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Research Article 研究报告

利用蛋白质组学研究新德里金属β-内酰胺酶 1 对鲍曼不动杆菌的影响

曾观娣，徐倩，刘婉婷，阳小燕，孙雪松*

暨南大学生命与健康工程研究院，功能蛋白质研究广东普通高校重点实验室，广东 广州 510632

摘要：【目的】比较临床分离的亲缘关系近的多药耐药鲍曼不动杆菌 MDR-ZJ06 (bla_{NDM-1}^-)和 ABC3229 (bla_{NDM-1}^+)的差异蛋白质组，以期发现新德里金属β-内酰胺酶 1 (New Delhimetallo-β-lactamase-1，NDM-1)对鲍曼不动杆菌生长代谢的影响。【方法】利用 2-DE 联合 MALDI-TOF MS/MS 技术鉴定差异表达蛋白，并在 GO 注析的基础上，对差异蛋白进行通路分析、功能分类和富集分析，并作出蛋白与蛋白相互作用网络。【结果】发现 ABC3299 相对于 MDR-ZJ06 有 51 个差异表达蛋白，其中 11 个蛋白表达上调，40 个蛋白表达下调，并且这些差异蛋白主要涉及降低碳代谢、氨基酸代谢、脂肪酸代谢和细胞壁合成，增加铁离子转运系统形成。【结论】这个结果揭示了 NDM-1 可能是通过减缓细菌自身的代谢，增加自身铁的摄取使细菌机体系统地抵抗抗生素从而达到耐药。

关键词：鲍曼不动杆菌，NDM-1，多药耐药，差异蛋白质组学

鲍曼不动杆菌是在医院流行的需氧革兰氏阴性菌[1]。临床很多感染与鲍曼不动杆菌直接相关，如尿路感染、继发性脑膜炎、创伤或烧伤感染、肺炎等[2–3]，David 首次发现在濒临灭绝的欧洲水貂身上鲍曼不动杆菌感染也会致命[4]。在 20 世纪 70 年代，鲍曼不动杆菌对大多数抗生素都敏感。然而，由于抗生素的滥用，鲍曼不动杆菌已经发展到能抵抗几乎所有常用抗生素[5]。在医院的重症监护病房的病人，多发生多药耐药鲍曼不动杆菌感染或致死[6]。

新德里金属β-内酰胺酶 1 (New Delhimetallo-β-lactamase-1，NDM-1)是由 bla_{NDM-1} 基因编码的产物，它能抵抗几乎所有的抗生素，甚至对公认的最后防线的抗生素——碳青霉素也有抵抗作用，因而增大了治疗的难度[7–8]。近年来，除了美国中部和南部以及南极洲外，bla_{NDM-1} 通过基因水平在世界快速地传播[7, 9–10]。此外，据报道在印度的饮用水和污水中也分离出了细菌产出的 NDM-1[11]。

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*通信作者。Tel：+86-20-85226165；Fax：+86-20-85227039；E-mail：tsunxs@jnu.edu.cn

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NDM-1 被划分为 B 类金属-β-内酰胺(Class B metallo-β-lactamase，MBL)[12–13]，它能通过切割β-内酰胺的酰胺键从而摧毁β-内酰胺类抗生素，除了单环β-内酰胺抗生素外，它能降解所有的β-内酰胺类抗生素[12]。

bla$_{NDM-1}$和其他抵抗喹诺酮类、磺胺类、大环内酯类和利福平的耐药基因通常位于同一质粒[9]，导致非常复杂的耐药机制。为了单一地研究 NDM-1 对鲍曼不动杆菌生长代谢的影响，我们选择了两个临床分离的多药耐药的鲍曼不动杆菌[14]：没有携带 *bla*$_{NDM-1}$的 MDR-ZJ06 和携带 *bla*$_{NDM-1}$的 ABC3229，利用蛋白质组学的方法研究 NDM-1 的影响。MLST (Mulitlocus sequence typing)分析显示这 2 个菌株仅有 1 个位点的差异，表明他们的亲缘关系非常近。联合 2-DE (Two-dimensional gel electrophoresis)和 MALDI-TOF MS/MS 技术分析 2 个菌株的差异蛋白质组学。基于 GO 注释，发现被 NDM-1 改变的生物通路。本研究是第一次通过蛋白质组学的方法去揭示 NDM-1 对细菌生长代谢的影响。

1 材料和方法

1.1 菌株来源与主要材料

鲍曼不动杆菌 MDR-ZJ06 和 ABC3229 是浙江大学俞云松教授课题组提供的，只有 ABC3229 携带 *bla*$_{NDM-1}$基因。LB 培养基配制所需的 Tryptone、Yeast extract 购自 OXOID 公司。尿素、硫脲、CHAPS、SDS 购自 Sigma 公司。IPG buffer 和覆盖液购自 Amersham Biosciences。DTT、IAA 购自 Roche 公司。咪唑购自 GeneBase 公司。

1.2 生长条件

MDR-ZJ06 和 ABC3229 都在 LB 培养基中，37 °C、220 r/min 过夜培养。准备新鲜的肉汤培养基 100 mL 以 1∶100 的体积比接种，过夜培养。当培养到稳定期早期(OD_{600}=1.6–1.7)时以 6000×g、20 min 收菌。

1.3 细菌生长曲线的测定

将过夜活化的细菌 *A. baumannii* AB3229、*A. baumannii* MDR-ZJ06 按照 1∶100 接种于 100 mL 的 LB 培养基中，37 °C、240 r/min 培养，每隔 1–2 h 测定 1 次 600 nm 处的吸光度，作好记录，用 Originpro 8.0 绘制生长曲线。

1.4 蛋白提取

用 1×PBS 缓冲液将收集到的细菌清洗 2 遍，最后用 1–2 mL 的溶解缓冲液[7 mol/L 尿素，2 mol/L 硫脲，4% CHAPS，15 mmol/L Tris-base，蛋白酶抑制剂(Roche，USA)，13 mmol/L DTT (Abcam)]将细菌重悬，反复冻融 3 次，在冰上超声 15 min。4 °C，13200×g 离心 30 min 去除没有破碎的细菌。通过 Bradford 法测蛋白浓度，并在–80 °C 冰箱中保存，备用。

1.5 双向凝胶电泳(2-DE)

将 100 μg 的蛋白溶解在水化液(7 mol/L 尿素，2 mol/L 硫脲，4% CHAPS，20 mmol/L DTT，0.5% IPG pH 4–7，0.002%溴酚蓝)中，使终浓度为 250 μL。以 1∶100 (*V*/*W*)加入核酸酶，混匀，在冰上放置 1 h，13000 r/min、30 min 离心。将处理好的蛋白样品加入清洗干净的胶条槽中，放入 pH 4–7 IPG 胶条，吸胀 30 min。加入 1 mL 覆盖油，盖槽盖。设置等电聚胶程序并电泳 30 V，12 h；500 V，1 h；1000 V，1 h；8000 V，4 h；2000 V，10 h。取出胶条，用电泳缓冲液清洗，放入平衡液 A (50 mmol/L Tris-HCl pH 8.8，6 mol/L 尿素，30%甘油，2% SDS，0.002%溴酚蓝储液，0.001% DTT)处理胶条 15 min，

再用平衡液 B (50 mmol/L Tris-HCl pH 8.8，6 mol/L 尿素，30%甘油，2% SDS，0.002%溴酚蓝储液，0.0025% IAA)平衡 15 min。将胶条置于 12.5%聚丙烯酰胺凝胶上，并用 0.5%低熔点琼脂糖封闭。按每块胶 15 mA 进行电泳，30 min 后再将电流设置为每块胶 30 mA 并跑到最后。电泳结束后用与质谱兼容的银染溶液对凝胶进行银染[15]。

1.6 图片的获取和 2-DE 的分析

用 Image Scanner (GE)扫描银染的胶，输出 tif 格式的图片，并用 ImageMaster 2D Platinum 6.0 对 *A. baumannii* MDR-ZJ06 (blaNDM-1-)和 ABC3229 (blaNDM-1+)进行图像分析，参数设置是同一位置两蛋白点差异在 1.5 倍或以上，差异蛋白质点由软件自动选择合适的蛋白位点后再由手动过滤，确定两蛋白位点确实存在显著性差异的蛋白质位点(大于 1.5 倍或以上)，才能用于质谱分析。

1.7 胶内酶解

手动挖取显著性差异蛋白质位点，并用胶内酶解的方法将胶内的蛋白切为短肽[16]。用质谱级胰蛋白酶(Promega)消化蛋白[17]。消化后，离心取上清到干净的新的离心管中，并在原离心管加入 30 μL 萃取液(67%乙腈，2.5% TFA)，在 4 °C 中超声 30 min，离心将上清转移到新的离心管中，并用真空泵干燥。

1.8 质谱分析和搜库鉴定

用 ABI 4800 plus MALDI TOF/TOF 质谱仪(Applied Biosystems, Foster City，CA)分析样品得到肽段信息。将肽段信息通过 MASCOT 引擎与鲍曼不动杆菌 MDR-ZJ06 的蛋白数据库进行搜索。肽段的搜库标准是：最多漏掉 2 个切割位点，可变修饰为半胱氨酸甲酰胺甲基化和甲硫氨酸氧化，无固定修饰。一级质谱的误差为 0.01%，二级质谱的误差为 0.2 Da。Protein Score C. I. %大于 95%，则认为鉴定结果是可信的。肽段鉴定的假阳性率(False discovery rate，FDR)低于 1%。

1.9 差异蛋白功能分类、富集分析和相互作用网络构建

基于 GO 注释，对差异蛋白进行通路分析、功能分类，并编写 R 语言程序对差异蛋白进行富集分析，最后利用 STRING 进行构建差异蛋白相互作用的网络[16](http://string-db.org/)。参数设置如下：生物体(Organism)：鲍曼不动杆菌 19606，可信度阈值(Confidence threshold)：0.70，不超 10 个关联度[18]。Cytoscape 网络图代表着分子间的相互作用。

2 结果和分析

2.1 NDM-1 对生长无影响

首先，利用紫外分光光度计测定了 *A. baumannii* AB3229 和 *A. baumannii* MDR-ZJ06 的生长曲线。从图 1 看到，两株菌的生长曲线基本重合，无明显差异，表明 NDM-1 对鲍曼不动杆菌的生长无影响。

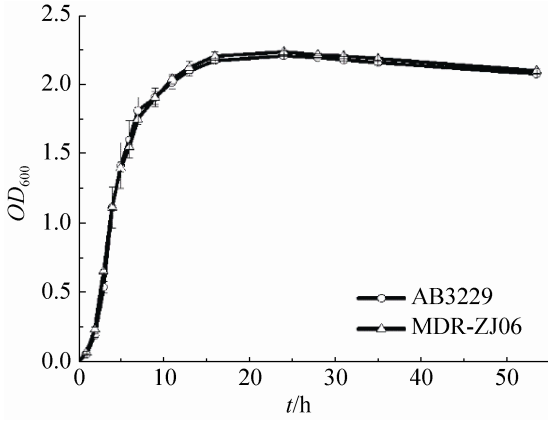

图 1. *A. baumannii* AB3229、*A. baumannii* MDR-ZJ06 生长曲线

Figure 1. The growth curves of *A. baumannii* AB3229 and MDR-ZJ06.

2.2 NDM-1 主要下调差异蛋白的表达

为了揭示 NDM-1 对鲍曼不动杆菌的影响，我们比较分析了临床分离的亲缘关系非常近的鲍曼不动杆菌 ABC3229 和 MDR-ZJ06 的蛋白质组学。鲍曼不动杆菌 ABC3229 包含的 $bla_{\text{NDM-1}}$ 基因产物能抵抗庆大霉素、丁胺卡那霉素、环丙沙星、米诺环素和替加环素[14]。为了弄清楚 NDM-1 是如何引起细胞代谢的改变，分别提取两株鲍曼不动杆菌的蛋白质，并用双向凝胶电泳对蛋白进行分离。

双向凝胶电泳的结果(图 2)显示这 2 个菌株的电泳图谱非常相似，进一步说明这两株菌株是高度同源的菌株。蛋白点多集中于 pH 5–6。通过 Progenesis SameSpots 分析，发现相对于鲍曼不动杆菌 MDR-ZJ06 而言，ABC3229 有 51 个显著性差异的蛋白位点，其中 11 个上调表达，40 个下调表达(表 1)，结果表明 NDM-1 主要降低蛋白的表达，但是铁转运系统的铁蛋白受体(Putative ferric siderophore receptor protein)却上调表达(3.9 倍)。

2.3 NDM-1 降低了细胞代谢

富集分析(图 3)富集了 17 个蛋白，其中参与到生物学过程中最多的 3 个蛋白为锌依赖性乙醇脱氢酶(Zn-dependent alcohol dehydrogenase，class III)、丝氨酸羟甲基转移酶(Serine hydroxymethyltransferase，SHMT)、酮脂酰辅酶 A 硫解酶(3-Ketoacyl-CoA thiolase)。锌依赖性乙醇脱氢酶是细胞内主要短链醇代谢的关键酶[19]，利用烟酰胺腺嘌呤二核苷酸(NAD)为辅酶催化伯醇与醛的可逆性反应，可有效消除体内外的甲醛，甚至在酵母中，发现乙醇脱氢酶可将糖类转化为酒精。SHMT 除了可催化 L-丝氨酸和甘氨酸相互转化[20–21]，还可以为很多生物合成反应提供一碳单位，更是氨基酸和核酸

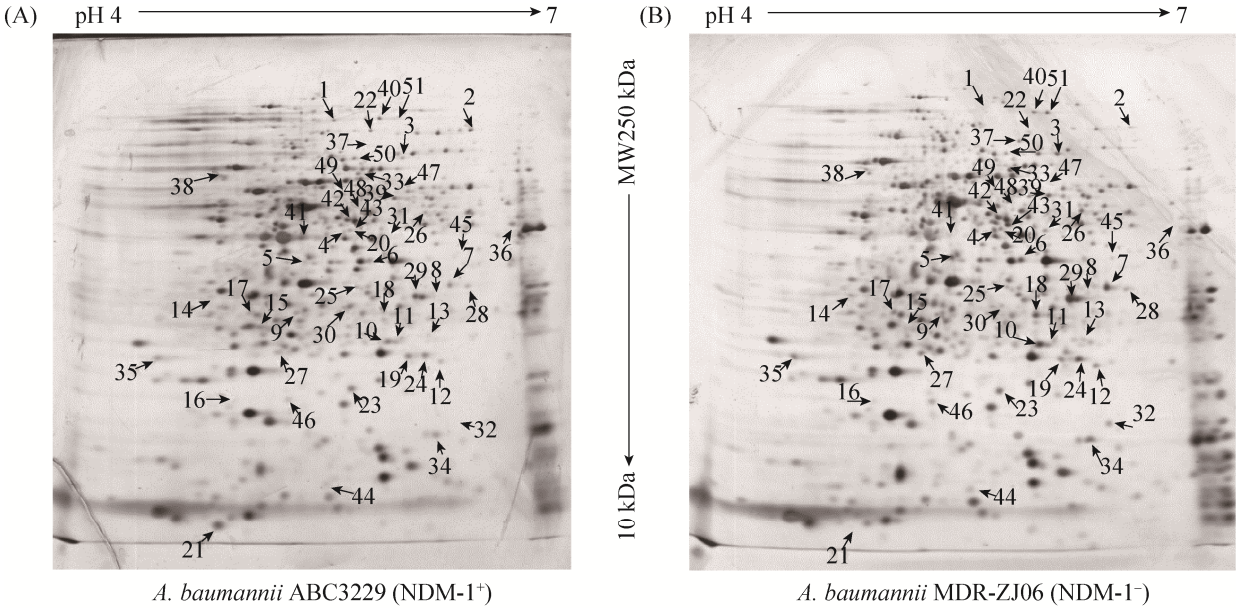

图 2. 双向电泳图

Figure 2. The 2-DE profiles of whole cell proteins extracted from *A. baumannii* ABC3229 (A) and *A. baumannii* MDR-ZJ06 (B).

表 1.　质谱鉴定到的 *A. baumannii* AB3229 相对于 *A. baumannii* MDR-ZJ06 差异表达蛋白功能分类

Table 1.　The detailed information of DEPs identified by MODI-TOF MS/MS

Spot No.[a]	Accession No.[b]	Protein name	Peptides[c]	Protein MW[d]	Protein pI[e]	F.D.[f]	Protein score C. I./%[h]
Carbohydrate metabolism							
2	gb|AEP05957.1	NAD-dependent aldehyde dehydrogenase	7	76692.2	5.94	–2.2	100
5	gb|AEP06361.1	Acetoin: 26-dichlorophenolindophenol oxidoreductase beta subunit	19	41096.1	5.90	–2.4	100
8	gb|AEP04952.1	Formyltetrahydrofolate deformylase	12	32673.8	5.74	–1.6	100
10	gb|AEP05548.1	Carbonic anhydrase	12	22128.2	5.61	–1.5	100
24	gb|AEP04987.1	Bifunctional 4-hydroxy-2-oxoglutarate aldolase/2-dehydro-3-deoxyphosphogluconate aldolase	8	21545.4	5.79	–1.6	100
40	gb|AEP07193.1	Putative bifunctional protein (MaeB)	14	82760.9	5.52	–1.5	100
42	gb|AEP06186.1	S-adenosyl methionine synthetase	13	41948.2	5.44	–1.5	100
43	gb|AEP04541.1	Putative polysaccharide biosynthesis protein	29	40413.1	5.46	–2.0	100
49	gb|AEP07159.1	Serine hydroxymethyltransferase	7	44966.7	5.44	+1.9	99.463
51	gb|AEP07395.1	Isocitrate dehydrogenase	9	82586.9	5.63	–2.0	99.995
Amino acid metabolism							
2	gb|AEP05957.1	NAD-dependent aldehyde dehydrogenase	7	76692.2	5.94	+2.2	100
5	gb|AEP06361.1	Acetoin:26-dichlorophenolindophenol oxidoreductase beta subunit	19	41096.1	5.90	–2.4	100
3	gb|AEP08253.1	Urocanate hydratase	28	61163.7	5.64	–2.1	100
19	gb|AEP07680.1	Putative intracellular protease/amidase	4	20995.6	5.61	–1.6	99.781
24	gb|AEP04987.1	Bifunctional 4-hydroxy-2-oxoglutarate aldolase/2-dehydro-3-deoxyphosphogluconate aldolase	8	21545.4	5.79	–1.6	100
30	gb|AEP07715.1	Gcv-like aminomethyltransferase	6	26768.6	5.49	+2.2	100
33	gb|AEP04591.1	Methylmalonate-semialdehydedehydrogenase,oxidoreductase protein	12	55046.3	5.40	–2.3	100
36	gb|AEP04808.1	3-ketoacyl-CoA thiolase	8	41061.9	6.09	+1.8	99.993
42	gb|AEP06186.1	S-adenosylmethionine synthetase	13	41948.2	5.44	–1.5	100
48	gb|AEP05897.1	Threonine dehydrogenase	8	41812.5	5.44	–1.7	100
49	gb|AEP07159.1	Serine hydroxymethyltransferase	7	44966.7	5.44	+1.9	99.463
Fatty acid metabolism							
2	gb|AEP05957.1	NAD-dependent aldehyde dehydrogenase	7	76692.2	5.94	+2.2	100
7	gb|AEP07759.1	Acetyl CoA carboxylase, beta subunit	18	32950.6	5.85	–2.2	100
18	gb|AEP05106.1	Acetyl CoA carboxylase alpha subunit	14	29622.0	5.60	–2.3	100
29	gb|AEP05034.1	NADH-dependent enoyl-ACP reductase	17	30996.9	6.00	–1.9	100
36	gb|AEP04808.1	3-ketoacyl-CoA thiolase	8	41061.9	6.09	+1.8	99.993
50	gb|AEP07777.1	Acyl-CoA dehydrogenase	18	65580.9	5.42	+1.7	100
38	gb|AEP05798.1	Alkyl hydroperoxide reductase subunit, FAD/NAD(P)-binding, detoxification of hydroperoxides	6	57261.3	4.98	–1.6	99.995
Nucleic acid metabolism							
8	gb|AEP04952.1	Formyltetrahydrofolate deformylase	12	32673.8	5.74	–1.6	100
11	gb|AEP08190.1	Orotate phosphoribosyltransferase	10	24057.6	5.59	–1.9	100
12	gb|AEP07873.1	Xanthine phosphoribosyltransferase	6	20806.2	5.83	–1.9	100

(待续)

(续表 1)

16	gb|AEP04475.1	Hypoxanthine phosphoribosyltransferase	13	19576.1	4.93	–1.5	100
Translation							
15	gb|AEP07282.1	Elongation factor P	3	21205.5	4.88	+2.0	99.868
Transcription							
21	gb|AEP04730.1	Regulatory protein for nitrogen assimilation by glutamine synthetase, regulates GlnL (NRII) and GlnE	4	12195.6	5.41	+5.9	99.966
25	gb|AEP05556.1	Esterase operon transcriptional regulator	20	33751.9	5.44	–1.5	100
Iron transport							
1	gb|AEP04976.1	Outer membrane receptor for monomeric catechols	10	81197.3	5.71	+2.1	100
28	gb|AEP08077.1	Osmolarity response regulator	23	28797.2	5.91	–1.7	100
22	gb|AEP04581.1	Putative ferric siderophore receptor protein	26	79483.2	5.70	+3.9	100
Cell wall synthesis process							
4	gb|AEP04540.1	Putative UDP-N-acetylglucosamine 2-epimerase	6	44276.0	5.79	–2.4	99.999
31	gb| AEP04542.1	Putative polysaccharide biosynthesis protein	17	40316.9	5.56	–1.9	100
Vitamin synthesis process							
6	gb|AEP06176.1	Biotin synthetase	13	37113.5	5.46	–1.9	100
17	gb|AEP07119.1	Thiamine biosynthesis protein, thiazole moiety	6	29636.3	5.09	–1.6	98.652
Others							
9	gb|AEP06368.1	Zn-dependent hydrolase, including glyoxylase	9	26025.7	5.20	–4.0	100
13	gb|AEP06219.1	Glucose-inhibited division protein B (methyltransferase)	8	23704.6	5.76	–1.7	100
14	gb|AEP04726.1	Conserve hypothetical protein	6	31192.6	4.83	–1.7	99.149
20	gb|AEP06847.1	Conserve hypothetical protein	10	37380.5	5.49	–1.6	99.996
23	gb|AEP04978.1	ATP-dependent Clp protease proteolytic subunit	16	22525.3	5.26	–1.4	100
26	gb|AEP06438.1	Zn-dependent alcohol dehydrogenase, class III	5	39225.7	5.64	–1.6	99.852
27	gb|AEP07535.1	Nitroreductase	19	22825.7	5.13	–1.6	100
32	gb|AEP08348.1	Gentamicin 3′-acetyltransferase (gentamicin acetyltransferase I) (aminoglycoside N(3′)-acetyltransferase I) (AAC (3)-I)	15	19375.9	5.78	–4.6	100
34	gb|AEP08000.1	Conserve hypothetical protein	11	18057.1	6.06	–1.4	100
35	gb|AEP07457.1	Putative porin protein associated with imipenem resistance	11	26489.1	4.80	–1.9	100
37	gb|AEP05088.1	Acyl-CoA synthetase (AMP-forming)/AMP-acid ligase II	10	60187.5	5.39	–2.6	100
38	gb|AEP05798.1	Alkyl hydroperoxide reductase subunit, FAD/NAD(P)-binding, detoxification of hydroperoxides	6	57261.3	4.98	–1.6	99.995
39	gb|AEP06215.1	Parvulin-like peptidyl-prolylisomerase	18	48998.5	6.35	–1.6	100
41	gb|AEP07733.1	Outer membrane protein	15	38426.5	5.32	+1.7	100
44	gb|AEP06279.1	Scaffold protein	4	13773.0	5.38	–1.8	100
45	gb|AEP07759.1	Oxidoreductase	10	39354.9	5.83	–1.8	100
46	gb|AEP08000.1	Rossman fold nucleotide-binding protein	7	20885.6	5.15	–1.8	100
47	gb|AEP06956.1	Putative kinase	22	49626.5	5.60	+1.7	100

a: The spot of 2-DE. b: The number of protein in NCBI. c: The number of peptide. d: The molecular weight of protein. e: The isoelectric point of protein. f: The fold is the ratio of protein expression levels in *A. baumannii* ABC3229 to *A. baumannii* MDR-ZJ06. h: The confidence level of peptides matching to protein.

转化的节点(连接点)。在辅酶 A 存在下，酮脂酰辅酶 A 硫解酶可将 β 酮酰基辅酶 A 硫解为乙酰辅酶 A 和酰基辅酶 A。三大营养物质糖、脂肪和蛋白质通过乙酰辅酶 A 汇聚成一条共同的代谢通路——三羧酸循环和氧化磷酸化，经过这条通路彻底氧化生成二氧化碳和水，释放能量用于 ATP 合成，用于细胞内的生命活动。乙酰辅酶 A 是合成脂肪酸、酮体等能源物质的前体物质；酰基辅酶 A 是脂肪酸合成和分解的活性代谢中间物，水解时生成脂肪酸和辅酶 A，从而参与到细胞的脂肪酸代谢。

富集分析(图 3、表 2)发现只有 2 个差异蛋白表达量上调，而这些差异蛋白主要参与了鲍曼不动杆菌的碳代谢、氨基酸代谢、脂肪酸代谢和核酸代谢等生物学过程，这说明 NDM-1 通过下调表达代谢通路的蛋白使细胞代谢减缓。从而推测耐药菌可能是通过减缓机体的代谢，慢慢调控机体对抗抗生素的能力，从而达到耐药。

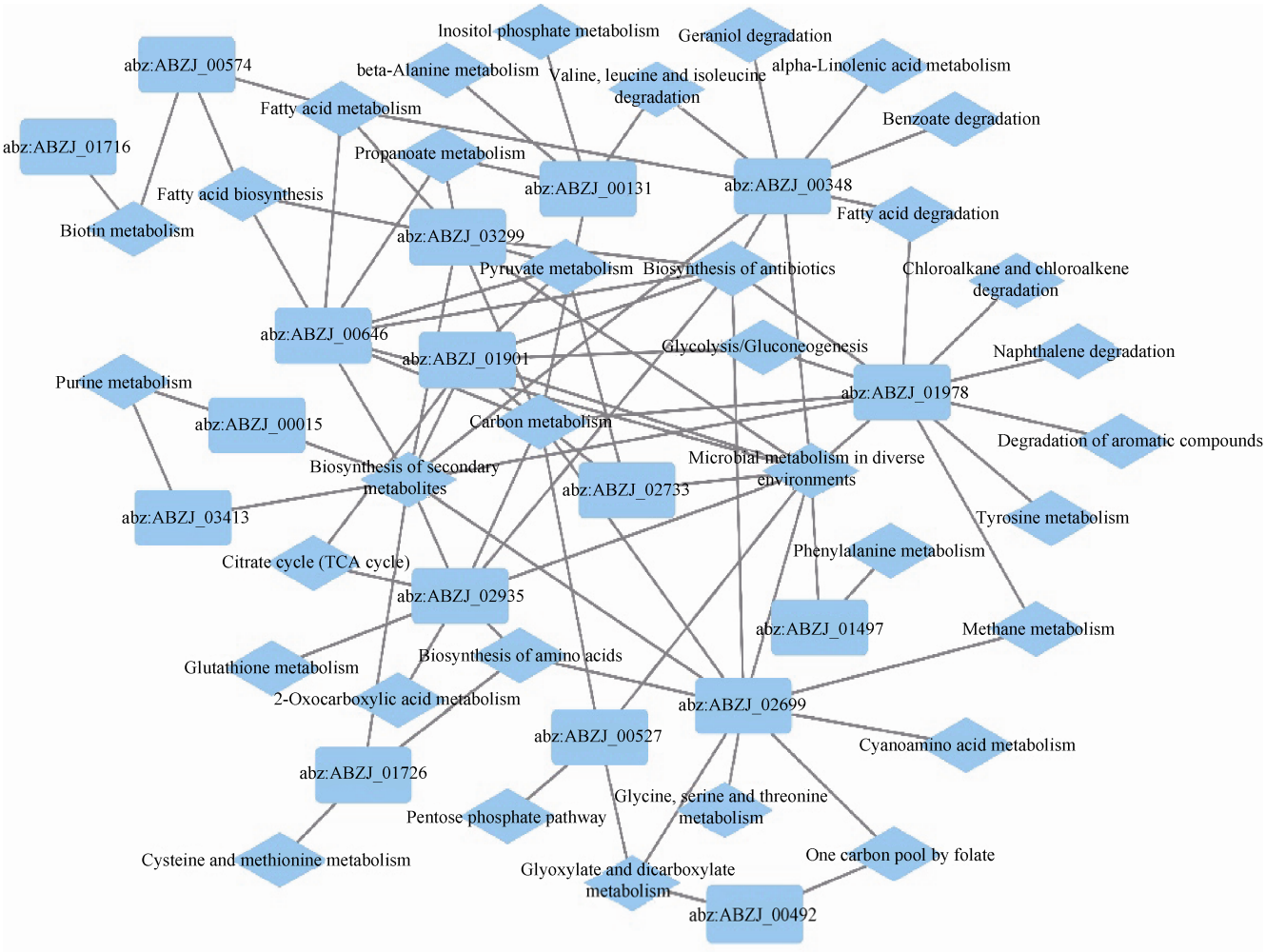

图 3. 差异蛋白富集分析

Figure 3. Enrichment analysis of DEPs. Rectangle represents the identified protein, lozenge represents the biological process.

表 2. 差异蛋白富集分析富集到的蛋白及参与的通路

Table 2. The biological process of DEPs

Spot No.	Accession No.	Protein name	Biological process	Count	F.D.
26	gb|AEP06438.1	Zn-dependent alcohol dehydrogenase, class III	Fatty acid degradation, Biosynthesis of antibiotics, Glycolysis/Glyconeogenesis, Carbon metabolism, Biosynthesis of secondary metabolites, Microbial metabolism in diverse environments, Methane metabolism, Tyrosine metabolism, Degradation of aromatic compounds, Naphthalene degradation, Chloroalkane and chloroalkene degradation	11	–1.6
49	gb|AEP07159.1	Serine hydroxymethyltransferase	Biosynthesis of antibiotics, Biosynthesis of secondary metabolites, Carbon metabolism, Biosynthesis of antibiotics, Microbial metabolism in diverse environments, Methane metabolism, Cyanoamino acid metabolism, One carbon pool by folate, Glycine, serine and threonine metabolism, Glyoxylate and dicarboxylate metabolism,	10	+1.9
36	gb|AEP04808.1	3-Ketoacyl-CoA thiolase	Fatty acid biosynthesis, Biosynthesis of secondary metabolites, Biosynthesis of antibiotics, Microbial metabolism in diverse environments, Fatty acid degradation, Benzoate degradation, alpha-Linolenic acid metabolism, Geraniol degradation, Valine, leucine and isoleucine degradation	9	+1.8
18	gb|AEP05106.1	Acetyl CoA carboxylase alpha subunit	Fatty acid biosynthesis, Fatty acid metabolism, Propanoate metabolism, Pyruvate metabolism, Biosynthesis of antibiotics, Carbon metabolism, Microbial metabolism in diverse environments, Biosynthesis of secondary metabolites	8	–2.3
7	gb|AEP07759.1	Acetyl CoA carboxylase, beta subunit	Fatty acid biosynthesis, Fatty acid metabolism, Propanoate metabolism, Pyruvate metabolism, Biosynthesis of antibiotics, Carbon metabolism, Microbial metabolism in diverse environments	7	–2.2
51	gb|AEP07395.1	Isocitrate dehydrogenase	Citrate cycle (TCA cycle), Biosynthesis of secondary metabolites, Carbon metabolism, Biosynthesis of antibiotics, Microbial metabolism in diverse environments, Biosynthesis of amino acids, 2-Oxocarbosylic acid metabolism	7	–2.0
5	gb|AEP06361.1	Acetoin:26-dichlorophenolindophenol oxidoreductase beta subunit	Pyruvate metabolism, Biosynthesis of antibiotics, Glycolysis/Glyconeogenesis, Microbial metabolism in diverse environments, Carbon metabolism, Biosynthesis of secondary metabolites, Citrate cycle (TCA cycle)	6	–2.4
33	gb|AEP04591.1	Methylmalonate-semialdehydedehydrogenase, oxidoreductase protein	Propanoate metabolism, beta-Alanine metabolism, Inositol phosphate metabolism, Valine, leucine and isoleucine degradation, Pyruvate metabolism	5	–2.3
24	gb|AEP04987.1	Bifunctional 4-hydroxy-2-Oxoglutarate aldolase/2-dehydro-3-deoxyphosphogluconate aldolase	Carbon metabolism, Microbial metabolism in diverse environments, Glyoxylate and dicarboxylate metabolism, Pentose phosphate pathway	4	–1.6
40	gb|AEP07193.1	Putative bifunctional protein (MaeB)	Pyruvate metabolism, Carbon metabolism, Microbial metabolism in diverse environments	3	–1.5
29	gb|AEP05034.1	NADH-dependent enoyl-ACP reductase	Biotin metabolism, Fatty acid biosynthesis, Fatty acid metabolism	3	–1.9
42	gb|AEP06186.1	S-adenosyl methionine synthetase	Biosynthesis of secondary metabolites, Biosynthesis of amino acids, Cysteine and methionine metabolism	3	–1.5
8	gb|AEP04952.1	Formyltetrahydrofolate deformylase	Glyoxylate and dicarboxylate metabolism, One carbon pool by folate	2	–1.6
12	gb|AEP07873.1	Xanthine phosphoribosyltransferase	Purine metabolism, Biosynthesis of secondary metabolites	2	–1.9
2	gb|AEP05957.1	NAD-dependent aldehyde dehydrogenase	Microbial metabolism in diverse environments, Phenylalanine metabolism	2	–2.2
16	gb|AEP04475.1	Hypoxanthine phosphoribosyltransferase	Purine metabolism, Biosynthesis of secondary metabolites	2	–1.5
6	gb|AEP06176.1	Biotin synthetase	Biotin metabolism	1	–1.9

2.4 差异蛋白相互作用网络

蛋白相互作用网络(图 4)显示除 1 个较大的网络外，只有小部分的蛋白形成 4 个小网络。最大的蛋白相互作用网络的节点蛋白 HMPREF0010_00676 是丙酮酸脱氢酶复合体的丙酮酸脱氢酶(Pyruvate dehydrogenase complex dihydrolipoamide acetyltransferase)，可催化丙酮酸氧化脱羧，将乙酰单位转移到辅酶 A 形成乙酰辅酶 A 和降低胞内的 NAD^{+}[22–23]。HMPREF0010_00308 是乙酰辅酶 A 羧化酶，生物素羧化酶亚基(Acetyl-CoA carboxylase, biotin carboxylase subunit)，催化乙酰辅酶 A+ATP+HCO_3^-→丙二酰辅酶 A+ADP+Pi 反应的生物素酶，也是降低体内酰辅酶 A 的途径。此外，共同伴侣蛋白 HscA 和 HscB 可促进铁硫簇受体蛋白成熟。结果表明 HscA 和 HscB 可促进铁硫簇蛋白成熟。

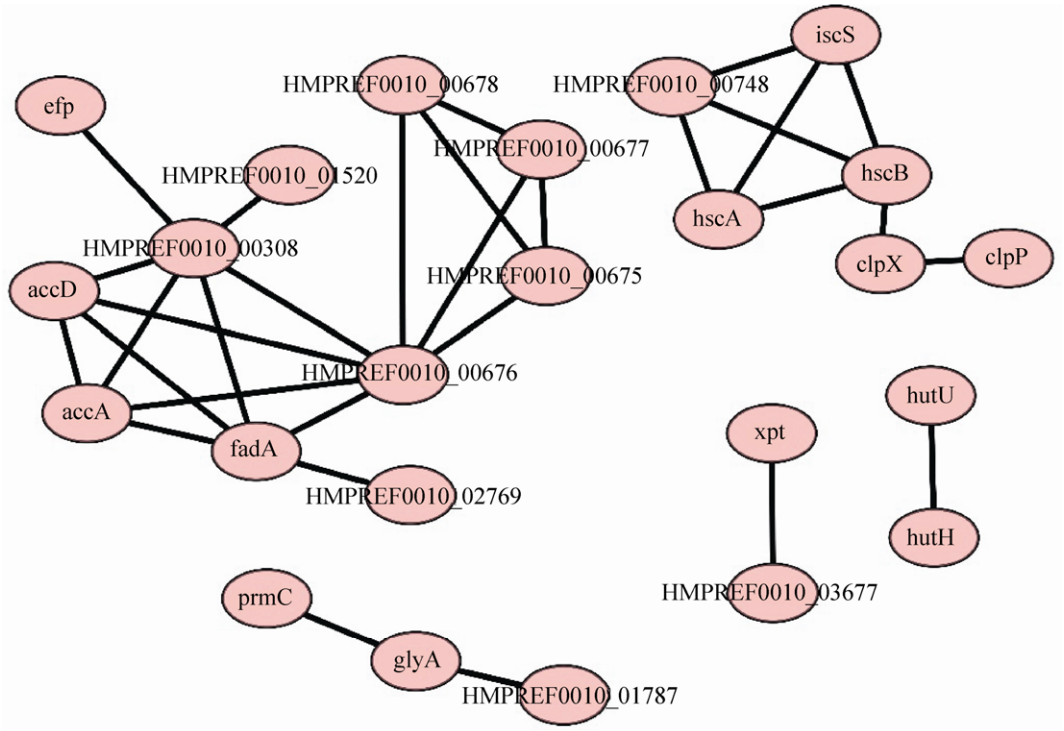

图 4. 差异蛋白相互作用网络

Figure 4. The protein-protein network of DEPs.

3 讨论

含有 bla_{NDM-1} 的鲍曼不动杆菌几乎能抵抗所有的抗生素。然而，bla_{NDM-1} 编码产物 NDM-1 如何影响鲍曼不动杆菌的代谢还是未知的。本研究通过全局分析 NDM-1 诱导细菌蛋白组学的改变以揭示 NDM-1 引起细菌耐药的原因。

NDM-1 虽然没有影响鲍曼不动杆菌的生长表型(图 1)，但是质谱的结果显示 NDM-1 降低了通路蛋白的表达(表 1)，从而使含 NDM-1 的细菌代谢减缓。这些通路蛋白(差异蛋白)主要参与到细菌碳代谢、氨基酸代谢、脂肪酸代谢、核酸代谢等。

碳代谢是细菌生存最主要的产能途径。有 10 个差异蛋白参与碳代谢途径，并被 bla_{NDM-1} 调节。三羧酸循环(Tricarboxylic acid cycle)是生物体最重要的能量代谢途径,并为细菌提供 NADH 和 $FADH_2$。在 ABC3229 中，三羧酸循环的限制酶——异柠檬酸脱氢酶(Isocitrate dehydrogenase)下调表达意味着在 NDM-1 存在的情况下，导致三羧酸减弱，从而使细菌产能减弱。

除了三羧酸循环外，脂肪酸代谢是生物体内另一个重要的供能途径。鉴定到 6 个与脂肪酸代谢相关的蛋白，都是α和β乙酰辅酶 A 的亚基，参与脂肪酸合成的第一步。在 NDM-1 存在时，鲍曼不动杆菌中这 2 个蛋白也下调表达。此外，NDM-1 的鲍曼不动杆菌的乙酰辅酶 A 脱氢酶(Acyl-CoA dehydrogenase)高表达，说明其负责的脂肪酸降解会加快。然而，烷基氢过氧化物还原亚基(Alkyl hydroperoxide reductase subunit)除了参与降解体内的脂肪酸，还在防御宿主的氧化应激方面起着至关重要的作用[24]。结果表明 NDM-1 降低了脂肪酸的合成，增加脂肪酸的降解，减弱了防御宿主的能力。

脂多糖(Lipopolysaccharide，LPS)是细菌毒力因子，是细菌致病的关键[25]。胞外的多糖聚β-(1-6)-N-乙酰氨基葡萄糖(Polysaccharide poly-beta-(1-6)-N-acetylglucosamine)在细菌细胞壁形成过程中扮演着一个重要的角色。在革兰氏阴性和革兰氏阳性细菌中，UDP-N-乙酰葡糖胺 2-差向异构酶(UDP-N-acetylglucosamine 2-epimerase)催化 UDP-N-acetylglucosamine (UDP-GlcNAc)和 UDP-N-acetylmannosamine (UDP-ManNAc)可逆的差向异构化[26–27]。这 2 个蛋白的活性形式 GlcNAc 和 ManNAc 在这个过程中产生，并参与细胞壁表面多糖的形成[27]，这些脂多糖保护细菌对抗宿主的免疫系统[28–29]。在鲍曼不动杆菌 ABC3229 中 UDP-GlcNAc 和 UDP-GlcNAc 下调表达，暗示着细胞壁多聚糖合成减少，从而使细胞壁合成也减缓，最终使含 NDM-1 的鲍曼不动杆菌的毒力也减弱。这也印证了之前研究的结论，MDR 菌株相对于 MDS 菌株毒力受损[30]。

铁是细菌生存和致病所必需的[31–34]。许多病原菌，比如链球菌分泌铁载体去螯合宿主的铁用于自身的生长。细菌表面特异受体吸收铁[35]。也有研究表明，在缺铁的情况下，相对于敏感株，耐多粘菌素的鲍曼不动杆菌生长严重受限[36]，加入铁后耐多粘菌素的鲍曼不动杆菌生长得更好，说明铁是耐药菌的生长必不可少的。Luciana F. Costa 发现鼠伤寒沙门氏菌通过摄取铁去帮助扩张宿主发炎的肠道，使宿主致病[31]。本研究发现铁载体(Ferric siderophore)在鲍曼不动杆菌中高表达(3.9 倍)，这些蛋白将会为鲍曼不动杆菌生长和致病募集更多的铁，将为鲍曼不动杆菌致病和抵抗抗生素作出贡献。

本研究发现含 NDM-1 会减弱细菌代谢和细胞壁的生物合成，从而减弱细菌的毒力。此外，还通过增加铁的摄入会提高细菌抵抗抗生素的能力，但是 NDM-1 是如何影响铁的摄取机制还是未知的。未来的研究将使用分子方法去揭示铁的摄取与细菌耐药的关系。本研究将会为 NDM-1 介导的多药耐药细菌抵抗抗生素提供一个更深的认识。

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Comparative proteomics reveals the role of NDM-1 in *Acinetobacter baumannii*

Guandi Zeng, Qian Xu, Wanting Liu, Xiaoyan Yang, Xuesong Sun*

Key Laboratory of Functional Protein Research of Guangdong Higher Education Institutes, Institute of Life and Health Engineering, College of Life Science and Technology, Jinan University, Guangzhou 510632, Guangdong Province, China

Abstract: [Objective] In order to investigate how NDM-1 affect the metabolism of *A. baumannii*, we analyzed the differentially expressed proteins (DEPs) between clinically isolated multidrug-resistant *A. baumannii* MDR-ZJ06 ($bla_{\text{NDM-1}}^{-}$) and ABC3229 ($bla_{\text{NDM-1}}^{+}$) which have a close genetic relationship. **[Methods]** We used 2-DE coupled with MALDI-TOF MS/MS to analyze the differentially expressed proteins (DEPs) between *A. baumannii* MDR-ZJ06 and ABC3229. Based on GO annotations, we analyze DEPs by KEGG, Function classification, enrichment analysis and protein-protein interaction network. **[Results]** We found that there were 51 DEPs, including 11 up-regulated proteins and 41 down-regulated proteins in ABC3229. These proteins were predominantly involved in reducing carbohydrate metabolism, amino acid metabolism, fatty acid metabolism, cell wall synthesis, and increasing of iron transport system formed. **[Conclusion]** These results uncover the effect of NDM-1 on bacteria may be resistant to antibiotics by slowing down the metabolism of bacteria and increasing the uptake of iron, thereby the bacteria will systemic resistance antibiotics.

Keywords: *Acinetobacter baumannii*, NDM-1, multidrug resistance, comparative proteomics

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*Corresponding author. Tel: +86-20-85226165; Fax: +86-20-85227039; E-mail: tsunxs@jnu.edu.cn

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