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卵巢高/低级别浆液性癌的定量蛋白质组学比较研究*

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摘要 目的：探讨卵巢高级别浆液性癌和低级别浆液性癌的差异表达蛋白，为阐明卵巢癌发生机制及寻找诊断和预后标志物的提供线索。**方法：**收集卵巢癌新鲜组织标本冻存于液氮中，经病理学确诊为高级别浆液性癌和低级别浆液性癌，两种类型各收集 15 例。应用 iTRAQ 定量蛋白质组学技术筛选及鉴定高/低级别浆液性癌的差异表达蛋白，并进行生物信息学分析。**结果：**卵巢高级别和低级别浆液性癌组织的定量蛋白质组学比较研究鉴定出差异表达蛋白 314 个，其中与低级别浆液性癌组比较，高级别浆液性癌组上调蛋白有 97 种，下调蛋白有 217 种。GO 分析显示这些差异蛋白在分子功能、生物学功能、细胞成分方面均具有一定分布特点。KEGG 分析显示这些差异蛋白涉及复杂的信号通路。**结论：**高/低级别浆液性癌之间存在差异表达蛋白，这些蛋白涉及复杂的功能和信号通路可能在两型卵巢癌发生机制及肿瘤生物学行为差异中具有重要意义。

关键词：卵巢癌；高级别浆液性癌；低级别浆液性癌；定量蛋白质组学

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Quantitative Proteomic Analysis of Differentially Expressed Proteins in High/low Grade Serous Ovarian Cancer*

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ABSTRACT Objective: To investigate the differentially expressed proteins between ovarian high-grade serous carcinomas and low-grade serous carcinomas, and to provide information for the elucidation of the pathogenesis of ovarian cancer, and for the identification of diagnostic and prognostic markers. **Methods:** Fresh tissue specimens of ovarian cancer were collected and kept in liquid nitrogen, which were diagnosed as high-grade serous carcinoma and low-grade serous carcinoma by both of pathologists, and 15 cases were collected in each of the two types. Screening and identification of differentially expressed proteins in serous carcinomas by iTRAQ quantitative proteomics technique and bioinformatics analysis. **Results:** Quantitative proteomics of high grade and low grade ovarian serous carcinoma showed that 314 differentially expressed proteins were identified . Compared with low-grade serous carcinoma group, high-grade serous carcinoma group have 97 up-regulated proteins , 217 down-regulated proteins. GO analysis showed that these differential proteins were distributed in molecular function, biological function and cell composition. KEGG analysis showed that these differential proteins were involved in complex signaling pathways. **Conclusion:** There are differentially expressed proteins between high and low serous carcinomas. These proteins are involved in complex functions and signaling pathways, which may play an important role in the pathogenesis of two type of ovarian cancer and biological differences in tumor behavior.

Key words: Ovarian cancer; High-grade serous carcinoma; Low-grade serous carcinoma; Quantitative proteomics

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前言

恶性上皮性肿瘤是卵巢肿瘤中最常见的类型，是妇科肿瘤中病死率最高的恶性肿瘤。目前有多个分级系统用于卵巢癌分级，如 FIGO 系统、WHO 系统、妇科肿瘤学组(Gynecologic Oncology Group，GOG)、通用分级系统(universal grading system)。近年来，一种二级分级系统用于卵巢癌并受到密切关注，该系统将卵巢癌分为低级别(Ⅰ型)和高级别(Ⅱ型)，该分级系统被认为应用方便、可重复性好，并且是基于不同类型间遗传学差异，尤其适用于浆液性癌。Ⅰ型为低级别浆液性腺癌，常由交界性肿瘤逐渐发生癌变，发展慢，较少进展为高级别肿瘤，预后较好。Ⅱ型为高级别浆液性腺癌，细胞异型性明显，大多无前驱病

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变,生长迅速,侵袭性强,预后差[1,2]。Ⅰ型及Ⅱ型上皮性卵巢癌在临床表现、预后等方面的差异提示二者可能具有不同的起源及遗传学改变,如Ⅰ型常伴有 KRAS 和 BRAF 基因突变,Ⅱ型常伴有 TP53 突变和 BRCA 基因异常[3]。为了进一步阐明低级别浆液性癌和高级别浆液性癌的分子遗传学差异,本研究采用定量蛋白质组学技术比较两型卵巢浆液性癌进行蛋白表达的差异。

1 材料与方法

1.1 实验样本

本研究所使用的卵巢癌组织标本取自湖南省肿瘤医院 2016 年 1 月至 8 月在妇瘤科行手术治疗的卵巢肿瘤患者,拟研究病例为临床诊断为卵巢癌并计划进行术中冷冻快速切片诊断的卵巢肿瘤患者,术前没有做过放疗、化疗等治疗。对于术中冰冻送检组织取一部分保存于液氮中,一部分经术中冷冻切片初步诊断为卵巢癌,再进行术后 HE 染色病理检查,以明确病理学类型。最终确定用于蛋白质组学的病例为高级别浆液性癌 20 例,低级别浆液性癌 20 例,患者年龄从 44-64 岁,中位年龄 53 岁。本研究所使用的 8 标 iTRAQ 试剂盒为 AB Sciex 公司(美国)产品。

1.2 实验方法[18]

1.2.1 iTRAQ 蛋白质组学技术 第一步进行细胞破碎及蛋白提取,将组织置于液氮中研磨,及至粉碎。加入裂解液,反复吹打裂解细胞。裂解后的细胞会释放出粘稠的物质,超声处理,冰上冷却,离心后收集上清液。第二步进行蛋白定量,取 BSA 制作标准曲线,双复管测定。575 nm 处测定吸光值。第三步蛋白酶切及 iTRAQ 试剂标记,第四步第一维高 pH-RP 液相分离,用第一维高 pH-RP 液相 A 相重溶,混匀上样,从线性梯度开始收集组分,反复循环接样;根据峰型和时间共收取 10 个组分,用 50%TFA 酸化,真空干燥后,进行第二维反相液质联用 RPLC-MS,肽段用样品溶解液(0.1%甲酸、2%乙腈)溶解,充分振荡涡旋,13200 rpm,4℃离心 10 min,上清转移到上样管中,进行质谱鉴定;样品通过 Dionex Ultimate3000 高效液相系统让多肽经过 C18 反向柱(100 μm i.d., 10 cm long, 3 μm resin from Michrom Bioresources, Auburn, CA)进行分离,分离后的肽段直接进入质谱仪 TripleTOF 5600 system (AB SCIEX)进行在线检测。广州辉骏生物技术有限公司为本研究提供蛋白质组学技术平台。

1.2.2 生物信息学分析 蛋白质检索软件为 Protein Pilot 4.0(AB Sciex),数据库下载网站链接:ftp://ftp.uniprot.org/pub/databases/uniprot/current_release/knowledgebase/proteomes/HUMAN.fasta.gz。对蛋白质鉴定基本流程为选出可信蛋白、根据分析模式组合数据、选出可分析蛋白、选出差异蛋白。本研究差异蛋白的选择以比值≥ 2 为上调蛋白标准,≤ 0.5 为下调蛋白标准。

1.2.3 基因本体分析(Gene Ontology, GO) 通过一套动态更新的标准词汇表来全面描述生物体中基因产物包括多肽和蛋白质的属性,分为分子功能、生物过程和细胞成分三部分。

1.2.4 京都基因与基因组百科全书(Kyoto Encyclopedia of Gene and Genome, KEGG) 在本研究中应用 KEGG 对差异表达蛋白所属信号通路进行分类研究。基本步骤如下,第一,打开 KEGG 结果文件夹;第二,点击 "index.html" 文件打开 KEGG 结果文件;第三,从打开的结果文件中选择与研究紧密相关或者感兴趣的通路;第四,展示所选择的通路。

2 结果

2.1 卵巢高/低级别浆液性癌中差异表达蛋白的定量蛋白质组学比较

应用 iTRAQ 蛋白质组学技术对卵巢高级别和低级别浆液性癌组织进行了蛋白组学比较分析,鉴定出可信蛋白(置信区间为 95%,即 unused score≥ 1.3)有 5588 种,以比值≥ 2 为上调蛋白标准,以≤ 0.5 为下调蛋白标准,共鉴定差异表达蛋白 314 个。与低级别浆液性癌组比较,高级别浆液性癌组上调蛋白共有 97 个,表 1 为差异最为显著的 30 种上调蛋白。与乳低级别浆液性癌组比较,高级别浆液性癌组下调蛋白共有 217 个,表 2 为差异最为显著的 30 种下调蛋白。

2.2 GO 分析卵巢高/低级别浆液性癌间差异表达蛋白

对差异表达蛋白的生物学过程进行 GO 分析,结果显示这些差异表达蛋白涉及 64 种生物学功能,其中包含蛋白数量最多前十种功能如图 1 所示,包括转运(transport)、细胞蛋白修饰过程(cellular protein modification process)、囊泡运输(vesicle-mediated transport)、细胞氮复合代谢过程(cellular nitrogen compound metabolic process)、免疫系统的过程(immune system process)、生物合成过程(biosynthetic process)、细胞分化(cell differentiation)、应急反应(response to stress)、信号转导(signal transduction)、解剖结构的发育(anatomical structure development)。其它细胞功能还包括了细胞粘附、细胞骨架结构、细胞死亡、细胞运动、细胞增殖、分解代谢过程、稳态过程、蛋白复合物的组装、小分子代谢过程等。

对差异表达蛋白进行细胞成分的 GO 分析,结果显示差异蛋白分属 26 种细胞成分,涉及蛋白数量最多的前十种细胞成分为核质(nucleoplasm)、细胞质囊泡(cytoplasmic vesicle)、细胞骨架(cytoskeleton)、胞外空间(extracellular space)、蛋白复合物(protein complex)、细胞核(nucleus)、质膜(plasma membrane)、细胞质(cytosol)、细胞质(cytoplasm)、胞外区(extracellular region),见图 2。其它细胞成分还包括内质网、高尔基体、线粒体、溶酶体、核染色质、内体、核膜等。

对差异表达蛋白进行分子功能的 GO 分析,结果显示差异蛋白的分子功能共有 35 种,根据涉及蛋白的数量最多的前十种分子功能为氧化还原酶活性(oxidoreductase activity)、肽酶活性(peptidase activity)、脂质结合(lipid binding)、DNA 结合(DNA binding)、酶调节活性(enzyme regulator activity)、RNA 结合(RNA binding)、结构分子活性(structural molecule activity)、酶结合(enzyme binding)、细胞骨架蛋白结合(cytoskeletal protein binding)、离子结合(ion binding),见图 3。其它分子功能还有 ATP 酶活性、信号转导活性、转录因子活性、解旋酶活性、跨膜转运蛋白活性、蛋白结合、核酸结合、异构酶活性、激酶活性、裂解酶活性、mRNA 结合、转运蛋白活性、组蛋白结合、转移酶活性等。

2.3 卵巢高/低级别浆液性癌差异性表达蛋白的 KEGG 通路分类分析结果

KEGG 通路分析结果显示差异表达蛋白与 197 条信号通路有关，包含蛋白数量最多的 10 条通路依次是代谢通路(30 种)、局部粘附(19 种)、紧密连接(11 种)、癌中的蛋白多糖(11 种)、细胞外基质受体相互作用(10 种)、补体和凝血级联反应(10 种)、PI3K-Akt 信号通路(10 种)、扩张型心肌病(10 种)、致心律失常性右室心肌病 (9 种)、阿米巴病 (9 种)、单纯疱疹感染(8 种)。其它通路包括蛋白质消化吸收、血管平滑肌收缩、肌动蛋白细胞骨架调节、DNA 复制、甘氨酸 / 丝氨酸和苏氨酸代谢、癌症通路等。

表 1 卵巢高级别浆液性癌较低级别浆液性癌的 30 种上调蛋白

Table 1 30 kinds of up-regulated proteins in high-grade ovarian serous carcinoma compared to low-grade serous carcinoma

Protein name	Acession num	Sequence coverage	Molecular weight	Isoelectric point	Ratio
Interferon-induced GTP-binding protein Mx1	sp|P20591|MX1_HUMAN	47.13	75.52	5.59	11.91
Hydroxymethylglutaryl-CoA synthase, cytoplasmic	sp|Q01581|HMCS1 HUMAN	15.77	57.29	5.21	8.43
Guanylate-binding protein 1	sp|P32455|GBP1_HUMAN	44.76	67.93	5.97	7.44
Signal transducer and activator of transcription 1-alpha/beta	sp|P42224|STAT1_HUMAN	52.47	87.33	5.74	7.28
DNA topoisomerase 2-alpha	sp|P11388|TOP2A_HUMAN	27.47	174.38	8.82	6.45
Stathmin	sp|P16949|STMN1_HUMAN	71.81	17.30	5.76	6.33
DNA replication licensing factor MCM2	sp|P49736|MCM2_HUMAN	31.53	101.89	5.34	6.30
Proliferation marker protein Ki-67	sp|P46013|KI67_HUMAN	18.94	358.69	9.48	6.13
Ferritin light chain	sp|P02792|FRIL_HUMAN	60.86	20.02	5.51	6.13
Galectin-4	sp|P56470|LEG4_HUMAN	21.21	35.94	9.21	5.82
Carbonic anhydrase 1	sp|P00915|CAH1_HUMAN	75.29	28.87	6.59	5.78
Hemoglobin subunit delta	sp|P02042|HBD_HUMAN	99.32	16.05	7.84	5.77
Amine oxidase [flavin-containing] A	sp|P21397|AOFA_HUMAN	39.19	59.68	7.94	5.68
Asparagine synthetase [glutamine-hydrolyzing]	sp|P08243|ASNS_HUMAN	24.60	64.37	6.39	5.59
Spectrin beta chain, erythrocytic	sp|P11277|SPTB1_HUMAN	41.18	246.46	5.14	5.42
DNA replication licensing factor MCM7	sp|P33993|MCM7_HUMAN	31.44	81.31	6.08	5.34
HLA class II histocompatibility antigen, DRB1-12 beta chain	sp|Q95IE3|2B1C_HUMAN	40.60	29.88	7.69	5.24
Antigen peptide transporter 2	sp|Q03519|TAP2_HUMAN	24.49	75.66	8.24	5.19
dCTP pyrophosphatase 1	sp|Q9H773|DCTP1_HUMAN	44.12	18.68	4.93	5.19
Proliferating cell nuclear antigen	sp|P12004|PCNA_HUMAN	43.68	28.77	4.57	5.03
Tryptophan--tRNA ligase, cytoplasmic	sp|P23381|SYWC_HUMAN	49.90	53.16	5.83	4.97
Probable ATP-dependent RNA helicase DDX58	sp|O95786|DDX58_HUMAN	30.65	106.59	6.03	4.55
Nuclear autoantigenic sperm protein	sp|P49321|NASP_HUMAN	32.68	85.24	4.26	4.40
DNA replication licensing factor MCM3	sp|P25205|MCM3_HUMAN	31.00	90.98	5.53	4.15
Interferon-induced protein with tetratricopeptide repeats	sp|P09914|IFIT1_HUMAN	39.75	55.36	6.75	4.11

Structural maintenance of chromosomes protein 2	sp|O95347|SMC2_HUMAN	27.95	135.65	8.54	4.08
Cellular retinoic acid-binding protein 2	sp|P29373|RABP2_HUMAN	67.76	15.69	5.42	3.83
Kunitz-type protease inhibitor 2	sp|O43291|SPIT2_HUMAN	17.86	28.23	8.68	3.81
E3 ubiquitin-protein ligase RNF213	sp|Q63HN8|RN213_HUMAN	22.13	591.40	6.05	3.79
Phosphoserine aminotransferase	sp|Q9Y617|SERC_HUMAN	40.00	40.42	7.56	3.75

表 2 卵巢高级别浆液性癌较低级别浆液性癌的 30 种下调蛋白

Table 2 30 kinds of down-regulated proteins in high-grade ovarian serous carcinoma compared to low-grade serous carcinoma

Protein name	Accession number	Sequence coverage	Molecular weight	Isoelectric point	Ratio
Serpin B3	sp|P29508|SPB3_HUMAN	22.56	44.56	6.35	0.03
Serotransferrin	sp|P02787|TRFE_HUMAN	81.09	77.06	6.81	0.05
Annexin A2	sp|P07355|ANXA2_HUMAN	89.97	38.60	7.57	0.05
Immunoglobulin heavy constant gamma 2	sp|P01859|IGHG2_HUMAN	76.30	35.90	7.66	0.05
Keratin, type I cytoskeletal 16	sp|P08779|K1C16_HUMAN	44.72	51.27	4.98	0.05
Tenascin-X	sp|P22105|TENX_HUMAN	36.89	458.21	5.05	0.06
Mucin-5B O	sp|Q9HC84|MUC5B_HUMAN	12.17	596.33	6.19	0.06
Tubulin polymerization-promoting protein family member 3	sp|Q9BW30|TPPP3_HUMAN	59.09	18.98	9.19	0.06
Neutrophil gelatinase-associated lipocalin	sp|P80188|NGAL_HUMAN	77.28	22.59	9.02	0.07
Cysteine and glycine-rich protein 1	sp|P21291|CSRP1_HUMAN	76.17	20.57	8.90	0.08
Prelamin-A/C	sp|P02545|LMNA_HUMAN	78.47	74.14	6.57	0.09
IgGFc-binding protein	sp|Q9Y6R7|FCGBP_HUMAN	12.92	572.01	5.14	0.09
Keratin, type I cytoskeletal 23	sp|Q9C075|K1C23_HUMAN	38.63	48.13	6.09	0.09
Keratin, type II cytoskeletal 5	sp|P13647|K2C5_HUMAN	39.66	62.38	7.58	0.09
Protein S100-A6	sp|P06703|S10A6_HUMAN	83.34	10.18	5.32	0.10
Keratin, type II cytoskeletal 6A	sp|P02538|K2C6A_HUMAN	47.16	60.04	8.09	0.10
Keratin, type I cytoskeletal 17	sp|Q04695|K1C17_HUMAN	62.50	48.10	4.97	0.10
Deleted in malignant brain tumors 1 protein	sp|Q9UGM3|DMBT1_HUMAN	15.67	260.73	5.18	0.10
Putative ciliary rootlet coiled-coil protein 2	sp|H7BZ55|CRCC2_HUMAN	47.83	185.87	5.42	0.11
Zinc finger protein 185	sp|O15231|ZN185_HUMAN	23.30	73.52	6.67	0.11
Tropomyosin beta chain	sp|P07951|TPM2_HUMAN	71.83	32.85	4.66	0.11
Filamin-A	sp|P21333|FLNA_HUMAN	73.37	280.73	5.70	0.12
Alpha-1-antitrypsin	sp|P01009|A1AT_HUMAN	82.54	46.73	5.37	0.12
Antithrombin-III	sp|P01008|ANT3_HUMAN	65.84	52.60	6.32	0.12
Collagen alpha-1(XIV) chain	sp|Q05707|COEA1_HUMAN	61.03	193.51	5.16	0.13
Periplakin	sp|O60437|PEPL_HUMAN	48.21	204.74	5.47	0.13
Complement factor H	sp|P08603|CFAH_HUMAN	39.81	139.09	6.21	0.13
Myosin-11	sp|P35749|MYH11_HUMAN	73.91	227.34	5.42	0.14
Band 4.1-like protein 2	sp|O43491|E41L2_HUMAN	56.21	112.59	5.34	0.14
Lactotransferrin	sp|P02788|TRFL_HUMAN	60.50	78.18	8.50	0.14

3 讨论

卵巢高级别和低级别浆液性癌代表了两种不同的疾病，具有不同的预后、检测与处理方法，并由此出现了卵巢癌发生的

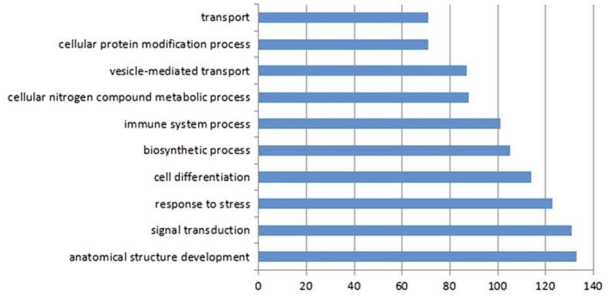

图 1 差异表达蛋白的生物学功能的 GO 分析

Fig.1 GO analysis of the biological functions of differentially expressed proteins

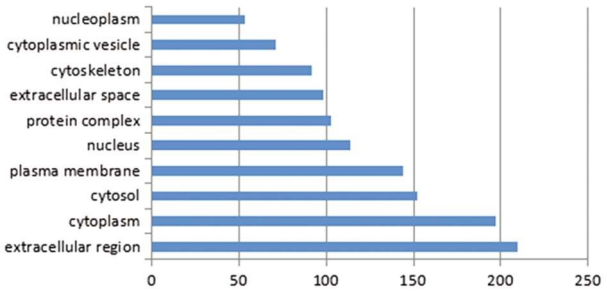

图 2 差异表达蛋白的细胞成分的 GO 分析

Fig.2 GO analysis of the cell composition of differentially expressed proteins

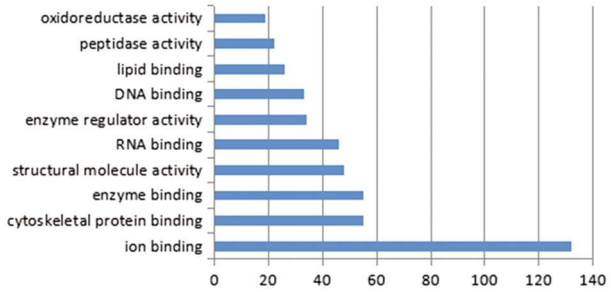

图 3 差异表达蛋白的分子功能的 GO 分析

Fig.3 GO analysis of the molecular function of differentially expressed proteins

二元论，对于高级别癌和低级别癌的发生机制的阐明以及在病理学上进行鉴别诊断及相关诊断标志物的研究显得尤为重要。基于蛋白质组学的高通量筛选的优点，本研究使用 ITRAQ 定量蛋白质组学比较研究卵巢高级别浆液性癌和低级别浆液性癌的差异表达蛋白。目前关于卵巢癌相关的蛋白质组学有一些报道，如高级别浆液性癌与浆液性囊腺瘤间的蛋白质组学和糖蛋白质组学研究[4]。高级别浆液性癌与健康人血清的蛋白芯片研究发现 30 多种抗原对卵巢癌的检测有一定意义[5]。高级别浆液性癌的基因组学和蛋白质组学研究涉及一些重要的科学问题如拷贝数的改变影响蛋白质组，与染色体不稳定有关的蛋白，不同基因组重排汇集的信号通路与生存期短密切相关的蛋白[6]。良性和恶性卵巢浆液性肿瘤的血清和组织标本蛋白质组研究发现载脂蛋白 A1 和血清转铁蛋白在卵巢癌的血清和组织中均较低[7]。此外，卵巢癌化疗耐药或化疗敏感性的组学研究也有报道[8-10]。但目前关于卵巢高级别浆液性癌和低级别浆液性癌的蛋白质组学研究少有报道。

通过 ITRAQ 定量蛋白质组学技术的筛选，本研究得到卵巢高级别浆液性癌和低级别浆液性癌的差异表达蛋白 314 种，以低级别癌为对照，高级别癌中上调蛋白 97 种，下调蛋白 217 种，并且对这些蛋白的细胞成分属性、分子功能和生物学功能进行分析以及其信号通路的归属。这些信息将为进一步大样本证实这些蛋白在高低级别中表达状态提供依据。目前关于卵巢高低级别浆液性癌的研究有一些报道，如低级别浆液性癌较高级别浆液性癌有更高的生存率和特殊的激素受体表达，如 ER、PR、AR 等[11]。EphA5 可能作为高低级别浆液性癌的标志物，其在高级别癌中表达更低[12]。TP53 突变在高级别浆液性卵巢癌中是常见的，TP53 突变可区分高、低级别浆液性癌，并作为以突变型 P53 为靶临床试验的重要标志物[13]。低级别浆液性癌有较高的患病率伴有 KRAS 和 BRAF 基因突变，但 TP53 基因突变率低，而高级别浆液性癌 TP53 突变率高[14]。B7-H4 是经常在浆液性卵巢癌中的表达，尤其是高级别浆液性癌中表达[15]。长链非编码 RNA asap1-it1、fam215a 和 linc00472 的表达在低级别肿瘤和早期病变中比高级别和肿瘤晚期更高[16]。高级别浆液性癌和低级别浆液性癌的肿瘤微环境及相关因子的表达也存在差别[17]。

本研究通过蛋白质组学比较研究筛选出的差异蛋白将更具有针对性，通过对其在不同类型卵巢癌中的功能进行研究，最终将发现一系列能有效鉴别高低浆液性癌的标志物。

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