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论著

N-端加flag标签增加P21Cip1/WAF1蛋白质稳定性

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摘要 背景与目的： 已知细胞分裂周期抑制因子P21通过蛋白酶体通路降解，其氨基端的泛素化可能参与了这一过程。Flag为一蛋白标签，常用于标记重组蛋白质，以便对靶蛋白进行生物学功能分析。本文旨在研究N-端加flag对P21蛋白质稳定性的影响。 材料与方法： 建立稳定表达外源flag-p21蛋白质的NIH3T3细胞株，应用蛋白印迹法 (WB) 检测NIH3T3细胞表达的flag-p21，并比较其与内源P21蛋白质半衰期的差异；确定阻断蛋白酶体水解通路对其降解过程的影响。 结果： NIH3T3细胞表达的内源P21蛋白质半衰期约30 min，而同一细胞表达的flag-p21融合蛋白半衰期则明显延长；用抑制剂MG-132阻断蛋白酶体水解通路后，P21蛋白质的量明显增加，但同一细胞内表达的flag-p21量却无明显改变。 结论： N-端加flag标签可增加P21蛋白质的稳定性。在对P21蛋白质的生物学功能进行研究时，应考虑N-端加flag对P21泛素化-蛋白酶体依赖的降解过程的影响。

关键词 P21Cip1/WAF1； Flag标签； 蛋白酶体； 降解

N-terminal Flag Tag Stabilizes P21Ci p1/WAF1 Protein

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Abstract BACKGROUND & AIM: N-terminal ubiquitination may play a role in p21Cip1/WAF1 degradation via proteasome-dependent proteolytic pathway. We studied the influence of adding a N-terminal flag tag on the stability of p21 protein. MATERIAL AND METHODS: NIH3T3 cell stably expressing flag-p21 was set up by retrovirus pWB3flag-p21 infection followed by Blasticidin selection. Half-life of p21 protein was tested by western blot with rabbit polyclonal anti-p21 antibody, and proteasome inhibitor MG-132 was employed to test the role of proteasome pathway on the degradation of p21 protein. RESULTS: Half-life of endogenous p21 in NIH3T3 cells was about 30 minutes, while the half-life of exogenous flag-p21 fusion protein expressed in the same tested cells was obviously increased. Although MG-132 significantly increased the amount of endogenous p21 protein, there was no detectable change of flag-p21 expression under the same condition. CONCLUSION: N-terminal ubiquitination gvas necessary for effective p21 proteasome-dependent degradation. That adding flag tag at the amino acid termini may stabilize p21 protein should be considered.

Keywords P21Cip1/WAF1 flag tag proteasome degradation

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