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Effects of portacaval shunt on gastric mucosa in cirrhotic portal hypertension in rats

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Abstract Objective: To observe the functional changes of the gastric mucosa in cirrhotic portal hypertension after portacaval shunt (PCS). Methods: After the model of hepatic cirrhotic portal hypertension was established in rats, the changes of the levels of gastric blood flow (GMBF), glycoprotein (GP) and prostaglandins (PGE_2 and 6-keto-$PGF_{1\alpha}$) were observed. Results: The levels of these parameters were significantly lower in the rats with hepatic cirrhotic hypertension than those of the control group. After PCS, they were gradually increased and were significantly higher than those before PCS in the 5th week postoperatively ($P < 0.01$). Conclusion: Under cirrhotic portal hypertension, the defensive capacity of the gastric mucosa was weakened to result in pathological changes and PCS can improve the microcirculation and function of synthesizing and secreting GP and PGs of the gastric mucosa and its defensive capacity was therefore increased.

Key words gastric mucosa; liver cirrhosis, portal hypertension; portacaval shunt; rat

抗人 C_{1-INH} McAb轻、重链可变区基因的克隆及其在 E. coli中表达的研究*

The cloning and expression in E. coli of V_H /V_L gene of anti-human C_{1-INH} McAb

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PCR技术是近年来发展起来的一种体外扩增特异 DNA片段的技术,不仅用于已知序列 DNA扩增,也用于未知序列,例如具有某些特定特点的免疫球蛋白可变区基因的扩增,大量扩增获得 IgV区基因,使体外表达某些新抗体小片段成为可能,例如,重链可变区(V_H)与轻链可变区(V_L)相连构成 Fv,而 Fv片段是具有抗体完全结合位点的最小片段,利用基因编码连接肽 Linker将 V_H和 V_L连接起来构成单链 Fv(sFv)等,大规模制备的 sFv无论在基础理论还是临床应用中均具有重要意义。本文报道了抗人 C_{1-INH} F_7轻、重链可变区基因的克隆,序列分析,sFv_7的表达载体构建和 F_7 sFv在大肠杆菌中的表达。

使用 V_H、V_L二对通用引物,应用 PCR技术分别由抗人 C_{1-INH}杂交瘤 F_7细胞株经反转录合成第一链 cDNA,扩增获得轻链、重链可变区基因。PCR产物定向克隆进测序载体 pUC18和 pUC19,分别由二个方向对 PCR产物进行双脱氧法序列测定。基因 DNA序列测定结果及由 DNA推导氨基酸序列分析表明: (1)抗人 C_{1-INH} F_7 V_H基因长为 342 bp,共编码 114个氨基酸,F_7V_H和 V_L DNA序列与小鼠 IgG重、轻链同源性达 65%~80%。(2)按 Kabat分类,抗人 C_{1-INH} F_7 V_H基因编码的氨基酸与小鼠 V_HII (A)亚组同源性为 76%,属于II (A)亚组,F_7V_H基因是由 V_H-D-J_H重排产生,而 F_7 V_L编码的氨基酸符合小鼠 κ链 IV亚组氨基酸结构特征,同源性为 73%,F_7V_L基因是由 V_κ-$J_{\kappa 1}$重排而成。

将获得的 V_H和 V_L基因片段构建在已有的分泌型表达载体中,中间以编码 $(Gly_4\ Ser)_3$ 15个氨基酸 Linker的基因相连,V_L 3′端含编码 $(His)_5$的基因片段,构建的表达载体 pIg F_7 sFv以 phoA为引导肽,编码引导肽、V_H Linker V_L、$(His)_5$和 SPA序列,在大肠杆菌 $DH_5\alpha$和 BL21中表达的是含 $(His)_5$和 SPA的 F_7 sFv融合蛋白,分子量为(33~34) KD,表达量占细菌外周质蛋白的 11.3%,占细菌细胞膜蛋白的 9%。抗原结合实验中,发现具有一定的结合活性。本研究获得的表达的产物为进一步 sFv的纯化,活性鉴定,奠定了基础。获得的 sFv可用于抗体的结构与功能研究,构象分析,深入探讨 C_{1-INH}的功能及其在补体经典激活途径中活化和调节的细节等。

关键词 PCR; C_{1-INH}; McAb; V_H; V_L;序列测定;序列分析;表达;大肠杆菌

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