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论著

幽门螺杆菌CagA基因的原核表达及抗原性测定

陈雄， 徐灿霞， 王芬， 沈守荣， 贾燕

中南大学湘雅三医院消化内科， 长沙 410013

摘要：

目的：合成胃癌相关幽门螺杆菌(Helicobacter pylori，H.pylori)的 CagA基因片段，建立原核表达系统，鉴定重组表达产物的抗原性。方法：选取前期研究确定的与胃癌相关的H.pylori CagA基因片段，优化设计并合成CagA基因，将合成的CagA基因从pUC57-CagA质粒中切出，用表达载体pET32a携带该基因转化宿主菌BL21(DE3)，通过氨苄青霉素抗性筛选和菌落PCR挑选出阳性克隆pET32a-CagA；以IPTG诱导含pET32a-CagA的宿主菌表达融合蛋白，经SDS-PAGE凝胶电泳分析CagA蛋白表达情况，用Western印迹鉴定融合蛋白的抗原性。结果：设计并人工合成了CagA基因片段，序列测定结果显示，合成的CagA基因序列与设计的序列（AF289435）基本一致；成功构建pET32a-CagA原核表达质粒，对应蛋白序列与AAG09884同源性为100%；含pET32a-CagA的BL21(DE3)宿主菌经IPTG诱导后能表达CagA融合蛋白，经SDS-PAGE凝胶电泳分析结果表明，融合蛋白相对分子质量大小与预期一致(45 kD)；Western印迹检测结果显示该融合蛋白可与抗H.pylori全菌抗体结合反应。结论：本实验构建的原核表达系统表达的CagA融合蛋白具有良好的抗原性，为临床胃癌相关H.pylori菌株的筛选和针对性治疗奠定了基础。

关键词： 幽门螺杆菌 CagA基因 原核表达 抗原性

Prokaryotic expression and antigenicity of the CagA gene in Helicobacter pylori

CHEN Xiong， XU Canxia， WANG Fen， SHEN Shourong， JIA Yan

Department of Gastroenterology， Third Xiangya Hospital， Central South University， Changsha 410013， China

Abstract:

ObjectiveTo synthesize the specific CagA gene segment of the gastric cancer idiotype Helicobacter pylori（H.pylori）,establish the prokaryotic expression system and identify the antigenicity sequence of recombination signals. MethodsWe selected the CagA fragment which was related to gastric cancer in our earlier research. The CagA gene segment was optimized and synthesized. The synthesized CagA gene was cut from the pUC57-CagA plasmid and then was carried by expression vector pET32a to be transformed into the host bacterium BL21(DE3). The positively cloned pET32a-CagA was selected by receptivity of aniline and colony PCR. The host bacterium with pET32a-CagA was induced by IPTG to express fusion protein. The expression of CagA protein was analyzed by SDS-PAGE gel electrophoresis and the antigenicity of fusion protein was examined by Western blot. Results CagA gene segment was designed and synthesized. The sequence of synthesis CagA gene segment was the same as the one designed before（AF289435）.We successfully constructed the plasmid of prokaryotic expression of the pET32a-CagA. Homology of the target CagA proteinum was 100%, the same as AAG09884. The host bacterium BL21 (DE3) containing pET32a-CagA could express CagA fusion protein after the IPTG induction. SDS-PAGE gel electrophoresis showed that the molecular weight of fusion protein was the same as expected (45 kD). Western blot showed that the fusion protein could be combined with the antibody of the whole bacterium of anti-H.pylori.ConclusionThe synthesized CagA fusion protein from the prokaryotic expression system has antigenicity. We hope to set the foundation for selecting the strain in H.pylori correlated to gastric cancer and corresponding therapy in clinical practice.

Keywords: Helicobacter pylori;CagA gene;prokaryotic expression;antigenicity

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通讯作者: xucanxia2000@hotmail.com

作者简介:

作者Email: 徐灿霞

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