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## 缺氧对PC12细胞中miR-210的表达及对细胞线粒体 得到:

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Title: Effect of hypoxia on miR-210 expression and mitochondrial function in PC12 cells

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关键词: [miR-210](#); [线粒体](#); [缺氧](#); [PC12细胞](#); [基因表达](#)

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摘要: 目的 研究缺氧环境下miR-210在PC12细胞中的表达、调控细胞应对缺氧的相关机制以及缺氧对线粒体功能的影响。 方法 PC12细胞在1% O<sub>2</sub>、94% N<sub>2</sub>、5% CO<sub>2</sub>条件下进行缺氧培养,采用CCK-8检测缺氧暴露后细胞生存率的变化;用qRT-PCR方法检测PC12细胞中miR-210的表达水平;用Western blot检测线粒体相关蛋白ISCU1/2和COX10的表达情况;采用流式细胞仪测定缺氧暴露后PC12细胞线粒体膜电位的变化;利用高效液相色谱法(HPLC)测定细胞中ATP、ADP和AMP的含量。 结果 缺氧暴露降低了PC12细胞存活率,缺氧24 h组和48 h组细胞存活率仅为(63.2±0.1)%和(48.7±0.4)%,显著低于正常对照组( $P<0.05$ )。利用罗丹明123和高效液相色谱法的检测结果表现出缺氧组较对照组线粒体膜电位下降,线粒体内ATP含量显著降低,而ADP、AMP含量显著升高。用qRT-PCR方法检测出在缺氧暴露后PC12细胞中miR-210的表达显著升高。Western blot结果提示ISCU1/2和COX10作为miR-210的靶基因,其表达显著降低( $P<0.05$ ),与缺氧暴露存在着时间-效应关系。 结论 缺氧导致PC12细胞线粒体功能障碍,缺氧诱导的miR-210高表达对其靶基因线粒体相关蛋白ISCU1/2和COX10的调控可能是重要途径之一。

Abstract: Objective To determine the expression of miR-210 in PC12 cells exposed to hypoxia and the regulatory mechanisms of hypoxia on mitochondria function. Methods PC12 cells were cultured in hypoxic condition (94%

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N<sub>2</sub>, 5% CO<sub>2</sub>, and 1% O<sub>2</sub>) at 37 °C. CCK-8 assay was carried out to analyze cell vitality. The expression of miR-210 was tested by qRT-PCR. Western blotting was used to detect the expression of mitochondrial proteins, iron-sulfur cluster assembly proteins (ISCU1/2) and cytochrome oxidase (COX10). Rhodamine 123- indicated mitochondrial membrane potential (MMP) was detected with flow cytometry. The concentrations of ATP, ADP and AMP in PC12 cells were measured by high performance liquid chromatography (HPLC). Results After hypoxia exposure for 24 h and 48 h, the viability of PC12 cells was decreased to (63.2±0.1)% and (48.7±0.4)% of normally cultured cells (P<0.05), respectively. The expression of miR-210 was up-regulated at 12, 24 and 48 h after hypoxia exposure. A sharp and transient increase of MMP at 12 h was followed by significant attenuation at 24 and 48 h. Cellular contents of ADP and AMP were increased while ATP level was declined. Western blot results showed the expression of ISCU1/2 and COX10, target genes of miR-210, was dramatically reduced after hypoxia challenge in an obvious time-effect relationship. Conclusion Hypoxia induces mitochondrial dysfunction in PC12 cells. The impact of hypoxia-upregulated miR-210 expression on its target genes, ISCU1/2 and COX10, may be one of the possible key pathways.

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