利用噬菌體展示技術製作並分析具特異性結合能力到卵巢腫瘤相關抗原 OVTA-1 的雞隻單株抗體片段

Generation and characterization of chicken antibody fragments against potential tumor-associated antigen, OVTA-1, by phage display technology

中文摘要

根據先前的研究，利用卵巢癌病人血清的抗體從 peptide library 進行篩選, 顯示出 OVTA-1 可能是卵巢癌的腫瘤相關蛋白。在本實驗中，我們主要目的是藉由免疫來亨雞和噬菌體展示技術來製作並篩選出具有與 OVTA-1 特異性結合反應的多株及單株抗體。OVTA-1 蛋白的完整 DNA 全長為 6072 bp，而本實驗所使用的為其中的部份片段 DNA，長度為 520 bp。此 520 bp DNA 在經過 PCR 放大後，選殖入 pGEX 質體後再利用大腸桿菌表現出與 GST 融合的 OVTA-1 蛋白。經由 Western blot 實驗結果顯示抗 GST 的抗體可辨認到此 GST-OVTA-1 融合蛋白。另外使用凝血蛋白酶分離 OVTA-1 和 GST 蛋白，由 SDS-PAGE 及 western blot 分析顯示 OVTA-1 蛋白的大小為~19kDa，這些結果証明此大腸桿菌表現的蛋白為 OVTA-1 的部份蛋白。來亨雞在經過 4 次的 GST-OVTA-1 融合蛋白肌肉注射之後，從雞蛋中純化出免疫球蛋白(IgY)並利用 SDS-PAGE 估算一顆雞蛋約可純化出 50-100 mg 的 IgY。在之後的 western blot 和 ELISA 的實驗顯示這些 IgY 多株抗體具有結合到 GST-OVTA-1 融合蛋白的能力。為了製作出可與 OVTA-1 蛋白結合的單株抗體庫，使用 PCR 連接雞隻產生的抗體輕鍊和重鍊 DNA 成為 scFv 的抗體片段，再經由 DNA 電泳可確認此抗體片段長度為 750-bp。之後將此 scFv DNA 片段選殖入 pComb3X 質體，送入 XL1-Blue 大腸桿菌，再藉由感染噬菌體來製做出多個 scFv 抗體基因庫，其中一個具有 1.1X105 個細胞株的基因庫。之後使用 phage panning 方法從所建構的抗體庫篩選出具有和 OVTA-1 蛋白結合能力的 scFv 菌株，並藉由 XL1-Blue 大腸桿菌表現出 10 個蛋白質大小為 28 kDa 的 scFv 抗體蛋白。利用 ELISA 和 western blot 分析結果顯示其中一些含有抗體片段的菌液具有和 OVTA-1 蛋白特異性結合的能力。另外在序列分析中顯示，其中 2 個菌株在 phage panning 過程中被富化，証明 phage panning 過程是成功的。這些出具有和腫瘤相關蛋白結合能力的抗體在將來可以幫助發展診斷或是具治療效果的抗體。

英文摘要

In previous studies, OVTA-1 was identified as a tumor-associated antigen identified in ovarian cancer patient. In this study, we aimed to generate polyclonal and monoclonal chicken antibodies with high affinity against OVTA-1 protein by using

phage display technology. The full length of OVTA-1 DNA is 6072 bp whose 520 bp DNA fragment was cloned into pGEX vector and was expressed as GST-OVTA-1 fusion protein. In western blot analysis, the result showed that the GST-OVTA-1 fusion protein can be detected by goat anti-GST antibody. Besides, both SDS-PAGE and western blot analysis indicated that OVTA-1 protein separated from GST is about 19 kDa. The result demonstrated that this E.coli-expressed protein is partial OVTA-1 protein. The Leghorn chicken was immunized by intramuscular injection with purified GST-OVTA-1 fusion protein. Polyclonal IgY antibodies were isolated from the collected eggs and were estimated to be 50-100 mg per egg by SDS-PAGE analysis. Moreover, GST-OVTA-1 fusion protein can be recognized by these polyclonal antibodies in western blot and ELISA analysis. To construct OVTA-1-specifc antibody libraries, overlap PCR was performed to generate a variety of 750 bp DNA fragments containing IgY heavy and light chain variable genes. These scFv DNA fragments were cloned into pComb3X vectors and then infected the XL1-Blue E.coli with VCSM13 phage. Several antibody libraries against OVTA-1 protein were obtained and one has 1.1x105 clones. The antibody library was performed with four rounds of phage panning in order to isolate antibody fragments against OVTA-1 protein. After panning, total phagemid DNA was introduced into TOP10F' E.coli and ten clones were selected for antibody expression. Our result indicated that the protein size of E.coli-expressed scFv antibody is about 28 kDa. These clones containing scFv antibody fragments were examined for their binding ability to OVTA-1 protein by ELISA and western blot analysis. Our result showed that several clones containing scFv can specifically bind to OVTA-1 protein. Besides, sequence analysis of these antibody fragments indicated that two particular clones were enriched through phage panning process. These antibody fragments with specific binding ability to tumor-associated antigens can be applied in the development of diagnostic or therapeutic agents in the future.