Differential Plasma Exosomal Long Non-Coding RNAs Expression Profiles and Their Emerging Role in Smokers and Vapers

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INTRODUCTION

- **Long non-coding RNA (lncRNA)** are diverse set of transcripts that play a critical role in biological processes like gene regulation, transcription, post-transcriptional modification, and chromatin remodeling.
- Studies have reported the presence of **lncRNA in the exosomes**.
- **Exosomes** are the smallest subtype of extracellular vesicles (30-150 nm in size) surrounded by phospholipid bilayer.
- They are known to be involved in regulating **cell-to-cell communication in lung pathologies** including lung cancer, COPD, asthma and IPF.
- Importantly, exosomal lncRNAs are being extensively investigated as potential diagnostic biomarkers, especially for cancers.
- **Cigarette smoking** has been found to alter lncRNA associated with activation of crucial metabolic pathway. Thus, It is likely that tobacco smoke exposure can alter the lncRNA profile within the lung tissue and can be involved in the pathogenesis of lung diseases.
- Smoking habits amongst adults and adolescents are not confined to combustible tobacco cigarettes only. In the last few decades, the popularity of **e-cigarette and related products** is on a rise amongst young adults in the US.
- We thus were interested in comparing how the lncRNA profiles vary amongst non-smokers, cigarette smokers and e-cig users.
- Successful completion of this project will **characterize the lncRNA content of the plasma-derived exosomes** from various smoking populations **to identify biomarkers relevant to pulmonary pathologies like COPD, asthma or IPF.**

HYPOTHESIS

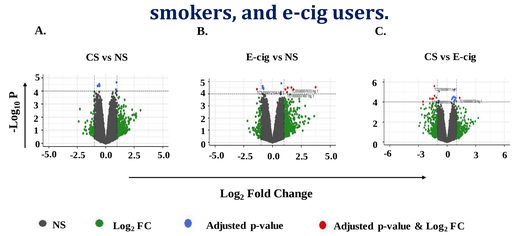
Plasma derived exosomal lncRNA profile is distinct amongst smokers, vapers and non-smokers and are responsible in regulating the inflammatory responses eventually leading to lung-related pathologies amongst these populations. .

METHODS

- **Ethics approval and consent to participate:** All the protocols and procedures were approved by the Institutional Review Board (IRB)/Research Subject Review Board (RSRB) committee at the University of Rochester Medical Center, Rochester, NY with an approval number CR00002635. All the study participants recruited for the study signed an informed written consent form before recruitment and sample collection.
- **Study design and subjects:** 20-25 ml of venous blood was collected by venipuncture from smokers (CS), vapers (E-cig), and non smokers (NS). In brief, n=6-8 subjects were selected in each group.
- **Plasma exosome isolation:** We employed commercially available kit from Norgen Biotek (Cat# 57400; Ontario, Canada) to isolate exosomes from human plasma. Plasma exosomes were isolated as per manufacturer's protocol. Transmission electron microscopy (TEM) was used to visualize and characterize the isolated exosomes and nanoparticle tracking analysis was performed to analyze the particle size and concentration.
- **Exosomal RNA extraction:** Total RNA from exosomes was isolated using Exosomal RNA isolation kit (Norgen Bioteck Corporation, Cat# 58000) per the manufacturer's protocol.
- **Library preparation and Microarray analysis:** GeneChip™ WT Pico kit (Cat# 902622 and 902623, Applied Biosystems, Foster city, CA) was employed for transcriptome profiling of the RNA isolated from various experimental groups. The library preparation and microarray hybridization were outsourced to the Microarray & NextGen Sequencing Core at the Center for Functional Genomics at SUNY Albany.
- **Statistical analysis:** The long non-coding RNA (lncRNA) data from two batches were first normalized and they analyzed in the R/Bioconductor. Volcano plots were generated to highlight significant lncRNAs with significant fold changes (> 2-fold changes) and raw P-values (less than 0.0001). Heatmaps were generated to show the lncRNA expressions in different groups. To examine the overlaps in identified lncRNAs from related group comparisons, *venn.diagram* function in R/Bioconductor was used.

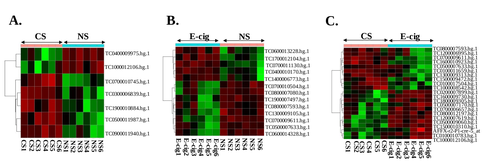
RESULTS

Volcano Plot showing distinct expression profiles of lncRNA from plasma exosomes from non-smokers, smokers, and e-cig users.



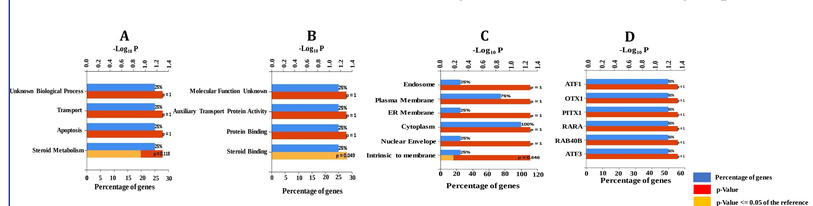
Volcano plot showing the relation between P-values (Y-axis) vs fold change (X-axis) in the differentially expressed lncRNAs amongst (A) Cigarette smokers vs. non-smokers, (B) E-cig users vs. Non-smokers, and (C) Cigarette smokers vs. E-cig users. Log_2 fold change (cut-off = ± 1, vertical lines) was plotted against the $-log_{10}$ p-value (cut-off = 4, horizontal line). Adjusted p-value is p-value <=0.0001.

Hierarchical cluster analyses of differentially expressed lncRNA. from plasma exosomes from non-smokers, smokers, and e-cig users.



Heat map showing differentially expressed lncRNAs that are significantly varies between (A) Non-smokers and cigarette smokers, (B) Non-smokers and E-cigarette users, and (C) Cigarette smokers and E-cig users. These lncRNAs were identified based on individual pairwise comparisons (with unadjusted raw *p*-value; $P < 0.05$). Each row represents individual lncRNA, and each column represents individual sample. The relative lncRNA expression is depicted according to the color scale as shown at the right side of the figure.

Gene enrichment analysis for the differentially expressed lncRNAs.



The top six enriched: (A) Biological process, (B) Molecular function, (C) Cellular component, and (D) Transcription factors for the significant lncRNAs and possible gene targets on pairwise comparisons between E-cigarette users vs. Non-smokers.

RESULTS

Significant differentially expressed lncRNAs on comparing E-cig users with non-smokers and their significance.

PROBEID	GENENAME	log2 Fold change	P. Value	Description
E-cig vs NS				
TC0400010170.hg.1	skusworbu	-0.854161017	3.15E-05	Putative Protein
TC0500007633.hg.1	lozorby	1.644179344	2.96E-05	RNA-binding protein like family member.
TC0600013228.hg.1	SLC2A12	-0.792272819	3.95E-05	Solute carrier family 2 member 12
TC0600014328.hg.1	OSTM1	0.764009224	1.32E-05	Osteoclastogenesis associated transmembrane protein 1
TC0700009611.hg.1	Transfer RNA-Cys (GCA) 1-1	1.115171958	4.41E-05	tRNA; Genhancer regulatory region
TC0700010504.hg.1	OSBPL3	0.721080223	6.85E-05	Oxysterol binding protein like 3
TC0700011130.hg.1	T324413 (miTranscriptome)	-0.862064073	2.30E-05	Unannotated
TC0800007080.hg.1	BNIP3L	3.736036439	2.80E-05	Bcl2 interacting protein 3 like
TC0800007593.hg.1	zoyberby	1.81372327	4.40E-05	Endonuclease reverse transcriptase family member
TC1300009105.hg.1	lnc-PCDH20-13	1.325335976	3.19E-05	RNA gene
TC1400006773.hg.1	miR4307	-0.779299012	3.36E-05	micro RNA
TC1700012104.hg.1	T156883 (miTranscriptome)	-1.361512953	4.10E-05	Unannotated
TC1900007497.hg.1	flarchoy	1.258862445	7.32E-05	Endonuclease reverse transcriptase

SUMMARY

- **13 lncRNA** gene loci showed altered expression among e-cig users as compared to NS.
- A **four-fold increase** in the TC0800007080.hg.1 gene locus that encodes **Bcl2 interacting protein 3 like-protein (BNIP3L)** was observed.
- Gene enrichment analyses revealed a significant change (p=0.049) in the expression of **lncRNAs involved in steroid binding** in the plasma-derived exosomes from e-cig users as compared to their non-smoking controls.

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