Circulating Salivary MiRNA as Potential Biomarkers for Determining Hand Food Mouth Disease in Children

Nyo Min[1*], Wong Anng Anng[2], Tan Natalie Woon Hui[2], Chong Chia Yin[2], Chu Justin Jang Hann[1]

[1.] Laboratory of Molecular RNA Virology and Antiviral Strategies. Department of Microbiology, Yong Loo Lin School of Medicine, National University Health System, 5 Science Drive 2, National University of Singapore, Singapore 117597, [2.] Infectious Disease Service, Department of Paediatric Medicine, KK Women's and Children's Hospital, 100 Bukit Timah Road, Singapore 229899

Background/Objective

Hand Food Mouth Disease or HFMD is one of the major health concerns in South East Asia countries in which serious cases result in fatality. Clinical diagnosis of such diseases are currently still limited to virus isolation, sera neutralization and polymerase chain reactions of viral targets. However former methods can be significantly time consuming and involve invasive sample retrieval. Circulating host miRNA from biofluid are emerging as a powerful diagnostic tool for detecting several diseases and here we propose a novel and non-invasive method for detecting HFMD using a panel of salivary miRNA as potential biomarkers.

Method

Pools of saliva (n=6) from HFMD patients and healthy patients are screened primarily using qPCR miRNA arrays from Exiqon and significantly dysregulated 10 miRNA are further validated with 26 patient samples using individual qPCR assays as secondary screen. Expression levels of each miRNA are examined and diagnostic values are determined. Gene union analysis is also carried out using DIANA tools with significant hits from primary screen.

Result

Primary screen using miRNA qPCR array reveals 51 dysregulated miRNA in HFMD patients from healthy individuals ($p<0.05$). Interestingly, enrichment analysis reveals P13K-Akt, MAPK, Wnt signaling and actin cytoskeleton regulation pathways are to be involved ($p<6.15e-26$) which are known to be EV71 triggered host pathogenesis processes. The secondary screening data are subjected to receiver operating characteristics (ROC) analysis and hsa-miR-221-3p (AUC 0.825, 0.657-0.993), hsa-miR-324-3p (AUC 0.642, 0.352-0.925) and hsa-miR-335-5p (AUC 0.692, 0.439-0.945) are found to significantly differentiate HFMD from healthy samples (95% CI). The three miRNA combined using binary logistic regression analysis results in 83% specificity and 90% sensitivity (AUC 0.925, 95% CI, 0.824-1).

Conclusion

This study established that saliva could be used as a reliable diagnostic medium for HFMD infections and a panel of three circulating miRNA could serve as a potential biomarker in discriminating HFMD from healthy patients.