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论文

无标记定量法研究冠心病不稳定性心绞痛血瘀证的差异蛋白质组

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摘要:

应用高解析离子淌度质谱(HDMS)与纳升级超高效液相色谱(UPLC)联用, 寻找冠心病不稳定性心绞痛血瘀证血浆差异蛋白, 探索冠心病不稳定性心绞痛血瘀证的蛋白质组学特点. 采用美国Agilent公司多克隆抗体亲和柱去除冠心病不稳定性心绞痛血瘀证患者和健康人血浆中6种丰度最高的蛋白后, 进行无标记定量蛋白质组(Label free proteome)分析. 结果表明, 本方法的离子强度变异系数小于5%, 保留时间变异系数小于3%, 具有良好的重现性. 动态范围约104数量级. 总蛋白数3843种, 差异倍数大于1.5倍的差异蛋白数25种, 上调蛋白数13种(包括心绞痛血瘀证患者独有蛋白3种), 下调蛋白数12种. ACTA1, ITIH3和 LBP仅在冠心病不稳定性心绞痛血瘀证患者血浆中表达, Haptoglobin, SAA, CP, C6, MYH11, APOH和ANXA6在冠心病不稳定性心绞痛血瘀证患者中高表达, 而HBB, HBA, HBE, HBD, HBG, HRG, IGHG, GSN和TF在冠心病不稳定性心绞痛血瘀证患者中低表达. 表达上调的差异蛋白根据功能可分为: (1) 急性时相反应蛋白; (2) 补体蛋白; (3) 细胞骨架蛋白; (4) 凝血相关蛋白. 表达下调的差异蛋白根据功能可分为: (1) 载脂蛋白; (2) 运输蛋白; (3) 抗凝血相关蛋白; (4) 免疫球蛋白; (5) 细胞骨架调控蛋白. 以上结果提示, 冠心病不稳定性心绞痛血瘀证可能属于一种炎症反应; 冠心病不稳定性心绞痛血瘀证患者可能同时存在心肌损伤、凝血因子异常、脂代谢紊乱与氧运输障碍; 这些方面相互影响, 互为因果. 这些差异蛋白可为研究或发现抗心绞痛药物作用的新靶标提供线索. 本研究结果表明, 非标记定量蛋白质组学(Label free proteomics)是疾病及证候生物标志物研究的一种有效手段.

关键词: 质谱; 心绞痛; 血瘀证; 无标记; 蛋白质组学; 生物标志物

Label Free Proteome Analysis of Plasma from Unstable Angina with Blood Stasis Syndrome Patients

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Abstract:

Label free LC-MS/MS method was used in seeking of unstable angina blood stasis syndrome(UABSS) differentially expressed plasma proteins. A polyclonal antibody affinity column, nanoAcquity UPLC and Synapt HDMS were used on plasma of two classes of samples(12 UABSS patients and 12 health volunteers). The results show that the method has a good reproducibility. The ionic strength coefficient of variability was less than 5%, retention time coefficient of variability was less than 3%. 3843 proteins were detected, among which 24 kinds of proteins differentially expressed large than 1.5 fold, include 11 down-regulated and 13 up-regulated(include 3 kinds of proteins only found in UABSS patients). The dynamic range was about 10^4. ACTA1, ITIH3 and LBP were only found in the plasma of UABSS patients, Haptoglobin, SAA, CP, C6, MYH11, APOH and ANXA6 were significantly highly expressed in the plasma of UABSS patients, while HBB, HBA, HBE, HBD, HBG, HRG, IGHG, GSN and TF were lowly expressed in the plasma of UABSS patients. These identified increased expressed proteins could be divided into four categories according to their functions: (1) acute phase reactive protein; (2) complement protein; (3) cytoskeletal protein; (4) blood coagulation protein. The decreased expressed proteins could be divided into five categories: (1) apolipoprotein; (2) transport protein; (3) anticoagulated blood protein; (4) immunoglobulin; (5) cytoskeletal modulin protein. In conclusion, UABSS may correlated with inflammatory reaction, lipid metabolic disorder, myocardial damage, blood coagulation factor abnormal, oxygen transport obstacle, and these differentially expressed proteins could provide clues for the study and discovery of new protein targets for antianginal drugs. The label free proteomics is an efficient method for the discovery of differentially expressed proteins of complicated sample.

Keywords: Mass spectrometry; Unstable angina; Blood stasis syndrome; Label free; Proteomics;

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参考文献:

[1]Silva J. C., Denny R., Dorschel C. A. et al.. Anal. Chem.[J], 2005, 77: 2187—2200
[2]Radulovic D., Jelveh S., Ryu S., et al.. Mol. Cell Proteomics[J], 2004, 3: 984—997
[3]Wiener M. C., Sachs J. R., Deyanova E. G., et al.. Anal. Chem.[J], 2004, 76: 6085—6096
[4]Lu P., Vogel C., Wang R., et al.. Nat. Biotechnol.[J], 2007, 25: 117—124
[5]Mallick P., Schirle M., Chen S. S., et al.. Nat. Biotechnol.[J], 2007, 25: 125—131
[6]Radulovic D., Jelveh S., Ryu S., et al.. Mol. Cell. Proteomics[J], 2004, 3: 984—997
[7]Wiener M. C., Sachs J. R., Deyanova E. G., et al.. Anal. Chem.[J], 2004, 76: 6085—6096
[8]ZHAO Hui-Hui(赵慧辉), WANG Shuo-Ren(王硕仁), WANG Wei(王伟). Journal of Beijing University of Traditional Chinese Medicine(北京中医药大学学报)[J], 2008, 31(1): 39—41
[9]Mateos-Cáceres P. J., García-Méndez A., López Farré A., et al.. J. Am. Coll. Cardiol[J], 2004, 44(8): 1578—1583
[10]ZHAO Hui-Hui(赵慧辉), WANG Wei(王伟), WANG Shuo-Ren(王硕仁), et al.. Chinese Archives of Traditional Chinese Medicine(中华中医药学刊)[J], 2008, 26(4): 724—726
[11]ZHAO Hui-Hui(赵慧辉), WANG Wei(王伟). Acta Chimica Sinica(化学学报)[J], 2009, 67(2): 167—173
[12]ZHAO Hui-Hui(赵慧辉), WANG Wei(王伟). Spectroscopy and Spectral Analysis(光谱学与光谱分析)[J], 2009, 29(6): 1647—1650
[13]ZHAO Hui-Hui(赵慧辉), WANG Wei(王伟), GUO Shu-Zhen(郭淑贞). Chinese Journal of Arteriosclerosis (中国动脉硬化杂志)[J], 2008, 16(7): 545—548
[14]SUN Ping(孙萍), LUO Guo-An(罗国安), QU Jun(曲峻). Chem. J. Chinese Universities(高等学校化学学报)[J], 2003, 24(12): 2169—2172
[15]JIANG Ning(蒋宁), ZHOU Wen-Xia(周文霞), ZHANG Yong-Xiang(张永祥), et al.. Chem. J. Chinese Universities(高等学校化学学报)[J], 2006, 27(8): 1462—1466
[16]WANG Ya-Dong(王亚冬), WU Jin-Dao(吴金道), JIANG Zhong-Li(江中立), et al.. Chem. J. Chinese Universities(高等学校化学学报)[J], 2007, 28(11): 2065—2072
[17]DONG Lei(董雷), JIANG Ning(蒋宁), ZHOU Wen-Xia(周文霞), et al.. Chem. J. Chinese Universities(高等学校化学学报)[J], 2007, 28(2): 274—277
[18]Martínez-Amat A., Boulaiz H., Prados J., et al.. Br. J. Sports. Med.[J], 2005, 39: 830—834
[19]Schumann R. R., Zweiger J., et al.. Clin. Chem. Lab. Med.[J], 1999, 37: 271—274
[20]Lamping N. R., Dettmer N. W. J., Schroeder D., et al.. J. Clin. Invest.[J], 1998, 101: 2065—2071
[21]Dobryszycka W.. Eur. J. Clin. Chem. Clin. Biochem.[J], 1997, 35(9): 647— 654
[22]Katayama T., Nakashima H., Yonekura T.. J. Cardiol.[J], 2003, 42: 49—56
[23]Ehrenw ld E., Chisolm G. M., Fox P. L.. J. Clin. Invest.[J], 1994, 93: 1493
[24]Yasojima K., Schwab C., McGeer E. G., et al.. Arterioscler Thromb. Vasc. Boil.[J], 2001, 21: 1214—1219
[25]Lin K. Y., Pan J. P., Yang D. L., et al.. Life Sci.[J], 2001, 69(6): 707—719
[26]Schousboel, Rasmussen M. S.. Int. J. Bionc.[J], 1988, 20: 787—792
[27]ZHANG Yi(张毅), CHEN Rui-Wen(陈蕊雯), SUN Shu-Han(孙树汉). Science in China(Life Sciences)(中国科学, C辑)[J], 2002, 32(2): 172—176
[28]Dvorin E., Corder L. N., Benson M. D., et al.. J. Biological Chemistry[J], 1986, 261(33): 15714—15718
[29]Recalde D., Ostos A. M., Badell E. A. L., et al.. Thromb. Vasc. Biol.[J]. 2004, 24: 756—761
[30]Vowinkel T., Mori M., Krieglstein C. F., et al.. J. Clin. Invest.[J], 2004, 114: 260—269
[31]Gupta R., Rastogi S., Nagar R., et al.. J. Assoc. Physicians India[J], 2000, 48(5) : 489
[32]ZHAO Hui-Hui(赵慧辉), HOU Na(侯娜), WANG Wei(王伟), et al.. Chinese Journal of Integrated Traditional and Western Medicine(中国中西医结合杂志)[J], 2009, 29(6): 489—492
[33]Ezeh B., Haiman M., Alber F. H., et al.. J. Lipid Res.[J], 2003, 44: 1523—1529
[34]Johnson B. D., Kip D. E., Marroquin O. C., et al.. Circulation[J], 2004, 109: 726—732
[35]Manttari M., Manninen V., Huttunen J. K., et al.. Eur. Heart J.[J], 1994, 15(12) : 1599—1603

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