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[研究简报]

SPAP2基因的克隆、融合表达及分离纯化

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关键词 SPAP2基因; 克隆; 融合表达
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SPAP2是免疫受体家族中包含 2个 ITIM (Immunoreceptor tyrosine-based inhibition motif)和 2个 ITAM (Immunoreceptor tyrosine-based activation motif)的跨膜蛋白质, SPAP2的细胞外部分包括 6个免疫球蛋白样的结构域, 细胞内部含有 2个 ITIM和 2个 ITAM[1]. SPAP2的基因定位于人类 1q21号染色体上, 包含 16个外显子, 由 734个氨基酸残基组成, 其细胞内片段有 137个氨基酸残基[2]. 当有细胞外信号刺激并激活 SPAP2时, 其细胞内部的 2个 ITIM和 2个 ITAM中的酪氨酸残基发生磷酸化, 诱导胞浆内的含有 SH2结构域 (SH2-domain containing)的 PTP和 PTK由胞浆向细胞膜迁移, 并且结合到磷酸化的 ITIM和 ITAM上, 继续进行下游信号转导[3].

本文构建的表达载体 pGex-2T-SPAP2CT在大肠杆菌中表达出可融性蛋白, 其分子量为 46 000, 经纯化后得到产率为 10%、纯度大于 90%的 GST-SPAP2CT蛋白.

1 实验部分

1.1 试剂与仪器 大肠杆菌 DE3-plysS, 质粒 pBluescript Ⅱ KS为本实验室保存, PCR模板 DNA和质粒 pGex-2T为吉林大学生命科学学院赵志壮教授惠赠, 各种限制性内切酶及 T_4 DNA连接酶系 TaKaRa公司产品, QIAquick Gel Extraction Kit购自德国 Qiagen公司, 其它试剂均为国产分析纯.

Q-Sepharose Fast Flow (0.18 cm ×15 cm), Sepharose 4B (GSH亲和层析柱, 0.12 cm ×2 cm)购自 Pharmacia公司.

1.2 实验过程 以 SPAP2的全长 DNA为模板, 利用 PCR方法扩增 SPAP2细胞内结构域基因, 克隆到载体 pBluescript Ⅱ KS上, 并构建可溶性融合蛋白表达载体 pGex-2T-SPAP2CT. 用 pGex-2T-SPAP2CT转化大肠杆菌 DE3-plysS, 进行融合蛋白质 GST-SPAP2CT的高效可溶性表达, 并利用 FFQ离子交换层析、GSH亲和层析纯化该融合蛋白质, 得到 GST-SPAP2CT, 进行 Western blot检测[4,5].

2 结果与讨论

2.1 目的基因的获得 以 SPAP2全长基因为模板, 通过 PCR扩增其细胞内片段的基因.

2.2 克隆载体的构建及其酶切和测序结果 克隆载体用 BamH Ⅰ和 EcoR Ⅰ双酶切后, 电泳显示出 3.0 kb和 0.4 kb两条带 (图 1), 测序结果表明, 除第 200个和 341个碱基处有 2个沉默突变外, 其余的碱基均相同, 证明已得到 SPAP2CT基因, 并已成功地与克隆载体连接.

2.3 表达载体的构建及其酶切鉴定 表达载体 pGex-2T-SPAP2CT经 BamH Ⅰ和 EcoR Ⅰ双酶切后, 电泳显示出 4.9 kb和 0.4 kb两条带, 表明目的基因与质粒 pGex-2T连接成功, 结果如图 2所示.

2.4 目的蛋白的可溶性表达 重组质粒转化 *E. coli* DE3-plysS, 用 IPTG诱导表达目的蛋白, 全菌电泳鉴定, 泳道在 45000和 66000之间出现新的条带, 与预计的目的蛋白分子量 46000大小一致

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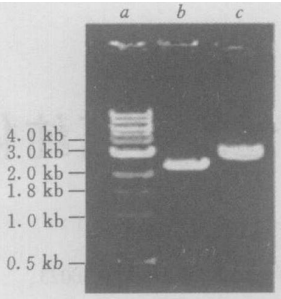

Fig. 1 Agarose gel electrophoresis of pKS-SPAP2CT cut by BamH Ⅰ and EcoR Ⅰ

a. Molecular weight marker; *b*. pKS-SPAP2CT; *c*. pKS-SPAP2CT cut by BamH Ⅰ and EcoR Ⅰ.

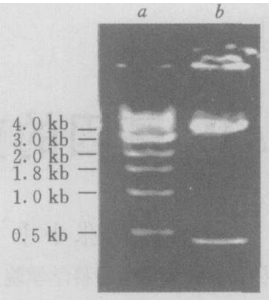

Fig. 2 Agarose gel electrophoresis of pGex-2T-SPAP2CT cut by BamH Ⅰ and EcoR Ⅰ

a. DNA Marker; *b*. pGex-2T-SPAP2CT cut by BamH Ⅰ and EcoR Ⅰ.

(图3). 经薄层扫描分析可知，目的蛋白占菌体蛋白的18%，未经诱导的细菌裂解物的上清液则没有这条带.

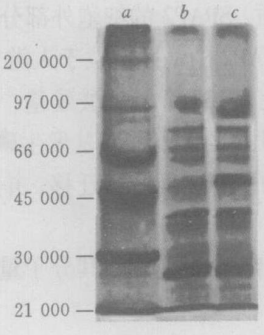

Fig. 3 SDS-PAGE of all proteins of pGex-2T-SPAP2CT

a. Molecular weight marker; *b*. pGex-2T-SPAP2CT uninduced; *c*. pGex-2T-SPAP2CT induced by IPTG.

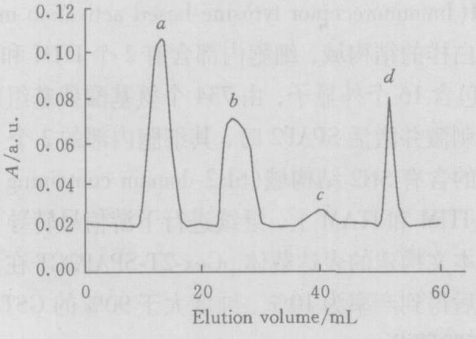

Fig. 4 Affinity chromatography of GST-SPAP2CT on glutathione sepharose 4B

a. Fraction flowing through glutathione sepharose 4B; *b*. fraction eluted with buffer 1; *c*. fraction eluted with buffer 2; *d*. fraction eluted with 10 mmol/L glutathione readuced.

2.5 纯化目的蛋白的SDS-PAGE分析 菌体蛋白经离子交换层析及亲和层析分离提纯，用不同成分的Buffer进行阶段洗脱，用紫外检测仪检测蛋白含量(图4). 图4中峰*d*为洗脱缓冲剂(Elution Buffer)的洗脱峰，对应于图5中的*e*泳带，由此可判断其洗脱液中含有较多的目的蛋白，经薄层扫描分析，鉴定其纯度在90%以上，利用考马斯亮蓝法，计算得到Elution Buffer的洗脱液中目的蛋白GST-SPAP2CT

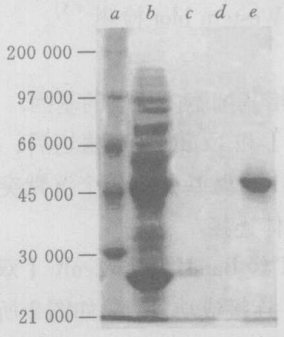

Fig. 5 Separation of GST-SPAP2CT on SDS gel protein samples separated by 10% SDS gels and then stained with Coomasie Brilliant Blue

a. Molecular weight marker; *b*. *a* from glutathione sepharose 4B; *c*. *b* from glutathione sepharose 4B; *d*. *c* from glutathione sepharose 4B; *e*. *d* from glutathione sepharose 4B.

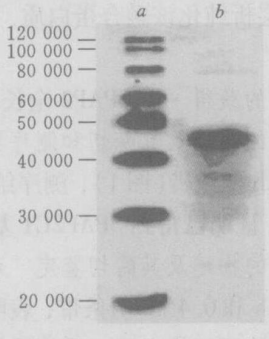

Fig. 6 Separation of GST-SPAP2CT on SDS gels and analysed by immunoblotting with anti-SPAP2CT(163)

a. Magic marker; *b*. GST-SPAP2CT.

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含量为 1.25 mg/mL.

2.6 Western blot验证 GST-SPAP2CT结果 纯化得到的 GST-SPAP2CT用 Anti-SPAP2CT(163)Western blot检测, *b*泳带的蛋白质分子量为 46 000, 证明此蛋白就是 GST-SPAP2CT. 用 Western blot验证 GST-SPAP2CT, 在 28 000～46 000之间有一些蛋白质与抗体(163)结合, 这些蛋白质是 GST-SPAP2CT的不完全合成产物和降解产物, 与 SPAP2有相同的结构域, 所以能够和层析柱上的 GSH结合(图 6).

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Cloning, Fusion Expression and Purification of SPAP2 Domain Gene

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Abstract SPAP2, a transmembrane protein, is an Ig family receptor containing both ITIMs (immunoreceptor tyrosine-based inhibition motifs) and ITAMs (immunoreceptor tyrosine-based activation motifs). The extracellular portion of SPAP2 contains six immunoglobulin-like domains and its intracellular segment has two ITAMs and two ITIMs. Sequence alignment with the genomic database reveals that the SPAP2 gene contains 16 exons and is localized at chromosome 1q21. SPAP2 is consisted of 734 amino acids, and the intercellular portion of SPAP2 contains 137 amino acids. Tyrosine-phosphorylated SPAP2 is specifically associated with SH2 domain-containing tyrosine kinases and SH2 domain-containing tyrosine phosphatases, which lead to the initiation of signal transduction. SPAP2CT gene was amplified by PCR with SPAP2 full-length DNA as the template and cloned to the pBluescript II KS vector. pGex-2T-SPAP2CT, the expression vector of dissoluble fusion protein, was constructed and transferred into *E. coli* of DE3-plysS. The fusion protein GST-SPAP2CT was expressed efficiently and purified by FFQ ion exchange chromatography and GSH affinity chromatography. The result indicates that we have constructed steady expression vector pGex-2T-SPAP2CT, which was expressed in *E. coli*. The molecular weight of the dissoluble fusion protein is 46 000, the productivity of GST-SPAP2CT protein is 10% and the purity is over 90% after the purification.

Keywords SPAP2 gene; Clone; Fusion expression

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