[1]李力力,游扬,周源,等.PNPase调控线粒体microRNA对线粒体DNA的保护作用[J].第三军医大学学报,2014,36(08):780-784.

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PNPase调控线粒体microRNA对线粒体DNA的保护作

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Title: Regulation of mitochondrial microRNAs by PNPase inhibition prevents mitochondrial DNA from damage *in vitro*

作者: 李力力; 游扬; 周源; 凌贤龙
第三军医大学新桥医院消化内科

Author(s): Li Lili; You Yang; Zhou Yuan; Ling Xianlong
Department of Gastroenterology, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China

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摘要: 目的 探讨抑制PNPase表达对线粒体microRNA（MitomiRs）表达的影响以及对线粒体DNA的保护作用。 方法 以人肝癌细胞SK-Hep1、HepG2和人骨肉瘤细胞U_2OS为研究对象，用带绿色荧光蛋白的（green fluorescent protein, GFP）PNPase shRNA慢病毒转染细胞，采用Western blot 方法检测PNPase表达。分离细胞线粒体、提取线粒体RNA、microRNA芯片检测下调PNPase前后线粒体microRNA（MitomiRs）种类的变化；Q-PCR检测mtDNA损伤频率、ELISA方法检测线粒体8-羟基脱氧鸟苷（8-OHdG）。 结果 激光共聚焦显微镜观察显示，SK-Hep1、HepG2和U2OS细胞转染空载体病毒及PNPase shRNA慢病毒转染后12～24 h可见明显的绿色荧光。当MOI（转染复数）=20时，3种细胞的慢病毒转染效率达80%以上。连续观察2周，转染效率仍维持在70%~80%。Western blot检测结果显示PNPase shRNA组细胞PNPase蛋白表达低于正常对照组及阴性对照组。microRNA芯片显示：PNPase shRNA后目的细胞MitomiRs表达谱发生明显变化，miR-30c-2-3p、miR-494、miR-1273g-3p、miR-4443表达上调，而miR-324-3p、miR-574-5p、miR-371b-5p、miR-6068、miR-21-5p表达下调。Q-PCR检测显示PNPase shRNA组细胞mtDNA损伤频率减少。ELISA结果显示PNPase shRNA组线粒体8-OHdG含量下降。 结论 抑制肿瘤细胞线粒体PNPase表达可导致MitomiRs表达谱发生变化，同时，肿瘤细胞mtDNA损伤减少，提示MitomiRs可能参与了mtDNA复制的调控。

Abstract: Objective To investigate the regulation of mitochondrial microRNAs (MitomiRs) by polynucleotide phosphorylase (PNPase) inhibition, and the possible protection

effects on mitochondrial DNA (mtDNA). Methods Human hepatocellular cell lines SK-Hep1 and Hep G2 and human bone sarcoma cell line U2OS were transfected by PNPase shRNA lentivirus with green fluorescent protein (GFP). PNPase expression was detected by Western blotting. Then the mitochondria were separated, and MitomiRs were extracted. MitomiRs were analyzed by microRNA chips before and after PNPase shRNA treatment. MtDNA damage frequency was assessed by Q-PCR, and mitochondrial 8-hydroxydeoxyguanosine (8-OHdG) was detected by ELISA. Results Visible green fluorescence was found in SK-Hep1, HepG2 and U2OS cells transfected with PNPase shRNA lentivirus in 12 to 24 h later. When the MOI (transfection plural) was 20, the transfection efficiencies of 3 kinds of cells were more than 80%. In the next 2 consecutive weeks, the transfection efficiencies maintained at 70% to 80%. PNPase expression in the cells transfected with PNPase shRNA lentivirus was lower than that in the normal control and negative control cells. MicroRNA chip analysis results showed the obvious changes of MitomiRs profiles in the PNPase shRNA-treated cells. MiR-30c-2-3p, miR-494, miR-1273-g-3p, and miR-4443 were upregulated, while miR-324-3p, miR-574-5p, miR-371-b-5p, miR-6068, and miR-21-5p were downregulated. MtDNA damage frequency was reduced in the cells transfected with PNPase shRNA, and the content of 8-OHdG in the mitochondria of those cells was lowered. Conclusion Inhibition of PNPase expression in tumor cell mitochondria results in changes of MitomiRs expression. At the same time, mtDNA damage is reduced, implying that MitomiRs may participate in the regulation of mtDNA replication.

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