以第二型登革熱病毒次基因體的細胞株篩選抑制登革熱病毒複製的藥物並以寡核甘酸微陣列的方式探討登革熱病毒在複製時所調控的宿主細胞基因

Dengue type II subgenomic replicon cell line: A tool for drug screening and searching for host gene regulation by microarray analysis

中文摘要

登革熱病毒 (Dengue virus) 屬於黃熱病毒科 (Flavivarida)，為正股 RNA 病毒，依抗原性的不同分為一、二、三、四型。感染任何一型病毒皆會引發出典型登革熱 (Dengue fever)的病症，嚴重者會造成出血型登革熱 (Dengue hemorrhagic fever) 症狀，甚至引發登革休克症候群 (Dengue shock syndrome)而造成 50~80% 的致死率，目前並沒有有效的疫苗及藥物治療。本論文的目的是以含有第二型登革熱病毒次基因體的細胞株篩選抑制登革熱病毒複製的藥物，並以 microarray 的方式探討登革熱病毒在複製時所調控的宿主細胞基因。我以一已知抑制 RNA 病毒藥物 ribavrin 處理第二型登革熱病毒次基因體的 D2RepLuf #1 細胞 48 小時發現 8 M ribavirin 可使嵌在次基因體的 firefly luciferase 報導基因的活性與次基因體的病毒NS3蛋白表現量都下降至未處理細胞的50% (ribavrin的EC50為8 M)，但是高達 30 M ribavirin 只會使細胞活性下降約 20% (ribavirin 的 TC50 >30 ?M) 。當以 10 M 的 ribavirin 處理受登革熱病毒感染的 BHK-21 細胞 48 小時後，可觀察到細胞存活的情形明顯比未受 ribavirin 處理的細胞佳，因而確立次基因體細胞株系統應該可用於抗病毒藥物的篩選。以此 D2RepLuf #1 細胞測試 6,400 種化合物的抑制登革熱病毒能力，發現 11 種化合物雖可抑制報導基因的活性但是並無法有效保護受病毒感染的細胞。另以含有第二型登革熱次基因體的 BHK-21 細胞 D2N 與 BHK-21 細胞 （為一倉鼠腎細胞株）以小鼠全基因晶片進行 microarray 實驗，分析出在含有登革熱病毒次基因體的細胞中有 53 個宿主基因會被正向調控，有 24 個基因會被負向調控。因倉鼠基因尚未完全解碼，我針對 3 個正向調控基因（Mtdth, Nox4, Ddb2）及 1 負向調控基因（Idh1）的 cDNA 在人、小鼠、大鼠、及倉鼠四個物種間做序列鑑定與比對，發現這四個基因在物種間的相似度高達 90%左右。因此物種基因序列的差距對 microarray 結果的正確性應該不至於造成大影響。日後可利用 quantitative PCR 確認的 microarray 實驗的發現。

英文摘要

Dengue virus belongs to the Flaviviridae family. Dengue virus has four serotypes that have similar clinical presentation. Infection with any serotype can course dengue fever, dengue hemorrhagic fever (DHF), or even dengue shock syndrome (DSS), and the death rate can be 50-80%. Currently, there is no vaccine or effective anti-Dengue virus drug available. In this thesis, I planed to use a stable baby hamster kidney-21 (BHK-21) cell line D2RepLuf #1 that harbored a persistently replicating Dengue virus type II subgenomic replicon and contained a firefly luciferase reporter gene for antiviral drug screening. The preliminary study with the D2RepLuf #1 cell showed that ribavirin, an RNA virus inhibitor, decreased the firefly luciferase activity and the viral nonstructural protein NS3 expression at a dosage dependent manner and that the EC50 and TC50 of ribavirin were 8 ?M and > 30 ?M, respectively. Additionally, treating the Dengue virus infected BHK-21 cell with 10 ?M ribavirin released the cell from cytopathic effect. Thus, the reported-based subgenomic replicon system appeared to be suitable for anti-Dengue drug screening. 6,400 compounds were then screened for the anti-Dengue virus activity. 11 compounds could attenuate the firefly luciferase activity of D2RepLuf #1 cell, but none of them prevented the cytopathic effect of Dengue virus infection. I used another Dengue virus subgenomic replicon baby hamster kidney-21 (BHK-21) cell line D2N to analyze host gene regulation by mouse oligonucleotide mircroarray approach. 53 genes were up regulated and 24 genes were down regulated in D2N cell in compared to the parental BHK-21 cell. To justify the use of mouse oligonucleotide microarray for hamster gene expression analysis, I amplified the cDNA of three up regulated genes Mtdth, Nox4, and Dsb2, and one down regulated gene Idh1 from the BHK-21 cell, and compared the sequence of 0.5 Kb region with the corresponding genes of human, mouse, and rat. The results show that these genes are highly homology among different species and the sequence similarity of each gene is ~90%. Quantitative-PCR will be carried out to confirm the findings of the microarray study.