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3TAT-DRBD融合蛋白的原核表达及其siRNA结合活性与穿膜功能的鉴定

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刁艳君 刘家云 马越云 苏明权 郝晓柯

第四军医大学 附属西京医院 全军临床检验医学研究所，陕西 西安 710032;第四军医大学 附属西京医院 全军临床检验医学研究所，陕西 西安 710032;第四军医大学 附属西京医院 全军临床检验医学研究所，陕西 西安 710032;第四军医大学 附属西京医院 全军临床检验医学研究所，陕西 西安 710032;第四军医大学 附属西京医院 全军临床检验医学研究所，陕西 西安 710032

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摘要：

目的： 构建3TAT-DRBD重组载体，表达和纯化融合蛋白，并对其siRNA结合活性和穿膜功能进行初步验证。 方法： 采用基因合成技术获取靶基因 3TAT-DRBD， 并克隆到原核表达载体pET-44b中；用IPTG诱导融合蛋白表达，镍亲和凝胶层析柱纯化融合蛋白，凝血酶切除标签，Western blotting鉴定。凝胶迁移阻滞实验验证DRBD和siRNA的结合能力，激光共聚焦显微镜观察TAT的穿膜能力。 结果： 限制性酶切和基因测序表明重组质粒pET-44b-3TAT-DRBD构建成功；IPTG诱导后3TAT-DRBD融合蛋白（含Nus标签和S标签）在大肠杆菌中高效表达，可溶性蛋白占菌体总蛋白约80%；成功切除融合标签并纯化了无标签的融合蛋白，经Western blotting鉴定其相对分子质量约为17 000；凝胶迁移阻滞实验证明，融合蛋白3TAT-DRBD 能有效结合靶向survivin基因的siRNA（survivin-siRNA）；激光共聚焦显微镜下可见，在TAT的介导下survivin-siRNA穿透胞膜进入前列腺癌PC3细胞的效率明显增高。 结论： 成功表达并纯化了具有siRNA结合活性与穿膜功能的3TAT-DRBD融合蛋白，为进一步3TAT-DRBD的功能研究及临床应用奠定了基础。

关键词：TAT DRBD 融合蛋白 siRNA 前列腺癌 结合活性 穿膜功能

Prokaryotic expression of 3TAT-DRBD fusion protein and identification of itssiRNA-binding activity and membrane-penetrating function

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Diao Yanjun Liu Jiayun Ma Yueyun Su Mingquan Hao Xiaoke

Institute for Clinical Laboratory Medicine of PLA, Xijing Hospital Affiliated to Fourth Military Medical University, Xi’ an 710032, Shaanxi, China;Institute for Clinical Laboratory Medicine of PLA, Xijing Hospital Affiliated to Fourth Military Medical University, Xi’ an 710032, Shaanxi, China;Institute for Clinical Laboratory Medicine of PLA, Xijing Hospital Affiliated to Fourth Military Medical University, Xi’ an 710032, Shaanxi, China;Institute for Clinical Laboratory Medicine of PLA, Xijing Hospital Affiliated to Fourth Military Medical University, Xi’ an 710032, Shaanxi, China;Institute for Clinical Laboratory Medicine of PLA, Xijing Hospital Affiliated to Fourth Military Medical University, Xi’ an 710032, Shaanxi, China

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Abstract:

Objective: To construct a recombinant vector 3TAT-DRBD expressing purified fusion protein, and to preliminary validate its siRNA-binding activity and membrane-penetrating function. Methods: The target gene 3TAT-DRBD was obtained by gene synthesis and cloned to prokaryotic expression vector pET-44b. The expression of fusion protein was induced by IPTG. The fusion protein was purified by Ni-NTA agarose, and cut by thrombin and detected by Western blotting analysis. The binding activity of DRBD was tested by EMSA and the cytomembrane penetrating activity of TAT was observed by confocal laser scanning microscopy (CLSM). Results: Restriction enzyme digestion and gene sequencing showed that the recombinant plasmid pET-44b-3TAT-DRBD was successfully constructed. The fusion protein (containing Nus and S tags) induced by IPTG was efficiently expressed in E. coli, with the soluble parts accounting for around 80% of the total proteins. The tags were successfully cut off and the fusion protein without tags was purified with a molecular weight of 17 000 Da identified by Western blotting. EMSA identified that the fusion protein 3TAT-DRBD could effectively bind siRNA targeting survivin gene (survivin-siRNA). The efficiency of survivin-siRNA penetrating into prostate cancer PC3 cells mediated by TAT was significantly increased under an observation of CLSM. Conclusion: 3TAT-DRBD fusion protein with siRNA-binding activity and cell membrane-penetrating function is successfully expressed and purified, lying a good basis for further functional research and clinical application of 3TAT-DRBD.

Keywords:TAT DRBD fusion protein siRNA prostate cancer binding activity membrane-penetrating function

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