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论文

免疫共沉淀检测人p65和SMRT蛋白间相互作用

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摘要：

目的 使用免疫共沉淀技术法检测人p65和SMRT蛋白在儿童急性髓细胞白血病（AML）细胞株THP-1中相互作用。方法 体外人工合成p65基因CDS区碱基序列和SMRT-C 645bp对应的基因片段，分别克隆至pUC57载体，经酶切、测序正确后，PCR扩增目的基因片段，酶切后亚克隆至带有HA标签和Myc标签的真核表达载体pCMV-HA和pCMV-Myc，测序、酶切鉴定正确后，共转染THP-1细胞，免疫共沉淀法检测二者间相互作用。结果 成功构建了人p65和SMRT蛋白真核表达载体pCMV-HA-p65和pCMV-Myc-SMRT，共转染THP 1细胞，抗HA和Myc多克隆抗体分别沉淀细胞裂解液，用抗Myc和HA单克隆抗体分别进行Western Blotting检测，可以检测到HA-p65和Myc-SMRT蛋白的表达。结论 成功构建了人p65和SMRT蛋白重组真核表达载体，在THP-1细胞株中表达后，免疫共沉淀证实p65和SMRT蛋白间存在相互作用，为进一步探讨儿童AML发病机制和白血病的靶向治疗提供理论依据。

关键词： 免疫共沉淀；p65；SMRT；髓细胞白血病

Interactions between p65 and SMRT by Co-Immunoprecipitation

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Abstract:

Objective To determine interactions between p65 and SMRT proteins in the acute myelogenous leukemia cell line (THP-1) by co-immunoprecipitation. Methods The human p65 gene and SMRT gene were synthesized in vitro and cloned into the pUC57 vector. Corresponding DNA was amplified by PCR and the PCR products were subcloned into eukaryotic vectors of pCMV-HA and pCMV-Myc after restriction enzyme digestion. Then, these recombined plasmids were confirmed by restriction enzyme digestion and DNA sequencing and co-transfected into THP-1 cell lines. The interactions between p65 and SMRT were detected by co-immunoprecipitation. Results Restriction enzymes digestion and DNA sequencing showed that the recombinant eukaryotic vectors of pCMV-HA-p65 and pCMV-Myc-SMRT were correctly constructed. After being immunoprecipitated by anti-HA and anti-Myc multi-antibodies, fusion proteins of HA-p65 and Myc-SMRT were identified by Western blotting with anti-Myc and anti-HA antibodies. Conclusion Recombinant eukaryotic vectors of pCMV-HA-p65 and pCMV-Myc-SMRT were successfully constructed. The interaction of P65 and SMRT should be identified by co-immunoprecipitation after co-expression in THP-1 cell line, which can serve as a tool for further study of the pathogenesis of AML in children and targeting therapy of leukemia.

Keywords: Co-immunoprecipitation; P65; SMRT; Myelocytic Leukemia

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