乳腺癌单克隆抗体 AF9 抗原的特性及分布*

蔡桂英　曾文杰

（华西医科大学生物化学教研室，成都 610041）

摘要　对抗人乳腺癌单抗 AF9 识别的抗原特性及分布进行了研究，结果表明 AF9 抗原是由糖、脂及蛋白质组成的复合蛋白质，不耐热；AF9 识别的抗原决定簇不存在于铁蛋白及癌胚抗原；蛋白质印迹检测表明 AF9 识别的抗原有 4 种成分，分子量分别为 51 000，56 000，67 000，73 000. 免疫组化 ABC 染色显示该抗原主要存在于乳腺癌细胞的胞浆及胞膜，在部分其它种类肿瘤组织中也可检测到，但在所检正常组织中未见到. AF9 抗原可能是新的乳腺肿瘤相关抗原.

关键词　乳腺癌，肿瘤相关抗原，单克隆抗体

单克隆抗体现已广泛用于乳腺肿瘤相关抗原的研究中，国外这方面的研究较多[1-3]，国内未见报道. AF9 是以乳腺癌原发灶细胞膜作免疫原制备的单克隆抗体，初步研究表明，AF9 与乳腺癌细胞膜呈阳性反应而与正常乳腺及肝细胞膜不反应. 为了对 AF9 抗原有一定的认识，作者对其特性进行了研究，并对其在人体正常组织及肿瘤组织中的分布进行了观察.

1　材料与方法

1.1　组织标本

各类肿瘤组织取自近年的手术切除标本，正常组织取自尸检标本，均经病理检查确诊.

1.2　AF9 抗体

将分泌特异性 AF9 抗体的杂交瘤细胞注入 BALB/C 小鼠腹腔，10d 后收集血清及腹水，以硫酸铵沉淀法纯化.

1.3　乳腺癌细胞膜抗原的提取

新鲜乳腺癌组织去除脂肪、结缔组织及坏死组织后，以 1∶10（W/V）在缓冲液（Tris 50mmol/L，蔗糖 250mmol/L，PMSF 0.5mmol/L，DDT 1mmol/L，pH 7.2）中匀浆，1000×g 4℃离心 10min，上清液于 7 5000×g 4℃离心 1h，沉淀溶解，超声波处理，测蛋白含量及 5′-核苷酸酶活性.

1.4　AF9 对应抗原性质的研究

用间接 ELISA 法观察高碘酸钠、甲醇、胰蛋白酶及煮沸 4 种因素对抗原活性的影响[4].

1.5　AF9 对应抗原与铁蛋白及癌坯抗原的相关性

用间接型竞争 ELISA 法检测这两种恶性肿瘤相关抗原与 AF9 抗原的关系.

1.6　蛋白质印迹检测

按 Laemmli 的方法[5]乳腺癌细胞膜抗原与标准分子量以 5%—20%梯度胶进行 SDS-PAGE 分析，凝胶再按 Towbin[6]的方法将蛋白质转移至硝酸纤维薄膜（Bio-Rad）上，用间接免疫过氧化物酶技术显色.

1.7　免疫组化 ABC 染色

除有 13 例乳腺癌标本为冰冻切片外其余组织标本均用常规石腊包埋、切片. 按 ABC 染色法（Vector）染色，以细胞膜或胞浆呈棕色为阳性细胞，按阳性细胞数及染色强度分四级标准判断结果，分别标为：－（无阳性细胞）、＋（棕色颗粒少，阳性细胞数少于 25%）、＋＋（棕色颗粒中量，阳性细胞数占 25%—50%）、＋＋＋（棕色颗粒多量，呈深棕色，阳性细胞数多于 50%）.

*国家自然科学基金资助项目（39070798）.

收稿日期：1993-11-10，修回日期：1994-03-18

2 结果

2.1 AF9 抗原的特性

2.1.1 乳腺癌细胞膜用 4 种相关因素处理后，AF9 抗原活性均有所降低（表 1，图 1），表明 AF9 对应抗原为糖、脂及蛋白质组成的复合蛋白质，且不耐热.

表 1 4 种因素对 McAb AF9 抗原的影响

处理因素	A_{492}	
	处理前	处理后
2.5mg/ml 胰蛋白酶 37℃ 1h	0.93	0.81
20mmol/L $NaIO_4$ 室温 1h	1.07	0.40
甲醇 0℃ 30min	1.06	0.65
煮沸 10min	1.00	0.57

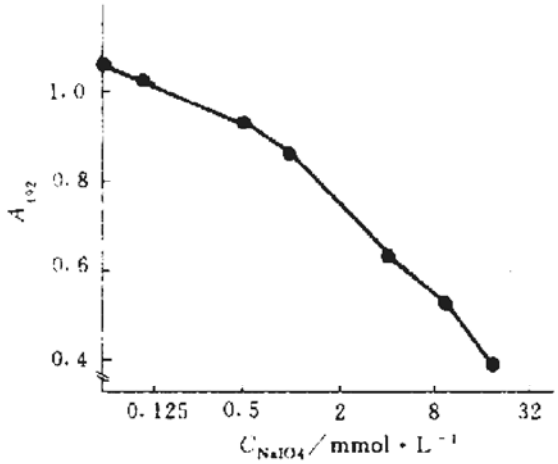

图 1 $NaIO_4$ 对 McAb AF9 抗原的影响

2.1.2 ELISA 竞争性抑制试验结果，铁蛋白及癌胚抗原对 AF9 与其抗原的结合无明显的抑制作用，光密度分别为 0.56 及 0.53，缓冲液对照为 0.50. 说明这两种恶性肿瘤相关抗原与 AF9 抗原没有结构上的必然联系.

2.1.3 蛋白质印迹的结果表明，AF9 识别的抗原有 4 条带，分子量分别为 51 000、56 000、67 000、73 000. 结果见图 2.

2.2 AF9 抗原的组织分布

用免疫组化 ABC 法对多种恶性肿瘤及正常组织进行了染色，结果见表 2. 免疫组化的染色结果还表明抗原分布于胞膜及胞浆，结果见图 3.

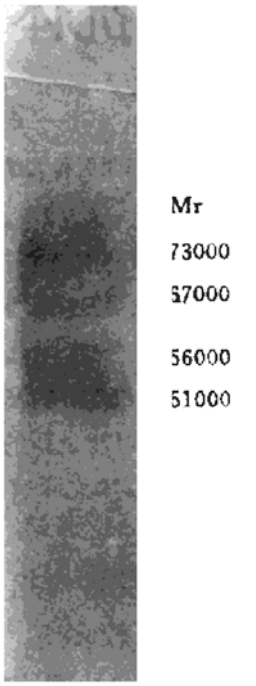

图 2 AF9 抗原的免疫印迹分析

表 2 McAb AF9 的免疫组化染色结果

组织	所检标本数	阳性数	染色强度			
			−	+	++	+++
乳腺癌	20	19	1	5	4	10
乳腺癌转移淋巴结	7	2	5	1		1
其它癌症						
卵巢	3	3		3		
胃	3	3			1	2
直肠	3	2	1	1		1
肝	3	1	2			1
肺	2	1	1			1
肾	3	0	3			
前列腺	2	0	2			
宫内膜	1	0	1			
甲状腺	2	0	2			
正常组织	5	0	5			

注：正常组织含正常乳腺、直肠、甲状腺、胃体及肛门皮肤.

AF9 与 20 例乳腺癌中的 19 例呈阳性反应，与卵巢癌、胃癌、直肠癌、肝癌、肺癌等有一定的交叉反应，而与肾癌、前列腺癌、宫内膜

癌、甲状腺癌及5例正常组织不反应.

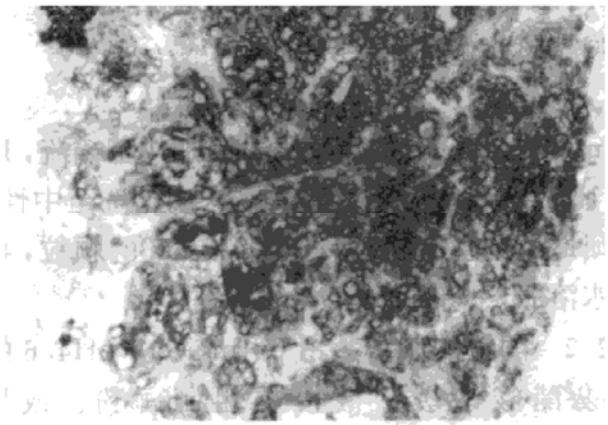

图3 McAb AF9对乳腺癌冰冻切片的免疫组化染色

3 讨 论

AF9是一个以乳腺癌原发灶细胞膜作免疫原制备的抗人乳腺癌单克隆抗体，属小鼠IgG_1类. 免疫组化结果显示AF9具有良好的肿瘤特异性，表明其识别的抗原与细胞恶变相关联，但为一种分布较为广泛的肿瘤相关抗原，可在多种肿瘤中检测到. 对其特性的进一步研究显示该抗原为糖、脂及蛋白质组成的复合蛋白质，由分子量为51000，56000，67 000，73 000的4条带组成，该抗原不耐热，被AF9识别的抗原决定簇不存在于铁蛋白及癌胚抗原. AF9抗原有可能是由这4条带组成的大分子化合物，也可能是相同蛋白质核心上表达不同的侧链.

目前国外的抗乳腺癌单克隆抗体识别的抗原大多数为高分子量的粘蛋白样糖蛋白，分子量为200 000—400 000[7]，AF9抗原显然不属于这一类. Garcia[8]等报道了一种分子量为52 000的雌激素调节蛋白，存在于胞浆及胞膜上，同时能通过外吐进入细胞培养液中，能在乳腺癌病人渗出液及血清中检测到，这点与AF9抗原不同，竞争性抑制试验表明AF9抗原不存在于乳腺癌病人血清中. Keydar[9]等报道的单抗H23识别一种据认为属于乳腺癌病毒产物的68 000糖蛋白，免疫组化染色结果只与乳腺癌组织反应而不与正常组织及其它肿瘤反应. 这两种成分均不与脂类相关联，故可以认为，AF9对应抗原可能是新的乳腺肿瘤相关抗原，对其深入研究将有助于了解乳腺癌的癌变因素，从而有助于乳腺癌的诊断及治疗.

参 考 文 献

1 Stacker S A, Thompson C, Riglar C *et al*. JNCI, 1985; **75**: 801
2 Pancino G, Charpin C, Osinaga E *et al*. Cancer Res, 1990; **50**: 7333
3 Peterson J A, Zava D T, Duwe A K *et al*. Hybridoma, 1990; **9**: 221
4 Canevari S, Fossati G, Balsari A *et al*. Cancer Res, 1983; **43**: 1301
5 Laemmli U K. Nature, 1970; **227**: 680
6 Towbin H, Stachelin T, Gordon J. Proc Natl Acad Sci USA, 1979; **6**: 4350
7 Mandeville R, Damas F, Amarouch A. Hybridoma, 1987; **6**: 441
8 Garcia M, Capony F, Derocq D. Cancer Res, 1985; **45**: 709
9 Keydar I, Chou C S, Hareuveni M *et al*. Proc Natl Acad Sci USA, 1989; **86**: 1362

酶固定化的新型载体——PF凝胶应用研究*

李源勋 叶庆玲

（河北大学化学系，保定 071002）

摘要 用对苯二酚和甲醛在酸催化下制得新一类凝胶，此凝胶价廉，易于制备，多孔、无毒、亲水性强；能选择性吸附多糖，而用于多糖与低聚糖及单糖的分离；可作为载体对多种酶及蛋白质给予固定，与蛋

*河北省科委资助课题. 收稿日期：1993-11-20，修回日期：1994-02-28

structure formation is the foundation of protein folding initiation. The paper presents the solution conformation of protein fragment and methods of conformation elucidation. and De novo design of peptide with secondary structure. Also, the application of these achievements in constructing the theoretical model of folding initiation and progress on research of protein folding initiation up to date are detailedly reviewed.

Key words framework model, secondary structure, de novo design, folding initiation

Peptide Library and Its Applications in Molecular Recognition. Qi Jie, Lu Zhibin, Wang Yuhong, Li Wei. (*Department of Molecular Biology, Jilin University, Changchun* 130023). *Prog. Biochem. Biophys.* (China), 1994; **21** (6): 513—517

Peptide library is a collections of peptides. The peptides display on the N-terminal of p Ⅲ or p Ⅷ coat protein of bacteriophage through gene cloning. This might be used in the fields associated with molecular recognition. such as: drug design, selection of enzymes inhibitor, vaccines selection, interaction of proteins, etc. Peptide library technique is a lately developed technique with high practical and theoretical value. Peptide library birth, development and potential applications in the future are reviewed.

Key words molecular recognition, peptide library, gene cloning

The Analgesic Domain of Interleukin-2. Xu Di, Jiang Chunlei, Zheng Zhongcheng, Fan Peifang, Sun Lanying, Liu Xinyuan, Song Chaoyou, You Zhendong, Wang Chenghai, Lu Changlin. (*Shanghai Institute of Biochemistry, Acadmia Sinica, Shanghai* 200031). *Prog. Biochem. Biophys.* (China), 1994; **21** (6): 518—520

Interleukin-2 (IL-2) is an important immune regulator. Recently it is found that IL-2 is also an analgesic molecule. Using the potassium iontophoresis as pain stimulus to induce tail-flick and taking the intensity of current (mA) at the moment of the response as the pain threshold (PT), it is reported that mutant 20 Leu-IL-2 (20Asp→Leu), which could not bind to the β subunit of IL-2 receptor and thus had no immune activity, could increase the PT of the rats, indicating that the analgesic and the immunal functions be related to two different domains in IL-2 molecule. Mutant 45Val-IL-2 (45Tyr→Val), which had immunal activity, had no analgesic effet, suggesting that the 45Tyr be crucial for the analgesic effect of IL-2 and the analgesic domain be located around the 45Tyr.

Key words interleukin-2, site-ditected mutagenesis, analgesia, structure-function studies, central nervous system

Characterization and Distribution of the Antigen Recognized by Breast Cacinoma McAb AF9. Cai Guiying, Zeng Wenjie. (*Department of Biochemistry, West China University of Medical Sciences, Chengdu* 610041). *Prog. Biochem. Biophys.* (China), 1994; **21** (6): 521—523

A preliminary study was made on the characterization and distribution in human tissues of AF9-recognized antigen. The results demonstrated that the antigen is a complex protein composed of carbohydrate, lipid and protein and susceptible to heating to 100℃. The determinant of AF9-recognized antigen was not present in the ferritin and carcinoembryonic antigen. Western blotting revealed that AF9-rec-

ognized antigen with molecular weights of 51, 56, 67 and 73 kD. Immunohistochemical staining showed that the antigen mainly existed in the cytoplasm and on the membrane of breast cancer cell, and also could be observed in some other tumors, but no staining was detected in normal tissues. The AF9-recognized antigen may be a new tumor-associated antigen.

Key words breast carcinoma, tumor-associated antigen, monoclonal antibody

Application of PF Gel as New Carrier for the Immobilization of Enzyme. Li Yuanxun, Ye Qingling. (*Department of Chemistry, Hebei University, Baoding* 071002). *Prog. Biochem. Biophys.* (China), 1994; **21** (6): 523—527

PF gel-type resin has been synthesised by polycondensation of hydroquinone with formaldehyde in the presence of acidic solution. The gel-type resin is cheap and easy to preduce, non-toxic, porous, hydrophilic, extremely stable. It is a effective carrier for the simple and rapid immobilization of various enzymes and proteins. The amounts of bound protein (BSA) and glycoamylase in one gramme of the dry carrier were 558mg and 330mg respectively, the activity recovery of 84% for immobilized glycoamylase was obtained. The coversion (%) for starch into glycoase, using immobilized glycoamylase is as high as 93%. A new kind of modified PF gel-type resins were synthesised by copolymerization of hydroquinone and some resorcin with formaldehyde. Thus PF and modified PF gel-type resins are superior carriers for immobilization of enzyme.

Key words phenolic-formaldehyde resin, hydroquinone-formaldehyde resin, immobilization, glycoamylase, bovine serum albumin (BSA)

Characterization of Modified Staphylococcal Protein A and Its Artificial Membrane on Substrate Surface. Lu Bin, Yie Ning, Wei Yu. (*Laboratory of Molecular and Biomolecular Electronics, Southeast University, Nanjing* 210018). *Prog. Biochem. Biophys.* (China), 1994; **21** (6): 528—532

A method is described for incorporation of water-soluble SpA into phospholipid monolayer using covalent SpA-stearate conjugates, and then transferring the monolayer on a pre-treated silica surface to form a SpA containing membrane. Stearic acid containing a reactive N-hydroxysuccinimide ester group is synthesized, and the derivative is reacted with SpA in a deoxycholate buffer. The modified SpA (m-SpA) has the solubility properties very similar to intergral membrane proteins. The CD results show that the content of β structure in the m-SpA is incresed in lower lipid coupling degree, but in higher modification the random coli content rapidly increased. After SpA is incorporated in the DPPA monolayer, unmodified SpA is readily ejected from the monolayer but m-SpA incorporates into the monolayer stably. The incorporation of the protein is proportional to the lipid coupling degree. The IgG binding ability of m-SpA decreases with the increase of the lipid coupling degree. The ability is further reduced about 20%—30% when m-SpA is incorporated in the membrane, which might be due to the incorporating procedure.

Key words SpA, lipid modification, conformation changes, artifical membrane, IgG binding activity

A Study of Combining Surroundings of Ca^{2+} in Fibrinolytic Principle of the Venoms in Agistrodon Acutus Using Tb^{3+} as a Fluorescent Probe. Xia Wensheng, Lu Jingfen, Liu