以噬菌體呈現法尋找人類肝白蛋白受體

Search for human albumin receptor using phage display system

計畫編號：NSC 90-2314-B-002-219-MH
執行期限：90 年 8 月 1 日至 91 年 7 月 31 日
主持人：李嘉哲　台大醫院內科

一、中文摘要

人類白蛋白是肝所製造的重要蛋白之一，它在維持血液滲透壓扮演重要角色，並作為各種荷爾蒙，蛋白質，脂肪，離子及藥物的載體。我們發現白蛋白的表現，會隨培養液中白蛋白的濃度的增加而減少。這顯示肝細胞可能含有白蛋白受體。我們先用噬菌體呈現法來尋找此受體，但是效果不佳，因此改用了細菌二雜交系統。我們找到了兩個可能的基因，分別為 phosphodiesterse 3B 及 ATP-binding cassette member 4。我們已選殖此基因，現正研究其功能中。

關鍵詞：白蛋白、受體、細菌二雜交系統

Abstract

Human serum albumin in one of major proteins that are produced by the liver. It plays an important role in maintaining intravascular oncotic pressure and acts as a carrier for various hormones, proteins, fatty acids, ions and drugs. We found that expression of albumin in hepatocyte or hepatocellular carcinoma cell line correlated inversely with the concentration of albumin in the culture medium. This implied that albumin receptor possibly existed in these cells. Initially, we used phage display system to find out this receptor, but failed. We switched to BacterioMatch two-hybrid system and revealed two candidate genes, phosphodiesterse 3B and ATP-binding cassette, member 4. We Have cloned these genes. We are currently defined their roles as possible receptor of albumin.

Keywords: Albumin, Receptor, BacterioMatch two-hybrid system

二、緣由與目的

Human serum albumin plays an important role in maintaining intravascular oncotic pressure and acts as a carrier for various hormones, proteins, fatty acids, ions and drugs. Kinetic studies for several of these substances suggest that uptake is mediated primarily by direct interaction of the albumin-ligand complex with the hepatocyte surface (1). Potential albumin receptors are also found in proximal tubule cells (2, 3, 4) endothelial cells (5), thymocyte (6) and cardiomyocyte (7). Albumin may act as a signalling molecule in proximal tubular cells, and may induce the expression of numerous pro-inflammatory genes (3). Most of the albumin-binding proteins (ABP) were found out by binding to ^{125}I-albumin. Only approximate molecular weight of these ABPs could be inferred. The exact nature of these ABPs has yet to be determined.

Recently, yeast two-hybrid system and phage display system have been used to study the interaction between proteins or between proteins and nucleic acids. The cDNA sequence of the fished-out protein can be determined quickly. In this project, we will utilize human liver cDNA T7 phage display system to find out proteins that interact with human albumin. Through the sequence and expression of proteins, the mechanism of albumin function can be further clarified.

三、結果與討論

We cultivated Huh7 cells and Chang liver cells in serum-free medium and add variable amount of albumin. After incubation for 48 hours, we extracted cellular RNA and checked for expression of albumin mRNA using quantitative PCR. We found that albumin mRNA expression decreased with increasing concentration of albumin in medium. This implied that liver cells response to extracellular albumin and this response could possibly through albumin-receptor interaction. Next we tried to fish out cellular protein that interacted with albumin by phage display system. During screening steps, albumin was used as a blocking agent to reduce non-specific reactions. Without albumin there were many false positive clones. After several trials, we abandon phage display system and switched to baceriomatch two-hybrid system. We cloned albumin-coding sequence 3' to the lambda Cl in pBT vector, and co-transfected into reporter bacteria with a liver cDNA library in pTRG (purchased from Stratagene), which contained RNA polymerase alpha 5' to the cDNA. Thirty clones was obtained, sequenced, and subjected to genbank search. We choosed clones that were in-frame to coding region, membrane protein and that appeared more than once. Two genes satisfied these criteria and are phosphodiesterse 3B (PDE3b) and ATP-binding cassette member 4 respectively. We cloned PDE3b genes, and are doing transfection experiment to see if it will be co-precipitated with albumin. We are also doing gene-knock-out experiment to see if suppression of PDE3b expression will abolish cell response to variation of albumin concentration.

PDE3 involved in control of lipolysis, glycogenolysis, myocardial contractility, smooth muscle relaxation, mesangial cell proliferation, and insulin and renin secretion. Whether it also interacts with albumin and acts as an albumin receptor remains to be determined.

四、計畫成果自評

We found cell expression of albumin correlated inversely with extracellular concentration of albumin. Although phage display system failed to find cellular gene interact with albumin as originally planned, we found alternative with bacteriomatch two-hybrid system. We have found two candidate genes and hopefully can determine whether they act as albumin receptor with further experiments.

五、參考文獻

1. Ockner RK, Weisiger RA, Gollan JL. Hepatic uptake of albumin-bound substances: albumin receptor concept. Am J Physiol 1983; 245:G13-8.

2. Brunskill NJ, Nahorski S, Walls J. Characteristics of albumin binding to opossum kidney cells and identification of potential receptors. Pflugers Archiv - European Journal of Physiology 1997; 433:497-504.

3. Brunskill NJ. Molecular interactions between albumin and proximal tubular cells. Experimental Nephrology 1998; 6:491-5.

4. Cessac-Guillemet AL, Mounier F, Borot C, et al. Characterization and distribution of albumin binding protein in normal rat kidney. American Journal of Physiology 1996; 271:F101-7.

5. Antohe F, Dobrila L, Heltianu C, Simionescu N, Simionescu M. Albumin-binding proteins function in the receptor-mediated binding and transcytosis of albumin across cultured endothelial cells. European Journal of Cell Biology 1993; 60:268-75.

6. Dobrila L, Serban G, Heltianu C. Identification of albumin-binding proteins of thymocyte plasmalemma . Bioscience Reports 1996; 16:425-38.

7. Popov D, Hasu M, Ghinea N, Simionescu N, Simionescu M. Cardiomyocytes express albumin binding proteins. Journal of Molecular & Cellular Cardiology 1992; 24:989

8. Dove SL, Joung JK, Hochschild A.

Activation of prokaryotic transcription
through arbitrary protein-protein contacts.
Nature. 1997; 386:627-30.

行政院國家科學委員會補助專題研究計畫成果報告

※※※※※※※※※※※※※※※※※※※※※※※※※※
※　　　　　　　　　　　　　　　　　　　　　　　　※
※　　以噬菌體呈現法尋找人類肝白蛋白受體　※
※　　　　　　　　　　　　　　　　　　　　　　　　※
※※※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：√個別型計畫　　□整合型計畫
計畫編號：NSC 90-2314-B-002-219-MH
執行期間：　90 年　8 月　1 日至　91 年　7 月　31 日

計畫主持人：李嘉哲
共同主持人：
計畫參與人員：

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

執行單位：台大醫院內科

中　華　民　國　　91 年　　10 月　　30 日