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論文名稱(中)	由重組桿狀病毒感染之 Sf 9 細胞株中表現及純化嚴重急性呼吸道症候群冠狀病毒之 S547 刺突蛋白片段並建立一個以細胞為基礎之抗病毒藥物篩選模式

論文名稱(英)	Expression and Purification of Severe Acute Respiratory Syndrome-Coronavirus Spike Protein S547 from Recombinant Baculovirus Infected Sf9 Cells and Establishment a Cell-Based Model for Anti-viral Drugs Screening
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關鍵字(英)	SARS Spike future system China data
摘要(中)	2002 年一新興呼吸道疾病-嚴重急性呼吸道症候群 (Severe Acute Respiratory Syndrome；SARS) 在短時間內於全世界發生，造成全球 8,098 個疑似病例其中有 774 個死亡病例。經各國通力合作，證實造成 SARS 的病原體為一全新之冠狀病毒 (Coronavirus)，因此 WHO 將此病毒正式命名為 SARS-Coronavirus (SARS-CoV)。SARS 流行期間主要採用三氮唑核? (ribavirin) 和皮質類固醇 (corticosteroid) 共同使用的治療方式，然而其卻無顯著治療效果，為避免 SARS 再次傳播固需要有效治療 SARS 藥物。SARS 傳播主要藉由空氣傳染途徑，以病毒刺突蛋白 (spike) 與宿主細胞表面之受器血管緊張素轉換?2 (Angiotensin converting enzyme 2；ACE2) 結合而染感宿主。本實驗最終目的為希望利用 SARS-CoV 的 spike 與 ACE2 間結合關係，以建構一個以細胞為基礎之安全藥物篩選平台。為了獲得 spike 蛋白質片段，於研究中利用重組桿狀病毒感染 Sf9 細胞，以獲得由第 16 個氨基酸至第 547 氨基酸序列之 spike 蛋白片段，特稱為 S547，並利用西方墨點法確認其大小約為 85 kDa。此外，針對 S547 上由六個組氨酸所組成之標誌 (His-Tag)，以 Ni2+-NTA 純化系統加以純化，並以 50 mM 至 90 mM 間各不同濃度之 imidazole 去除非專一性蛋白以加強純化效果。為了確認 ACE2 在細胞株之表現，實驗中以 Vero E6 做為標的細胞，利用反轉錄連鎖聚合?反應、西方墨點法以及免疫螢光染色法分析，證明 ACE2 可分佈與表現於 Vero E6 細胞表面。為了確認 S547 可與 Vero E6 進行鍵結，以免疫螢光染色法證明 S547 蛋白確實會結合到 Vero E6 細胞上。為了建立安全之抗病毒藥物篩選平台，初步以 Vero E6 培養於 96 孔培養盤中，加入 S547 之後以 Anti-His Tag monoclonal antibody 進行 ELISA 分析，結果發現 S547 在此以細胞為基礎之模式中，確實能與 Vero E6 上所表現之 ACE2 結合。未來，當成熟建立此模式後，將應用於抗病毒藥物之篩選。
摘要(英)	In 2002, a new contagious disease had been found in Southern China and the disease spread widely and resulted in 8098 cases and 774 deaths. This disease was characterized as an atypical pneumonia symptom. The World Health Organization defined it as severe acute respiratory syndrome (SARS). The pathogen of SARS is a new coronavirus and named as SARS-coronavirus (SARS-CoV). Combination of ribavirin and corticosteroid is the most frequent antiviral treatment for SARS. However, the unobvious curative and annoying side effects for these medicines, it is urgent to find new anti-SARS drugs. SARS-CoV spreads mainly via respiratory routes and infects host cells by binding of viral spike protein to cellular angiotensin converting enzyme-2 (ACE2). The aim of thesis is to express the spike protein fragment and establish an anti-viral drug screening model. In the present study, I have obtained a recombinant baculovirus that carried a cDNA encoding SARS-CoV spike proteins from 16 a.a. to 547 a.a. Then a histidine Tag fusion protein (85 kDa), named as S547, was purified from recombinant baculoviruses infected sf9 cells by

	the Ni2+-NTA system. The results of reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting indicated that ACE2 expression could be detected in Vero E6 cells. The data of immunofluorescent staining demonstrated that S547 proteins can bind to Vero E6 cells. Moreover, I have set up the cell-based ELISA to prove that S547 could bind to Vero E6 cells in a dose-dependant manner. In the future, the cell-based ELISA model will be applied for screening anti-viral drugs.
論文目次	
參考文獻	http://www.fhcrc.org/labs/hahn/methods/biochem_meth/biorad_assay.pdf Sambrook , Joseph, Russell , David W , 1957- David William (2001) Molecular Cloning : A Laboratory Manual (Cold Spring Harbor, New York. : Cold Spring Harbor Laboratory Press) 3rd pp8.24 ppA8.40-A8.47 Pharmingen (1999). Baculovirus Expression Vector System Manual (San Diego: Pharmingen) 6th pp1-14 pp31-43 Qiagen (2001). Ni-NTA Magnetic Agarose Beads Handbook (Qiagen) pp7-12 Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, J. R., and Hilgenfeld, R. (2003). Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. Science 300, 1763-1767. Babcock, G. J., Esshaki, D. J., Thomas, W. D., Jr., and Ambrosino, D. M. (2004). Amino acids 270 to 510 of the severe acute respiratory syndrome coronavirus spike protein are required for interaction with receptor. J Virol 78, 4552-4560. Boehm, M., and Nabel, E. G. (2002). Angiotensin-converting enzyme 2--a new cardiac regulator. N Engl J Med 347, 1795-1797. Bosch, B. J., van der Zee, R., de Haan, C. A., and Rottier, P. J. (2003). The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. J Virol 77, 8801-8811. Chang, Y. J., Liu, C. Y., Chiang, B. L., Chao, Y. C., and Chen, C. C. (2004). Induction of IL-8 release in lung cells via activator protein-1 by recombinant baculovirus displaying severe acute respiratory syndrome-coronavirus spike proteins: identification of two functional regions. J Immunol 173, 7602-7614. Chen, J., Miao, L., Li, J. M., Li, Y. Y., Zhu, Q. Y., Zhou, C. L., Fang, H. Q., and Chen, H. P. (2005). Receptor-binding domain of SARS-Cov spike protein: soluble expression in E. coli, purification and functional characterization. World J Gastroenterol 11, 6159-6164. Cinatl, J.,

Morgenstern, B., Bauer, G., Chandra, P., Rabenau, H., and Doerr, H. W. (2003). Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. Lancet 361, 2045-2046. De Clercq, E. (2004). Antiviral drugs in current clinical use. J Clin Virol 30, 115-133. De Clercq, E. (2006). Potential antivirals and antiviral strategies against SARS coronavirus infections. Expert Rev Anti Infect Ther 4, 291-302. Drosten, C., Gunther, S., Preiser, W., van der Werf, S., Brodt, H. R., Becker, S., Rabenau, H., Panning, M., Kolesnikova, L., Fouchier, R. A., Berger, A., Burguiere, A. M., Cinatl, J., Eickmann, M., Escriou, N., Grywna, K., Kramme, S., Manuguerra, J. C., Muller, S., Rickerts, V., Sturmer, M., Vieth, S., Klenk, H. D., Osterhaus, A. D., Schmitz, H., and Doerr, H. W. (2003). Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 348, 1967-1976. Eickmann, M., Becker, S., Klenk, H. D., Doerr, H. W., Stadler, K., Censini, S., Guidotti, S., Masignani, V., Scarselli, M., Mora, M., Donati, C., Han, J. H., Song, H. C., Abrignani, S., Covacci, A., and Rappuoli, R. (2003). Phylogeny of the SARS coronavirus. Science 302, 1504-1505. Fouchier, R. A., Kuiken, T., Schutten, M., van Amerongen, G., van Doornum, G. J., van den Hoogen, B. G., Peiris, M., Lim, W., Stohr, K., and Osterhaus, A. D. (2003). Aetiology: Koch's postulates fulfilled for SARS virus. Nature 423, 240. Groneberg, D. A., Hilgenfeld, R., and Zabel, P. (2005). Molecular mechanisms of severe acute respiratory syndrome (SARS). Respir Res 6, 8. Guan, Y., Zheng, B. J., He, Y. Q., Liu, X. L., Zhuang, Z. X., Cheung, C. L., Luo, S. W., Li, P. H., Zhang, L. J., Guan, Y. J., Butt, K. M., Wong, K. L., Chan, K. W., Lim, W., Shortridge, K. F., Yuen, K. Y., Peiris, J. S., and Poon, L. L. (2003). Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science 302, 276-278. Hamming, I., Timens, W., Bulthuis, M. L., Lely, A. T., Navis, G. J., and van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 203, 631-637. Ho, J. C., Ooi, G. C., Mok, T. Y., Chan, J. W., Hung, I., Lam, B., Wong, P. C., Li, P. C., Ho, P. L., Lam, W. K., Ng, C. K., Ip, M. S., Lai, K. N., Chan-Yeung, M., and Tsang, K. W.. (2003). High-dose pulse versus nonpulse corticosteroid regimens in severe acute respiratory syndrome. Am J Respir Crit Care Med 168, 1449-1456. Hofmann, H., Geier, M., Marzi, A., Krumbiegel, M., Peipp, M., Fey, G. H., Gramberg, T., and Pohlmann, S. (2004a). Susceptibility to SARS coronavirus S protein-driven infection correlates with expression of angiotensin converting enzyme 2 and infection can be blocked by soluble receptor. Biochem Biophys Res Commun 319, 1216-1221. Hofmann, H., Hattermann, K., Marzi, A., Gramberg, T., Geier, M., Krumbiegel, M., Kuate, S., Uberla, K., Niedrig, M., and Pohlmann, S. (2004b). S protein of severe acute respiratory syndrome-associated coronavirus mediates entry into hepatoma cell lines and is targeted by neutralizing antibodies in infected patients. J Virol 78, 6134-6142. Holmes, K. V. (2003). SARS coronavirus: a new challenge for prevention and therapy. J Clin Invest 111, 1605-1609. Jones, B. M., Ma, E. S., Peiris, J. S., Wong, P. C., Ho, J. C., Lam, B., Lai, K. N., and Tsang, K. W. (2004). Prolonged disturbances of in vitro cytokine production in patients with severe acute respiratory syndrome (SARS) treated with ribavirin and

steroids. Clin Exp Immunol 135, 467-473. Klempner, M. S., and Shapiro, D. S. (2004). Crossing the species barrier--one small step to man, one giant leap to mankind. N Engl J Med 350, 1171-1172. Koren, G., King, S., Knowles, S., and Phillips, E. (2003). Ribavirin in the treatment of SARS: A new trick for an old drug? Cmaj 168, 1289-1292. Ksiazek, T. G., Erdman, D., Goldsmith, C. S., Zaki, S. R., Peret, T., Emery, S., Tong, S., Urbani, C., Comer, J. A., Lim, W., Rollin, P. E., Dowell, S. F., Ling, A. E., Humphrey, C. D., Shieh, W. J., Guarner, J., Paddock, C. D., Rota, P., Fields, B., DeRisi, J., Yang, J. Y., Cox, N., Hughes, J. M., LeDuc, J. W., Bellini, W. J., and Anderson, L. J. (2003). A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 348, 1953-1966. Kuba, K., Imai, Y., and Penninger, J. M. (2006). Angiotensin-converting enzyme 2 in lung diseases. Curr Opin Pharmacol 6, 271-276. Kuiken, T., Fouchier, R. A., Schutten, M., Rimmelzwaan, G. F., van Amerongen, G., van Riel, D., Laman, J. D., de Jong, T., van Doornum, G., Lim, W., Ling, A. E., Chan, P. K., Tam, J. S., Zambon, M. C., Gopal, R., Drosten, C., van der Werf, S., Escriou, N., Manuguerra, J. C., Stohr, K., Peiris, J. S., and Osterhaus, A. D. (2003). Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet 362, 263-270. Li, W., Moore, M. J., Vasilieva, N., Sui, J., Wong, S. K., Berne, M. A., Somasundaran, M., Sullivan, J. L., Luzuriaga, K., Greenough, T. C., Choe, H., and Farzan, M. (2003). Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426, 450-454. Marra, M. A., Jones, S. J., Astell, C. R., Holt, R. A., Brooks-Wilson, A., Butterfield, Y. S., Khattra, J., Asano, J. K., Barber, S. A., Chan, S. Y., Cloutier, A., Coughlin, S. M., Freeman, D., Girn, N., Griffith, O. L., Leach, S. R., Mayo, M., McDonald, H., Montgomery, S. B., Pandoh, P. K., Petrescu, A. S., Robertson, A. G., Schein, J. E., Siddiqui, A., Smailus, D. E., Stott, J. M., Yang, G. S., Plummer, F., Andonov, A., Artsob, H., Bastien, N., Bernard, K., Booth, T. F., Bowness, D., Czub, M., Drebot, M., Fernando, L., Flick, R., Garbutt, M., Gray, M., Grolla, A., Jones, S., Feldmann, H., Meyers, A., Kabani, A., Li, Y., Normand, S., Stroher, U., Tipples, G. A., Tyler, S., Vogrig, R., Ward, D., Watson, B., Brunham, R. C., Krajden, M., Petric, M., Skowronski, D. M., Upton, C., and Roper, R. L. (2003). The Genome sequence of the SARS-associated coronavirus. Science 300, 1399-1404. Miura, H. S., Nakagaki, K., and Taguchi, F. (2004). N-terminal domain of the murine coronavirus receptor CEACAM1 is responsible for fusogenic activation and conformational changes of the spike protein. J Virol 78, 216-223. Nicholls, J. M., Poon, L. L., Lee, K. C., Ng, W. F., Lai, S. T., Leung, C. Y., Chu, C. M., Hui, P. K., Mak, K. L., Lim, W., Yan, K. W., Chan, K. H., Tsang, N. C., Guan, Y., Yuen, K. Y., and Peiris, J. S. (2003). Lung pathology of fatal severe acute respiratory syndrome. Lancet 361, 1773-1778. Peiris, J. S., Guan, Y., and Yuen, K. Y. (2004). Severe acute respiratory syndrome. Nat Med 10, S88-97. Ren, X., Glende, J., Al-Falah, M., de Vries, V., Schwegmann-Wessels, C., Qu, X., Tan, L., Tschernig, T., Deng, H., Naim, H. Y., and Herrler, G. (2006). Analysis of ACE2 in polarized epithelial cells: surface expression and function as receptor for severe acute respiratory syndrome-associated coronavirus. J Gen Virol 87, 1691-1695. Rota, P. A., Oberste, M. S., Monroe, S. S., Nix, W. A., Campagnoli, R., Icenogle, J.

P., Penaranda, S., Bankamp, B., Maher, K., Chen, M. H., Tong, S., Tamin, A., Lowe, L., Frace, M., DeRisi, J. L., Chen, Q., Wang, D., Erdman, D. D., Peret, T. C., Burns, C., Ksiazek, T. G., Rollin, P. E., Sanchez, A., Liffick, S., Holloway, B., Limor, J., McCaustland, K., Olsen-Rasmussen, M., Fouchier, R., Gunther, S., Osterhaus, A. D., Drosten, C., Pallansch, M. A., Anderson, L. J., and Bellini, W. J.. (2003). Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300, 1394-1399. Shemetilo, I. G., and Mallabiu, G. A. (1975). [Treatment of patients with epicondylitis of the arm by means of novocaine electrophoresis, medium frequency rectified sinusoidal currents, and centimeter band microwaves]. Vopr Kurortol Fizioter Lech Fiz Kult, 369-370. Spiga, O., Bernini, A., Ciutti, A., Chiellini, S., Menciassi, N., Finetti, F., Causarono, V., Anselmi, F., Prischi, F., and Niccolai, N. (2003). Molecular modelling of S1 and S2 subunits of SARS coronavirus spike glycoprotein. Biochem Biophys Res Commun 310, 78-83. Stadler, K., Masignani, V., Eickmann, M., Becker, S., Abrignani, S., Klenk, H. D., and Rappuoli, R. (2003). SARS--beginning to understand a new virus. Nat Rev Microbiol 1, 209-218. Wang, P., Chen, J., Zheng, A., Nie, Y., Shi, X., Wang, W., Wang, G., Luo, M., Liu, H., Tan, L., Song, X., Wang, Z., Yin, X., Qu, X., Wang, X., Qing, T., Ding, M., and Deng, H. (2004). Expression cloning of functional receptor used by SARS coronavirus. Biochem Biophys Res Commun 315, 439-444. Wong, C. K., Lam, C. W., Wu, A. K., Ip, W. K., Lee, N. L., Chan, I. H., Lit, L. C., Hui, D. S., Chan, M. H., Chung, S. S., and Sung, J. J. (2004a). Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol 136, 95-103. Wong, S. K., Li, W., Moore, M. J., Choe, H., and Farzan, M. (2004b). A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J Biol Chem 279, 3197-3201. Wu, C. J., Jan, J. T., Chen, C. M., Hsieh, H. P., Hwang, D. R., Liu, H. W., Liu, C. Y., Huang, H. W., Chen, S. C., Hong, C. F., Lin, R. K., Chao, Y. S., and Hsu, J. T. (2004a). Inhibition of severe acute respiratory syndrome coronavirus replication by niclosamide. Antimicrob Agents Chemother 48, 2693-2696. Wu, C. Y., Jan, J. T., Ma, S. H., Kuo, C. J., Juan, H. F., Cheng, Y. S., Hsu, H. H., Huang, H. C., Wu, D., Brik, A., Liang, F. S., Liu, R. S., Fang, J. M., Chen, S. T., Liang, P. H., and Wong, C. H. (2004b). Small molecules targeting severe acute respiratory syndrome human coronavirus. Proc Natl Acad Sci U S A 101, 10012-10017. Xiao, X., Chakraborti, S., Dimitrov, A. S., Gramatikoff, K., and Dimitrov, D. S. (2003). The SARS-CoV S glycoprotein: expression and functional characterization. Biochem Biophys Res Commun 312, 1159-1164. Xu, Y., Liu, Y., Lou, Z., Qin, L., Li, X., Bai, Z., Pang, H., Tien, P., Gao, G. F., and Rao, Z. (2004). Structural basis for coronavirus-mediated membrane fusion. Crystal structure of mouse hepatitis virus spike protein fusion core. J Biol Chem 279, 30514-30522. Yuan, K., Yi, L., Chen, J., Qu, X., Qing, T., Rao, X., Jiang, P., Hu, J., Xiong, Z., Nie, Y., Shi, X., Wang, W., Ling, C., Yin, X., Fan, K., Lai, L., Ding, M., and Deng, H. (2004). Suppression of SARS-CoV entry by peptides corresponding to heptad regions on spike glycoprotein. Biochem Biophys Res Commun 319, 746-752. Zhang, R., Guo, Z., Lu, J., Meng, J., Zhou, C., Zhan, X., Huang, B., Yu, X., Huang, M., Pan, X., Ling, W., Chen, X., Wan, Z., Zheng, H.,

	Yan, X., Wang, Y., Ran, Y., Liu, X., Ma, J., Wang, C., and Zhang, B. (2003). Inhibiting severe acute respiratory syndrome-associated coronavirus by small interfering RNA. Chin Med J (Engl) 116, 1262-1264. Zhang, Y., Li, J., Zhan, Y., Wu, L., Yu, X., Zhang, W., Ye, L., Xu, S., Sun, R., Wang, Y., and Lou, J. (2004). Analysis of serum cytokines in patients with severe acute respiratory syndrome. Infect Immun 72, 4410-4415. Zhaori, G. (2003). Antiviral treatment of SARS: can we draw any conclusions? Cmaj 169, 1165-1166. Ziebuhr, J. (2004). Molecular biology of severe acute respiratory syndrome coronavirus. Curr Opin Microbiol 7, 412-419.
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