利用噬菌體展現技術分離可辨認嚴重急性呼吸道症候群

冠狀病毒的雞隻抗體研究

Chicken antibodies against SARS-CoV identified by phage display technology

中文摘要

當我們在面對嚴重急性呼吸道症候群冠狀病毒 (SARS-CoV) 的威脅時，最擔心的主要問題是缺乏有效的診斷以及治療的藥劑。因此，藉著將噬菌體展現技術應用於雞隻免疫的系統上，我們成功地篩選到了對於嚴重急性呼吸道症候群冠狀病毒的棘狀蛋白具有特異性結合力的單株抗體。首先，我們先藉由大腸桿菌表現出了十段重組的棘狀蛋白片斷分子，接著用這些重組的棘狀蛋白免疫雞隻後，建構出了二個單鏈抗體 (scFv) 的基因庫，分別爲具有短的聯結胜肽基因庫以及具有長的聯結胜肽基因庫，而這二個基因庫的大小則分別爲 5 × 107 以及 9 × 106。接著，在經過四次的篩選以及富化的過程後，利用個別所分離出的單鏈抗體展現噬菌體以及單鏈抗體分子來尋找位於棘狀蛋白上的抗原決定位區域。在將個別篩選出的抗體基因序列與雞隻的胚源基因 (germline) 做比對後發現，五個經過篩選後所得到的單鏈抗體展現噬菌體菌株在抗體基因上的互補決定區域 (CDR) 有 31%到 62%的變異性。然而，其中的 4S1 噬菌體對於棘狀蛋白上的胺基酸區域 456 到 650 這片段具有特異性的結合反應，並且此噬菌體也被證明了可以有效地結合上經由嚴重急性呼吸道症候群冠狀病毒所感染後的非洲綠猴腎細胞株(Vero E6 cells)。另一方面，我們也利用大腸桿菌所表現出的單鏈抗體來尋找具抗原性的抗原決定位。Ssc35 以及 Lsc18 這二個單鏈抗體除了對於病毒所感染後的細胞株具有高度特異性的結合反應外，在抗體基因的比對上我們也發現了相似於在單鏈抗體展現噬菌體菌株上所呈現的結果，即在抗體互補決定區域中均呈現出高度的基因變異。除此之外，我們也使用了考馬斯藍染色 (Coomassie blue staining) 以及西方墨點法 (Western blotting) 來分析這些單鏈抗體的表現。不過，值得注意的是，這二個單鏈抗體所辨認到的抗原決定位區域是同樣位於棘狀蛋白上胺基酸 750 到 1000 的片段，然而這個所找到的抗原決定位區域卻是不同於利用噬菌體 4S1 所找到的區域。對於這相異的結果，其原因目前仍需要更近ㄧ步的實驗來確認。總結而論，我們的實驗證明了利用噬菌體展現技術來表現雞隻單鏈抗體可以有效地製造出對於嚴重急性呼吸道症候群冠狀病毒的棘狀蛋白具有高度特異性結合力的分子，並且在未來的臨床與科學研究上這項技術將可被廣泛的應用。

英文摘要

The major concern for severe acute respiratory syndrome (SARS), caused by the SARS-associated coronavirus (SARS-CoV), is the lack of diagnostic and therapeutic

agents. Using phage display technology in a chicken system, high-affinity monoclonal antibody fragments against the SARS-CoV spike protein (S) were isolated and characterized. We expressed ten truncated S protein gene fragments in Escherichia coli cells. Following the immunization of chickens with these recombinant S proteins, two single-chain variable fragment (scFv) antibody libraries were established with short and long linkers to contain 5 × 107 and 9 × 106 transformants, respectively. After four rounds of panning selection, the scFv-expressing phages and scFv antibody molecules of individual clones were used to identify antigenic epitopes on S protein. In a comparison of nucleotide sequences with the chicken germline gene, five reactive scFv-expressing phage clones differ from 31% to 62% in complementarity determining regions. Of which, one dominant phage (4S1) had strong binding to a fragment located between amino acid residues 456 to 650 of S protein. This particular phage also showed significant binding to SARS-CoV-infected Vero E6 cells. When the soluble scFv antibodies was applied for antigenic mapping, two clones (Ssc35 and Lsc18) reacted strongly with SARS-CoV-infected Vero cells, and that significant sequence variation of these two clones in the complementarity-determining regions was also seen as that in scFv-expressing phage. In addition, the scFv expression was visualized by Coomassie blue staining, and detected by Western blot analysis. However, it is notable that those two specific scFv antibodies recognized the same region of the S protein spanning amino acid residues 750 to 1000, which differs from that identified by scFv-expressing S1 clone. The reason for this discrepant epitopic mapping by scFv displayed on phage surface and soluble scFv antibodies is presently unknown and needs further investigation. In conclusion, the results suggest that the chicken scFv phage display system can be a potential model to produce high-affinity binders against the SARS-CoV S protein and this knowledge can be applied in clinical or academic research.