Type: Poster Presentation

Final Abstract Number: 51.023
Session: Emerging Infectious Diseases
Date: Friday, April 4, 2014
Time: 12:45-14:15
Room: Ballroom

Investigation of potential cell binding sites on nipah virus attachment glycoprotein

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Background: Nipah virus (NiV), an emerging zoonotic paramyxovirus, is highly pathogenic with unusual broad host tropism. NiV gains entry into the host cells through a pH-independent membrane fusion event which requires the concerted action of the two membrane glycoproteins, attachment (G) and fusion (F) glycoprotein. This viral entry event is initiated when the G glycoprotein attaches to host cellular receptor, ephrin-B2 or ephrin-B3. The present study aims to map the virus-host interaction, particularly the regions on NiV G that are important for NiV direct binding to cells by using phage display system.

Methods & Materials: Five truncated fragments of NiV G extracellular domain were generated by RT-PCR and cloned into phagemid vector, pCANTAB5E. These recombinant phagemids were transformed into *Escherichia coli* TG1 for display of truncated NiV G on M13 phage g3p minor coat protein. The binding efficacy of recombinant phages displaying the different regions of NiV G to three NiV susceptible cell lines and the cells protein: African green monkey kidney cells (Vero), human lung fibroblast cells (MRC-5) and human monocytes (THP-1), were evaluated by phage ELISA.

Results: A library of recombinant phages displaying truncated NiV G was successfully generated, with a representative titer of at least 10^{10} cfu/ml. Recombinant phages displaying region of NiV G consisting of amino acids 498-602 (G498-602) demonstrated highest binding to three different cell lines (2.7 x 10^4 cfu/ml to Vero cells; 6.8 x 10^3 cfu/ml to MRC-5 cells; 8.7x10^3 cfu/ml to THP-1 cells) and the binding was dose-dependent. Binding of recombinant phages to Vero cells protein was also the highest for G498-602 phages. The region of NiV G from amino acids 71-181 (G71-181), on the other hand, showed highest binding to MRC-5 and THP-1 cells protein.

Conclusion: This study demonstrated the first direct binding of NiV G to cells by using phage display system, with findings suggesting that the region of NiV G from amino acids 498-602 plays an important role in NiV direct attachment to cells.

http://dx.doi.org/10.1016/j.ijid.2014.03.891

Type: Poster Presentation

Final Abstract Number: 51.024
Session: Emerging Infectious Diseases
Date: Friday, April 4, 2014
Time: 12:45-14:15
Room: Ballroom

Detection of bat coronaviruses in the bat population in Taiwan

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Background: The outbreak of severe acute respiratory syndrome (SARS) during 2002 to 2003 and the emerging of the Middle East respiratory syndrome (MERS) 10 years later have drawn much needed attention to the investigations for the natural reservoirs of coronaviruses, especially bats. Because bats can migrate for a very long distance and roost within human community, it is very important to assess the risk of coronaviral host transition and the outbreaks of emerging coronavirus by investigating the prevalence of bat coronavirus (BtCoV). Bat coronaviruses have been detected around the world, including the countries surrounding Taiwan, such as Japan, China, Hong Kong, Philippines, and Thailand.

Methods & Materials: To fill the gap of knowledge on the ecology of BtCoVs in Taiwan, individual feces were collected from 49 net-captured bats during 2013. The conserved region of RNA-dependent RNA polymerase (RdRp) gene was targeted by reverse transcription-polymerase chain reaction in the extracted viral RNA in the fecal samples.

Results: Coronaviral RNA was detected in 10 out of 17 (59%) fecal samples from endemic bat species *Rhinolophus monoceros* in Tainan City, 8 out of 14 fecal samples (57%) from migrating bat species *Scotophilus kuhlii* in Changhua County, 2 out of 12 (17%) fecal samples from endemic bat species *Miniopterus schreibersii fuliginosus* in New Taipei City, and 1 out of 6 (17%) fecal samples from endemic bat species *Kerivoula sp.* in I-Lan County. Sequencing and phylogenetic analysis by BLASTn and MEGA5 software using the neighbor-joining method indicated that the detected BtCoVs all belong to genus Alphacoronavirus. The sequences of PCR products (RdRp) showed over 95% identity to species *Rhinolophus BtCoV HKU2*, *Scotophilus BtCoV 512*, or *Miniopterus BtCoV 1*.

Conclusion: The findings of this study suggest that BtCoVs exit in the bat population endemically in at least four bat species in Taiwan and further CoV genomics and surveillance studies in bats throughout Taiwan are necessary to understand the role of BtCoVs to public health.

http://dx.doi.org/10.1016/j.ijid.2014.03.892