NOVEL IMMUNOASSAY APPROACH TO INVESTIGATE microRNA BIOMARKERS IN ACUTE MYOCARDIAL INFARCTION

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Introduction

MicroRNAs (miRNAs) are small non-coding RNA molecules playing an important regulatory role in gene translation through silencing or degradation of target mRNAs. They are involved in a wide range of biological processes, including differentiation and proliferation, metabolism, hemostasis, apoptosis or inflammation, and also in pathophysiology of many diseases. Numerous studies have suggested circulating miRNAs as promising diagnostic and prognostic biomarkers of CVD. Monitoring the level of specific miRNAs together with protein-based biomarkers may represent an efficient tool for diagnosis of CVD and prognosis estimation.

The aim of our study was to investigate potential predictive value of selected miRNAs. A cohort of 140 samples of patients with acute myocardial infarction (AMI) and 100 samples of healthy persons were obtained from the PRAGUE-18 study.

Method

Recently, we have introduced miREIA, a novel method for quantification of miRNA based on enzyme immunoassay format. The novel approach involves hybridization of miRNA isolated from a patient sample to complementary biotinylated DNA oligonucleotide probe. The DNA/RNA hybridization mixture is transferred onto a stationary solid phase coated with monoclonal antibody specific to perfectly matched RNA/DNA-biotin hybrids. In the next step, the solid phase is washed and subsequently incubated with streptavidin-HRP conjugate. Finally, the resulting complexes are visualized by chromogenic substrate 3’,3’-5’,5’-tetramethylbenzidine (TMB).

miRNA quantification

A) miREIA: miR-223 normalized to miR-126

<table>
<thead>
<tr>
<th>Sample</th>
<th>miR-223-3p (amol/µl)</th>
<th>miR-126-3p (amol/µl)</th>
<th>miR_223_normalized_to_miR_126</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>100</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>Sample 2</td>
<td>200</td>
<td>200</td>
<td>2.0</td>
</tr>
<tr>
<td>Sample 3</td>
<td>300</td>
<td>300</td>
<td>3.0</td>
</tr>
<tr>
<td>Sample 4</td>
<td>400</td>
<td>400</td>
<td>4.0</td>
</tr>
</tbody>
</table>

B) Dilution linearity

Two microRNAs from whole blood samples were serially diluted.

C) Calibration range

The calibration range of the miREIA-223-3p assay is 0.39-12.5 amol/µl.

D) Array of miRNA

For evaluation of diagnostic potential of normalized miR-223-3p, we constructed the receiver operating characteristic (ROC) curves. The ROC curves reflected strong separation between groups with and without acute myocardial infarction, with an AUC of 0.76, a sensitivity of 76.3 and specificity of 71.9.

Conclusion

We are introducing a novel method for absolute quantification of microRNA in clinical samples without reverse transcription and amplification steps.

miREIA method exhibits excellent analytical characteristics and strong correlation with qRT-PCR method.

This method is bringing an opportunity to analyze miRNA using conventional available immunoassay equipment and thus speed-up utilization of miRNA biomarkers in clinical and laboratory practice.

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References:

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