HIV-1 CRF07_BC毒株gp41 NHR结构域N51的表达及结构分析(点击查看pdf全文)

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Title: Expression, structure and antigenicity analysis of N51 derived from the N-terminal heptad repeat domain in gp41of HIV-1CRF07_BC strain

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关键词: 人免疫缺陷病毒1型; CRF07_BC; gp41; NHR结构域; N51基因; 结构分析

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摘要: 目的对来源于HIV-1中国流行株CRF07_BC的包膜糖蛋白gp41NHR结构域的N51进行表达和结构及抗原性分析。方法运用重叠延伸PCR方法扩增出N51Fd基因，将其插入真核表达载体pFUSE-hIgG1-Fc2，并进行核苷酸序列测定。利用生物信息学软件、圆二色谱法、免疫印迹法对表达的N51FdFc-BC重组蛋白进行结构和抗原性分析。结果成功构建pFUSE/N51Fd-BC表达载体，并在真核表达体系实现了目的蛋白的高效表达。免疫印迹结果显示该重组蛋白大小约为35000，可与抗HIV-1gp41N/C多肽的抗体反应。生物信息学分析显示N51FdFc-BC重组蛋白相对分子质量为34315.1，等电点PI为7.59，且形成了无规则卷曲结构，易于与抗体反应，可作为抗原。圆二色谱的分析与生物信息学软件预测的结果一致。结论N51FdFc-BC重组蛋白具有无规则卷曲结构，可适合作为HIV-1亚单位疫苗的免疫原。

Abstract: ObjectiveTo express N51 derived from the N-terminal heptad repeat (NHR) domain in gp41 of the HIV-1 CRF07_BC strain and analyze its molecular structure and antigenicity.MethodsOverlapping PCR was used to amplify the DNA fragment encoding N51Fd gene, which was then subcloned into the vector pFUSE-hIgG1-Fc2. The construct was confirmed by DNA sequencing. The structure and antigenicity of the recombinant protein N51FdFc-BC were analyzed using bioinformatic software, circular dichroism, and Western blotting.ResultsA recombinant expression vector pFUSE/N51Fd-BC was successfully constructed. N51FdFc-BC recombinant protein with a relative molecular mass of about35000was effectively expressed in mammalian293T cells and could be recognized by rabbit antibodies against HIV-1gp41N/C peptides as shown by Western blotting. Bioinformatic analysis showed that the recombinant protein

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N51FdFc-BC, with a relative molecular mass of34315.1and a PI of7.59, formed a secondary structure of random coil to allow its interactions as an antigen with antibodies.
Circular dichroism measurement confirmed the random coil structure of N51FdFc-BC protein.ConclusionThe recombinant protein N51FdFc-BC has a random coil structure and can be used as an immunogen for development of HIV-1subunit vaccine.

参考文献/REFERENCES

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