

应用蛋白质组学技术筛选胃癌耐药相关蛋白质*

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摘要 胃癌多药耐药性是临床胃癌化疗失败最主要的原因之一, 但其分子机制仍然不太清楚. 为了寻找新的胃癌耐药相关的蛋白质, 揭示胃癌多药耐药的分子机制, 以胃癌细胞 SGC7901 和长春新碱诱导的耐药胃癌细胞 SGC7901/VCR 为研究对象, 应用二维凝胶电泳 (two-dimensional electrophoresis, 2-DE) 技术分离两种细胞的总蛋白质, 图像分析识别差异表达的蛋白质点, 基质辅助激光解吸电离飞行时间质谱 (matrix-assisted laser desorption/ionization time of flight mass spectrometry, MALDI-TOF-MS) 及电喷雾电离串联质谱 (electrospray ionization tandem mass spectrometry, ESI-Q-TOF) 对差异表达的蛋白质点进行鉴定, 蛋白质印迹和实时 RT-PCR 验证部分差异蛋白质在两株细胞中的表达水平, 反义核酸转染技术分析 HSP27 (heat shock protein 27, HSP27) 高表达与 SGC7901/VCR 耐药的相关性. 得到了分辨率较高、重复性较好的两株细胞系的二维凝胶电泳图谱, 质谱分析共鉴定了 24 个差异蛋白质点, 蛋白质印迹和实时 RT-PCR 验证了部分差异蛋白的表达水平, 反义寡核苷酸抑制 HSP27 表达能增加 SGC7901/VCR 对长春新碱的敏感性. 研究结果不仅提示这些差异蛋白质如 HSP27, Sorcin 等可能与胃癌的多药耐药相关, 而且为揭示胃癌细胞的多药耐药性产生机制提供了线索.

关键词 长春新碱, 胃癌, 多药耐药, 蛋白质组, 二维凝胶电泳, 质谱, 免疫印迹, 定量 PCR, 反义核苷酸

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肿瘤细胞的多药耐药性 (multidrug resistance, MDR) 是指肿瘤细胞接触一种化疗药物并对其产生耐药后, 同时对其他化学结构和作用机制不同的化疗药物亦产生耐药^[1]. 胃癌是常见的恶性肿瘤之一, 占各种肿瘤死因的第二位. 胃癌细胞的多药耐药性是胃癌化疗失败最主要的原因之一, 深入研究胃癌的多药耐药性无疑有着重要的理论和临床意义.

以往的研究虽然揭示了一些胃癌多药耐药产生的机理^[2~4], 但是这些研究多数是从某个或某几个基因出发, 胃癌多药耐药的分子机理远未阐明. 而且这些研究多集中在 DNA 和 RNA 水平, 由于 DNA 和 RNA 水平的变化并不能完全反映蛋白质水平的变化^[5]. 因此, 有必要在蛋白质整体水平对胃癌多药耐药产生的机理进行研究. 蛋白质组学的出现及其研究技术的发展和完善为在蛋白质整体水平上研究多药耐药产生的机制成为可能. 为此, 本文以胃癌细胞 SGC7901 和长春新碱诱导的耐药胃癌细胞 SGC7901/VCR 为对象^[1], 应用二维凝胶电泳

和质谱技术筛选和鉴定胃癌耐药相关的蛋白质, 以揭示胃癌的多药耐药性产生机制, 为胃癌多药耐药性的逆转提供新靶点.

1 材料与方法

1.1 材料

1.1.1 细胞株 胃癌细胞 SGC7901 和长春新碱诱导的多药耐药胃癌细胞 SGC7901/VCR 系第四军医大学消化科樊代明教授惠赠. SGC7901/VCR 生长于含 1.0 mg/L 长春新碱的培养液中维持其耐药表型.

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1.1.2 试剂. 丙烯酰胺、甲叉 - 双丙烯酰胺、三羟甲基氨基甲烷、甘氨酸、十二烷基磺酸钠(SDS)、尿素、CHAPS、IPG 缓冲液 pH 3~10、24 cm 固相化 pH 梯度干胶条购自 Amersham Pharmacia 公司; 二硫苏糖醇(DTT)、碘乙酰胺、TPCK 处理的胰蛋白酶、铁氰化钾、三氟乙酸(TFA)、碳酸氢铵、硫代硫酸钠、乙腈、CCA 购自 Sigma 公司; 鼠抗人 HSP27、HSC70、VDAC2、Sorcina 单克隆抗体, 辣根过氧化物酶标记羊抗鼠二抗购自 Santa Cruz 公司; SYBR Green I 混合染料购自 Takara 公司.

1.2 方法

1.2.1 二维凝胶电泳. 收集对数生长期的 SGC7901 和 SGC7901/VCR 细胞, 加入细胞裂解液 (8 mol/L 尿素, 4% CHAPS, 40 mmol/L Tris-HCl, 65 mmol/L DTT) 室温裂解 2 h, 12 000 r/min 4℃ 离心 45 min, 取上清即为细胞总蛋白质. 蛋白质定量试剂盒 2D Quantification kit (Amersham Biosciences) 测定蛋白质浓度, -80℃ 冻存备用. 0.25 mg 细胞总蛋白质与水化液 (8 mol/L 尿素, 4% CHAPS, 40 mmol/L Tris-HCl, 18 mmol/L DTT, 0.5% IPG pH 3~10 缓冲液, 痕量溴酚蓝) 混合至总体积为 450 μ l. 蛋白质样本于 IPGphor 等电聚焦仪 (Amersham Biosciences) 按如下条件: 30 V 水化 14 h 后经 500 V 1 h、1 000 V 1 h、8 000 V 10 h 进行等电聚焦. 等电聚焦结束后分别于 10 ml 平衡 A 液 (50 mmol/L Tris-HCl、pH 8.8, 6 mmol/L 尿素, 30% 甘油, 0.2% DTT, 痕量溴酚蓝) 和 10 ml 平衡 B 液 (50 mmol/L Tris-HCl、pH 8.8, 6 mmol/L 尿素, 30% 甘油, 3% 碘乙酰胺, 痕量溴酚蓝) 各平衡 15 min. 平衡后的 IPG 胶条转移至 12% SDS- 聚丙烯酰胺凝胶电泳 (SDS-PAGE) 胶上端, 在 Ettan DALT II 垂直电泳槽 (Amersham Biosciences) 上进行第二向垂直电泳. 电泳结束后, 参照 “Blue silver” 染色方法^[6]对 2-D 进行考马斯亮蓝染色. 二维凝胶电泳分析重复 3 次.

1.2.2 图像分析. 应用 ImageScanner 扫描仪 (Amersham Biosciences) 扫描染色的 2-D 胶. PDQuest 2-DE 软件比较分析 SGC7901 与 SGC7901/VCR 细胞二维电泳图谱的差异, 选取表达水平相差 2 倍以上的蛋白质点进行质谱分析.

1.2.3 基质辅助激光解吸电离飞行时间质谱 (MALDI-TOF-MS) 分析. 从胶中切取差异蛋白质点于 1.5 ml EP 管中, 50% 乙腈和 50 mmol/L 碳酸氢铵脱色 30 min, 乙腈脱水冷冻抽干. 加入 10 μ l

TPCK 处理的胰蛋白酶 (0.1 mg/L) 冰上吸胀 40 min, 37℃ 酶解 12 h, 30 μ l 萃取液 (100% 乙腈: 5% 三氟乙酸 1:1) 萃取 60 min, 重复萃取 1 次. 将萃取液收集于 0.5 ml EP 管、冷冻浓缩至 10 μ l, 取 0.5 μ l 样品与 1 μ l CCA 基质液混合, 点样于不锈钢板, 在 MALDI-TOF-MS 质谱仪 (Applied Biosystem Voyager-DE STR Biospectrometry™ Workstation System 4307, ABI 公司产品) 上进行分析. MALDI-TOF-MS 分析采用反射模式, 正离子谱测定, 离子源加速电压 20 000 V, 反射电压比 1.12, N₂ 激光波长 337 nm, 脉冲宽度 3 ns, 离子延迟提取 100 ns, 真空度 4×10^{-7} Torr, 质谱信号单次扫描累加 50 次, 使用胰蛋白酶自降解峰 m/z 842.50 和 m/z 2211.10 作为内校正, 获得肽质量指纹图谱 (PMF). Mascot 软件检索 SwissProt 数据库鉴定蛋白质.

1.2.4 电喷雾电离串联质谱 (ESI-Q-TOF) 分析. 为保证蛋白质鉴定结果的准确性, 所有差异蛋白质点均进行 ESI-Q-TOF (Q-TOF micro™, Micromass 公司产品) 分析. 所有测定均在正离子方式下进行. 雾化气体为氮气, 碰撞气体为氦气. 源温 80℃, 锥孔电压 50 V. TOF 加速电压为 0.2 kV, MCP 检测器电压为 2.7 kV, 当进行 LC-ESI-MS/MS 自动分析时, 毛细管电压为 3 000 V. 测定结果仪器以 peaklist 文件形式给出, 通过 Mascot 软件检索 SwissProt 数据库鉴定蛋白质点, 并得到部分肽片段测序.

1.2.5 蛋白质印迹验证差异蛋白质的表达水平. 收集对数生长期的 SGC7901 和 SGC7901/VCR 细胞, 加入细胞裂解液 (50 mmol/L Tris-HCl、pH 7.5, 150 mmol/L NaCl, 1 mmol/L EDTA, 0.5 % Triton X-100, 0.5% NP40, 1 倍 protease inhibitor cocktail) 冰上裂解 30 min, 12 000 r/min 4℃ 离心 15 min 后, 取上清即为细胞总蛋白质, Bradford 方法测定蛋白质浓度. 15 μ g 总蛋白质进行 10% SDS-PAGE 分离, 蛋白质电转移至 PVDF 上, 印迹膜用 5% 脱脂牛奶室温封闭 2 h, 1:1 000 稀释的鼠抗人 HSP27, HSC70, VDAC2 或 Sorcina 一抗室温温育 1 h, PBS 洗涤 3 次, 每次 10 min. 1:3 000 稀释的羊抗鼠二抗室温温育 1 h, PBS 洗涤 3 次, ECL 试剂发光及显影. 实验重复 3 次.

1.2.6 Real-time RT-PCR 验证差异蛋白质的表达水平. 收集对数生长期的 SGC7901 和 SGC7901/VCR 细胞, 用 TRIzol 试剂盒 (GIBCO 公司产品) 提取总 RNA. 2 μ g 总 RNA 用逆转录试剂盒 (Promega 公司

产品)合成 cDNA. Real-time RT-PCR 反应在 LightCycler system 荧光 PCR 仪(Roche 公司产品)上进行, 反应体系如下: 2 μ l cDNA, 2 μ l SYBR Green 1 混合染料, 0.5 μ l 10 μ mol/L 上游引物, 0.5 μ l 10 μ mol/L 下游引物, 1 μ l 25 mmol/L MgCl₂

和 14 μ l H₂O. 实验结果自动以 CT 值给出, 相对表达量计算按照 Livak 等^[7]的 2^{- $\Delta\Delta$ CT}方法进行计算. 实验重复 3 次. 所用引物及 real-time RT-PCR 反应条件见表 1 和 2.

Table 1 Primers used in real-time RT-PCR analysis

Gene ^{a)}	Primer sequence	Gene	Primer sequence
Sorcin	S 5' tgcgtgaggatacaaac 3'	PKM2	S 5' aatcacgctggataacgc 3'
	AS 5' accgaaggcaagaggac 3'		AS 5' catcaaacctcgaaccc 3'
PPI	S 5' tggcaagaccagcaagaa 3'	PDX3	S 5' tattcagcaccagtctc 3'
	AS 5' gggagggaacaaggaaaa 3'		AS 5' cccaaccaccattctt 3'
IDH3A	S 5' caaggacctggaggaaag 3'	VDAC2	S 5' ctgggaacagaaatcgc 3'
	AS 5' aaaggcaaacacagcaat 3'		AS 5' agccctcataaccaagac 3'
HSP27	S 5' aaggatggcgtgtggaga 3'	PP	S 5' tattgctatgattgacg 3'
	AS 5' gaggaggaaacttgggtggg 3'		AS 5' cacagggtggtggtaaa 3'
GAPDH	S 5' aatcccatcaccattctcc 3'	HSC70	S 5' tttagcgacactgaacgg 3'
	AS 5' catcacgccacagtctcc 3'		AS 5' atcttggtagcctgacg 3'

a)PPI: Peptidylprolyl isomerase A; IDH3A: Isocitrate dehydrogenase[NAD] alpha precursor; PKM2: Pyruvate kinase, isozymes M2; PDX3: Peroxiredoxin 3; PP: Inorganic pyrophosphatase; VDAC2: Voltage-dependent anion-selective channel protein 2; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; HSP27: Heat shock protein 27; HSC70: Heat shock cognate 71 ku protein.

Table 2 Experimental protocol used for real-time RT-PCR analysis

Segment number	Temperature target/℃	Hold time/s	Slope/(℃·s ⁻¹)	Application mode
Program: Denaturation			Cycles: 1	
1	95	10	20	None
Program: PCR			Cycles: 40-50	
1	95	4	20	None
2	x ^{a)}	20	20	None
3	72	15	20	Single
Program: Melting			Cycles: 1	
1	95	0	20	None
2	60	10	20	None
3	95	0	0.2	Constant
Program: Cooling			Cycles: 1	
1	40	10	20	None

^{a)} Sorcin 51℃; PPI 52℃; PP 49℃; IDH3A 52℃; VDAC2 49℃; PDX3 50℃; PKM2 53℃; GAPDH 55℃; HSP27 56℃; HSC70 51℃.

1.2.7 HSP27 反义寡核核酸转染 SGC7901/VCR 细胞. 针对 HSP27 基因翻译起始区的全硫代修饰的 HSP27反义寡核苷酸链由 Takara 公司合成, 序列为 5' GGGACGCGGCGCTCGGTCAT 3', 作为对照的错义寡核苷酸链序列为 5' CAGCGCTGACA-ACAGTTTCAT 3'. 寡核苷酸转染细胞按脂质体转

染试剂盒 Oligofectamine (Invitrogen 公司产品) 的说明进行. 简要步骤如下: 以 10⁵/ml 密度接种 SGC7901/VCR 细胞于 6 孔和 96 孔培养板 (分别用于表达分析和 MTT 检测), 当细胞达到 50%融合时进行转染, 寡核苷酸的终浓度为 200 nmol/L. 转染 24 h 后, 蛋白质印迹及 real-time RT-PCR 检测

HSP27的表达情况. 转染 4 h 后加入含不同浓度长春新碱的培养基继续培养 24 h, MTT 方法检测细胞生存率, 以分析 HSP27 高表达与胃癌细胞耐药的关系. 实验重复 3 次.

2 结 果

2.1 SGC7901 与 SGC7901/VCR 细胞蛋白质 2-DE 图谱的建立

建立了分辨率较高、重复性较好的 SGC7901

与 SGC7901/VCR 细胞蛋白质的二维电泳图谱. 应用 PDQuest 2-DE 软件分析 SGC7901 与 SGC7901/VCR 细胞二维电泳图谱, 分别可检测到大约 1 100 个蛋白质点, 每株细胞 3 张 2-D 胶的平均胶比较 (组间比较) 共有 24 个差异表达的蛋白质点. SGC7901 与 SGC7901/VCR 细胞典型的二维电泳图谱见图 1. 图 2 为局部放大的部分差异表达的蛋白质点.

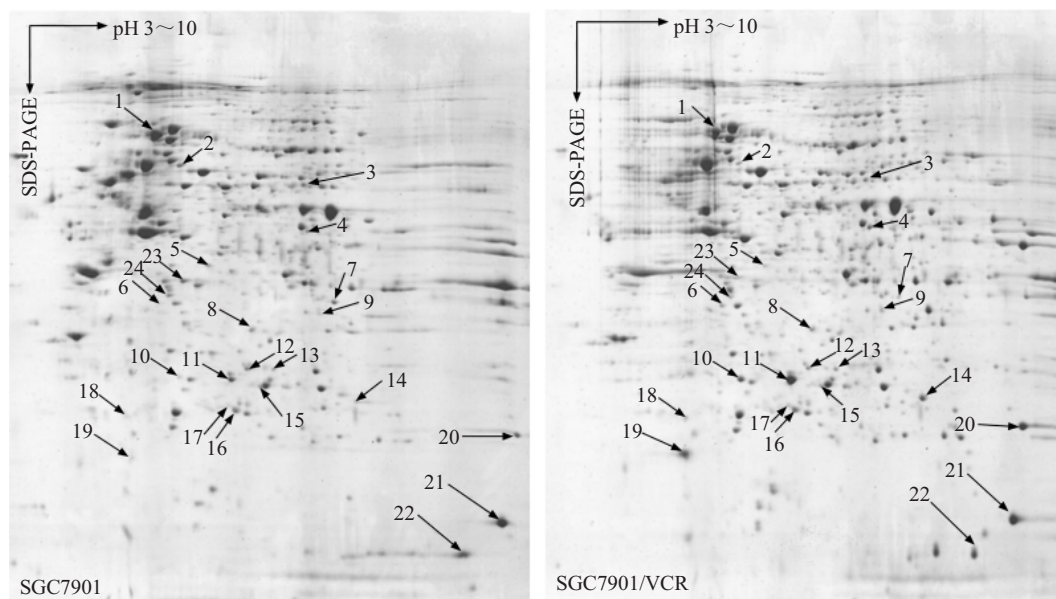


Fig. 1 2-DE maps of SGC7901 and vincristine-resistant SGC7901/VCR

24 differential expression proteins spots are labeled with arrows, and the data on the differential expression proteins are presented in the Table 3.

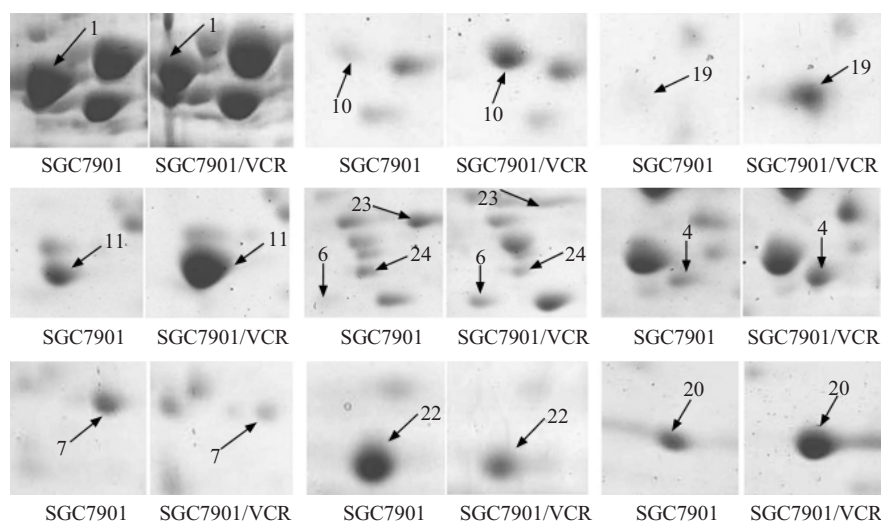


Fig. 2 Close-up image of partial differential expression protein spots between SGC7901/VCR and SGC7901

2.2 差异蛋白质点的质谱鉴定

从胶中切取 24 个差异蛋白质点，胶内酶解后

分别进行 MALDI-TOF-MS 和 ESI-Q-TOF 分析，获取肽质量指纹图谱及肽序列标签，数据库搜寻鉴定

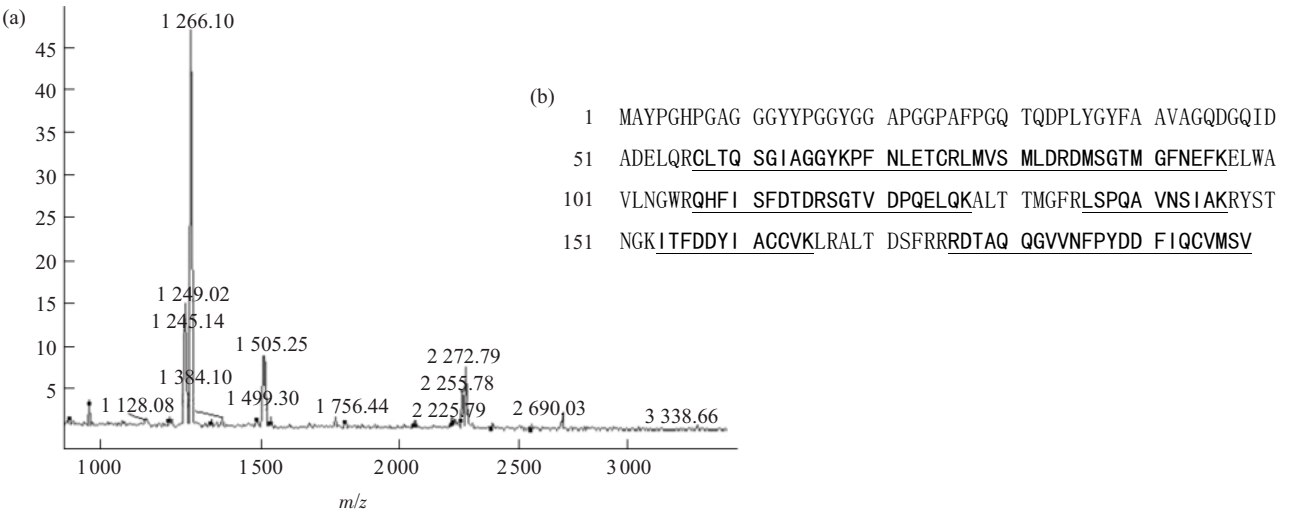


Fig. 3 MALDI-TOF-MS analysis of differential protein spot 19

(a) The MALDI-TOF-MS mass spectrum of spot 19 was shown, which was identified as the sorcin according to the matched peaks. (b) Protein sequence of sorcin was shown, and matched peptides were indicated in bold font and underlined.

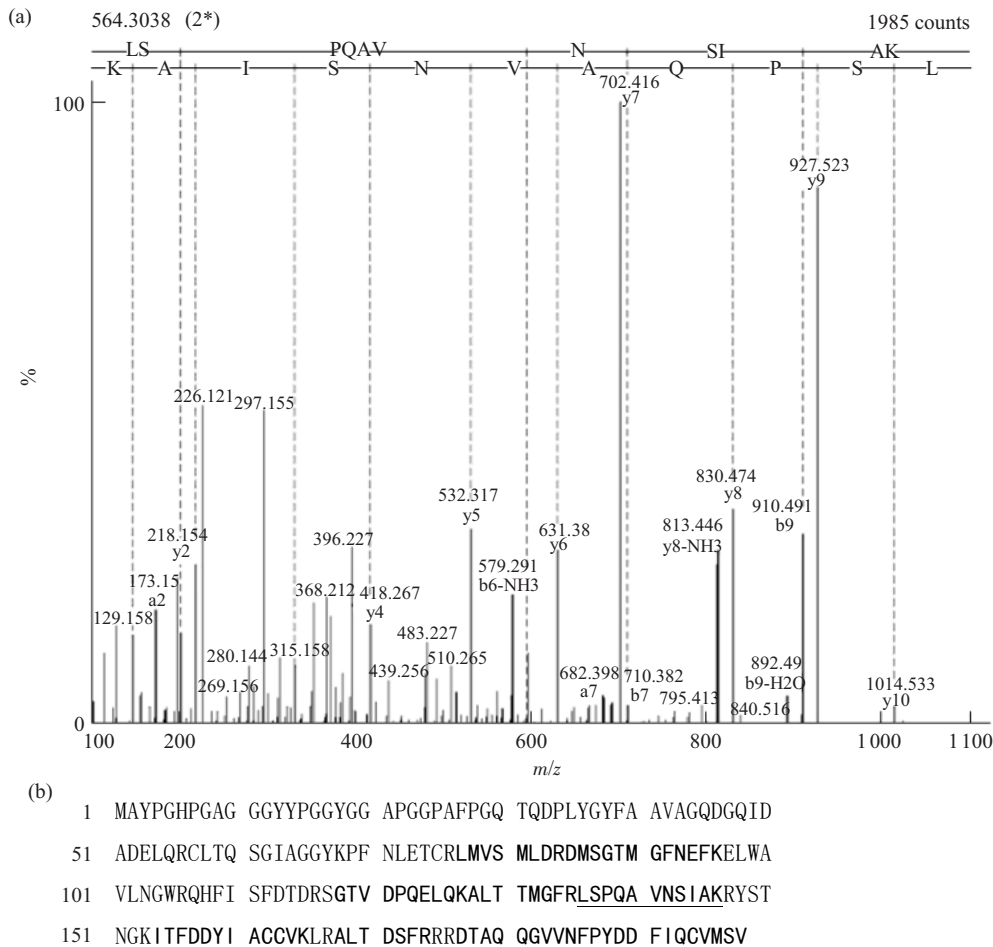


Fig. 4 ESI-Q-TOF analysis of differential protein spot 19

(a) The amino acid sequence of a doubly charged peptide with m/z 564.30 was identified as LSPQAVNSIAK from mass differences in the y-fragment ions series. (b) Protein sequence of sorcin was shown, and LSPQAVNSIAK fragmentation matched with residues 136~146 of sorcin was underlined.

蛋白质. 19 号蛋白质点鉴定结果见图 3 和 4. 该差异蛋白的肽质量指纹图谱检索 SwissProt 数据库显示为 Sorcin 蛋白, ESI-Q-TOF 分析亦显示, 来自该蛋白质的肽片段 (m/z 564.30) 序列 LSPQAVNSIAK 为 Sorcin 蛋白氨基酸序列的一部分. 所有 24 个差异蛋白质点均得到鉴定, 其详细信息见表 3. 与 SGC7901 比较, 11 个蛋白质即 NADP-dependent isocitrate dehydrogenase, inorganic pyrophosphatase, VDAC2 protein, heat shock protein 27 (HSP27), sorcin, mixture of HSP27 和 HADH2 protein, HADH2 protein, phosphoglycerate

mutase 1, peroxiredoxin 3, glyoxalase I 和 peroxiredoxin 1 在 SGC7901/VCR 表达上调. 而 13 个蛋白质即 heat shock cognate 71 ku protein (HSC70), T-complex protein 1 epsilon subunit, mixture of pyruvate kinase isozymes M2 和 fascin 1, isocitrate dehydrogenase [NAD] alpha precursor, uracil DNA glycosylase, ECH1 protein, endoplasmic reticulum protein 29 precursor, platelet-activating factor acetylhydrolase isoform Ib beta subunit 30 ku, BCL2-associated athanogene 2, cofilin non-muscle isoform, peptidylprolyl isomerase

Table 3 MALDI-TOF-MS and ESI-Q-TOF-MS identified proteins in SGC7901/VCR vs. SGC7901 comparison

Spot	Accession number ^{a)}	Protein name	Molecular mass/u	pI	Expression in SGC7901/VCR	Function
1	P11142	Heat shock cognate 71 ku protein ^{b)}	71 082	5.37	↓ ^{c)}	Chaperone
2	P48643	T-complex protein 1, epsilon subunit	60 100	5.45	↓	Chaperone
3	P14618/Q16658	Pyruvate kinase, isozymes M2/Fascin 1	58 447/55 151	7.95/6.84	↓	Metabolism/Structure
4	O75874	NADP-dependent isocitrate dehydrogenase	46 944	6.34	↑ ^{c)}	Metabolism
5	P50213	Isocitrate dehydrogenase [NAD] alpha precursor	40 022	6.47	↓	Metabolism
6	Q15181	Inorganic pyrophosphatase	35 962	5.95	↑	Metabolism
7	P13051	Uracil DNA glycosylase	35 698	8.22	↓	Repair of DNA damage
8	Q8WVX0	ECH1 protein	36 078	8.47	↓	Antioxidant
9	P45880	VDAC2 protein ^{b)}	30 849	6.81	↑	Signal transduction
10	Q96EI7	Heat shock protein 27 ^{b)}	22 427	7.83	↑	Chaperone
11	Q96EI7/Q99714	Heat shock protein 27/ HADH2 protein ^{b)}	22 427/26 196	7.83/6.73	↑	Chaperone/Metabolism
12	P30040	Endoplasmic reticulum protein 29 precursor	29 032	6.77	↓	Metabolism
13	P18669	Phosphoglycerate mutase 1	28 900	6.67	↑	Metabolism
14	Q99714	HADH2 protein ^{b)}	261 966.73	6.73	↑	Metabolism
15	Q29459	Platelet-activating factor acetylhydrolase, isoform Ib, beta subunit 30 ku	25 724	5.57	↓	Metabolism
16	P30048	Peroxiredoxin 3	26 107	7.04	↑	Antioxidant
17	Q86w59	BCL2-associated athanogene 2	23 928	6.25	↓	Chaperone
18	Q04760	Glyoxalase I	20 995	5.12	↑	Antioxidant
19	Q30626	Sorcin	20 617	5.11	↑	Calcium-binding
20	Q06830	Peroxiredoxin 1	22 300	8.27	↑	Antioxidant
21	P23528	Cofilin, non-muscle isoform	18 700	8.22	↓	Chaperone
22	P62937	Peptidylprolyl isomerase A	18 228	7.68	↓	Chaperone
23	P05388	Ribosomal protein P0	34 424	5.42	↓	Signal transduction
24	P62879	G protein beta subunit	38 061	5.75	↓	Signal transduction

^{a)} Swiss-Prot accession number. ^{b)} Heat shock cognate 71 ku protein (HSC70); Heat shock protein 27 (HSP27); HADH2 protein (3-hydroxyacyl-CoA dehydrogenase type II); VDAC2 protein (Voltage-dependent anion-selective channel protein 2). ^{c)} ↑ showed that spot intensity $\geq$ 2-fold increased in SGC7901/VCR in comparison to SGC7901; ↓ showed that spot intensity $\geq$ 2-fold decreased in SGC7901/VCR in comparison to SGC7901.

A, ribosomal protein P0 和 G protein beta subunit 在 SGC7901/VCR 表达下调. 基于数据库查询结果, 这些蛋白质按功能大致可以分为 6 类: 钙结合蛋白, 分子伴侣, 与解毒和 DNA 修复相关蛋白质, 代谢相关酶, 细胞结构相关蛋白质, 信号传导相关蛋白质.

2.3 差异蛋白质点的表达水平验证

为了验证比较蛋白质组学的结果, 应用蛋白质印迹及实时 RT-PCR 检测部分差异蛋白质在 SGC7901 和 SGC7901/VCR 细胞中的表达水平. 蛋白质印迹分析显示, HSP27, sorcin, VDAC2 蛋白在 SGC7901/VCR 细胞中表达上调, 而 HSC70 蛋白表达下调(图 5). 图 6 为代表性实时 RT-PCR 结果, sorcin 基因 mRNA 在 SGC7901/ VCR 细胞中表达上调约 1 004 倍, 其他基因 mRNA 表达水平的检测结果见表 4. 蛋白质印迹及实时 RT-PCR 检

测结果与比较蛋白组学研究结果一致.

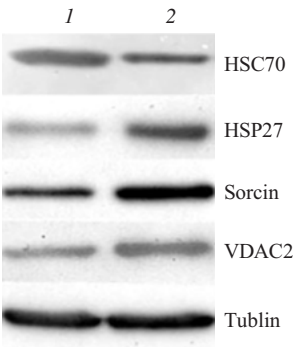


Fig. 5 A representative Western blot analysis of HSP27, sorcin, VDAC2, and HSC70 in SGC7901 and vincristine-resistant SGC7901/VCR

Compared with SGC7901, SGC7901/VCR had an obvious up-regulation of HSP 27, sorcin and VDAC2, and a marked down-regulation of HSC70. 1: SGC7901; 2: SGC7901/VCR.

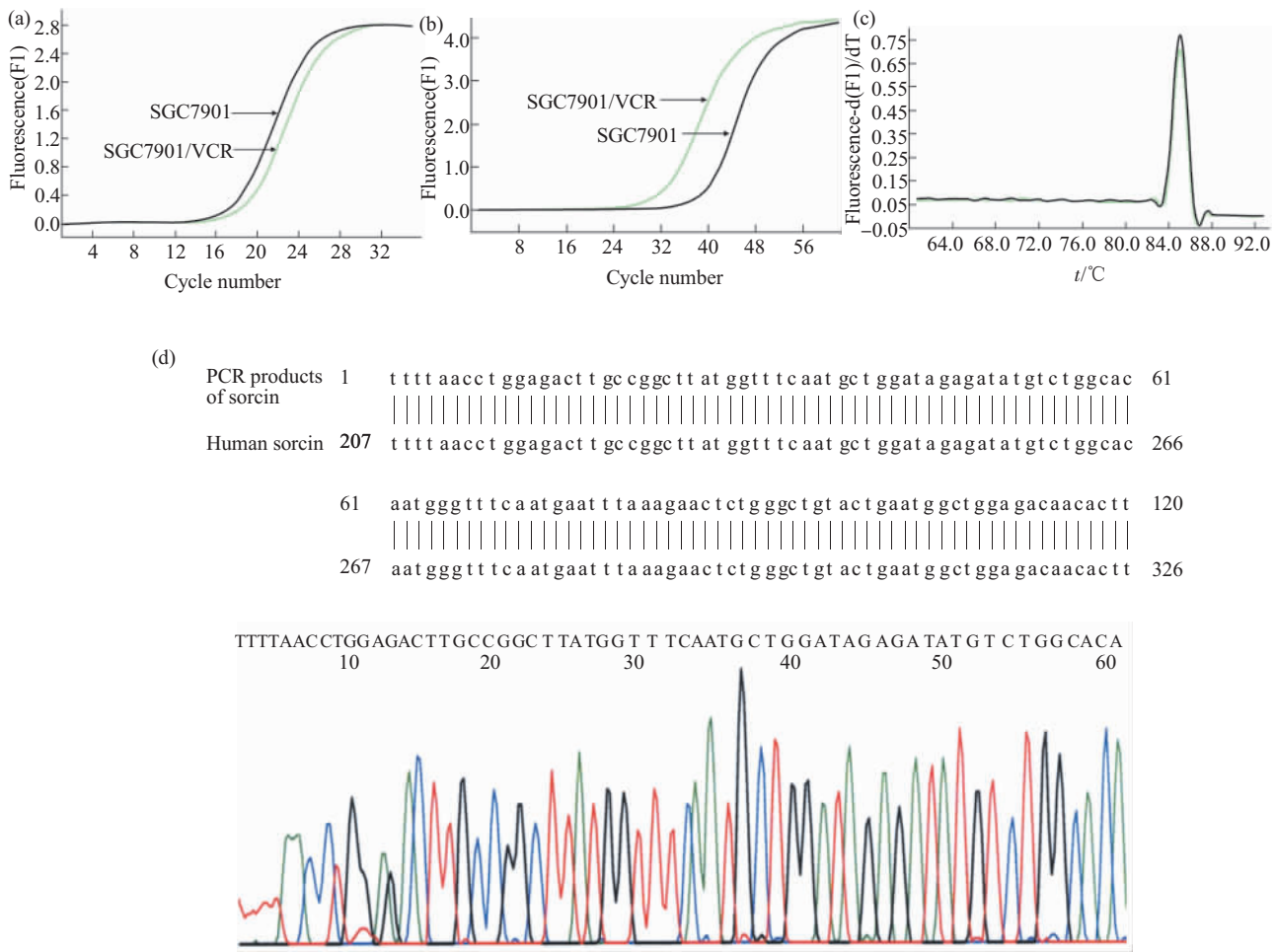


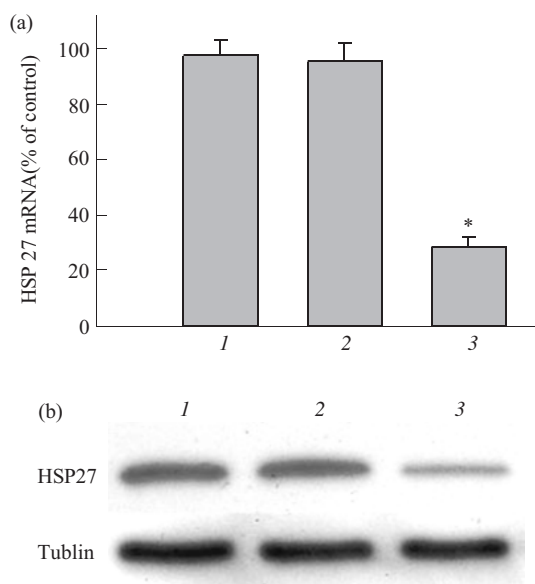
Fig. 6 A representative real-time RT-PCR analysis for the mRNA level of sorcin in SGC7901/VCR and SGC7901 (a) Graph showed that mRNA level of GADPH between SGC7901/VCR and SGC7901 had very little difference. (b) Graph showed that mRNA level of sorcin between SGC7901/VCR and SGC7901 had significant difference, which was higher in SGC7901/VCR than that in SGC7901. (c) Graph showed the melting curve of representative PCR products of sorcin. (d) PCR products of sorcin were confirmed by DNA sequencing. Graph showed partial nucleotide sequence of sorcin.

Table 4 The relative mRNA expression levels of partial differential proteins in SGC7901/VCR vs. SGC7901 comparison

Gene	SGC7901/VCR vs. SGC7901
Sorcin	mean (1 004.60 ± 70.73) folds increased in SGC7901/VCR
PPI	mean (5.79 ± 1.90) folds decreased in SGC7901/VCR
IDH3A	mean (9.24 ± 1.35) folds decreased in SGC7901/VCR
PKM2	mean (2.03 ± 0.19) folds decreased in SGC7901/VCR
PDX3	mean (2.21 ± 0.13) folds increased in SGC7901/VCR
VDAC2	mean (2.54 ± 0.87) folds increased in SGC7901/VCR
PP	mean (1 252.01 ± 219.80) folds increased in SGC7901/VCR
HSP27	mean (4.89 ± 0.56) folds increased in SGC7901/VCR
HSC70	mean (4.56 ± 0.79) folds decreased in SGC7901/VCR

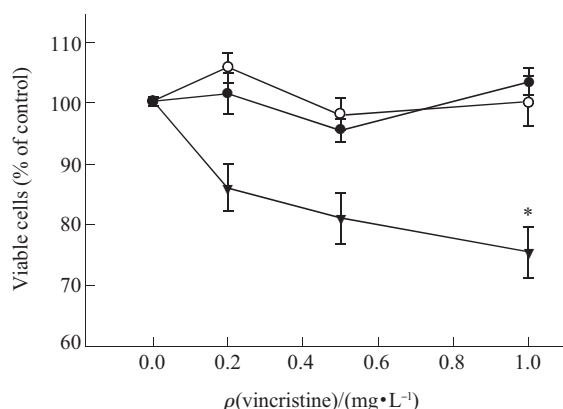
2.4 反义核酸转染分析 HSP27 高表达与胃癌细胞耐药的关系

为了探讨 HSP27 高表达与 SGC7901/VCR 耐药的关系, 先采用 HSP27 反义核酸抑制 HSP27 在 SGC7901/VCR 细胞中的表达, 再采用 MTT 方法分析转染的 SGC7901/VCR 细胞对化疗药物的敏感性. 实时 RT-PCR 和蛋白质印迹分析结果显示,

**Fig. 7** Sequence-specific inhibition of HSP27 expression by HSP27 antisense oligonucleotides (ASO) in SGC7901/VCR cells

(a) and (b) graphs showed that treatment of SGC7901/VCR cells with HSP 27 ASO significantly reduced the levels of HSP27 mRNA and protein, respectively, whereas HSP27 mRNA and protein expressions were not significantly suppressed by scrambled ASO. Data of real-time RT-PCR analysis are presented as the ($\bar{x} \pm s$). *, $P \leq 0.05$ differ from scrambled ASO and oligofectamine control by *t*-test. Oligofectamine, cells treated with oligofectamine only. 1: Oligofectamine; 2: Scrambled ASO; 3: HSP27 ASO.

HSP27 反义寡核苷酸显著抑制 HSP27 mRNA 和蛋白质的表达, 而错义寡核苷酸 (scrambled ASO) 和脂质体 (oligofectamine) 对 HSP27 表达无明显作用 (图 7). MTT 分析结果显示, HSP27 反义寡核苷酸显著增强 SGC7901/VCR 细胞对长春新碱的化疗敏感性, 而错义寡核苷酸和脂质体对 SGC7901/VCR 的耐药性无明显影响 (图 8). 这提示抑制 HSP27 的表达可逆转 SGC7901/VCR 细胞的多药耐药性.

**Fig. 8** MTT assay detected the effect of HSP27 ASO treatment on vincristine chemosensitivity in SGC7901/VCR cells

Graph showed that HSP27 ASO could significantly decrease the cell viability of SGC7901/VCR cells incubated with vincristine. The experiment was repeated in triplicate. Points, mean viable cells (% of control) from three experiments; bars, SE. *, $P \leq 0.05$ differ from scrambled ASO and oligofectamine control by *t*-test. Oligofectamine, cells treated with oligofectamine only. ●—●: Oligofectamine; ○—○: Scrambled ASO; ▼—▼: HSP27 ASO.

3 讨 论

肿瘤的多药耐药机制十分复杂, 涉及细胞膜药物外排增多使细胞内药物浓度降低, 药物在细胞内

的亚细胞分布发生改变使药物不能到达靶位置, 细胞内解毒系统、DNA 损伤修复系统及抗凋亡系统的能力增强. 已发现的耐药相关蛋白有 p170 糖蛋白, 多药耐药相关蛋白, 肺耐药蛋白, 谷胱甘肽 S 转移酶, 拓扑异构酶, 蛋白激酶 C, 乳腺癌耐药相关蛋白, 超氧化物歧化酶等. 但以上研究结果都不能完全解释肿瘤多药耐药现象. 因此, 有必要采用新方法和开展肿瘤多药耐药性研究.

蛋白质组学是后基因组时代的一门新学科, 其目的是从整体的角度分析细胞内动态变化的蛋白质组成成分, 了解蛋白质之间的相互作用和相互联系, 揭示蛋白质功能和生命活动规律^[8]. 蛋白质组学的出现及其理论和技术的发展和完善为研究肿瘤细胞的多药耐药机制、筛选肿瘤耐药相关蛋白提供了全新的手段. 胃癌是常见的恶性肿瘤之一, 占各种肿瘤死因的第二位. 胃癌细胞的多药耐药性是胃癌化疗失败最主要的原因之一. 虽然人们对胃癌多药耐药进行了大量的研究^[2~4], 但其发生的分子机制仍然不完全清楚. 为此, 本研究以长春新碱诱导的耐药胃癌细胞株 SGC7901/VCR 和非耐药胃癌细胞株 SGC7901 为对象, 采用蛋白质组学技术比较两种细胞蛋白质表达谱的差异, 寻找胃癌耐药相关的蛋白质. 研究成功地鉴定出 24 种与胃癌耐药相关的蛋白质, 这些蛋白质按功能大致可以分为六类: 钙结合蛋白, 分子伴侣, 与解毒和 DNA 修复相关的蛋白质, 代谢相关酶, 细胞结构相关蛋白, 信号传导相关蛋白.

钙结合蛋白如可溶性耐药相关性钙结合蛋白 sorcin (soluable resistance-related calcium-binding protein, sorcin)等在 SGC7901/VCR 细胞中高表达. Sorcin 是一种胞浆蛋白, 具有典型的 “E-F hand” 钙结合位点, 广泛分布于正常组织, 其中心肌组织含量最为丰富. Meyers 等^[9]的研究认为超过 50% 的多药耐药性的细胞株有 sorcin 的高表达, 而且只要细胞株能够维持其多药耐药现象, 一般都伴随有 sorcin 的高表达, 这说明 sorcin 与多药耐药现象相关. Lee^[10]建立了耐长春新碱 (VCR) 的淋巴瘤耐药株 HOB1/VCR, DNA 印迹检测发现仅在高浓度的 VCR 作用下才有 sorcin 基因的扩增, 而且 sorcin 基因不随 *mdr1* 基因的扩增而扩增, 因此, 认为 sorcin 基因的扩增和 mRNA 的表达是在高浓度 VCR 作用下产生的, 其研究结果也提示 sorcin 与 *mdr1* 相关性不高, 很可能在多药耐药性产生中单独起作用的. Parekh^[11]将 sorcin 基因 cDNA 分别转

染卵巢癌细胞株和乳腺癌细胞株, 诱导出了它们对紫杉醇的低水平抗药性, 虽然 sorcin 基因转染没有诱导出高水平的抗药性, 但这是 sorcin 参与多药耐药性形成的有力证据. 本研究发现胃癌耐药细胞株 SGC7901/VCR 高表达 sorcin, 说明 sorcin 高表达与胃癌细胞耐药密切相关. 尽管 sorcin 在多种肿瘤耐药细胞株中高表达, 但在胃癌耐药细胞株中高表达在国内外尚未见到报道.

分子伴侣如热休克蛋白 27 (heat shock protein 27, HSP27)等在 SGC7901/VCR 细胞中高表达. HSP27 的功能十分复杂, 它不仅作为分子伴侣参与蛋白质的折叠组装和运输, 亦与肿瘤多药耐药关系密切. Wu 等^[12]将 HSP27 cDNA 转染小鼠胚胎干细胞, 高表达 HSP27 的细胞获得抗顺铂以及镉、砷和汞化合物的能力. Lavoie 等^[13]的研究显示, HSP27 可以减少组织间活性氧, 增加谷胱甘肽水平, 参与细胞解毒功能. 本研究中 HSP27 不仅在 SGC7901/VCR 细胞中高表达, 而且反义核酸抑制 HSP27 的表达能增强 SGC7901/VCR 细胞对长春新碱的敏感性, 说明 HSP27 高表达与 SGC7901/VCR 细胞的多药耐药性有关.

本研究所识别的与胃癌细胞耐药相关蛋白还有细胞解毒修复系统相关蛋白, 如过氧化物酶 3 (peroxiredoxin 3, PDX3)、过氧化物酶 1 (peroxiredoxin 1, PDX1)、乙二醛酶 (glyoxalase I)、尿嘧啶 DNA 糖苷酶 (uracil DNA glycosylase) 等, 代谢相关酶, 如异柠檬酸脱氢酶前体 (isocitrate dehydrogenase 3 alpha precursor)、丙酮酸激酶 M2 (pyruvate kinase, isozymes M2)、无机磷酸酶 (PP protein) 等, 信号传导蛋白如 G protein beta subunit 和 VDAC2 等, 以及细胞结构相关蛋白. 这些蛋白质与胃癌多药耐药关系有待进一步研究.

在本研究中, 建立了 SGC7901/VCR 及 SGC7901 细胞的二维电泳图谱, 识别并鉴定了两种细胞之间的 24 个差异蛋白质, 并应用蛋白质印迹及实时 RT-PCR 验证了部分差异蛋白质的表达水平. 反义核酸转染证实了 HSP27 的高表达与 SGC7901/VCR 细胞耐药性相关. 这些资料为研究胃癌细胞的多药耐药性产生机制提供了新线索.

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Identification of Multidrug Resistance Related Proteins in Human Gastric Cancer Cells Using Proteomics Technique*

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Abstract Resistance to anticancer drugs is one major problem preventing effective chemotherapy of gastric cancer, but the molecular mechanisms of multidrug resistance (MDR) of gastric cancer is not completely clear. In order to find out new MDR related proteins of gastric cancer, two-dimensional gel electrophoresis (2-DE) was used to separate the total proteins of vincristine-resistant human gastric cancer cell line SGC7901/VCR and its parental cell line SGC7901, PDQuest software was applied to analyze 2-DE images, and the differential protein spots between the two cell lines were identified by both MALDI-TOF-MS and ESI-Q-TOF-MS. Then the differential expressional levels of partial identified proteins were detected by Western blot analysis and real-time RT-PCR. And the effect of HSP27 on the development of MDR of SGC7901/VCR was determined by antisense oligonucleotides (ASO) technique. The well-resolved, reproducible 2-DE patterns of SGC7901/VCR and SGC7901 were established. All the 24 differential proteins were identified, and the differential expression levels of the partial proteins between the two cell lines were confirmed by Western blot analysis and real-time RT-PCR. The suppression of HSP27 expression by HSP27 ASO could enhance vincristine chemosensitivity in SGC7901/VCR. These differential proteins such as HSP27 and sorcin may be related to MDR in SGC7901/VCR cells. The data will be valuable for further to study the mechanism of MDR in human gastric cancer.

Key words vincristine, gastric cancer, multidrug resistance, proteome, two-dimensional gel electrophoresis, mass spectrometry, immunoblotting, real-time RT-PCR, antisense oligonucleotides

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