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论著

宫颈癌细胞中NRDR选择性剪接新亚型表达质粒的构建及原核表达

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摘要 背景与目的： 构建宫颈癌细胞中视黄醇脱氢/还原酶_辅酶II依赖性视黄醇脱氢/还原酶(NRDR)及其选择性剪接亚型NRDRB1原核表达载体，并在BL21_AI大肠杆菌中表达融合蛋白。 材料与方法： 用RT_PCR检测宫颈癌组织中NRDR、NRDRB1表达，RACE方法克隆NRDRB1全长cDNA，运用Gateway表达系统将NRDR、NRDRB1编码区序列构建到表达载体，转化到大肠杆菌中表达，表达的融合蛋白通过金属离子亲和层析纯化。 结果： 在宫颈鳞癌组织中发现NRDR新的选择性剪接亚型NRDRB1，构建了NRDR、NRDRB1原核表达载体，在BL21_AI中表达出氨基端含6×His标签的重组蛋白，诱导表达4 h后目的蛋白可占总蛋白量的30%～50%，经一步亲和层析得到了高纯度的NRDR及NRDRB1重组蛋白。 结论： 在大肠杆菌中获得高效表达的NRDR、NRDRB1蛋白，为进一步研究其功能提供了实验材料。

关键词 NADP依赖性视黄醇脱氢/还原酶 选择性剪接； 宫颈癌； 重组表达

Expression and Purification of NADP(H)_dependent Retinol Dehydrogenase/reductase(NRDR) in Cervical Carcinoma

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Abstract BACKGROUND AND AIM: To express NRDRB1 in prokaryotic expression system for detecting enzyme activity and preparing polyclonal antibody. MATERIALS AND METHODS: We investigated the mRNA expression of NRDRB1 in cervical squamous carcinoma using RT_PCR. The total NRDR and NRDRB1 sequences were cloned and the coding regions were constructed to the Gateway_based expression vector (pDEST 17), which was transformed into the Escherichia coli(BL21_AI)for protein expression. The recombinant proteins were purified by affinity chromatography. RESULTS: We identified a novel alternatively spliced variant, NRDRB1, in HeLa cell and human cervical squamous carcinoma cells, characterized by a complete deletion of exon 3. The expression vectors of NRDR and NRDRB1 were constructed. The proteins, harvested at the optimal time point (4 hours after induction), were successfully expressed with a 30－50％ expression leve1. The homogeneous proteins were obtained by a one_step affinity chromatography. CONCLUSION: Recombinant NRDR and NRDRB1 from human cervical squamous carcinoma cells were expressed effectively in BL21_AI by pDEST 17.

Keywords NRDR alternative splicing cervical carcinoma recombinant expression

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