MICRORNAS AS STABLE CIRCULATING BIOMARKERS FOR LUNG CANCER DIAGNOSIS

Cheng-Chi Yen(嚴正棋)1, Chun-Liang Shih(施鈞喨)2, Chiuan-Chian Chiou(邱全芊)1,2

1Department of Medical Biotechnology and Laboratory Science and 2Institute of Biomedical Science
Chang Gung University, Taiwan

Abstract

Background: Lung cancer is the leading cause of cancer mortality in the world, but there are no satisfactory biomarkers for lung cancer detection. MicroRNAs are small non-coding RNAs that regulate the gene expressions at the posttranscription level. Many microRNAs are dysregulated and shows specific expression patterns in human cancer. The aim of this study is to profile circulating microRNAs in lung cancer patients and identify potential microRNA markers for lung cancer diagnosis. Method: Total RNAs from plasma of 5 non-small cell lung adenocarcinoma patients and 2 healthy volunteers were extracted and subjected to microRNA profiling with Illumina Human miRNA microarray. Microarray data were normalized with Global median and analyzed with One-Way ANOVA provided in Partek Genomics Suite. The microRNAs with significant higher level (p < 0.05 and fold change >4) in lung cancer patients were identified as marker candidates. Independent set of plasma samples (from 27 cancer patients and 27 volunteers) were used for validation of these markers with quantitative RT-PCR. Results: Principle Component Analysis (PCA) showed different profiles of plasma microRNA in lung cancer patients and healthy volunteers. Four microRNA candidates with higher level in lung cancer patients were identified. Further screening with clinical samples confirmed that the levels of two microRNAs, miR-148b* and miR-33a were significantly higher in lung cancer patients. Conclusion: Our results demonstrated that dysregulated microRNAs can be found in plasma of lung cancer patients and that these cancer-specific microRNAs may have potential to be used as diagnostic markers for lung cancer.
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Result 1. The profiles of microRNAs were different between NSCLC and control samples.

Table 1. Characteristics of non-small cell lung cancer for miRNA microarray

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<th>Subject</th>
<th>Gender</th>
<th>Age</th>
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<th>Day 1</th>
<th>Day 15-24</th>
<th>Day 35</th>
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</table>

Figure 1. Analysis of microarray data for microRNAs in NSCLC and control. (A) The intensity of microarray data for each samples (normalized with global median). Green bars are the healthy volunteers and blue bars are the lung cancer patients. (B) Principle component analysis (PCA) of the microarray data. Lung cancer patients (blue plot) and healthy volunteers (green plot) were separated in different groups.

Result 2. Four microRNAs candidates with higher level in plasma of lung cancer patients were identified.

Figure 2. 2D cluster analysis across lung cancer and healthy volunteers samples (left) and microRNA reporters (top). Hierarchical trees (top and left) and a heat map (bottom) are displayed. The green squares represent for downregulated miRNAs and the red squares, upregulated miRNAs.

Figure 3. The intensity of four microRNA candidates, miR-33a, miR-134, miR-148b* and miR-376b, on the microarray, shown as dot plot. Blue spots are the healthy volunteers and red spots are the lung cancer patient samples.

Result 3. Screening clinical samples by quantitative RT-PCR confirmed that the levels of two microRNAs, miR-148b* and miR-33a, are significantly higher in lung cancer patients.

Figure 4. The expression level of candidate microRNAs in an independent set of clinical samples (from 27 lung cancer patients and 27 control), measured with qRT-PCR. The level of miR148b*(A) and miR-33a (D) were significantly higher in lung cancer patients (P = 0.0049 and 0.0245, respectively). However, the level of miR-376b*(B) and miR-134 (C) have no difference in both groups. MiR-16 was used as a reference gene. ΔCp is (miR-16 Cp value) – (candidate miRNAs Cp value).

Conclusion

1. Our results demonstrated that dysregulated microRNAs can be found in plasma of lung cancer patients.
2. We established the technique of microRNAs detection in plasma by RT-qPCR.
3. These cancer-specific microRNAs may have potential to be used as diagnostic markers for lung cancer.