To Select the Appropriate Reference Gene for Normalizing the Quantitative Data to Assess MicroRNAs in Plasma Samples of Patients with Gastric Cancer

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Abstract

Introduction & Aim: Circulating microRNAs are promising biomarkers in diagnosis and assessment of cancerous patients. Quantitative Real-time PCR assay is a sensitive test for evaluating the levels of miRNAs expression. Nevertheless, there is no concurrence on selecting appropriate reference genes for qPCR analysis of miRNAs in circulation. Therefore, the current study aimed to select a suitable reference gene for normalizing the RT-qPCR assay results in plasma samples of patients with gastric cancer.

Methods: Based on previously published studies, three molecules SNORD47, U6 RNA, and miR-103 were selected as the candidate reference genes. After RNA extraction from plasma samples of 40 patients with gastric cancer and 40 healthy individuals, expression levels of these molecules were evaluated using Real-time PCR method.

Results: The results showed that the developed assays are able to diagnose their specified targets by a suitable linear range. By comparing patients and control groups, although the expression levels of miR-103 molecule were not equal between the two

groups (p= 0.017), SNORD47 and U6 RNAs had similar expression levels. However, the variations of SNORD47 expression were lower that U6 RNA.

Conclusion: Based on the results of the current study, the SNORD47 molecule has a stable expression levels in plasma samples of patients with gastric cancer and normal individuals and can be used as an appropriate reference gene for normalizing the quantitative data of qPCR assay.

Keywords: Gastric cancer, miRNAs, RT-qPCR, Reference gene