DOCTORAL THESIS

Proteomics method development and application for interaction of influenza virus and cells

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Proteomics Method Development and Application for Interaction of Influenza Virus and Cells

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Declaration

I hereby declare that this thesis represents my own work which has been done after registration for the degree of PhD at Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma or other qualifications.

Signature:

Date: January 2015

Abstract

Influenza virus H1N1 is a huge threat on human health. Influenza occurs with seasonal variations and reaches peak prevalence in winter, with many people killed worldwide every year. In the research of interaction between influenza virus and cells, four major parts were in the range of our consideration, namely the proteins of virus, the proteome of host cell, the method of proteomic and the potencial medicine related with those significant proteins.

Hemagglutinin (HA), as an envelope protein, plays an important role in influenza A virus. It was found that HA has a series of isoforms in two dimensional gels in this study. For the investigation of HA, firstly, virus was purified by sucrose density-gradient centrifugation, followed by the separation of virus proteins through electrophoresis method, and then these proteins were digested by different enzymes and analyzed through MALDI-TOF MS and ESI-Q-TOF MS. Database searching was used for identification of sequences. The results of the virus samples digested by different enzymes were compared, and the isoforms of HA were proved to be related with the glycan and their glycosylation sites.

A novel strategy of stable-isotope N-phosphorylation labeling was developed for peptide *de novo* sequencing and protein quantification based on organic phosphorus chemistry. Different from other stable-isotope labeling reagents that needed to be activated in advance for peptide coupling, N-phosphorylation labeling reagents were activated in situ to form labeling intermediates with high activity and selectivity targeting on N-terminus and -amino group of lysine under various reaction conditions. The obtained results showed excellent correlation of the measured ratios to theoretical ratios with errors that ranging from 0.5 to 6.7 %

and relative standard deviation of less than 10.6 %, indicating the reproducibility and preciseness of the developed method. The method development based on organic phosphorus chemistry offered a new approach for quantitative proteomics by using novel stable-isotope labeling reagents.

A method combining hydrazide chemistry, stable isotope labeling and mass spectrometry analysis was developed and applied to study glycoproteins of H1N1 (A/Purto Rico/8/1934) infected cell line (A549). The result showed that some glycoproteins were significant in influenza virus infected cells. In these glycoproteins, RPC1_HUMAN, RHG25_HUMAN , RPTOR_HUMAN, ARHGC_HUMAN, ROCK1_HUMAN, DOCK3_HUMAN were down-regulated. Protein named TITIN_HUMAN, DESP_HUMAN, PTN13_HUMAN were up-regulated.

High dose of *N*-acetylcysteine (NAC) was recently reported for a therapy of H1N1 influenza pneumonia. NAC was used as a small-molecule organic probe to investigate the protein expression of human lung carcinoma cell line (A549) infected by influenza virus H1N1. The obtained results showed that NAC kept cells away from apoptosis. Virus-infected cells were arrested in G0/G1 phase. The lowest cell population of G0/G1 phase was detected when the cells were treated by 10 mM NAC for one day. Software analysis showed that 4 proteins had close relationship. The results indicated that NAC as a small-molecule probe might effect the proteins expression of A549 cells infected by the H1N1 virus.

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