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/Puerto 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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# Table of Contents

Declaration........................................................................................................... I  
Abstract...............................................................................................................II  
Acknowledgements.............................................................................................IV  
Table of Contents...............................................................................................V  
List of Tables.......................................................................................................X  
List of Figures......................................................................................................XII  
List of Abbreviations......................................................................................... XVII  

Chapter 1 Introduction.......................................................................................1  
  1.1 Overview of influenza A virus.................................................................1  
  1.2 Proteomics in influenza virus research.................................................4  
  1.3 Potential medicine study in influenza virus infection.........................8  

Chapter 2 Identification of hemagglutinin isoforms difference of influenza A virus H1N1 ........................................................................................................10  
  2.1 Introduction.............................................................................................10  
  2.2 Materials and methods..........................................................................11  
    2.2.1 Virus cultivation................................................................................11  
    2.2.2 Isolation of virus by ultracentrifugation...........................................12  
    2.2.3 SEM detection, PNGase F digest and PAGE electrophoresis ......12  
    2.2.4 In-gel digestion................................................................................13  
    2.2.5 MALDI-TOF MS analysis...............................................................14  
    2.2.6 Nanoelectrospray-ESI-MS/MS analysis.........................................15  
  2.3 Result......................................................................................................15  
    2.3.1 Purification of influenza virus H1N1.............................................16
Chapter 3 Stable-isotope N-phosphorylation labeling for peptide de novo sequencing and protein quantification based on organic phosphorus chemistry

3.1 Introduction.................................................................28
3.2 Materials and methods..................................................34
  3.2.1 Materials and reagents.............................................34
  3.2.2 Synthesis of deuterium-labeled dimethyl phosphite ............34
  3.2.3 Trypsin digestion.....................................................35
  3.2.4 N-phosphorylation of standard peptide and tryptic peptides.....36
  3.2.5 Mass spectrometry analysis........................................36
  3.2.6 Nano LC-chip/Q-TOF MS analysis.................................37
  3.2.7 Protein identification and quantification..........................38

3.3 Results and discussion..................................................39
  3.3.1 Selection of phosphorus chemistry...............................39
  3.3.2 Stable isotopic N-phosphorylation labeling of standard peptide and proteins.........................................................41
  3.3.3 Peptide de novo sequencing......................................50
  3.3.4 Quantification of standard peptide and protein..................54
  3.3.5 Quantification of protein mixtures by nano LC-chip/TOF MS....57
  3.3.6 Validation of deuterium atom effect of labeled peptides.........62
Chapter 4 Identification and quantification of N-linked glycoproteins of influenza virus infected cells using hydrazide chemistry, stable isotope labeling and mass spectrometry

4.1 Introduction

4.2 Materials and methods
   4.2.1 Materials
   4.2.2 Standard proteins preparation
   4.2.3 Virus and cell line culture
   4.2.4 Separation of N-glycosylation protein
   4.2.5 N-phosphorylation of tryptic peptides
   4.2.6 Mass spectrometry analysis
   4.2.7 Nano LC-Chip Q-TOF MS
   4.2.8 Protein identification, quantification and bio-information analysis

4.3 Result and discussion
   4.3.1 Standard protein testing
   4.3.2 Glycoprotein separation and quantification
   4.3.3 Relationship with those different proteins

4.4 Chapter summary

Chapter 5 Proteomics study of N-acetylcysteine response in H1N1-infected cells by using mass spectrometry

5.1 Introduction

5.2 Materials and methods
   5.2.1 Virus and cell line culture

VII