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LETTER TO THE EDITOR Open Access

# Small non-coding RNA biomarkers in sputum for lung cancer diagnosis

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**Abstract**

The early detection of lung cancer can reduce the mortality. However, there is no effective means in clinical settings for noninvasively detecting lung cancer. We previously developed 3 sputum miRNA biomarkers and 2 sputum small nucleolar RNA (snoRNA) biomarkers that can potentially be used for noninvasively diagnosing lung cancer. Here we evaluate the individual and combined applications of the two types of biomarkers in different sets of lung cancer patients and controls. Combined analysis of the miRNAs and snoRNAs has a synergistic effect with 89 % sensitivity and 89 % specificity, and may provide a useful tool for lung cancer early detection.

## Background

Non-small cell lung cancer (NSCLC), primarily caused by cigarette smoking, is the leading cause of cancer-related mortalities [1]. There are two major types of NSCLC: adenocarcinoma (AC) and squamous cell carcinoma (SCC). The early detection of NSCLC may decrease the mortality [1, 2]. However, there is no effective and noninvasive means for the early detection [3]. Sputum is a noninvasively and easily accessible body fluid that contains exfoliated bronchial epithelial cells [4]. Molecular study of sputum could detect the molecular abnormalities in the bronchial airways that reflect those existing in primary lung tumors, and thus provides a noninvasive approach for NSCLC detection [5].

Small non-coding RNAs (ncRNAs) mainly consist of microRNAs (miRNAs) and small nucleolar RNAs (snoRNAs), and play an important role in the pathogenesis of various cancers [6–16]. There is significant interest in the development of the tumor-related ncRNAs as biomarkers for cancer diagnosis [17]. We have identified a panel of three sputum miRNA biomarkers (miRs-21, 31, and 210) with 82.9 % sensitivity and 87.8 % specificity and a panel of two snoRNA sputum biomarkers (snoRDs-66 and 78) with 74.6 % sensitivity and 83.6 % specificity for lung cancer early detection [18–20]. Since lung cancer is a heterogeneous disease featuring field defects in the airway of smokers [21, 22], a single biomarker type can't achieve the sensitivity and specificity required for clinically diagnosing NSCLC. Because miRNAs and snoRNAs have highly and actively different roles in tumorigenesis, integrating the miRNA and snoRNA-based biomarkers may improve the performance of the biomarkers for NSCLC detection. Here we evaluate the individual and combined applications of the two different types of ncRNAs for the early detection of lung cancer.

## Findings

With a protocol approved by Institutional Review Board of the University of Maryland Medical Center Center, we collected sputum from 316 NSCLC patients and 528 cancer-free smokers. Of the 316 lung cancer patients, 103 were diagnosed with stage I NSCLC. We used the 103 stage I NSCLC patients as cases. From the cancer-free subjects, we randomly selected 117 individuals as control cases. The 103 stage I NSCLC cases and 117 cancer-free smokers were randomly split into a training set (Table 1) and an internal testing set (Table 2).

We determined expressions of the five ncRNAs (miRs-21, 31, and 210, and snoRDs-66 and 78) by quantitative reverse transcriptase PCR (qRT-PCR) in the sputum samples [18, 23–27]. The panel of three miRNAs (miRs-21, 31, and 210) and panel of two

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**Table 1** Characteristics of lung cancer patients and cancer-free smokers of a training set

| | NSCLC cases ($n = 46$) | Controls ($n = 55$) | *P*-value |
|---|---|---|---|
| Age | 65.28 (SD 11.27) | 67.65 (SD 11.34) | 0.35 |
| Sex | | | 0.38 |
| Female | 18 | 22 | |
| Male | 28 | 33 | |
| Race | | | 0.08 |
| White | 30 | 36 | |
| African American | 16 | 19 | |
| Pack-years | 44.79 (Range, 5–172) | 43.45 (Range, 5–109) | 0.38 |
| FEV1/FVC | 0.45–0.79 | 0.43–0.80 | 0.10 |
| Nodule size (cm) | 4.79 (Range, 95.25) | 1.29 (Range, 56.76) | <0.01 |
| Stage, all are stage I | | | |
| Histological type | | | |
| Adenocarcinoma | 25 | | |
| Squamous cell carcinoma | 21 | | |

*Abbreviations*: *NSCLC* non-small cell lung cancer

snoRNAs (snoRDs-66 and 78) had a receiver operating characteristic (ROC) curve (AUC) value of 0.90 and 0.86, respectively (Fig. 1). Interestingly, combined use of the five ncRNAs produced 0.94 AUC (Fig. 1), being significantly higher than that of the panel of three miRNAs (0.90) or the panel of two snoRNAs (0.86) ($p < 0.05$). Furthermore, combined analysis of the five ncRNAs had higher sensitivity (89.13 % vs. 82.61 % or 73.91 %) and specificity (89.09 % vs. 85.45 % or 83.64 %) compared with the individual panels (All $P < 0.05$). The expression level of the five ncRNAs was associated with smoking history and size of PN of participants (All $P < 0.05$). The expression level of sputum miR-21 was more closely related with AC ($P < 0.05$), whereas miR-210 was associated with SCC ($P < 0.05$). However, overall, the panel of the five ncRNA biomarkers didn't exhibit special association with a histological type of the NSCLC cases, and the age, gender, ethnicity, and forced expiratory volume 1 (FEV1)/forced vital capacity (FVC) of the participants (All $P > 0.05$). The validation of the ncRNA biomarkers in a testing cohort confirmed that combined study of miRNAs and snoRNAs in sputum had a synergistic effect for the early detection of NSCLC.

**Table 2** Characteristics of lung cancer patients and cancer-free smokers of a testing set

| | NSCLC cases ($n = 57$) | Controls ($n = 62$) | *P*-value |
|---|---|---|---|
| Age | 64.26 (SD 12.37) | 66.69 (SD 10.88) | 0.36 |
| Sex | | | 0.39 |
| Female | 22 | 23 | |
| Male | 35 | 39 | |
| Race | | | 0.09 |
| White | 37 | 40 | |
| African American | 20 | 22 | |
| Pack-years | 43.89 (Range, 5–170) | 42.64 (Range, 5–112) | 0.39 |
| FEV1/FVC | 0.46–0.78 | 0.44–0.79 | 0.09 |
| Nodule size (cm) | 4.89 (Range, 96.22) | 1.54 (Range, 54.89) | <0.01 |
| Stage, all are stage I | | | |
| Histological type | | | |
| Adenocarcinoma | 31 | | |
| Squamous cell carcinoma | 26 | | |

*Abbreviations*: *NSCLC* non-small cell lung cancer

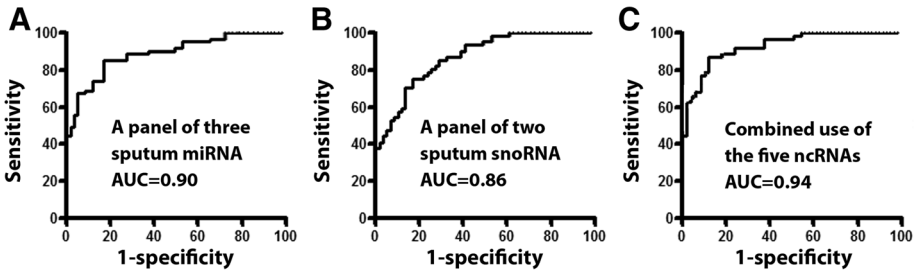


**Fig. 1** Combined analysis of miRNAs and snoRNAs in sputum has a synergistic effect for lung cancer detection. **a** ROC curve of a panel of three sputum miRNA biomarkers (miRs-21, 31, and 210) shows an AUC of 0.90 for differentiating NSCLC patients from the cancer-free subjects in terms of sensitivity and specificity. **b** a panel of two snoRNA sputum biomarkers (snoRDs-66 and 78) creates an AUC of 0.86 for distinguishing NSCLC patients from the cancer-free subjects. **c** combined study of the three miRNAs and two snoRNAs in sputum yields 0.94 AUC, which is significantly higher than that of any single type of ncRNAs used alone ($P < 0.05$) for lung cancer detection

## Conclusion

Combined study of the miRNAs and snoRNAs has higher sensitivity and specificity compared with a single type of the ncRNA biomarkers, providing a noninvasive approach for lung cancer early detection. Nevertheless, a prospective project is required for validating the utility.

## Ethical statements

No concern.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

YS and FJ conducted the experiments and participated in data acquisition and interpretation. MAG participated in data interpretation. HF conducted data analysis. FJ conducted study design, coordination, and prepared the manuscript. All authors read and approved the final manuscript.

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