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特别策划

毛细管整体柱在线二维分离系统应用于人体软骨的蛋白质组分析

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摘要 将已建立的 7 cm 柱长的磷酸基团强阳离子交换富集整体柱与85 cm柱长的C12烷基反相整体柱结合的在线二维分离平台应用于软骨提取蛋白的蛋白质组分析。对20 μg软骨提取蛋白的酶解产物进行14个盐梯度的分级, 然后对14个馏分进行反相色谱梯度分离及串联质谱鉴定, 成功地鉴定得到了7434个独立肽段对应的1901个非冗余蛋白质。对所鉴定到的蛋白质进行定位分类, 结果表明鉴定到的大部分蛋白质是来自于软骨细胞内部的低丰度蛋白质, 这对于许多关节类疾病的研究有重要意义。

关键词 整体柱 在线二维分离 蛋白质组学 软骨组织

Application of online two-dimensional separation system using monolithic columns for proteome analysis of human cartilage

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Abstract

In Shotgun proteome analysis, where nano-flow is adopted to increase the sensitivity as well as extremely complicated samples such as proteolytic digest are inevitably confronted, monolithic capillary columns are widely used to improve the liquid chromatography separation performance. It is known that cartilage contains extensive amounts of extracellular matrix (ECM), in which collagens and aggrecans being the most abundant macromolecules. It is obvious that the high content of ECM components causes a challenge in the comprehensive proteome analysis of cartilage. In this study, a 7 cm×150 μm i.d. phosphate strong cation exchange (SCX) monolithic capillary column was coupled with an 85 cm×75 μm i.d. C12 reversed-phase monolithic capillary column for online two-dimensional separation of 20 μg tryptic digest of proteins extracted from human cartilage. After 14 salt steps fractionation and following gradient separation coupled with tandem mass spectrometry detection, finally 7434 unique peptides, corresponding to 1901 distinct proteins were positively identified. Then, the identified proteins were analyzed by Gene Ontology (GO), and it was found that most of the identified proteins were come from articular chondrocytes with low abundance, which is important for the researches of articular diseases.

Key words monolithic column online two-dimensional separation proteomics cartilage tissue

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