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Use of miR-33a in human plasma as a potential biomarker for severe Dengue infection

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MicroRNAs (miRNAs) are small noncoding RNAs about 22 nucleotides long that can regulate the gene expression at mRNA level by RNA silencing. As such, differential expression of miRNA leading to regulation of gene expression during various infections has been investigated as potential biomarkers for many diseases. Perturbations in lipid homeostasis and cellular imbalance of cholesterol and fatty acid metabolism has been implicated in Dengue infections. Intronic microRNA, miR33a, located within the sterol regulatory element-binding protein-2 and -1 genes, has been shown to regulate cholesterol homeostasis in concert with their host genes. In fact, miR33a has shown differential expression in cultured cells infected with Dengue virus. Therefore, we evaluated the expression level of miR33a in human plasma to evaluate its potential as a biomarker for severe Dengue infection.

Total RNA was purified from plasma from six EDTA blood samples collected with consent from healthy people using mirVana microRNA isolation kit (Applied Biosciences). Plasma was separated within one hour of sample collection and was stored at -80 °C. Purified RNA was used for subsequent 3'polyadenylation of the mature microRNA and cDNA synthesis with oligo-dT primers with a universal tag sequence using miScript II RT Kit (Qiagen). Similarly, negative control experiments for genomic DNA were carried out with same amount of RNA without any reverse transcriptase in the cDNA synthesis reaction (-RT). Presence of miR33a in plasma was confirmed by quantitative real-time PCR using the mature miRNA33a nucleotide sequence with thiamidine in place of uracils as the forward primer and a universal primer with universal tag sequence in the oligo-dT primer as the reverse primer. Target specificity of the miR33a specific primer was confirmed by NCBI BLASTN suite. Quantitative real-time PCR using miScript SYBR Green PCR Kit (Qiagen) was performed using diluted cDNA at an annealing temperature of 58 °C for 40 cycles followed by melting curve analysis. Negative control reactions were carried out in parallel with same volume of water and -RT as template. miR33a in human plasma was detected above threshold 1.0 at cycle number (Ct) 35. The Ct number is higher due to the low abundance of RNA in plasma. miR33a was consistently detected above threshold at the given Ct values while no amplification was detected in the negative control experiments containing water or -RT as template, indicating the absence of primer dimer formation and genomic DNA. Presence of a single peak in the melting curve confirmed single product amplification. Therefore, plasma is a good source for detection of miR33a to evaluate differential expression in severe Dengue.

Keywords: microRNA, miR33a, Plasma biomarker

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