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研究论文

新型抗HIV-1重组导向制剂SL41在毕赤酵母中的表达及活性实验

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摘要 以酵母分泌型表达载体pPIC9k为基础, 通过一段柔性连接肽Linker构建含有人源化抗HIV-1 gp41单链抗体(ScFv41)和免疫诱导因子葡萄球菌肠毒素A(*staphylococcal enterotoxin* A, SEA)的融合表达质粒pPIC9k-SL41, 线性化后, 采用电转化法整合入巴斯德毕赤酵母GS115中, 经His+MutS表型鉴定、PCR分析以及G418筛选获得高拷贝重组转化子. 摇瓶培养、甲醇诱导表达、SDS-PAGE和Western Blot分析结果表明, 目的蛋白得到良好表达, 表达量最高可达到47.9 mg/L. 目的蛋白经初步纯化后, 用于制备的HIV-1感染靶细胞复制模型进行抗体亲和力测定、细胞结合活性测定和细胞杀伤活性研究, 结果显示, 目的蛋白能够很好地与靶细胞模型中的HIV-1外膜蛋白gp160发生结合反应, 并可介导特异的CTL反应, 对靶细胞具有明显的杀伤活性, 表明获得了具有生物活性的抗HIV-1重组导向制剂.

关键词 HIV-1 重组导向制剂 单链抗体 金黄色葡萄球菌肠毒素A(SEA) 分泌表达 毕赤酵母

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Expression and Activities of Recombinant Directing Agent SL41 Against HIV-1 in *Pichia pastoris*

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Abstract The humanize anti-HIV-1 single chain Fv fragment(ScFv) gp41 gene and super antigen staphylococcal exterotoxin A(SEA) gene were fused *via* soft linker peptide, and cloned into expression vector pPIC9k. The recombinant plasmid was linearized and transferred into Pichia pastoris strains GS115 by electroporation. High copies of trransformants were obtained with Mut^S and Mut^+ phenotype identification, PCR amplification and screening of G418. After flask culture and inducing expression by methanol, the targeted protein was well expressed and performed via SDS-PAGE and Western Blot. The highest production was 47.9 mg/L when different parameters were optimized. The primarily-purified protein was analyzed with antibody affinity assay, cellular binding activity and cellular killing activity using the HIV-1 infection target cell models prepared by our laboratory. The results suggest that the constructed anti-HIV-1 gp41 recombinant agent SL41 has a good biological activity and specific CTL cytotoxicity activity. This research will provide a potential values for AIDS treatment and the settled solid foundation for clinical trials.

Key words HIV-1 Recombinant directing agent Single chain Fv fragment Staphylococcus extoxin

A(SEA) Secrete expression *Pichia pastoris*

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