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Polo样激酶3针对P73蛋白的磷酸化位点分析 点此下载全文

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摘要：

目的：探讨P73蛋白上存在的能够被polo样激酶3（polo like kinases 3，Plk3）磷酸化的结构域或位点，并分析Plk3对P73介导肿瘤细胞凋亡的影响。方法：免疫共沉淀法检测COS 7细胞中Plk3与P73蛋白之间的相互作用，荧光免疫染色法检测Plk3与P73蛋白在细胞中的定位。制备不同的P73缺失突变体GST融合蛋白，体外点突变法构建GST P73(1~130)点突变的P73(T86A)（第86位苏氨酸点突变为丙氨酸）质粒，体外磷酸化实验分析P73中被Plk3磷酸化的结构域或位点。通过检测PARP蛋白的裂解分析Plk3对P73介导人宫颈癌HeLa细胞凋亡的影响。结果：Plk3与P73蛋白之间存在相互作用，Plk3与P73蛋白共定位于COS 7细胞核中。制备获得不同的P73缺失突变体GST融合蛋白，Plk3在P73蛋白N端第63~113位氨基酸残基之间磷酸化P73蛋白。GST P73(1~130)融合蛋白第86位苏氨酸点突变为丙氨酸(T86A)之后，不影响GST P73(1~130)蛋白的磷酸化状态。Plk3可抑制P73介导的HeLa细胞凋亡。结论：Plk3通过与P73蛋白结合，诱导P73蛋白N端第63~113位氨基酸磷酸化，但第86位苏氨酸并非Plk3的特异作用位点;此外Plk3抑制P73介导的HeLa细胞凋亡。

关键词：polo样激酶3(Plk3) P73 磷酸化 位点 凋亡 宫颈癌细胞

Analysis of phosphorylated sites of P73 protein by polo like kinases 3 Download Fulltext

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Abstract:

Objective:To investigate the structural domains and sites of P73 which can be phosphorylated by polo like kinases 3 (Plk3), and to analyze the effect of Plk3 on P73 mediated apoptosis. Methods: Co immunoprecipitation experiment was used to examine the interaction between Plk3 and P73. Immunofluorescence was used to examine the localization of Plk3 and P73 in cells. Different deletion mutants of GST P73 fusion protein were prepared. A site mutation plasmid of GST P73 (1 130) was constructed by converting threonine86 to alanine86 (T86A) and was named GST P73 (1 130) (T86A). The phosphorylated domains and sites of P73 by Plk3 were determined by in vitro phosphorylation assay. The effect of Plk3 on P73 mediated apoptosis of HeLa cells was examined by cleaved PARP detection. Results: Plk3 could interact with P73; Plk3 and P73 co located in the cell nuclei. Different deletion mutants of GST P73 fusion protein were successfully prepared, and Plk3 phosphorylated P73 at N terminal 63 113 amino residues. Point mutation (T86A) of GST P73 (1 130) fusion protein could not influence the phosphorylation status of P73 by Plk3. Furthermore, Plk3 inhibited P73 mediated apoptosis in HeLa cells. Conclusion: Plk3 can interact with and phosphorylate P73 at N terminal 63 113 amino residues (but not at the 86 threonine), thereby inhibiting P73 mediated apoptosis of HeLa cells.

Keywords:polo like kinases 3（Plk3） P73 phosphorylation site apoptosis cervical cancer cell

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