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APE1和p53蛋白在A549细胞和HeLa细胞中的结合作

《第三军医大学学报》[ISSN:1000-5404/CN:51-1095/R] 卷: 35 期数: 2013年第21期 页码: 2314-2319 栏目: 论著 出版日期: 2013-11-15

Title: Confirming direct association between APE1 and p53 protein in A549 and HeLa cells

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关键词: APE1蛋白; p53蛋白; 相互作用

Keywords: apurinic/apyrimidinic endonuclease 1; p53; interaction

分类号: R394-33; R394.3; R730.23

文献标志码: A

摘要: 目的 探讨脱嘌呤/脱嘧啶核酸内切酶 (apurinic/apyrimidinic endonuclease, APE1)和p53蛋白内源性和外源性相互结合作用。 方法 将重组质粒pET42a-hAPE1转染*E.coli* BL21(DE3)，应用IPTG诱导GST-APE1蛋白表达，鉴定后经金属螯合层析技术纯化，通过Western blot鉴定纯化蛋白，得到GST-APE1融合蛋白。免疫共沉淀(Co-Immunoprecipitation, Co-IP)和GST蛋白沉降实验分别检测内源性和外源性APE1和p53蛋白结合作用，免疫荧光标记结合激光共聚焦显微镜技术确定APE1和p53蛋白亚细胞定位。 结果 转染质粒经IPTG诱导表达，应用SDS-PAGE发现GST-APE1在预期位置64×10^3处存在阳性条带，“Ni”柱纯化后得到GST-APE1融合蛋白，通过Western blot鉴定。免疫共沉淀和GST蛋白沉降实验证实APE1和p53存在内源性和外源性结合作用。激光共聚焦显微镜观察免疫荧光标记的APE1和p53蛋白均为核浆共表达蛋白，氧化应激处理后，蛋白逐渐由胞浆向胞核移位，二者共定位于核膜处较明显。 结论 APE1和p53蛋白在A549和HeLa细胞中存在内源性和外源性结合作用,可能与其转录调控及肿瘤放化疗疗效有关。

Abstract: Objective To investigate the endogenous and exogenous interaction between apurinic/apyrimidinic endonuclease 1 (APE1) and p53 in A549 and HeLa cells. Methods Recombinant plasmid pET42a-APE1 was transformed into *E. coli* BL21(DE3), and the expression of APE1was induced and identified with IPTG. The expressed GST-APE1 fusion protein was purified by affinity

chromatography with Ni^{2+} column and identified by Western blotting. The interaction between APE1 and p53 protein was identified by co-immunoprecipitation (Co-IP) and GST pull down assay. Immunofluorescence assay and laser scanning confocal microscopy was used to observe the subcellular localization of the 2 proteins. Results Highly expressed hAPE1 fusion protein by pET42a-hAPE1 was obtained in *E.coli* BL21(DE3) after IPTG induction. The GST-APE1 fusion protein with molecular weight of 64×10^3 by SDS-PAGE was purified by affinity chromatography with Ni^{2+} column and identified by Western blotting. Through Co-IP and GST pull down assay, APE1 was confirmed to interact with p53 endogenously and exogenously. Immunofluorescence assay and laser scanning confocal microscopy indicated that the 2 proteins were located in the nuclear and cytoplasm, and then transformed from the cytoplasm to the nuclear membrane after oxidative stress. Conclusion There are endogenous and exogenous interaction between APE1 and p53 protein in A549 and HeLa cells, which might be associated with transcriptional regulation and efficiency of tumor radiotherapy and chemotherapy.

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更新日期/Last Update: 2013-11-06