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研究论文

斜纹夜蛾铁硫亚基蛋白的表达及功能鉴定

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Expression and function identification of the Rieske iron-sulfur protein from the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae)

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全文: PDF (5771 KB) HTML (1 KB) 输出: BibTeX | EndNote (RIS) 背景资料

摘要 铁硫亚基蛋白(rieske iron-sulfur protein, RISP) 是线粒体复合物Ⅲ的关键蛋白亚基之一， 在呼吸链电子传递过程中起到重要作用。本研究通过RT-PCR克隆得到斜纹夜蛾*Spodoptera litura* RISP基因*SlitRISP*的ORF， 构建了pET32a-*SlitRISP*原核表达载体， SDS-PAGE和Western blot检测结果显示， *SlitRISP*原核表达蛋白以包涵体的形式存在于菌体沉淀中， 且RISP抗体可成功用于该蛋白的免疫印迹检测。为了进一步鉴定SlitRISP在斜纹夜蛾离体细胞系SL-1中的功能， 通过向细胞内转染siRNA， 利用RNAi技术沉默SL-1中的*SlitRISP*。qRT-PCR结果表明， 分别经50 nmol/L和100 nmol/L siRNA处理48 h后， SL-1中*SlitRISP*的表达均几乎完全被抑制； Western blot结果显示， SL-1中*SlitRISP*含量显著低于CK。当SL-1 *SlitRISP*被成功沉默后， 通过检测SL-1线粒体膜电位、 细胞ATP含量和细胞增殖抑制率鉴定RISP在线粒体电子传递过程中的重要作用。流式细胞仪测定结果表明，经50 nmol/L和100 nmol/L siRNA处理24 h后， SL-1线粒体膜电位相对于CK分别降低23.52%和11.32%， 而处理48 h后，SL-1线粒体膜电位则分别升高5.58%和27.66%； siRNA处理24 h和48 h后， SL-1 ATP含量相对于CK分别降低82.71%和84.50%， 最终导致SL-1细胞增殖抑制率分别为53.64%和67.94%。这些结果表明SlitRISP在SL-1中参与线粒体膜电位的形成和细胞ATP的合成。介于RISP在线粒体电子传递链中的重要作用， 其可能成为新型杀虫作用靶标， 这可为研制新型呼吸抑制剂提供参考。

关键词： 斜纹夜蛾 铁硫亚基蛋白 原核表达 蛋白功能 SL-1细胞

Abstract： The Rieske iron-sulfur protein (RISP) is a key protein subunit of mitochondrial complex Ⅲ, which plays an important role in the respiratory electron transport chain. The opening reading-frame (ORF) of *SlitRISP* was cloned by RT-PCR from *Spodoptera litura* for the construction of prokaryotic expression vector pET32a-*SlitRISP*. SDS-PAGE and Western blot analysis showed that the prokaryotic protein SlitRISP was mainly present as inclusion body in the bacteria precipitate, and the antibody of RISP could be applied to the immunoblot analysis of SlitRISP successfully. In order to identify the function of SlitRISP in the cultured cell line SL-1 of *S. litura*, RNAi was used to silence *SlitRISP* by transfecting siRNA into SL-1 cells. The qRT-PCR result showed that at 48 h after the SL-1 cells were treated with 50 nmol/L and 100 nmol/L siRNA, respectively, the expression levels of SlitRISP mRNA were all inhibited effectively compared with the control. The Western blotting result showed that the content of SlitRISP in SL-1 cells was obviously lower than the control. The mitochondial membrane potential (MMP), ATP content and inhibition rate of cell proliferation were detected based on the obvious silence of *SlitRISP* in SL-1 cells to identify the important roles of RISP in the electron-transport chain of mitochondria. The changes of MMP in SL-1 cells were monitored by flow cytometry (FCM), which decreased by 23.52% and 11.32% at 24 h after the SL-1 cells were treated with 50 nmol/L and 100 nmol/L siRNA, respectively, compared with control. However, at 48 h after the SL-1 cells were treated with 50 nmol/L and 100 nmol/L siRNA, the MMP increased by 5.58% and 27.66%, respectively. The ATP content in SL-1 cells at 48 h after treatment with 50 nmol/L and 100 nmol/L siRNA was decreased by 82.71% and 84.50%, respectively, measured by luminometer. Owning to the suppression of ATP synthesis by siRNA in SL-1 cells, the inhibition rates of cell multiplication reached to 53.64% and 67.94%, respectively, at 48 h after the SL-1 cells were treated with 50 nmol/L and 100 nmol/L siRNA. These results demonstrate that the RISP plays an important role in the MMP formation and ATP synthesis in SL-1 cells. As RISP plays important roles in the electron-transport chain of mitochondria, it could become a new target for pest control, and this may provide reference for developing new respiration inhibitors.

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Key words: *Spodoptera litura* Rieske iron-sulfur protein prokaryotic expression protein function; *Spodoptera litura cultured* cell line (SL-1)

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