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论著

含Aβ_{1-15}的嵌合型HBcAg颗粒抗原的制备及其免疫原性分析

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摘要:

目的: 原核表达并纯化含β- 淀粉样肽(β-amyloid peptide, Aβ) 氨基段15 肽(Aβ1-15) 和删除c/e1表位的截断型HBcAg 的融合蛋白,观察其形成的病毒样颗粒,检测其免疫原性,为阿尔茨海默病(Alzheimer'sdisease, AD) 基因工程疫苗的研究提供基础。方法:将合成的Aβ1-15 基因连接于HBcAg 的1~71 的3′端,再将HBcAg 的88~144 位氨基酸的基因片断连接于Aβ_{1-15} 的基因的3′端,构建重组质粒pUC/c-Aβ_{15}-c,将重组基因亚克隆于原核表达载体pET-28a(+) 中,构建表达质粒pET/ c-Aβ_{15}-c。异丙基-β-*D*- 硫代吡喃半乳糖苷(isopropyl β-*D*-1-thiogalactopyranoside, IPTG) 诱导、SDS-PAGE 和考马斯亮蓝染色检测重组基因的表达。表达的融合蛋白(命名为CA15C) 纯化后,透射电镜观察病毒样颗粒的形成。以病毒颗粒抗原CA15C 腹腔注射免疫昆明小鼠,间接ELISA 法检测小鼠血清中抗-Aβ 抗体的滴度。结果:经酶切鉴定、DNA 序列测定证实,目的基因重组于表达质粒之中,与理论设计相符。诱导表达后,在细菌裂解液的上清和沉淀中均可见表达蛋白CA15C,以沉淀中为多,约占细菌沉淀总蛋白的40%。电镜下可见纯化后的CA15C 形成直径约30 nm 的病毒样颗粒。昆明小鼠经CA15C 免疫5 次后,其血清中抗-Aβ 抗体的滴度可达1:10000,且检测不到抗-HBc 抗体。结论:原核表达制备的含Aβ_{1-15} 和HBcAg 的融合蛋白CA15C,可形成病毒样颗粒,有较强的免疫原性。

关键词: β- 淀粉样肽(Aβ) HBcAg 融合蛋白 病毒样颗粒 基因工程疫苗

Prokaryotic expression and immunogenicity of the chimeric HBcAg containing Aβ_{1-15}

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Abstract:

Objective: To construct a recombinant prokaryoticexpression plasmid pET/ c-Aβ_{15}-c, and evaluate the immunogenicity of its encoded fusion protein as expressed in *E.coli*.
Methods: The gene fragment HBc$_{88-144}$ was amplified by PCR and subcloned to pUC19. The synthetic, double-strand Aβ_{1-15} gene was inserted downstream of HBc$_{1-71}$ in pGEMEX/c$_{1-71}$. After restriction enzyme digestion, c$_{1-71}$- Aβ15 was spliced to HBc$_{88-144}$, yielding the recombinant gene c-Aβ_{15}-c; that gene was subcloned into pET-28a(+). The fusion protein (CA15C) expressed in the transformed *E.coli* BL21 was induced with isopropyl β-*D*-1-thiogalactopyranoside (IPTG) and analyzed by SDS-PAGE. The virus-like particle (VLP) formed by fusion protein CA15C was observed with transmission electric microscope (TEM). Four Kunming (KM) mice were given intraperitoneal injections of CA15C, and the anti-Aβ antibody elicited was detected by indirect ELISA.
Results: The sequence of the recombinant gene was confirmed by restriction enzyme digestion and DNA sequencing. After IPTG induction, the fusion protein was expressed, mainly in the sediment from the bacterial lysate. The expression level was 40% of total protein in the sediment. The CA15C could form VLP. After 5 rounds of immunization, the titer of anti-Aβ antibody in the sera of KM mice reached 1:10000, while the anti-HBc antibody was undetectable.
Conclusion: Recombinant c-Aβ_{15}-c gene can be expressed in *E.coli*. The expressed protein can form VLPs and has a strong immunogenicity.

Keywords: β-amyloid peptide (Aβ) HBcAg fusion protein virus-like particle (VLP) genetic engineering vaccine

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