Expression Changes in Putative Target Genes of Differentially Expressed miRNA as Early Biomarkers for Severe Dengue

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Dengue fever is caused by a flavivirus transmitted by mosquitoes. Primary infection of dengue mostly causes mild dengue fever (DF) characterized by headache, retro orbital pain, body pain, nausea, vomiting, joint pains and weakness. Severe manifestations of dengue, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) also shows similar symptoms during the early stages of infection. After 3-5 days from fever onset, DHF patients manifest plasma leakage, elevated hematocrit and pleural effusions. Lack of proper medication or vaccines for dengue fever and inability to distinguish severe dengue from DF during the early stages of infection renders this disease life threatening. Early diagnosis and disease management can alleviate DHF related complications. Therefore, biomarkers that distinguish DHF during the acute phase of infection can help reduce mortality. In our previous studies, we evaluated the differential expression of five miRNAs during the acute phase of infection including hsa-miR-150, which showed significant ($p<0.05$) expression changes with the disease severity. Since the main function of miRNA is to regulate target gene expression at post-transcriptional level, we evaluated the expression levels of four target genes of those miRNA in peripheral blood cells (PBC) collected from 20 DF (male-70% and female-30%) and 20 DHF (male-85% and female-15%) patients (based on evidence of plasma leakage by ultrasonography) who tested positive for NS1 antigen within four days of fever onset (acute phase) by qRT-PCR. Relative expression of EZh2, ABCA1, DNMT3a and RIP140 were evaluated against GAPDH as the reference gene. EZh2 showed over 2-fold downregulation ($P<0.05$) in DHF patients compared to DF patients. Based on logistic regression analysis of ΔCq values, EZh2 expression within 4 days from fever onset may be useful as a biomarker for progression from DF to DHF with an area under the receiver operating characteristic curve (AUC) of 0.76, sensitivity of 0.80 and specificity of 0.65 at 2.69 ($P<0.05$). DNMT3a, RIP140 and ABCA1 did not show significant differential expression during the acute phase of infection between DF and DHF patient samples. EZh2 also showed significant ($P<0.05$) downregulation within 4 days from fever onset in patients with platelet count <100,000 cells/mm^3 ($n=31$) compared to those with platelet count >100,000 cells/mm^3 ($n=9$) during the course of infection. Therefore, EZh2 expression may also serve as a biomarker for disease severity marked by low platelet count. This analysis is limited by relatively small sample size and a disproportionate number of male subjects. However, the calculated sample size with 95% CI at 80% power for EZh2 expression as a marker to predict disease outcome is 34 (17 each). The data was confirmed normally distributed based on q-q plot and Shapiro-Wilk test ($P>0.05$).

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