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研究论文

缺氧预处理诱导心肌细胞蛋白质组变化的初步研究

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摘要 缺氧预处理(hypoxia preconditioning, HPC)可模拟缺血预处理(ischemic preconditioning, IPC)对缺血/再灌注心肌的保护作用, 涉及细胞内众多分子事件. 本工作旨在采用双向电泳和质谱分析等蛋白质组分析技术, 发现缺氧预处理后心肌细胞蛋白质整体表达上的变化, 初步分析其与缺氧预处理心肌保护作用的关系. 将原代培养的SD乳鼠心肌细胞分为2组(n=6): (1)缺氧预处理组(HPC): 将细胞置缺氧仓内短暂缺氧20 min进行缺氧预处理(HPC), 制备心肌细胞蛋白提取物; (2)对照组(control): 细胞置于培养箱内持续常氧孵育至实验结束, 提取蛋白. 采用双向凝胶电泳和图像扫描, 经蛋白样本分离和考马斯亮蓝染色后比较分析, 选取3个差异表达蛋白点进行胶内酶切、肽质量指纹图谱分析和数据库检索. 双向电泳可分离约529±45个蛋白质, 点匹配率约为78%±7.5%. 18种蛋白质在HPC后发生明显表达差异, 其中12种蛋白质表达降低, 6种表达增高. 经质谱分析鉴定出的3种蛋白质分别为myosin light polypeptide 3, nucleoside diphosphate kinase (NDPK)和calreticulin (CRT). 缺氧预处理引起心肌细胞蛋白质组变化, 初步发现其中myosin light polypeptide 3表达下调、nucleoside diphosphate kinase和calreticulin表达增加, 可能通过调节心肌细胞的收缩性、激活G蛋白、调节细胞内Ca^{2+}浓度而保护心肌. 本工作通过研究缺氧预处理延迟保护过程中心肌内源性蛋白表达水平的变化, 有助于从细胞水平探讨预处理延迟保护机制.

关键词 缺氧预处理 心肌细胞 蛋白质组 双向电泳 质谱

分类号

Pilot Study of Proteomic Changes of Cardiomyocyte Induced by Hypoxia Preconditioning

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Abstract Proteomic differential expression of cardiomyocyte after hypoxia preconditioning was investigated and protein spots with significant difference were identified using 2-D electrophoresis (2-DE) and mass spectrometry to understand the intracellular mechanism of preconditioning. Cultured cardiomyocytes of neonatal Sprague-Dawley rat have been divided into two groups: hypoxia preconditioning (HPC), and control (C) ones. After protein extraction, two-dimensional polyacrylamide gel electrophoresis, Coomassie Brillant Blue R-250 staining, followed by image analysis, was used to screen protein patterns of normal and cardiomyocytes after HPC for quantitative and qualitative differences in protein expression. Three protein spots with significant difference were picked out and subjected to in-gel digest and MALDI-TOF for indetification. More than 529 protein spots were detected on each CBB R-250-stained gel and a match rate 78% ±7.5% was achieved. The results also showed that 18 protein spots displayed quantitative changes in expression after HPC, of which, 12 proteins were decreased in abundance and 6 proteins showed higher expression. After mass spectrometry analysis, three protein spots were identified as myosin light polypeptide 3, nucleoside diphosphate kinase and calreticulin. HPC induced proteomic changes in cardiomyocyte. The decreased expression of myosin light polypeptide 3, and the increased expression of nucleoside diphosphate kinase and calreticulin induced by hypoxia preconditioning may be involved in the cardioprotection via decreasing contractibility of cardiomyocytes, activating G protein, and decreasing calcium concentration. It is helpful to discuss mechanism of preconditioning on cell level that can investigate proteomic differential expression of cardiomyocyte after hypoxia preconditioning.

Key words hypoxia preconditioning cardiomyocyte proteome two-dimensional electrophoresis mass spectrometry

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