dependent and time-dependent secretion of SEAP was found.

Conclusion: These results indicate the SEAP reporter assay can be used to substitute the plaque assay for DENVs titration, which can be a platform for antibody or drug selection against DENV infection.

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Effect of dengue virus infection on insulin pathway

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Background: Dengue virus (DENV) is a single, positive-stranded RNA virus from the family *Flaviviridae*. To date, vaccine and antiviral drugs for DENV infection is still not available due to the incomplete understanding of the underlying molecular mechanism involved during infection. This project aims to study the virus-host interplay by investigating insulin pathway that is activated during DENV infection.

Methods: Insulin pathway-related genes primers were designed and tested. DENV (clinical isolates) infection on HEK-293 cells was performed at one multiplicity of infection. The total RNA of infected and mock-infected cells was extracted after 48 hr post infection and reverse-transcribed into cDNA. The final product was then served as templates for real-time PCR in a 96-well plate containing pre-dispensed gene specific primer sets. The gene expression profiling of DENV-infected cells were compared to that of mock-infected cells.

Results: The real-time PCR result showed that the activation of the selected genes was serotype-specific. In general, insulin pathway-related genes were up-regulated. When compared to dengue fever (DF), the level of gene activation in dengue haemorrhagic fever (DHF) virus isolates was much higher for serotype-1 and -3 but lower for serotype-2 and -4. From the list of activated genes, we are interested to know whether the level of leptin protein in human sera could be exploited as biomarker for differentiating DF and DHF/dengue shock syndrome (DSS). Human leptin ELISA kit was employed to screen leptin protein level in DENV patients' sera. Results indicated that leptin protein level in patients' sera was higher during febrile phase than recovery phase. Patients infected by DENV-1 and -2 generally showed high level of leptin protein in the sera (higher than 2 fold increase) than healthy control sera. DENV-2 patients' sera showed higher level of leptin protein in DHF cases as compared to DF cases. However, DENV-3 infected patients' sera showed higher level of leptin protein in DF cases than DHF case instead. More human serum samples are tested for statistical analysis.

Conclusion: It is important to understand the interplay between DENV infection and insulin pathway because diabetes could be a predisposing factor for more severe complications and appropriate treatment should be given.

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E6-D25E of HPV16 Asian variant shows high potential of p53 degradation and miR-21 induction

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Background: Human papillomavirus type 16 (HPV 16), which is the most prevalent HPV genotype, has well-documented DNA sequence variants that show different geographical distributions and different oncogenic potentials. In Asian women, HPV16 Asian variant (HPV16As) with E6-D25E is the most common risk associated with development of cervical cancer. MicroRNA 21 (miR-21) is identified as an oncomir and over-expressed in a wide variety of cancers including cervical cancer. The oncogenic role of miR-21 in the cellular processes is implied to anti-apoptosis with target network of p53. This study aimed to evaluate the oncogenic potential of E6-D25E HPV16As comparing with E6 of HPV16 prototype by focusing on ability of p53 degradation.

Methods: E6 gene of HPV16 prototype and HPV16As isolated from cervical cancer samples were inserted into pcDNA3.2 expression vector. After transfection of the constructed vectors into HPV negative C33A cell line, the expressed E6 proteins were detected by western blot and the E6 protein function was determined for E6 protein interaction with E6AP protein by immunoprecipitation assay. p53 degradation, induction of miR-21 and mRNA PTEN level were investigated by transfection of these vectors into the C33A cells and determined by western blot and quantitative real time PCR. The ability of p53 degradation was evaluated in cyclohexamide treated C33A cell line.

Results: All expressed E6 proteins showed E6AP protein binding activity. This result suggested that the E6 with D25E of HPV16As variant still maintains carcinogenicity similar with E6 prototype. Interestingly, lower level of p53 was observed in C33A transfected with E6-D25E gene and correlated with the ability of p53 degradation at 2 hr after transfection that showed ~50% more than E6 prototype. The E6-D25E also induced the miR-21 expression higher than the E6 prototype with significant difference ($p<0.001$). The PTEN mRNA level was not significant different by induction with E6-D25E and E6 prototype. This up-regulation of miR-21 may support anti-apoptosis ability.

Conclusion: This study demonstrated that E6-D25E of HPV16As can elicit earlier p53 degradation and higher up-regulation of miR-21 gene than E6 prototype and suggested that oncogenic potential of E6-D25E may involve in persistent infection and cervical cancer progression.

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