

doi: 10.13241/j.cnki.pmb.2017.12.010

胰腺癌潜在肿瘤标志物的探索及在胰腺癌中的应用*

乔勃伟 殷若哲 方 诚 吕 行 唐明尧 付 航 陈 勇[△]

(第四军医大学西京医院肝胆胰脾外科 陕西 西安 710032)

摘要 目的:探索胰腺癌新的潜在标志物,建立夹心法 ELISA 体系,并初步应用于胰腺癌患者的血清检测。**方法:**应用基质辅助激光解吸电离飞行时间质谱(MALDI-TOF-MS)技术对胰腺癌患者术前术后血清进行分析,提纯分析差异蛋白并命名为 DAP44,通过杂交瘤技术制备出抗 DAP44 单克隆抗体,用 HRP 标记法标记抗体,间接 ELISA 法检测抗体滴度,用制备出的抗体对胰腺癌组织和癌旁组织进行组化染色,采用夹心 ELISA(DAS-ELISA)法制备抗 DAP44 检测试剂盒,检测胰腺癌病人和正常人血清 DAP44 值,比较两者差异。**结果:**对差异蛋白进行肽段测序和生物信息分析,并融合了 3 株能稳定分泌抗 DAP44 单克隆抗体的杂交瘤细胞 (2D6H5, 1E4D6, 5B8H12), 3 株杂交瘤细胞分泌的抗体效价均在 107 以上,通过抗体配对筛选确定以 2D6H5 为包被抗体, 1E4D6 为酶标抗体时, DAS-ELISA 法敏感性最高。两株抗体组化染色结果显示:癌组织 DAP44 表达量远高于癌旁。DAS-ELISA 法标准曲线线性范围在 0.78-25 ng/mL,检测下线为 0.78 ng/mL,此方法检测到的胰腺癌病人和正常人血清 DAP44 平均含量分别为 19.707 ± 1.464 和 10.653 ± 2.221 ,两者之间有统计学差异($P < 0.001$)。**结论:**DAP44 可能作为潜在的胰腺癌肿瘤标志物,建立的抗 DAP44 DAS-ELISA 法体系能够初步用于胰腺癌的临床诊断和疗效评估指标。

关键词:DAP44;基质辅助激光解吸电离飞行时间质谱;DAS-ELISA;胰腺癌

中图分类号:R-33; R446.62; R735.9 **文献标识码:**A **文章编号:**1673-6273(2017)12-2241-06

Detection of Potential Tumor Markers in Pancreatic Carcinoma and Its Application in Pancreatic Cancer*

QIAO Bo-wei, YIN Ruo-zhe, FANG Cheng, LV Xing, TANG Ming-yao, FU Hang, CHEN Yong[△]

(Xijing Hospital affiliated to The Fourth Military Medical University, Xi'an, Shaanxi, 710032, China)

ABSTRACT Objective: To find the potential tumor marker of pancreatic cancer and establish its sandwich ELISA system, and apply it to the detection of the serum of patients with pancreatic cancer. **Methods:** MALDI-TOF-MS was used to analyze the serum of preoperative and postoperative pancreatic cancer patients. The DAP44 protein was purified and purified by hybridoma technique. The anti-DAP44 monoclonal antibody was prepared by hybridoma technique. The antibody titer was detected by HRP labeling method. Antibody titer was detected by indirect ELISA. Histopathological staining of pancreatic cancer tissue and adjacent tissue was performed with the prepared antibodies. Anti-DAP44 kit was prepared by sandwich ELISA (DAS-ELISA) Pancreatic cancer patients and normal serum DAP44 values, compared the difference. **Results:** The differential protein peaks of preoperative and postoperative pancreatic cancer were detected and collected. Peptide sequencing and bioinformatics analysis were performed on the differentially expressed proteins. Three hybridoma cells stably secreting monoclonal antibodies against DAP44 (2D6H5, 1E4D6, 5B8H12, 3) hybridoma cells secreted antibody titers above 107, by antibody matching screening to determine 2D6H5 as coating antibodies, 1E4D6 for the enzyme labeled antibody. The results of histochemical staining showed that the expression of DAP44 in cancer tissues was much higher than that in adjacent tissues. The linear range of DAS-ELISA was 0.78-25 ng / ml and 0.78ng / ml, respectively. The detection of DAP44 in pancreatic cancer and The average content of DAP44 in normal group was 19.707 ± 1.464 and 10.653 ± 2.221 , respectively. There was significant difference between the two groups ($P < 0.001$). **Conclusion:** DAP44 could be used as a potential marker for the pancreatic cancer. The established anti-DAP44 DAS-ELISA system could be used for the clinical diagnosis and assessment of curative effect of pancreatic cancer.

Key words: DAP44; MALDI-TOF-MS; DAS-ELISA; Pancreatic cancer

Chinese Library Classification(CLC): R-33; R446.62; R735.9 **Document code:** A

Article ID: 1673-6273(2017)12-2241-06

前言

胰腺癌是 5 年生存率 <7% 的最具侵袭性的恶性肿瘤之一,由于诊断不足和治疗效果不佳,严重威胁人类的健康^[1]。据有关

* 基金项目:国家自然科学基金项目(81372607)

作者简介:乔勃伟(1988-),硕士研究生,主要研究方向:肝胆胰肿瘤,电话:18710905307, E-mail: 542583435@qq.com

[△] 通讯作者:陈勇,教授,主任医师,博士生导师,研究方向:肝胆胰肿瘤, E-mail: shuxi988@163.com

(收稿日期:2016-11-22 接受日期:2016-12-15)

组织估计,胰腺癌在未来十年内将发展成为人类癌症相关死亡的第二大原因^[2]。胰腺癌治疗的唯一有效手段是早期行根治性手术^[3]。然而,由于早期症状无特异性以及早期诊断手段不足,只有约 15-20%的患者能够及时行根治性手术,而晚期病人生存率低于 30%。与正常人相比,胰腺癌患者的预期寿命降低了 98%^[4]。如果能够早期发现胰腺癌,行根治性手术,病人的生存率将大幅提高^[5]。因此,早期诊断对于胰腺癌病人而言至关重要^[6]。

胰腺细胞从初始的恶性克隆到癌症诊断往往需要多年的时间^[7]。一方面,由于胰腺的解剖位置导致在早期并无特异性症状的出现,患者无法及时就诊;另一方面,现有的诊断技术如 B 超、CT、MRI、MRCP、ERCP、血液检查等对早期胰腺癌筛选缺乏特异性^[8]。因此,探索新的胰腺癌肿瘤标志物对胰腺癌的治疗有着重要的意义。本课题采用 MALDI-TOF-MS 技术对胰腺癌病人术前、术后血清进行分析,发现差异蛋白,通过肽段测序,人工合成该多肽,并根据生物信息分析命名为 DAP44,制备出单克隆抗体,建立起抗 DAP44 DAS-ELISA 体系,对临床胰腺癌病人血清进行检测,结果如下。

1 材料与方法

1.1 实验材料

1.1.1 试剂与器械 MALDI-TOF-MS 质谱仪(Bruker 公司),层析柱及填料(TOSOH 公司),HT、HAT、DMEM 培养基和胎牛血清(Hyclone 公司),羊抗鼠 HRP 酶标二抗、TMB 显色液、聚乙二醇、弗式佐剂(Sigma 公司),96 孔酶标板(Costar 公司),其他生化试剂为国产试剂。

1.1.2 临床血清样本 50 份胰腺癌病人术前、术后血清来自西京医院肝胆外科,胰腺癌病人与正常人血清各 40 份来自西京医院检验科。

1.1.3 抗原合成及实验动物 DAP44 抗原由上海吉尔公司测序,并合成,纯度大于 98%,BALB/c 小鼠购自第四军医大学实验动物中心,SP2/0 小鼠骨髓瘤细胞株来自第四军医大学生物技术中心实验室。

1.2 实验方法

1.2.1 DAP44 发现、测序及合成 用 MALDI-TOF-MS 技术对 50 名胰腺癌术前、术后血清进行质谱分析,发现表达差异蛋白后,对该峰值蛋白进行收集,送至上海吉尔公司进行氨基酸序列测定,用固体合成法合成 DAP44。同时,将此 DAP44 表位序列与锁孔蛋白(KLH)偶联成免疫原 DAP44-KLH 多肽。

1.2.2 抗 DAP44 抗体的制备和纯化 用免疫原 DAP44-KLH 多肽免疫 BALB/c 小鼠,取小鼠脾细胞与 SP2/0 细胞融合,经过多次单克隆筛选,获得杂交瘤细胞。将杂交瘤细胞注射进 BALB/c 小鼠腹腔,制备出小鼠腹水抗体,通过二乙氨基乙基-葡聚糖 A-50(DEAE-Sephadex A-50)柱对抗体进行纯化。采用间接 ELISA 法对纯化抗体进行抗体效价和特异性检测。

1.2.3 辣根过氧化物酶标记抗体 采用简易过碘酸钠法对纯化抗体进行标记。步骤如下:(1)称取 5 mg HRP 溶于 1 mL 蒸馏水中。(2)将上述溶液中加入 0.2 mL 新配置的 0.1M NaIO₄ 溶液,室温下避光搅拌 30 min。(3)将混匀后溶液装入透析袋中,用 1 mM, pH4.4 的醋酸钠缓冲液进行透析,4℃过夜。(4)次日用 20 μL 10.2M, pH9.5 碳酸盐缓冲液调整 pH 到 9.0-9.5,立即加

入 10 mg 纯化抗体,室温避光轻轻搅拌 2 h。(5)加新配的 0.1 mL NaBH₄(4 mg/mL),混匀,再置 4℃ 2 h。(6)再将其装入透析袋中,用 0.15M pH7.4 PBS 透析,4℃过夜。(7)搅拌下缓慢加入等体积饱和硫酸铵,置 4℃ 1 h。(8)用 3000 rpm 离心上述溶液 30 min,弃上清。用半饱和硫酸铵洗沉淀物 2 次后,将其溶于少量 0.15M pH7.4 的 PBS 中。(9)将上述溶液装入透析袋中,用 0.15M pH7.4 的 PBS 缓冲液透析,去除铵离子后,10000 rpm 离心 30 min 去除沉淀物,取得上清液即为酶结合物,加甘油后分装,冰冻保存。用双向琼脂扩散试验和直接 ELISA 法对酶结合物进行定性和效价测定。

1.2.4 抗 DAP44 DAS-ELISA 法测定流程 (1)包被:用 PH9.6 0.05 碳酸盐包被抗体 2D6H5 (10 μg/mL),100 μL/孔,对照组 100 μL 包被液,4℃过夜,(2)洗板:倒掉孔内液体,加洗涤缓冲液,静置 5 min,在吸水毛巾上拍尽缓冲液,如此三次,(3)封闭:用 5%的脱脂奶粉加满每孔,37℃水中孵育 2 h,洗板三次,如步骤(2),(4)加样:选取 8 孔,第一孔滴加 25 ng/mL DAP44,一次倍比稀释,第 8 孔浓度 0.78 ng/mL,设置副孔,结果做标准曲线,在剩余孔中设置空白孔,阴性和加样孔,100 μL/孔,37℃水中孵育 1 h,洗板 3 次,方法同上,(5)加 1E4D6-HRP:空白孔滴加抗体稀释液 100 μL,余加 100 μL 1E4D6-HRP(1:1000),置于 37℃水浴锅孵育 1 h,洗板 3 次,(6)显色:滴加 TMB 100 μL/孔,避光置于 37℃水浴箱 15 min,(7)终止:滴加 2 M 100 μL H₂SO₄至每孔,(8)测定结果:酶标仪设置检测波长 450 nm,调零空白孔后读取各孔 OD 值。(9)阳性判定:OD 值大于阴性空 2.1 倍视为阳性。

1.2.5 临床血清样本检测 用上述方法检测各 40 份的胰腺癌和正常人血清 DAP44 的 OD 值。

1.3 统计学方法

采用 SPSS19.0 软件对实验数据进行处理和统计学分析,以均数±标准差($\bar{x} \pm s$)表示计量资料,用独立样本 t 检验进行两组比较,P<0.05 认为差异有统计学意义。

2 结果

2.1 DAP44 的发现、测序和合成

如图 1 所示,利用 MALDI-TOF-MS 质谱技术对胰腺癌患者术前和术后血清糖蛋白进行质谱分析,发现胰腺癌患者的术前血清中有一个明显的高强度峰值,而在术后峰值较低。对此峰值蛋白进行提取测序,测序结果如下:Met-Leu-Glu-Thr-Leu-Arg-Glu-Arg-Leu-Leu-Ser-Val-Gln-Gln-Asp-Phe-Thr-Ser-Gly-Leu-Lys-Thr-Leu-Ser-Asp-Lys-Ser-Arg-Glu-Ala-Lys-Val-Lys-Ser-Lys-Pro-Ser-Trp-Leu-Asn-Gln-Glu-Gln-Phe。发现其为一段长度为 44 个氨基酸的多肽,该多肽的前 26 个氨基酸序列与 DTNBP1 编码的精神分裂症相关蛋白 Dysbindin-1 的 N 端序列完全一致,提示该多肽的编码基因可能与 DTNBP1 高度同源,故命名为 DTNBP1 Associated Peptide (DAP44)。

2.2 DAP44 单克隆抗体效价的测定

用间接 ELISA 法对融合的三株杂交瘤细胞产生的腹水进行检测,结果如表 1 所见,抗体效价分别是:2D6H5 株 10⁸、1E4D6 株 10⁸、5B8H12 株 10⁷。

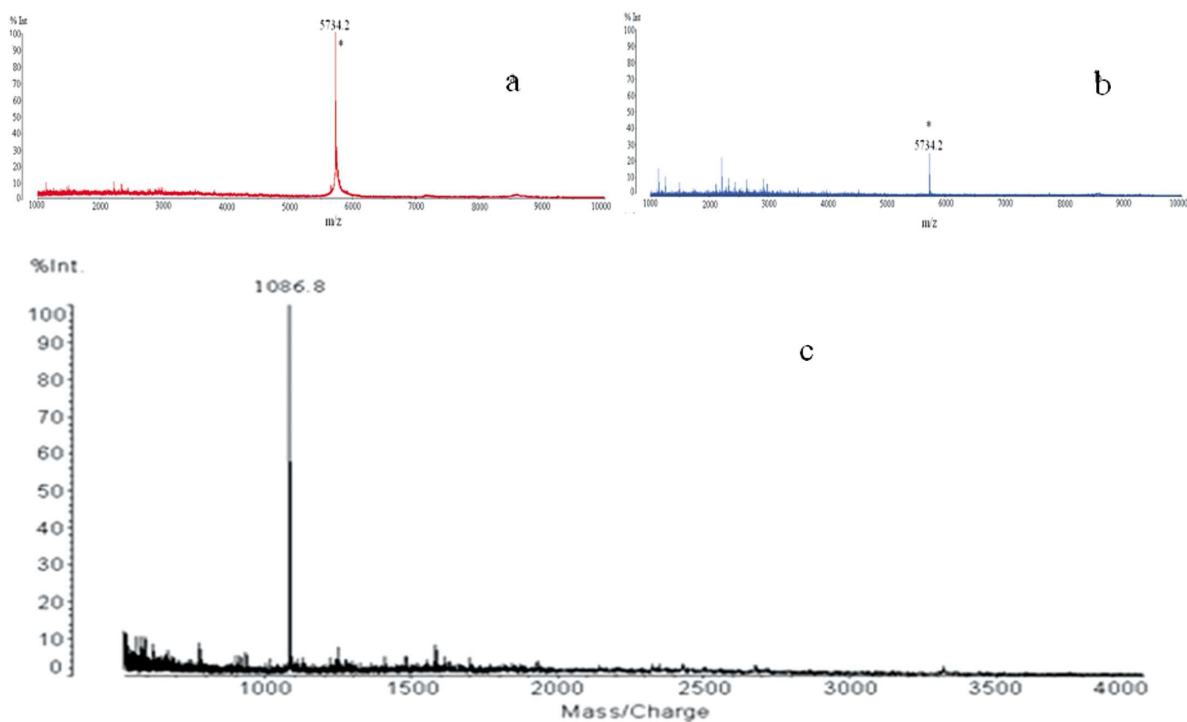


图 1 a,b 为胰腺癌术前,术后质谱,c 差异蛋白质谱峰

Fig.1 a, b for pancreatic cancer before and after mass spectrometry, c differential protein spectrum peak

表 1 BALB/c 小鼠腹水抗体效价测定

Table 1 BALB/c mice ascites antibody titer determination

Dilution ratio	Anti-DAP44 antibody		
	2D6H5	1E4D6	5B8H12
1:101	3.006	2.998	2.972
1:102	2.975	2.943	2.961
1:103	2.897	2.903	2.884
1:104	2.707	2.877	2.520
1:105	2.456	2.521	1.623
1:106	1.062	1.221	0.910
1:107	0.514	0.515	0.227
1:108	0.241	0.229	0.144
Negative control	0.074	0.061	0.069
Blank control	0.021	0.018	0.030

Note: positive determination: OD value is 2.1 times higher than the negative control. Coated DAP44 10ug / ml 100 μ L / well.

2.3 配对抗体的筛选

用 DAS-ELISA 法及正交实验筛选配对抗体,结果如表 2 所示,当以 2D6H5 为包被抗体,1E4D6 为酶标抗体时,检测 DAP44 的 OD 值最大,表现出好的敏感度。

2.4 组化染色

用 2D6H5 和 1E4D6 单克隆抗体分别对胰腺癌组织和癌旁组织进行组化染色,结果如图 2 所示,胰腺癌组织 DAP44 的表达高于癌旁组织。

2.5 DAS-ELISA 法体系条件的确立

2D6H5 为包被抗体,1E4D6-HRP 为酶标抗体,采用棋盘滴

定法确立 DAS-ELISA 法的条件。结果如表 3 所示:当 2D6H5 浓度为 10 μ g/mL,1E4D6-HRP 为 1:1000 时,OD 值为 2.948, DAS-ELISA 法体系检测 DAP44 的敏感性和特异性最高。

2.6 标准曲线的绘制

用上述建立的 DAS-ELISA 法体系检测 DAP44,将 DAP44 按照初始浓度为 50 ng/mL 倍比稀释 8 次,最终浓度为 0.39 ng/mL,测 OD 值如表 4 所示,当 DAP44 浓度在 0.75~25 ng/mL 时,检测值有良好的梯度,且结果都为阳性。根据上述结果,以 OD450 值为横坐标,DAP44 浓度为纵坐标绘制标准曲线如图 3 所示,曲线拟合度 $R^2=0.9992$ 。

表 2 配对抗体的筛选结果
Table 2 Results of paired antibody screening

Coated antibody	HRP-McAb	OD value	Control
2D6H5	1E4D6	2.435	0.056
	5B8H12	1.422	0.054
1E4D6	2D6H5	1.855	0.057
	5B8H12	0.938	0.057
5B8H12	2D6H5	0.893	0.051
	1E4D6	0.689	0.053

Note: Detection of antigen: DAP44 10 μg/mL, 100 μL/well.

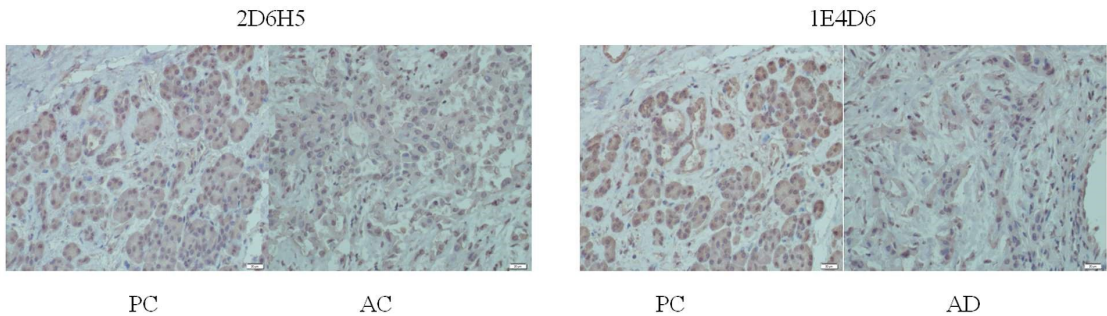


图 2 组化染色(× 40)

Fig.2 Histochemical staining

Note: PC: pancreatic cancer;AC:Adjacent to the cancer.

表 3 包被和酶标抗体浓度确定结果
Table 3 Results of coating and enzyme-labeled antibody concentration determinations

Coated antibody(μg/mL)	HRP-McAb			Control
	1:500	1:1000	1:2000	
20	3.001	2.998	2.602	0.055
10	3.027	3.031	2.126	0.052
5	1.723	1.476	0.828	0.053

Note: Detection of antigen: DAP44 10μg/mL, 100 μL/well.

表 4 DAS-ELISA 法检测 DAP44 上下限
Table 4 DAS-ELISA detection of DAP44 upper and lower limits

Concentration(ng/ml)	50	25	12.5	6.25	3.13	1.57	0.78	0.39	Negative
OD value	2.64	2.65	1.41	0.71	0.46	0.31	0.22	0.13	0.06

Note: The hole OD values are taken 7-hole average.

2.7 临床血清检测

用建立的 DAS-ELISA 法体系检测胰腺癌和正常人血清各 40 例,检测结果如表 5。40 例胰腺癌病人 DAP44 的平均含量为 19.707± 1.464,正常人的平均含量为 10.653± 2.221,两组之间有统计学意义(P<0.001),如图 4 所示。

3 讨论

胰腺癌由于早期缺乏明显症状,被诊断时多已经处于进展期^[9]。不幸的是,目前还没有有效的影像学和检验学方法能够特异的检测出早期胰腺癌,现在常用的肿瘤标志物是碳水化合物抗原 19-9(CA19-9),但是研究表明其特异性不高,所以寻找新

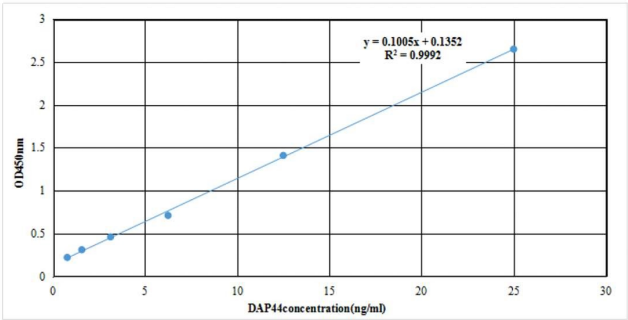


图 3 DAS-ELISA 法标准曲线

Fig.3 DAS-ELISA standard curve

表 5 DAS-ELISA 法检测胰腺癌和正常人血清
Table 5 DAS-ELISA detection of the serum of pancreatic cancer and normal pancreas

PC				NC			
Concentration	ODvalue	Concentration	ODvalue	Concentration	ODvalue	Concentration	ODvalue
19.745	2.119	21.024	2.248	11.226	1.263	0.952	0.231
17.924	1.936	21.964	2.342	0.939	0.231	12.003	1.341
21.635	2.309	20.458	2.191	0.987	0.234	10.054	1.145
18.985	2.043	19.652	2.111	10.346	1.175	0.974	0.233
17.998	1.944	18.304	1.975	12.012	1.342	10.876	1.228
22.001	2.346	16.021	1.743	11.367	1.277	11.224	1.263
20.153	2.161	21.904	2.336	0.942	0.231	0.988	0.235
20.364	2.182	19.213	2.066	0.981	0.234	10.248	1.165
19.456	2.091	18.326	1.977	10.021	1.142	10.795	1.221
18.354	1.981	19.887	2.134	0.986	0.234	0.981	0.234
18.621	2.006	20.846	2.231	10.007	1.141	12.345	1.376
17.999	1.944	21.859	2.332	11.168	1.270	11.482	1.289
18.243	1.968	17.879	1.932	0.994	0.235	10.978	1.238
19.426	2.087	21.058	2.252	12.018	1.343	10.634	1.204
20.324	2.178	19.541	2.099	11.961	1.337	11.425	1.283
18.397	1.984	22.023	2.348	0.982	0.234	0.954	0.231
18.032	1.947	18.496	1.994	10.957	1.236	10.006	1.141
19.312	2.076	19.548	2.101	12.215	1.363	10.725	1.216
21.369	2.283	20.318	2.177	0.968	1.233	11.009	1.242
17.201	1.864	20.008	2.146	10.637	1.204	0.994	0.235

Note: PC: pancreatic cancer; NC: normal control.

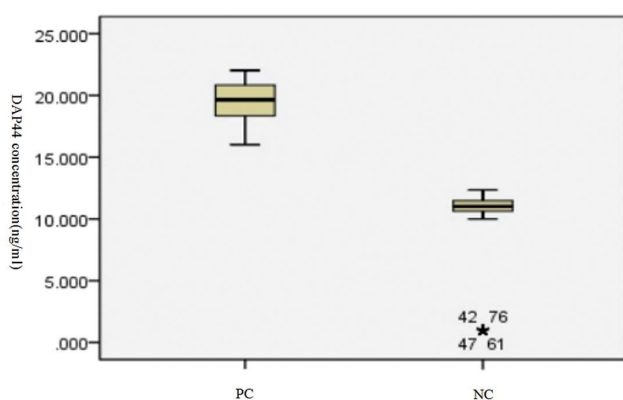


图 4 血清样本 DAP44 浓度($P<0.001$)

Fig.4 Serum sample DAP44 concentration

Note: PC: pancreatic cancer; NC: normal control.

的特异性和敏感性较高的标志物非常迫切^[10]。目前,多通过基因组学、转录组学、蛋白组学及代谢组学等技术寻找新的特异性和敏感性高的肿瘤标志物^[11]。通过上述技术发现了过表达的 microRNA^[12]、表观遗传的改变如循环核小体的甲基化^[13]、游离核算如 ctDNA^[14]及循环胰腺细胞(CPCs)^[15]等,但是,这些新的发现并没有改善胰腺癌病人的生存率,世界各地的专家们仍在协

同合作,希望能找到检测胰腺癌的可靠的生物标记物^[16]。

本课题通过 MALDI-TOF-MS 质谱技术对胰腺癌患者术前术后血清进行分析,发现其血清存在差异表达的蛋白峰,对此峰蛋白进行提前分析测序和生物信息分析,证实此蛋白是一段由 44 个氨基酸组成的多肽,此多肽的编码序列与精神分裂症相关蛋白的编码基因 DTNBP1 可能高度同源,故命名为 DAP44。DTNBP1 被认为是精神分裂症最可能易感的基因之一^[17],目前对它的研究多集中于其编码蛋白 Dysbindin-1 功能上面。有研究证明 Dysbindin-1 能够减弱 neclin 对 p53 转录活性的抑制作用^[18],也有研究显示 Dysbindin-1 能够作为检测胰腺癌的新的肿瘤标志物,并优于 CA19-9^[19],但是我们在 TCGA 数据库对胰腺癌和正常人的 DTNBP1 的表达进行对比分析,发现两组之间 DTNBP1 的表达并没有统计学差异。因此,DAP44 对胰腺癌检测具有一定的研究意义。

本研究在质谱分析的基础上合成了 DAP44,制备出抗 DAP44 单克隆抗体,并对胰腺癌组织和癌旁组织进行了组化染色,发现癌组织中 DAP44 的表达强于癌旁组织,同时成功建立起了能检测 DAP44 的双夹心 ELISA 体系,并检测了胰腺癌和正常人血清 40 例,结果显示胰腺癌患者血清 DAP44 的表达水平明显高于正常人,差异有统计学意义。因此,我们初步认

为 DAP44 是胰腺癌的一个潜在血清生物标志物。我们下一步将扩大检测样本量,同时与 CA19-9 进行对比研究,对胰腺癌患者的诊断、临床治疗以及预后进行长期随访,进一步去验证 DAP44 在胰腺癌诊断中的价值和意义。

参考文献(References)

- [1] Hessmann E, S A Johnsen, J T Siveke, et al. Epigenetic treatment of pancreatic cancer: is there a therapeutic perspective on the horizon? [J]. Gut, 2016, 10(1136): 1468-1488
 - [2] Tumas J, K Kvederaviciute, M Petrulionis, et al. Metabolomics in pancreatic cancer biomarkers research [J]. Med Oncol, 2016, 33(12): 133-147
 - [3] Baines A T, P M Martin, C J Rorie, et al. Current and Emerging Targeting Strategies for Treatment of Pancreatic Cancer[J]. Prog Mol Biol Transl Sci, 2016, 144(11): 277-310
 - [4] Carrato A, A Falcone, M Ducreux, et al. A Systematic Review of the Burden of Pancreatic Cancer in Europe: Real-World Impact on Survival, Quality of Life and Costs [J]. J Gastrointest Cancer, 2015, 46(3): 201-211
 - [5] Wang W, Z Sun, J Deng, et al. Integration and analysis of associated data in surgical treatment of gastric cancer based on multicenter, high volume databases [J]. Chinese Journal of Gastrointestinal Surgery Chin J Gastrointest Surg, 2016, 19(2): 179-185
 - [6] Liu J B and M S Baker, Surgical Management of Pancreatic Neuroendocrine Tumors[J]. Surg Clin North Am, 2016, 96(6): 1447-1468
 - [7] Yachida S, S Jones, I Bozic, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer [J]. Nature, 2014, 467(31): 1114-1127
 - [8] Takai E, S Yachida. Circulating tumor DNA as a liquid biopsy target for detection of pancreatic cancer[J]. World J Gastroenterol, 2016, 22(38): 8480-8488
 - [9] Martinez-Useros J, J Garcia-Foncillas. Can Molecular Biomarkers Change the Paradigm of Pancreatic Cancer Prognosis? [J]. Biomed Res Int, 2016, 20(16): 4873-4899
 - [10] Herreros-Villanueva M, L Bujanda. Non-invasive biomarkers in pancreatic cancer diagnosis: what we need versus what we have [J]. Ann Transl Med, 2016, 4(7): 134-157
 - [11] Yee N S. TRPM8 Ion Channels as Potential Cancer Biomarker and Target in Pancreatic Cancer [J]. Adv Protein Chem Struct Biol, 2016, 10(4): 127-155
 - [12] Bloomston M, Frankel W L, Petrocca F, et al. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis [J]. JAMA, 2007, 29(7): 1901-2228
 - [13] Bauden M, D Pamart, D Ansari, et al. Circulating nucleosomes as epigenetic biomarkers in pancreatic cancer [J]. Clin Epigenetics, 2015, 21(7): 106-120
 - [14] Singh N, Gupta S, Pandey RM, et al. High levels of cell-free circulating nucleic acids in pancreatic cancer are associated with vascular encasement, metastasis and poor survival [J]. Cancer Invest, 2015, 33(9): 78-85
 - [15] Kulemann B, Pitman M B, Liss AS, et al. Circulating tumor cells found in patients with localized and advanced pancreatic cancer [J]. Pancreas, 2015, 44(7): 547-560
 - [16] Chari S T, Kelly K, Hollingsworth M A, et al. Early detection of sporadic pancreatic cancer: summative review [J]. Pancreas, 2015, 44(4): 693-712
 - [17] Straub R E, Jiang T H, C J MacLean, et al. Genetic variation in the 6p22.3 gene DTNBP1, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia [J]. Am J Hum Genet, 2002, 71(2): 337-348
 - [18] Ma X, E Fei, C Fu, et al. Dysbindin-1, a schizophrenia-related protein, facilitates neurite outgrowth by promoting the transcriptional activity of p53 [J]. Mol Psychiatry, 2011, 16(11): 1105-1116
 - [19] Guo X, X Lv, C Fang, et al. Dysbindin as a novel biomarker for pancreatic ductal adenocarcinoma identified by proteomic profiling [J]. Int J Cancer, 2016, 139(8): 1821-1829
-
- (上接第 2337 页)
- [11] Jurgens C Y, Goodlin S, Dolansky M, et al. Heart Failure Management in Skilled Nursing Facilities, A Scientific Statement From the American Heart Association and the Heart Failure Society of America [J]. Journal of Cardiac Failure, 2015, 21(4): 263-299
 - [12] Khan H, Kunutsor S, Rauramaa R, et al. Cardiorespiratory fitness and risk of heart failure: a population-based follow-up study [J]. European Journal of Heart Failure, 2014, 16(2): 180-188
 - [13] Wei Y, Liu X, Hou L, et al. Qiliqiangxin affects L type Ca^{2+} current in the normal and hypertrophied rat heart [J]. Evidence-based complementary and alternative medicine: eCAM, 2011, 2012(2): 131-830
 - [14] Ding M, Yang P. Effects of Qiliqiangxin capsule on chronic congestive heart failure in patients [J]. Heart, 2010, 96(Suppl 3): A103-A104
 - [15] Saltzman H E. Arrhythmias and Heart Failure [J]. Cardiology Clinics, 2014, 32(1): 125-133
 - [16] Chyu J, Fonarow G C, Tseng C H, et al. Four-variable risk model in men and women with heart failure [J]. Circulation Heart Failure, 2014, 7(1): 88-95
 - [17] Dery A S, Hamilton L A, Starr J A. Nebivolol for the treatment of heart failure [J]. Expert Opinion on Investigational Drugs, 2011, 20(12): 1733-1746
 - [18] Nakayama M, Fuse K, Sato M, et al. Cardiogenic shock induced by bisoprolol in a patient with CYP2D6 variant. A case report [J]. Journal of Cardiology Cases, 2015, 11(4): 109-112
 - [19] Rau T, Düngen H, Edelmann F, et al. Impact of the β 1-Adrenoceptor Arg389Gly Polymorphism on Heart-Rate Responses to Bisoprolol and Carvedilol in Heart-Failure Patients [J]. Clinical Pharmacology & Therapeutics, 2012, 92(1): 21-28
 - [20] Madamanchi C, Alhosaini H, Sumida A, et al. Obesity and natriuretic peptides, BNP and NT-proBNP: Mechanisms and diagnostic implications for heart failure [J]. International Journal of Cardiology, 2014, 176(3): 611-617
 - [21] Zou Y, Lin L, Ye Y, et al. Qiliqiangxin inhibits the development of cardiac hypertrophy, remodeling, and dysfunction during 4 weeks of pressure overload in mice [J]. Journal of Cardiovascular Pharmacology, 2012, 59(3): 268-280