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E-钙粘蛋白和S100钙结合蛋白A4在乳腺癌组织中的表达及其与病理特征的关系*

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摘要 目的:探讨E-钙粘蛋白(E-cadherin)和S100钙结合蛋白A4(S100A4)在乳腺癌组织中的表达及其与病理特征的关系。**方法:**选取2010年3月-2017年12月期间梅州市人民医院收集的乳腺癌石蜡块标本50例为乳腺癌组,另选取同时期病理检查为良性肿瘤的石蜡块标本38例为对照组,应用免疫组化法检测E-cadherin和S100A4在乳腺癌组与对照组的表达情况,分析E-cadherin和S100A4与乳腺癌病理特征的关系,并采用Spearman相关性分析方法分析E-cadherin和S100A4的相关性。**结果:**E-cadherin在乳腺癌组中的阳性表达率低于对照组,S100A4阳性表达率高于对照组(*P<0.05*)。E-cadherin和S100A4阳性表达率与乳腺癌患者的年龄、是否绝经及病灶直径无关(*P>0.05*),有淋巴结转移、临床分期为III-IV期、病理分级为III级的乳腺癌患者中E-cadherin阳性表达率低于无淋巴结转移、临床分期为I-II期、病理分级为I-II级者,而有淋巴结转移、临床分期为III-IV期、病理分级为III级的乳腺癌患者中S100A4阳性表达率高于无淋巴结转移、临床分期为I-II期、病理分级为I-II级者(*P<0.05*)。经Spearman相关性分析显示,E-cadherin阳性表达与S100A4阳性表达呈负相关(*P<0.05*)。**结论:**S100A4在乳腺癌组织中呈现高表达,E-cadherin乳腺癌组织中呈现低表达,且其与患者淋巴结转移、临床分期及病理分级存在一定的相关性。

关键词:乳腺癌;E-钙粘蛋白;S100钙结合蛋白A4;表达;淋巴结转移;关系
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Expression of E- cadherin and S100 Calcium Binding Protein A4 in Breast Cancer Tissues and Its Relationship with Pathological Features*

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ABSTRACT Objective: To investigate the expression levels of E-cadherin (E-cadherin) and S100 calcium binding protein A4 (S100A4) in breast cancer tissues and its relationship with pathological features. **Methods:** A total of 50 cases of breast cancer specimens from Meizhou People's Hospital from March 2010 to December 2017 were selected as breast cancer group, and 38 cases of benign paraffin block specimens from pathological examination during the same period were selected as control group. Immunohistochemical method was used to detect the expression of E-cadherin and S100A4 in breast cancer group and control group. The relationship between E-cadherin and S100A4 and pathological characteristics of breast cancer was analyzed. The correlation between E-cadherin and S100A4 was analyzed by Spearman correlation analysis method. **Results:** The positive expression rate of E-cadherin in breast cancer group was lower than that in control group,and the positive expression rate of S100A4 in breast cancer group was higher than that in control group (*P<0.05*). The positive expression rates of E-cadherin and S100A4 were independent of the age, whether menopause and lesion size of breast cancer patients (*P>0.05*). The positive rate of E-cadherin expression in breast cancer patients with lymph node metastasis, III-IV stage of clinical stage and III grade of pathological grade was lower than that without lymph node metastasis, I-II stage of clinical stage and I-II grade of pathological grade, the positive expression rate of S100A4 of the former was higher than that of the latter (*P<0.05*). Spearman correlation analysis showed that the positive expression of E-cadherin was negatively correlated with the positive expression of S100A4 (*P<0.05*). **Conclusion:** The expression of S100A4 in breast cancer tissues is high and the expression in E-cadherin breast cancer

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tissues is low, and there is a certain correlation between lymph node metastasis, clinical stage and pathological grading.

Key words: Breast cancer; E- cadherin; S100 calcium binding protein A4; Expression; Lymph node metastasis; Relationship

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前言

乳腺癌是临床常见的女性恶性肿瘤,近年来其发病率呈逐年升高的趋势,且发病人群趋于年轻化,严重威胁着女性的健康安全,给患者的生活与工作带来不良影响[1-3]。目前临床上对该病的发病机制尚不明确,据相关研究显示,某些钙结合蛋白和趋化因子可能在乳腺癌的发生与发展过程中发挥着重要的作用[4,5]。S100 钙结合蛋白 A4(S100 calcium binding protein A4,S100A4)是一种 S100 蛋白,其广泛参与细胞转录因子组成、细胞磷酸化及细胞骨架组成等,并能够促进肿瘤细胞的转移、增殖及侵袭等[6,7]。E- 钙粘蛋白(E-cadherin)是连接钙依赖性细胞的黏附因子[8,9]。有研究显示,E-cadherin 的基因转录、突变及翻译异常,可以对 E-cadherin 在乳腺组织中的正常表达产生抑制作用,致使乳腺癌的发生[10,11]。本次研究主要探讨 E-cadherin 和 S100A4 在乳腺癌组织中的表达及其与病理特征的关系,旨在为临床防治乳腺癌提供参考依据。现整理结果如下。

1 资料与方法

1.1 一般资料

选取 2010 年 3 月 -2017 年 12 月期间梅州市人民医院收集的乳腺癌石蜡块标本 50 例为乳腺癌组,纳入标准:(1)经病理诊断为浸润性乳腺癌;(2)均在本院接受手术治疗;(3)术前未接受化疗、放疗治疗者。排除标准:(1)伴有其他恶性肿瘤者;(2)临床资料不完整者;(3)生存期 <3 个月者。乳腺癌组患者年龄 35-79 岁,平均(51.87± 14.12)岁;绝经 34 例,未绝经 16 例;有淋巴结转移 21 例,无淋巴结转移 29 例;临床分期:Ⅰ期 14 例,Ⅱ期 22 例,Ⅲ期 10 例,Ⅳ期 4 例;病理分级:Ⅰ级 18 例,Ⅱ级 19 例,Ⅲ级 13 例;病灶直径≤ 2.0 cm19 例,病灶直径 >2.0 cm 31 例。另选取同时期病理检查为良性肿瘤的石蜡块标本 38 例为对照组,对照组年龄 38-80 岁,平均(52.53± 15.92)岁。两组年龄对比差异无统计学意义(*P>0.05*),说明组间可进行比较,本研究通过梅州市人民医院伦理委员会批准。

1.2 方法

应用免疫组化检测 E-cadherin 和 S100A4 在乳腺癌组织中的表达:以良性乳腺组织为对照组,应用石蜡块标本进行连续切片,切片的厚度控制在 4 μm,应用枸橼酸缓冲液对抗原进行 5-10 min 的修复,在牛血清白蛋白中封闭 2h,然后应用磷酸缓冲盐溶液(phosphate buffer saline,PBS)洗涤 3 次,每次洗涤 5 min,应用浓度为 0.01 mmol/L 的 PBS 缓冲液作为替代一抗,E-cadherin 鼠抗人单克隆抗体与兔抗人 S100A4 多克隆抗体购自北京中杉金桥生物技术有限公司,免疫组化试剂盒由江苏泰州生物科技制药公司提供,应用二氨基联苯胺(diaminobenzidine,DAB)显色配置,应用苏木紫进行复染,树胶进行封片,在显微镜下观察。

1.3 阳性判断标准[12]

E-cadherin 蛋白表达以细胞膜呈棕黄色颗粒为阳性,S100A4 表达以细胞膜、细胞液呈棕黄色或黄色为阳性。根据显微镜下阳性细胞计数:0 分:无阳性细胞,1 分:阳性细胞比例 1%-25%;2 分:阳性细胞比例 26%-50%;3 分:阳性细胞比例 51%-75%;4 分:阳性细胞比例 >75%;根据染色强度进行计分:0 分:无色;1 分:淡黄色;2 分:棕黄色;3 分:褐色。染色强度与阳性细胞百分比乘积≥ 3 分 E-cadherin 或 S100A4 为阳性表达,<3 分 E-cadherin 或 S100A4 为阴性表达。

1.4 观察指标

对比 E-cadherin 和 S100A4 在乳腺癌组与对照组中的表达情况,分析 E-cadherin 和 S100A4 阳性表达与乳腺癌病理特征的关系,并分析 E-cadherin 和 S100A4 的相关性。

1.5 统计学方法

采用 SPSS19.0 统计学软件,计量资料均以($\bar{x}\pm s$)的形式表示,经 t 检验分析,计数资料以%的形式表示,经 x^2 检验分析,E-cadherin 和 S100A4 的相关性分析采用 Spearman 相关分析,以 *P<0.05* 时表明差异有统计学意义。

2 结果

2.1 E-cadherin 和 S100A4 在乳腺癌组与对照组中的表达情况对比

E-cadherin 在乳腺癌组中的阳性表达率低于对照组,S100A4 在乳腺癌组中的阳性表达率高于对照组(*P<0.05*),见表 1。

表 1 E-cadherin 和 S100A4 在乳腺癌组与对照组中的表达情况对比[n(%)]

Table 1 Comparison of E-cadherin and S100A4 expressions in breast cancer group and control group[n(%)]

Groups	n	E-cadherin		S100A4	
		+	-	+	-
Breast cancer group	50	15(30.00)	35(70.00)	40(80.00)	10(20.00)
Control group	38	20(52.63)	18(47.37)	2(5.26)	36(94.74)
x^2		4.617		20.137	
P		0.032		0.000	

2.2 E-cadherin 和 S100A4 阳性表达与乳腺癌病理特征的关系分析

E-cadherin 和 S100A4 阳性表达率与乳腺癌患者的年龄、是否绝经及病灶直径无关(*P*>0.05),有淋巴结转移、临床分期为 III-IV 期、病理分级为 III 级的乳腺癌患者中 E-cadherin 阳性表达率低于无淋巴结转移、临床分期为 I-II 期、病理分级为 I-II 级者,有淋巴结转移、临床分期为 III-IV 期、病理分级为 III 级的乳腺癌患者中 S100A4 阳性表达率高于无淋巴结转移、临床分期为 I-II 期、病理分级为 I-II 级者(*P*<0.05),见表 2。

表 2 E-cadherin 和 S100A4 阳性表达与乳腺癌病理特征的关系分析

Table 2 Relationship between E-cadherin and S100A4 expression and pathological characteristics of breast cancer

Pathological features		n	E-cadherin positive	x^2	*P*	S100A4 positive	x^2	*P*
Age(years)	≤ 50	22	7(31.82)	0.062	0.804	17(77.27)	0.183	0.669
	>50	28	8(28.57)			23(82.14)		
Menopause	yes	34	10(29.41)	0.018	0.895	27(79.41)	0.023	0.880
	no	16	5(31.25)			13(81.25)		
Lymph node metastasis	yes	21	3(14.29)	5.352	0.021	20(95.24)	5.255	0.022
	no	29	12(41.38)			20(68.97)		
Clinical stages	I-IIstage	36	14(38.89)	13.912	0.004	26(72.22)	4.861	0.027
	III-IVstage	14	1(7.14)			14(100.00)		
Pathological grading	I-IIgrade	37	14(37.84)	4.163	0.041	27(72.97)	4.392	0.036
	IIIgrade	13	1(7.69)			13(100.00)		
Lesion diameter(cm)	≤ 2.0	19	6(31.58)	0.036	0.849	15(78.95)	0.021	0.884
	>2.0	31	9(29.03)			25(80.65)		

2.3 E-cadherin 和 S100A4 阳性表达相关性分析

经 Spearman 相关性分析显示,E-cadherin 阳性表达与 S100A4 阳性表达呈负相关(r=-0.281,*P*=0.018)。

3 讨论

乳腺癌患者的预后与乳腺癌浸润程度、病理类型及淋巴结转移等存在显著相关性。肿瘤细胞的转移与侵袭是肿瘤进展的主要原因,也是导致癌症患者死亡的高危因素[13-15]。近年来,较多的乳腺癌患者在通过早期诊断及规范的手术治疗后生存率得到了提高,但是仍有部分患者发生肿瘤转移及侵袭,而且生存率较低[16-18]。肿瘤发生及发展的关键在于抑癌基因与癌基因调节功能的失衡[19,20],因此癌基因分子靶向治疗成为大多数学者研究乳腺癌治疗方法的重点,同时乳腺癌转移与侵袭机制也是目前临床学者研究的热点。

E-cadherin 是机体重要的上皮细胞标志物,据相关研究显示,E-cadherin 表达的完全缺失或者部分缺失与乳腺癌高侵袭性、乳腺癌的低分化及临床预后差具有密切的相关性,给予乳腺癌患者外源性的 E-cadherin 虽然不能够逆转间质细胞表型,但对乳腺癌的转移具有一定的抑制作用[21-23]。S100A4 蛋白是一种钙离子结合蛋白,具有双螺旋结构,参与了细胞的分化与增殖、肿瘤的侵袭、转移、细胞内外信号传递、细胞间黏附等过程,在较多恶性肿瘤的发展中发挥了重要的介导作用[24-26]。在本次研究中 E-cadherin 在乳腺癌组织阳性表达降低,S100A4 的阳性表达升高,通过分析 E-cadherin 与 S100A4 的阳性表达率与临床病理特征的关系,结果显示 E-cadherin 和 S100A4 阳性表达率与乳腺癌患者的年龄、是否绝经及病灶直径无关,有淋巴结转移、临床分期为 III-IV 期、病理分级为 III 级的乳腺癌患者中 E-cadherin 呈现低表达,S100A4 则在患者以上临床病理参数中呈现高表达,分析其原因主要是因为 E-cadherin 是一种跨膜糖蛋白,能够维持细胞间的粘附力,进而发挥抑制肿瘤侵袭与转移的作用,E-cadherin 失活或者表达降低时,细胞间的黏附作用降低或者出现急性紊乱,促进肿瘤的转移,同时还能够促进肿瘤细胞的分化与侵袭[27,28]。S100A4 是一种重要的转移相关基因,该基因对肿瘤的侵袭与转移具有促进作用,在啮齿类肿瘤细胞中均可发现 S100A4、E-cadherin 的表达,两者细胞间粘附力分别参与了正负向调节,所以临床分期、病理分级越高,且在转移性较强的肿瘤中 S100A4 表达升高而 E-cadherin 的表达降低[29,30]。经 Spearman 相关性分析显示,E-cadherin 阳性表达与 S100A4 阳性表达呈负相关,说明 E-cadherin 阳性表达与 S100A4 阳性表达存在一定的联系,分析其原因主要是因为 E-cadherin 阳性表达降低,可促进乳腺癌的发生,因此使 S100A4 在肿瘤细胞中的表达升高。

综上所述,E-cadherin 对乳腺癌患者淋巴结转移及肿瘤进展具有抑制作用,S100A4 对乳腺癌的发展具有促进作用,二者存在负相关关系,在今后的临床治疗中可联合检测 E-cadherin 与 S100A4 来评估患者的病情,为临床治疗提供方向。

参 考 文 献(References)

[1] Rajakumar T, Pugalendhi P, Thilagavathi S. Protective Effect of Allyl Isothiocyanate on Glycoprotein Components in 7,12-dimethylbenz(a) anthracene Induced Mammary Carcinoma in Rats [J]. Indian J Clin Biochem, 2018, 33(2): 171-177

[2] Ishiba T, Oda G, Nakagawa T, et al. A Case of HER2 Positive Occult

Breast Cancer Presenting as Swollen Axillary LymphNodes [J]. Gan To Kagaku Ryoho, 2018, 45(3): 533-535

[3] Ledet MM, Anderson R, Harman R, et al. BB-Cl-Amidine as a novel therapeutic for canine and feline mammary cancer via activation of the endoplasmic reticulum stress pathway [J]. BMC Cancer, 2018, 18 (1): 412

[4] Xu H, Li M, Zhou Y, et al. S100A4 participates in epithelial-mesenchymal transition in breast cancer via targeting MMP2[J]. Tumour Biol, 2016, 37(3): 2925-2932

[5] 郑榕英,张爱龙,肖雪明,等.乳腺癌易感基因 1、E- 钙黏蛋白、p120 在乳腺浸润性导管癌组织的表达及其临床意义[J].中华实验外科杂志, 2017, 34(11): 1950-1953
Zheng Rong-ying, Zhang Ai-long, Xiao Xue-ming, et al. Expression and clinical significance of breast cancer susceptibility gene 1, E-cadherin and p120 in infiltrating ductal breast carcinoma tissue[J]. Chinese Journal of Experimental Surgery, 2017, 34(11): 1950-1953

[6] Chen A, Wang L, Li BY, et al. Reduction in Migratory Phenotype in a Metastasized Breast Cancer Cell Line via Downregulation of S100A4 and GRM3[J]. Sci Rep, 2017, 7(1): 3459

[7] Kau S, Miller I, Tichy A, et al. S100A4 (metastasin) positive mesenchymal canine mammary tumour spheroids reduce Tenascin C synthesis under DMSO exposure in vitro [J]. Vet Comp Oncol, 2017, 15(4): 1428-1444

[8] Elisha Y, Kalchenko V, Kuznetsov Y, et al. Dual role of E-cadherin in the regulation of invasive collective migration of mammary carcinoma cells[J]. Sci Rep, 2018, 8(1): 4986

[9] Li D, Lo W, Rudloff U. Merging perspectives: genotype-directed molecular therapy for hereditary diffuse gastric cancer (HDGC) and E-cadherin-EGFR crosstalk[J]. Clin Transl Med, 2018, 7(1): 7

[10] Jiang W, Li Y, Ou J, et al. Expression analysis of E-cad and vascular endothelial growth factor in triple-negative breast cancer patients of different ethnic groups in western China [J]. Medicine (Baltimore), 2017, 96(42): e8155

[11] Peretti AS, Dominguez D, Grimes MM, et al. The R-Enantiomer of Ketorolac Delays Mammary Tumor Development in Mouse Mammary Tumor Virus-Polyoma Middle T Antigen (MMTV-PyMT) Mice[J]. Am J Pathol, 2018, 188(2): 515-524

[12] 王可铮,吴迪,张令波,等.整合素与 EGFR 受体家族相互作用在乳腺癌中的作用研究进展 [J]. 现代生物医学进展, 2015, 15(36): 7197-7200, 7036
Wang Ke-zheng, Wu Di, Zhang Ling-bo, et al. Progress in the Role of Integrin and EGFR Family Interaction in Breast Cancer [J]. Progress in Modern Biomedicine, 2015, 15(36): 7197-7200, 7036

[13] Yuan H, Wang X, Shi C, et al. Plac1 Is a Key Regulator of the Inflammatory Response and Immune Tolerance in Mammary Tumorigenesis[J]. Sci Rep, 2018, 8(1): 5717

[14] Zhang C, Winnard PT Jr, Dasari S, et al. Label-free Raman spectroscopy provides early determination and precise localization of breast cancer-colonized bone alterations [J]. Chem Sci, 2017, 9(3): 743-753

[15] Lozza L, Fariselli L, Sandri M, et al. Partial breast irradiation with CyberKnife after breast conserving surgery: a pilot study in early breast cancer[J]. Radiat Oncol, 2018, 13(1): 49

[16] Chung JM, Jung Y, Kim YP, et al. Identification of the Thioredoxin-Like 2 Autoantibody as a Specific Biomarker for Triple-Negative Breast Cancer[J]. J Breast Cancer, 2018, 21(1): 87-90

[17] Lee SB, Sohn G, Kim J, et al. Chronological Improvement in Survival of Patients with Breast Cancer: A Large-Scale, Single-Center Study[J]. J Breast Cancer, 2018, 21(1): 70-79

[18] Yuan CL, Liu ZH, Zou N, et al. Relationship between expression of CXCR7 and NF-κB in breast cancer tissue and occurrence of breast cancer and lymphatic metastasis [J]. Saudi J Biol Sci, 2017, 24(8): 1767-1770

[19] Romanoff A, Schmidt H, McMurray M, et al. Who Is Ordering MRIs in Newly Diagnosed Breast Cancer Patients?[J]. Am Surg, 2018, 84 (3): 351-357

[20] Xu Y, Wang W, Wang M, et al. High Salt Intake Attenuates Breast Cancer Metastasis to Lung [J]. J Agric Food Chem, 2018, 66(13): 3386-3392

[21] Niit M, Arulanandam R, Cass J, et al. Regulation of HC11 mouse breast epithelial cell differentiation by the E-cadherin/Rac axis [J]. Exp Cell Res, 2017, 361(1): 112-125

[22] Bontempo A, Ugalde-Villanueva B, Delgado-González E, et al. Molecular iodine impairs chemoresistance mechanisms, enhances doxorubicin retention and induces downregulation of the $CD44^{+}/CD24^{+}$ and E-cadherin+/vimentin+ subpopulations in MCF-7 cells resistant to low doses of doxorubicin[J]. Oncol Rep, 2017, 38(5): 2867-2876

[23] El Sharouni MA, Postma EL, van Diest PJ. Correlation between E-cadherin and p120 expression in invasive ductal breast cancer with a lobular component and MRI findings[J]. Virchows Arch, 2017, 471 (6): 707-712

[24] Egeland EV, Boye K, Park D, et al. Prognostic significance of S100A4-expression and subcellular localization in early-stage breast cancer[J]. Breast Cancer Res Treat, 2017, 162(1): 127-137

[25] Ismail TM, Bennett D, Platt-Higgins AM, et al. S100A4 Elevation Empowers Expression of Metastasis Effector Molecules in Human Breast Cancer[J]. Cancer Res, 2017, 77(3): 780-789

[26] Gómez-Contreras P, Ramiro-Díaz JM, Sierra A, et al. Extracellular matrix 1 (ECM1) regulates the actin cytoskeletal architecture of aggressive breast cancer cells in part via S100A4 and Rho-family GTPases[J]. Clin Exp Metastasis, 2017, 34(1): 37-49

[27] Memni H, Macherki Y, Klayech Z, et al. E-cadherin genetic variants predict survival outcome in breast cancer patients [J]. J Transl Med, 2016, 14(1): 320

[28] Halilovic A, Bulte J, Jacobs Y, et al. Brief fixation enables same-day breast cancer diagnosis with reliable assessment of hormone receptors, E-cadherin and HER2/Neu [J]. J Clin Pathol, 2017, 70(9): 781-786

[29] Brilliant YM, Brilliant AA, Sazonov SV. Epithelial cadherins and associated molecules in invasive lobular breast cancer[J]. Arkh Patol, 2017, 79(1): 12-18

[30] Gründker C, Bauerschmitz G, Schubert A, et al. Invasion and increased expression of S100A4 and CYR61 in mesenchymal transformed breast cancer cells is downregulated by GnRH [J]. Int J Oncol, 2016, 48(6): 2713-2721