cancer and its treatment.

Materials and methods

Serum from NSCLC patients (Bioreclamation, NY) and healthy donors (Asterand, Detroit, MI) was analyzed. The control group comprised total RNA from healthy donor serum samples. Group 1 comprised total RNA from NSCLC serum samples. Total RNA from 200 µl serum was purified with the miRNeasy Serum/Plasma Kit. The RT² PreAMP cDNA Synthesis Kit and RT² lncRNA PreAMP Primer Mix for Human Cancer PathwayFinder 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. Real-time PCR was carried out on an Applied Biosystems® 7900HT Sequence Detection System. The QIAGEN® PCR Array Analysis Tool was used for gene expression analysis.

Results

A genomic contamination control was used to assure that no genomic DNA contamination was present, which could affect the result (Cq>30). With preamplification, 3 out of 5 reference genes (B2M, RPLP0 and RN7SK) were consistently detected at a high level, and their geometric mean was used as a reference for normalization. Use of the Human RT² lncRNA Cancer PathwayFinder PCR Array allowed the detection of signal from 41 cancer-related lncRNAs (average Cq<30) in NSCLC serum samples – thus, the RT² lncRNA Cancer PathwayFinder PCR Array and PreAMP cDNA Synthesis 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 serum samples. Among these increased lncRNAs in cancer samples, two, PVT1 and RMRP, were significantly upregulated (77-fold and 24-fold, p<0.01, Cq<25 in cancer samples) (Figure 1). These significant changes in the serum of NSCLC samples, demonstrated the possibility of using lncRNAs as lung cancer biomarkers.

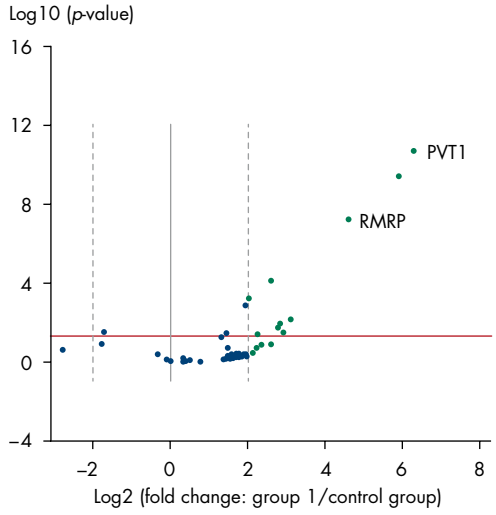

Figure 1. Successful detection of lncRNAs in serum samples with preamplification. Volcano plot of lncRNA gene expression changes in NSCLC serum samples compared with healthy donor serum samples. **Y**: p-value; **X**: Log2 (fold change).

Discussion and conclusions

Identification of potential lung cancer related lncRNA biomarkers is challenging due to limited starting material and low lncRNA content. Using the miRNeasy Serum/Plasma Kit to purify total RNA, followed by preamplification with the RT² PreAMP cDNA Synthesis Kit and RT² lncRNA PreAMP Primer Mix for Human Cancer PathwayFinder overcomes these challenges, enabling reliable detection of lncRNAs from human serum samples and non-biased expression analysis. Profiling of expression changes using the RT² Human Cancer PathwayFinder lncRNA PCR Array

focuses on the most promising lncRNAs linked to cancer (Table 1). Identification of significant expression changes that correlate with disease state will highlight lncRNAs that could potentially be used as biomarkers for blood-based lung cancer screening.

Table 1. lncRNAs profiled using the RT² lncRNA PreAMP Primer Mix for Human Cancer PathwayFinder, listed by functional gene grouping.

Functional Gene Group	Functional Gene Subgroup	lncRNAs
Oncogenes	-	ACTA2-AS1, AFAP1-AS1, BANCR, BCAR4, BLACAT1, CBR3-AS1, CCAT1, CCAT2, CRNDE, H19, HAND2-AS1, HEIH, HIF1A-AS2, HNF1A-AS1, HOTAIR, HOTTIP, HOXA-AS2, HULC, JADRR, KCNQ1OT1, LINC00152, LINC00963, LSINCT5, LUCAT1, MALAT1, MIR155HG, MIR17HG, PCA3, PCAT1, PCGEM1, POU5F1P5, PRNCR1, PVT1, SNHG16, SPRY4-IT1, SUMO1P3, TERC, TRERNA1, TUG1, UCA1, XIST
Tumor Suppressor Genes	-	ADAMTS9-AS2, CAHM, DLEU2, DLX6-AS1, GACAT1, GAS5, GAS6-AS1, GNAS-AS1, LINC00261, LINC00312, MEG3, MIR31HG, MIR7-3HG, NAMA, NEAT1, PTCSC1, PTCSC3, PTENP1, TERC, TUSC7, WT1-AS, ZFAS1
Cancers	Bladder Cancer	BLACAT1, H19, MALAT1, MEG3, SNHG16, TERC, TUG1, UCA1
	Breast Cancer	BCAR4, GAS5, H19, HIF1A-AS2, HOTAIR, JADRR, LSINCT5, MIR31HG, MRPL23-AS1, TRERNA1, UCA1, XIST, ZFAS1
	Cervical Cancer	MEG3
	Colorectal Cancer	CCAT1, CCAT2, CRNDE, H19, HOTAIR, MALAT1, PCAT1, PVT1, SNHG16, TUSC7
	Endometrial Cancer	H19, HOTAIR
	Esophageal Squamous Cell Carcinoma	CBR3-AS1, HOTAIR
	Gallbladder Cancer	MALAT1
	Gastric Cancer	CCAT1, CDKN2B-AS1, GACAT1, H19, HOTAIR, HULC, LINC00152, LINC00261, LINC00312, MEG3, PVT1, SUMO1P3, TERC
	Glioma	H19, HOTAIR, MEG3
	Kidney Cancer	AFAP1-AS1, DGCR5, HIF1A-AS2, WT1-AS
	Laryngeal Squamous Cell Carcinoma	HOTAIR, MALAT1
	Leukemia	DLEU2, GAS5, H19, HOTAIR, HOXA-AS2, MALAT1, MEG3, TERC, TUG1, XIST
	Liver Cancer	H19, HEIH, HOTAIR, HOTTIP, HULC, KCNQ1OT1, MALAT1, MEG3, MIR7-3HG, PANDAR
	Non-Small Cell Lung Cancer	BANCR, CCAT2, GAS6-AS1, HOTAIR, MALAT1, MEG3
	Other Lung Cancers	ACTA2-AS1, LUCAT1
	Melanoma	BANCR, HOTAIR, PTENP1
	Multiple Myeloma	MEG3, PVT1
	Nasopharyngeal Carcinoma	HOTAIR, LINC00312
	Neuroblastoma	HAND2-AS1, SNHG16
	Oesophageal Squamous Cell Carcinoma	LINC01234
	Osteosarcoma	TUG1
	Ovarian Cancer	H19, HOTAIR, LSINCT5, WT1-AS, XIST
	Pancreatic Cancer	GAS5, HOTAIR, PVT1
	Pituitary Cancer	MEG3

Functional Gene Group	Functional Gene Subgroup	lncRNAs
	Prostate Cancer	CBR3-AS1, GAS5, HOTAIR, LINC00963, MALAT1, PCA3, PCAT1, PCGEM1, PRNCR1, PVT1, TERC, XIST
	Rhabdomyosarcoma	RMST
	Sporadic Pediatric Adrenocortical Tumors	KCNQ1OT1
	Testicular Cancer	XIST
	Thyroid Cancer	NAMA, PTCSC3
	Other Cancer-Related lncRNAs	AIRN, EMX2OS, FTX, HIF1A-AS1, HOTAIRM1, HOXA11-AS, IPW, KRASP1, LINC00538, LINC00887, LINC01233, NBR2, NRON, RMRP, RPS6KA2-AS1, TSIX

See the full information for this array at **www.qiagen.com/RT2lncRNAPCR-Array**.

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