Hypermethylation of microRNA gene: potential in the diagnosis of lung cancer

I. Pronina, M. Gubenko, A. Burdennyy, V. Loginov

Laboratory of Pathogenomics and Transcriptomics, Institute of General Pathology and Pathophysiology, Moscow, Russian Federation

Background

Non-small cell lung cancer (NSCLC) is one of the most common human cancers, and the search for new biomarkers for detecting NSCLC at early stages is relevant.

MicroRNAs (miRNAs) function as post-transcriptional regulators of the expression of protein-coding genes, including those associated with oncogenesis. Their expression can change in response to changes in the methylation of the CpG island adjacent to or overlapping the miRNA gene.

The methylation of miRNA genes may be used as a cancer biomarker.

The aim of this work was to study the level of methylation of promoter CpG-islands of miRNA genes as promising NSCLC markers.

Methods

Methylation analysis of 80 paired (tumour/normal) NSCLC samples was carried out using quantitative methyl-specific PCR. For each paired sample, the level of methylation (%) was obtained.

Statistical analysis was performed in the R software environment using the nonparametric Mann-Whitney U-test, multiple comparisons (the Benjamini–Hochberg correction and the FDR value). The optimal marker system was selected based on the results of the ROC analysis carried out using the resource (http://www.biosoft.hacettepe.edu.tr/easyROC/); marker sets were evaluated by sensitivity, specificity, and AUC on ROC curves.

Results

The methylation status of 10 miRNA genes was studied, significant differences were established between methylation levels in tumour samples and histologically normal tissue of NSCLC patients for 8 genes: *MIR124-1/3, MIR125B-1, MIR127, MIR129-2, MIR137, MIR339* and *MIR375* ($p<0.001$) (Fig. 1).

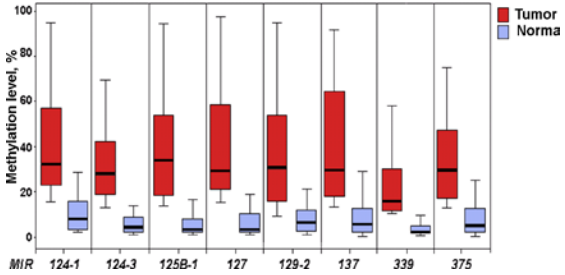

Fig. 1. *The methylation levels of 8 miRNA genes in tumour and histologically normal tissue samples from the same patients with NSCLC.*

Significant correlations were found between the methylation frequency of *MIR125B-1, MIR137, MIR124A-3* miRNA genes with cancer stage ($p=0.001$, *0.004* and *0.001*, respectively) (*Fig. 2A*) and *MIR125B-1* with metastasis ($p=0.005$) (*Fig. 2B*).

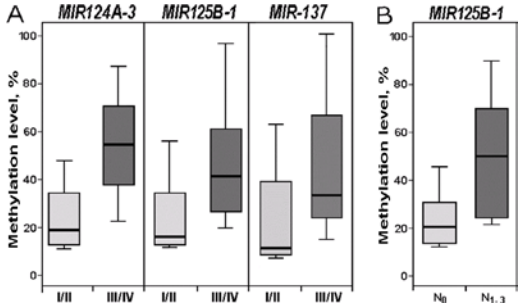

Fig. 2. *Correlations of miRNA gene methylation level with NSCLC progression: **A**, clinical stage; **B**, metastases in the lymph nodes.*

An effective marker system of 4 genes (*MIR125B-1, MIR137, MIR129-2, MIR375*) was determined by ROC analysis for the diagnosis of NSCLC at early stages with high clinical sensitivity (90%) and specificity (90 %); AUC value > 0.9 (*Fig.3*).

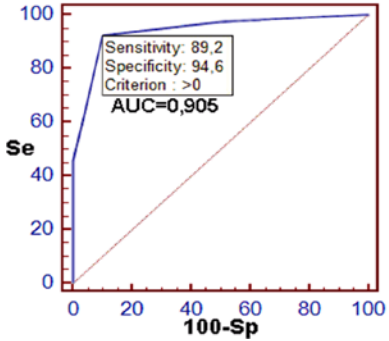

Fig. 3. *ROC analysis for the methylation index for a system of 4 markers. Se - sensitivity; 100-Sp is the proportion of false positive results. criterion – ROC-curve cutoff criterion.*

Conclusions

Thus, new hypermethylated miRNA genes can be used as potential biomarkers for the early diagnosis of NSCLC.

The study was supported by the state task FGFU-2022-0007 of the Ministry of Science and Higher Education of the Russian Federation.

For correspondence: e-mail zolly_sten@mail.ru