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·临床研究·

转录因子粒状头样2在乳腺癌组织中的表达及其与患者临床病理特征和预后的关系

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[摘 要] **目的:**探讨转录因子粒状头样2(GRHL2)在乳腺癌组织中的表达及其与患者临床病理特征和预后的关系,为乳腺的临床治疗寻找新的靶点。**方法:** 选取2010年1月至2017年1月在新乡市中心医院普外二科诊疗并经病理确认的初发乳腺癌患者88例的癌组织及相应的癌旁组织。免疫组织化学染色法检测GRHL2在乳腺癌组织及相应的癌旁组织中的表达,并分析GRHL2的表达水平与患者临床病理特征的关系;通过分析TCGA乳腺癌临床数据,探究GRHL2的表达与乳腺癌患者预后的关系。**结果:**乳腺癌组织中GRHL2的表达率(75.00%)明显高于癌旁组织(36.36%)(*P*<0.01)。TCGA数据库中114例正常乳腺组织以及1 097例原发性乳腺癌组织中GRHL2的表达结果显示,原发性乳腺癌组织中GRHL2的表达高于正常乳腺组织,差异有统计学意义(*P*<0.01)。GRHL2的表达与乳腺癌TNM分期、组织学分级、HER2表达、淋巴结转移状态等相关(均*P*<0.05);通过分析TCGA数据库中1 979例低表达GRHL2乳腺癌患者与1 972例高表达GRHL2乳腺癌患者无复发生存期(RFS)结果显示,高表达GRHL2的乳腺癌患者RFS患者显著缩短(*HR*=1.24,95%CI:1.11～1.38,*P*<0.01);GRHL2表达差异对TNBC乳腺癌患者的RFS无明显影响(*HR*=1.30,95%CI: 0.89～1.88,*P*=0.170);GRHL2的表达差异对ER$^+$乳腺癌患者的RFS无明显影响(*HR*=1.17,95%CI:0.76～1.78,*P*=0.470);但是高表达GRHL2的HER2$^+$乳腺癌患者其RFS显著低于低表达GRHL2的HER2$^+$乳腺癌患者(*HR*=1.72,95%CI:1.11～2.68,*P*=0.015)。**结论:** GRHL2在乳腺癌组织中表达升高,并与HER2表达、组织学分级、TNM分期、淋巴结转移密切相关,在乳腺癌发生发展过程中发挥重要作用,提示不良预后。

[关键词] 乳腺癌;粒状头样2;病理特征;预后
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Expression of transcription factor grainyhead-like-2 in breast cancer tissues and its relationship with clinicopathological features and prognosis of patients

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[Abstract] Objective: To detect the expression of GRHL2 (grainyhead-like-2) in breast cancer tissues and to explore its correlation with clinicopathological characteristics and prognosis of breast cancer (BC) patients, aiming to find new therapeutic target for breast cancer. **Method:** A total of 88 pairs of BC tissues and corresponding para-cancerous tissues from patients with primary BC that treated and pathologically confirmed at the Second Department of General Surgery, Xinxiang Central Hospital from January 2010 to January 2017 were collected for this study. The expression of GRHL2 in BC tissues and para-cancerous tissues was examined with IHC, and the association between GRHL2 and clinicopathological characteristics of BC patients was analyzed. Moreover, the correlation between GRHL2 and prognosis of BC patients was investigated by analyzing TCGA clinic data for BC. **Result:** The expression of GRHL2 was significantly higher in BC tissues (75.00%) compared with para-cancerous tissues (36.36%) (*P*<0.01); Based on the results of GRHL2 expression in 114 cases of normal breast tissues and 1 097 cases of primary breast cancer tissues in TCGA database, the expression of GRHL2 in primary BC tissues was significantly higher than that in normal breast tissues (*P*<0.01). GRHL2 expression was associated with BC TNM stage, histological grade, HER2 status and lymphnode metastasis status (all *P*<0.05); TCGA database showed that the RFS of 1 979 BC patients with high GRHL2 expression was significantly shorter than that of the 1 972 cases of BC patients with low GRHL2 expression (*HR*=1.24, 95%CI:1.11-1.38, *P*<0.01); GRHL2 expression exerted no significant effect on RFS of TNBC patients or

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ER$^+$ BC patients (TNBC: *HR*=1.30，95%CI: 0.89-1.88，*P*=0.170; ER$^+$: *HR*=1.17, 95%CI: 0.76-1.78，*P*=0.470); however, the RFS of HER2$^+$ BC patients with high GRHL2 expression was significantly shorter than that of HER2$^+$ BC patients with low GRHL2 expression (*HR*=1.72, 95%CI: 1.11-2.68, *P*=0.015). **Conclusion**: Expression level of GRHL2 was up-regulated in BC tissues, and was associated with BC TNM stage, histological grade, HER2 status and the lymphnode metastasis status. GRHL2 plays an important role in the generation and development of BC, indicating poor prognosis.

[Key words] breast cancer (BC); grainyhead-like-2(GRHL2); pathological characteristics; prognosis

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乳腺癌是女性发病率最高的恶性肿瘤，同时也是女性死亡率最高的恶性肿瘤之一[1-2]。目前影响乳腺癌临床诊疗的主要细胞分子标志物包括：细胞增殖抗原标志物Ki-67、雌激素受体（estrogen receptor，ER）、孕激素受体（progesterone receptor，PR）和人表皮生长因子受体2（human epidermal growth factor receptor-2，HER-2）以及一些其他乳腺癌预后预测分子[3]。但是由于不同类型乳腺癌基因表达的差异性，导致其对治疗的敏感性也存在差异，复发转移时有发生[4]。积极探寻乳腺癌诊断和治疗的新靶点，将会为乳腺癌临床治疗提供新的策略，推动乳腺癌个体化、精准化治疗[5]。粒状头样2（grainyhead-like-2, GRHL2）转录因子属于粒状头样GRHL转录因子家族中的一员，在维持上皮细胞形态和功能的过程中发挥重要作用[6]。研究表明，GRHL2在多种恶性肿瘤中表达上调，包括肝癌[7]、口腔上皮鳞状细胞癌[8]、胃癌[9]等肿瘤。目前，GRHL2在乳腺癌领域中的研究仍相对较少。在小鼠模型中，上调GRHL2的表达会促进乳腺癌细胞的增殖和转移[10]。但GRHL2在乳腺癌中的表达水平，以及与临床病理特征和预后的关系仍不清楚。本研究检测了GRHL2在正常乳腺组织和乳腺癌组织中的表达差异，以及GRHL2的表达是否与乳腺癌临床病理特征之间存在联系；同时通过TCGA乳腺癌临床数据，分析GRHL2表达与乳腺癌预后的联系，旨在探讨GRHL2在乳腺癌中的表达及与患者预后的关系，为乳腺癌的临床诊疗探寻新的靶点和预后预测因子。

1　资料与方法

1.1　临床资料

选取2010年1月至2017年1月在新乡市中心医院普外二科诊疗并经病理诊断确认的初发乳腺癌患者88例。所有病例均为女性，年龄41～76岁，平均（55.12±9.38）岁。所有病例均在术中留取乳腺癌组织及相应的癌旁组织，离体后迅速用甲醛溶液固定，石蜡包埋保存。本研究均经患者知情同意，并通过医院伦理委员会批准。

1.2　免疫组化法检测乳腺癌和癌旁组织中GRHL2蛋白的表达

免疫组织化学染色均由本院病理科医师完成。GRHL2的免疫组化结果根据染色强度和阳性细胞比例对结果进行判定[11]。（1）组织染色强度：没有着色、强黄色、黄褐色/棕黄色分别赋予0～3分；（2）组织阳性细胞比例：按<5%、5%～24%、25%～49%、50%～75%和>75%分别赋予0～4分；（3）根据（1）×（2）得分：0～2分为阴性，>3分为阳性。

1.3　通过Atlas肿瘤基因组数据分析GRHL2的表达与乳腺癌患者预后的关系

GRHL2的表达水平是在The Cancer Genome Atlas (TCGA)数据库通过UALCAN analysis tool进行分析[12]，GRHL2表达与乳腺癌预后分析是通过Kaplan-Meier Plotter，采用User selected probe set，use 35 dataset，Split patients by: median。GRHL2的表达水平是通过软件设置的中位表达作为cut-off值，大于中位表达定义为“High”，小于中位表达定义为“Low”。RFS是指从手术当天起到复发日期[13]。

1.4　统计学处理

采用SPSS 19.0统计学软件进行统计学分析，计数资料用率值表示，组间比较采用χ^2检验，生存分析采用Kaplan-Meier分析法以及Log-rank分析法，以*P*<0.05或*P*<0.01表示差异有统计学意义。

2　结　果

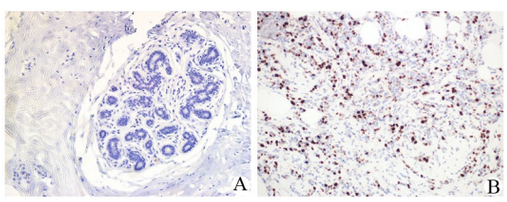
2.1　GRHL2在乳腺癌组织中呈高表达

免疫组织化学染色结果（图1、表1）显示，乳腺癌组织中GRHL2表达率（75.00%）明显高于癌旁组织（36.36%）（*P*<0.01）。同时分析TCGA数据库中114例正常乳腺组织以及1 097例原发性乳腺癌组织中GRHL2的表达水平，结果显示，原发性乳腺癌组织中GRHL2的表达高于正常乳腺组织，差异有统计学意义（*P*<0.01，图2）。

2.2　乳腺癌组织中GRHL2蛋白的表达与临床病理特征之间的关系

乳腺癌组织GRHL2蛋白的表达随着TNM分期的升级而表达升高（*P*<0.05）；乳腺癌组织学分级分升高也会引起GRHL2的表达升高（*P*<0.05）；淋巴结转移的患者GRHL2的表达升高（*P*<0.05）；HER$^+$的乳腺癌患者GRHL2的阳性率也明显升高，差异均有统计

学意义（P<0.05）；GRHL2的表达与患者年龄、肿瘤大小、病理分型、ER和PR的状态无关（P>0.05）。见表2。



A: Low expression level of GRHL2 in breast para-cancerous tissues; B: High expression of GRHL2 in breast cancer

图1 乳腺癌组织中GRHL2的表达高于癌旁组织（免疫组化，×200）

Fig.1 Expression of GRHL2 in breast cancer is higher than that in para-cancerous tissues (Immunohistochemical, ×200)

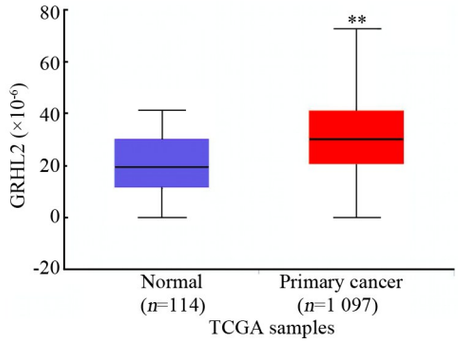
2.3 GRHL2表达与乳腺癌患者预后的关系

通过分析TCGA数据库中1 979例低表达GRHL2乳腺癌患者与1 972例高表达GRHL2乳腺癌患者无复发生存期（relapse free survival，RFS）之间的差异，结果显示，高表达GRHL2的乳腺癌患者RFS患者显著缩短，差异有统计学意义（HR=1.24，95%CI: 1.11～1.38，P<0.01；图3A）。同时分别分析TCGA数据库中ER^+、$HER2^+$、TNBC乳腺癌组织GRHL2的表达与RFS之间的关系，结果显示，GRHL2表达差异对TNBC乳腺癌患者的RFS无明显影响（HR=1.30，95%CI: 0.89～1.88，P=0.170；图3B）；GRHL2的表达差异对ER^+乳腺癌患者的RFS无明显影响（HR=1.17，95%CI:0.76～1.78，P=0.470；图3C）；但是高表达GRHL2的$HER2^+$乳腺癌患者其RFS显著低于低表达GRHL2的$HER2^+$乳腺癌患者（HR=1.72，95%CI: 1.11～2.68，P=0.015；图3D）。

表1 GRHL2在乳腺癌组织和癌旁组织中的表达差异（n）

Tab.1 The difference of GRHL2 Expression between breast cancer tissues and Para-cancer tissues (n)

Group	N	GRHL2 [n(%)]			
		Positive	Negative	χ^2	P
Cancer tissue	88	66（75.00）	22（25.00）	26.621	<0.001
Para-cancerous tissue	88	32（36.36）	56（63.64）		



$^{**}P$<0.01 *vs* Normal group

图2 乳腺癌组织中GRHL2的表达高于正常乳腺组织

Fig.2 Expression of GRHL2 in breast cancer is higher than that in normal breast tissue

2.4 88例入组乳腺癌患者癌组织GRHL2的表达水平与RFS的关系

对88例乳腺癌患者进行了预后随访，并对不同表达水平GRHL2与RFS的关系进行了分析，结果（图4）显示，66位高表达GRHL2的乳腺癌患者中位RFS为56.31个月，而低表达患者则为86.53个月[HR=2.84 95%CI:1.27～4.96，P=0.011]。

3 讨 论

近年来乳腺癌的发病率逐年升高并呈现年轻化的趋势，对全球女性健康造成严重威胁[14]。虽然乳腺癌有多个治疗靶点，但耐药始终是难以摆脱的临床难题[15]。探寻乳腺癌治疗的新靶点，将会为乳腺癌的治疗提供新的策略[16-17]。GRHL2在多种恶性肿瘤中表达升高，促进肿瘤细胞的增殖，导致不良预后[18]，但GRHL2在乳腺癌中的作用研究目前还比较少。GRHL2在乳腺癌临床组织水平的研究和随访，可能会进一步揭示其在乳腺癌发生发展过程中的重要作用。有文献[10]报道，上调小鼠模型中GRHL2表达可以促进乳腺癌细胞的增殖和转移，下调乳腺癌细胞系中GRHL2的表达可以抑制乳腺癌细胞的上皮细胞间质转化（epithelial-to-mesenchymal transition，EMT），从而抑制乳腺癌细胞的转移[19]。

本研究首先通过对88例乳腺癌患者癌和癌旁组织进行免疫组化染色分析，发现GRHL2在乳腺癌组织中的表达显著高于癌旁组织，提示GRHL2可能在乳腺癌中表达升高。对GRHL2的表达与乳腺癌临床病理特征关系进行分析中发现，GRHL2的表达在

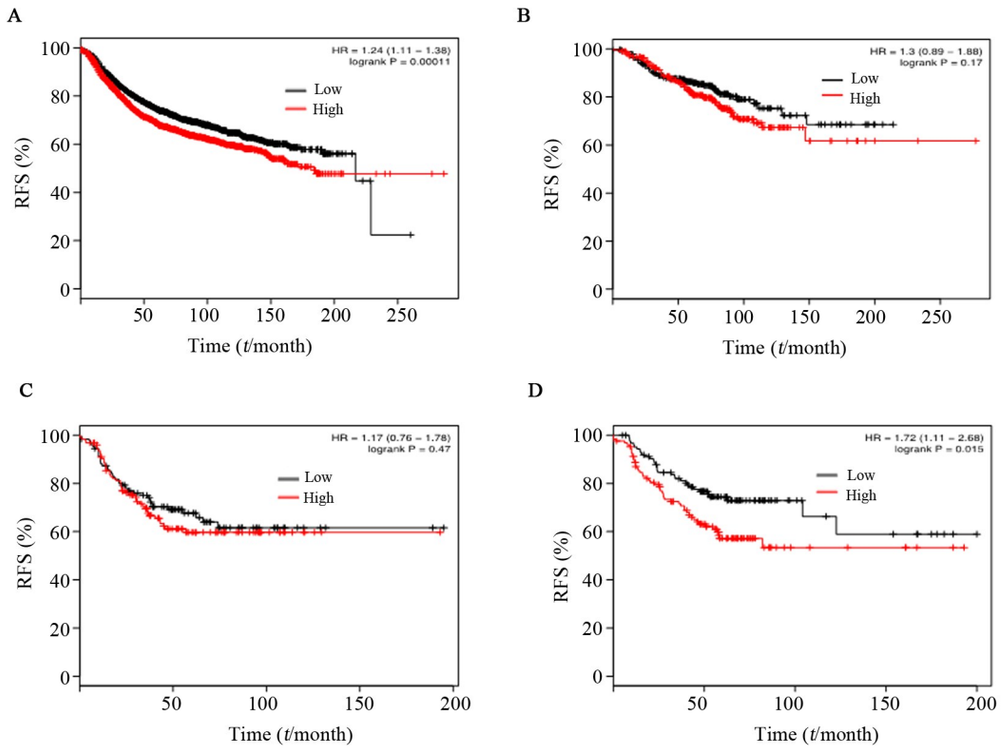
HER$^+$乳腺癌中表达显著升高；GRHL2的表达与乳腺癌组织学分级、TNM分期以及淋巴结转移呈正相关。考虑到样本量比较小的问题，进一步挖掘了TCGA数据中有效的乳腺癌病例信息，结果显示，乳腺癌组织中GRHL2的表达高于正常乳腺组织，这与本次免疫组化结果完全一致。通过分析TCGA数据库中GRHL2的表达对乳腺癌患者RFS的影响，结果显示，高表达GRHL2的乳腺癌患者RFS较低表达患者显著降低，GRHL2可能成为乳腺癌患者的一个预后预测因子。同时GRHL2的表达会显著降低HER2$^+$乳腺癌患者的RFS，而对ER$^+$以及TNBC乳腺癌患者的RFS无明显影响，这也基本证实了本次乳腺癌免疫组化结果，提示GRHL2更可能在HER2乳腺癌中发挥重要的促进作用。

表2 GRHL2在乳腺癌组织中的表达及其与临床病理特征的关系

Tab.2 The relationship between the expression of GRHL2 in breast cancer and the clinicopathological characteristics

Feature	*N*	GRHL2[*n*(%)]		χ^2	*P*
		Positive	Negative		
Age(*t*/a)				0.287	0.592
≥55	63	39(61.90)	24(38.10)		
<55	25	17(68.00)	8(32.00)		
Tumor size(*l*/cm)				0.857	0.355
>2	37	25(67.57)	12(32.43)		
≤2	51	39(76.47)	12(23.53)		
Pathogenic type				0.050	0.824
Invasive breast cancer	80	72(90.00)	8(10.00)		
Other type	8	7(87.50)	1(12.50)		
TNM stage				4.532	0.033
I-II	49	27(55.10)	22(44.90)		
III-IV	39	30(76.92)	9(23.08)		
Tumor grade				5.928	0.015
I-II	65	35(53.85)	30(46.15)		
III	23	19(82.60)	4(17.40)		
Lymph status				6.425	0.011
Positive	30	21(70.00)	9(30.00)		
Negative	58	25(43.10)	35(56.90)		
ER status				0.285	0.594
Positive	41	23(56.10)	18(43.90)		
Negative	47	29(61.70)	18(38.30)		
PR status				0.158	0.690
Positive	43	24(55.81)	19(44.19)		
Negative	45	27(60.00)	18(40.00)		
HER2 status				4.641	0.031
Positive	20	17(85.00)	3(15.00)		
Negative	68	40(58.82)	28(41.18)		

综上所述，GRHL2在乳腺癌组织中表达升高，与HER2阳性状态、组织学分级、TNM分期、淋巴结转移密切相关，并提示不良预后。其中GRHL2更可能在HER2$^+$乳腺癌的发生发展过程中扮演重要角色。GRHL2有望为临床乳腺癌的治疗提供新的靶点以及预后预测分子。



A: The RFS of breast cancer patients with high expression of GRHL2 was significantly shorter than that of patients with low expression of GRHL2; B: The difference of GRHL2 expression had no significant effect on RFS in TNBC breast cancer patients; C: The difference of GRHL2 expression has no significant effect on RFS of ER^+ breast cancer patients; D: In $HER2^+$ breast cancer, the increase of GRHL2 expression will significantly reduce RFS (219288 at is the gene ID of GRHL2 in TCGA database)

图3 乳腺癌组织中GRHL2的表达水平与RFS的关系

Fig.3 Relationship between GRHL2 expression level and RFS in breast cancer

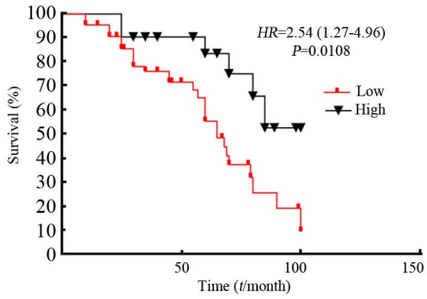


图4 88例入组乳腺癌患者癌组织GRHL2的表达水平与RFS的关系

Fig.4 Relationship between patients' RFS and GRHL2 expression in breast cancer from 88 cases of enrolled breast cancer patients

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