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论著

马蹄内翻足胎大鼠胫骨后肌组织的蛋白质组学分析

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摘要 目的 寻找马蹄内翻足畸形相关蛋白。方法 提取全反式维A酸诱导的单纯马蹄内翻足畸形胎大鼠及正常对照胎大鼠胫骨后肌群总蛋白，进行双向电泳，考马斯亮蓝染色；经PDQuest软件分析，选择重复出现的差异点，质谱分析鉴定蛋白。结果 获得了分辨率及重复性均好的双向电泳图谱。畸形胎大鼠与正常对照比较，有蛋白质点缺失、额外增加及明显上调或下调。质谱鉴定发现畸形胎大鼠肌组织慢骨骼肌肌钙蛋白T(sTnT)缺失，X连锁凋亡蛋白抑制因子(XIAP)下调，羧酸酯酶AY034877额外表达。结论 畸形胎大鼠胫骨后肌组织与正常胎大鼠比较存在蛋白质组差异，畸形胎大鼠sTnT，XIAP及羧酸酯酶AY034877表达改变，可能与马蹄内翻足畸形相关。

关键词 马蹄内翻足 蛋白质组 模型,动物
分类号 Q7,R363.25

Proteomic analysis of tibia-fibulae musculature in rat fetus with talipes equinovarus

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Abstract
AIM To identify the particular proteins in the pathogenesis of talipes equinovarns(clubfoot)-like deformities. **METHODS** Pregnant rats were given all trans-retinoic acid 135 mg·kg^{-1} at d 10 of gestation by a single intragastric infusion. Tibia-fibulae musculatures were removed from rat fetus on gestation d 21. Proteins were extracted with lysis solution (5 mol·L^{-1} urea, 2 mol·L^{-1} thiourea, 20 g·L^{-1} CHAPS, 20 g·L^{-1} SB3－10, 40 mmol·L^{-1} Tris, 0.2% Bio-Lyte 3－10), 20 mL·g^{-1} tissue. For two-dimensional electrophoresis, immobilized pH gradient gel (IPG, pH 3－10 and pH 5－8) iso-electric focusing was set as the first dimension, while 12% SDS polyacrylamide gel electrophoresis as the second dimension. Loaded sample sizes were 0.5 mg (350 μL) for each pH 3－10 IPG strip and 1.0 mg for pH 5－8 IPG strip. Following electrophoresis, gels were stained with Coomassie brilliant blue. The images were analyzed with a PDQuest 7.0 software package. Spots with significant differences were subjected to mass spectrometry analysis. The peptide mass fingerprints were identified with protein databases (Swiss-Prot and NCBInr) using different softwares including PeptIdent, Mascot and MS-Fit. **RESULTS** Compared with those of the normal controls, samples of model rat fetus showed gaining and losing some spots with increase or decrease in intensity for a number of protein spots. A total of eight protein spots showed significant differences, and peptide mass fingerprints were acquired for seven among them. Three protein spots were identified by more than two softwares to give similar results, *i.e.*, loss of slow skeletal muscle troponin T(sTnT), X-linked inhibitor of apoptosis(XIAP) and extra expression of carboxylesterase AY034877. **CONCLUSION** Proteomic difference between model and normal rat fetus can be well presented with 2-D electrophoresis. Expression change of sTnT, XIAP and carboxylesterase AY034877 may be related to the pathogenetic mechanisms of talipes equinovarus.

Key words talipes equinovarus proteome model animal

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