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氨基酸转运载体 SLC1A5 在胃癌组织中的表达及其临床病理学意义*

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摘要 目的:研究氨基酸转运载体溶质载体家族 1 成员 5(Solute Carrier Family 1 Member 5, SLC1A5)蛋白在胃癌组织中的表达情况,并探讨其与胃癌临床病理特征及预后的相关性。**方法:**收集进展期胃癌组织及对应癌旁组织 90 例,应用免疫组化技术检测 SLC1A5 在上述组织中的表达情况,并统计分析其表达与胃癌临床病理特征及预后的关系。同时通过基因数据库分析 SLC1A5 在胃癌组织和癌旁组织中表达情况及其对胃癌患者预后的影响。**结果:**与癌旁组织相比,胃癌组织中 SLC1A5 表达明显上调($P<0.0001$)。数据库研究也显示 SLC1A5 在胃癌组织中表达明显上调(GSE 65801, $P=0.0046$; GSE 63809, $P<0.0001$; GSE 27342, $P=0.0147$)。胃癌组织中 SLC1A5 高表达与肿瘤大小($P<0.05$)、肿瘤浸润深度($P<0.01$)、淋巴结转移($P<0.05$)、TNM 分期($P<0.05$)和 Ki-67($P<0.01$)相关,而与年龄、性别、肿瘤位置及分化程度均无显著相关性($P>0.05$)。胃癌组织中 SLC1A5 表达强度与患者预后相关,表达越高,患者预后越差(总体生存率, $P=0.0131$; 无复发生存率, $P=0.0293$)。数据库分析也显示 SLC1A5 高表达可明显缩短患者的总体生存期(GSE 14210, $P=0.011$; GSE 22377, $P=0.0015$)和无进展生存期(GSE 14210, $P=0.0095$; GSE 22377, $P=0.0012$)。**结论:**SLC1A5 蛋白表达在胃癌组织中明显上调,且与肿瘤大小、肿瘤浸润深度、淋巴结转移及 TNM 分期有关。SLC1A5 高表达与胃癌患者预后不良密切相关。

关键词:胃癌;溶质载体家族 1 成员 5;诊断;预后

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Expression of SLC1A5 in the Gastric Cancer and Its Correlation with the Clinicopathological Features of Gastric Cancer*

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ABSTRACT Objective: To detect the expression of Solute Carrier Family 1 Member 5 (SLC1A5) in the gastric cancer (GC) and analyze its relationship with the clinicopathological features and prognosis of GC patients. **Methods:** 90 cases of advanced GC tissue were selected as the experimental group, and corresponding paracancerous tissues were selected as the control group. Immunohistochemistry was used to detect the protein expression of SLC1A5 in 90 cases of advanced gastric cancer and its adjacent tissues. The relationship between the expression of SLC1A5 and clinicopathological features as well as the prognosis of GC patients was analyzed. By using gene database, we also analyzed the expression of SLC1A5 in GC tissues and adjacent tissues and its relationship with the prognosis of GC. **Results:** Compared to the control group, the expression of SLC1A5 was significantly increased in the advanced GC ($P<0.0001$). Statistical analysis by using GSE database also showed that the expression of SLC1A5 was significantly increased in the GC tissues compared to that of the paracancerous tissues (GSE 65801, $P=0.0046$; GSE 63809, $P<0.0001$; GSE 27342, $P=0.0147$). The expression of SLC1A5 in the GC tissue was correlated with the tumor size ($P<0.05$), depth of invasion ($P<0.01$), lymph node metastasis ($P<0.05$), TNM stage ($P<0.05$) and Ki-67 index ($P<0.01$). It had no significant correlation with the age, sex, tumor location and degree of differentiation. Overexpression of SLC1A5 in the GC tissue was associated with the poor prognosis (overall survival rate, $P=0.0131$; non-recurrence survival rate, $P=0.0293$). Meanwhile, database analysis also showed that higher expression level of SLC1A5 in GC was associated with the poor prognosis, which could significantly shorten the overall survival time (GSE 14210, $P=0.011$, GSE 22377, $P=0.0015$), and the progression free survival time of GC patients (GSE 14210, $P=0.0095$; GSE 22377, $P=0.0012$). **Conclusion:** SLC1A5 protein expression in the GC tissue was significantly upregulated, and it may be involved in the development of advanced GC. High expression of SLC1A5 was associated with the poor prognosis of GC patients.

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前言

溶质载体家族 1 成员 5 (Solute carrier family 1 member 5, SLC1A5)是氨基酸转运载体家族中的重要一员,主要转运多种小分子中性氨基酸。近年来,不少研究显示 SLC1A5 在多种肿瘤组织和细胞中呈高表达,包括乳腺癌^[1,2]、结肠癌^[3]、肺癌^[4,5]、黑色素瘤^[6]、神经母细胞瘤^[7]、胶质母细胞瘤^[8]、前列腺癌^[9]、胰腺癌^[10]、舌癌^[11]、子宫内膜癌^[12]、肝细胞癌^[13]、食管鳞癌^[14,15]、喉癌^[16]、肾癌^[17]、急性髓性白血病^[18]、多发性骨髓瘤^[19]。此外,研究还显示 SLC1A5 的高表达与肿瘤恶性生物学特征和预后不良预后有关^[11,14,17,20-22],SLC1A5 可作为预估恶性肿瘤预后的重要指标。本研究采用免疫组化技术检测 90 对进展期胃癌病理组织及对应癌旁组织中 SLC1A5 的表达情况,并分析其表达强度与胃癌临床病理特征及预后之间的关系。同时,通过基因数据库检索统计进一步分析 SLC1A5 在胃癌组织和癌旁组织中表达情况,并分析其对胃癌患者生存期的影响。

1 资料与方法

1.1 一般资料

采集行进展期胃癌切除术的胃癌组织及对应癌旁组织标本 90 例,时间跨度为 2013 年 1 月至 2013 年 06 月,标本采集自我院及南京鼓楼医院。所选患者均未在术前进行化疗、放疗等。在胃癌组织标本恶性肿瘤边缘处切取胃癌组织,在距胃癌组织边缘至少 5 cm 以上处切取癌旁组织。在 90 例患者中,男性有 69 例,女性有 21 例。年龄区间在 37 岁至 83 岁之间,平均年龄为 62.3 岁。胃癌的临床诊断标准及病理分期参考相应文献^[23]。同时我们运用数据库分析了 SLC1A5 在胃癌组织和癌旁组织中的表达情况及与患者预后的关系。数据来源于美国国立生物技术信息中心 (National Center for Biotechnology Information,NCBI),GEO (Gene Expression Omnibus) 数据库 (<https://www.ncbi.nlm.nih.gov/geo/>)。

1.2 试剂

兔抗人 SLC1A5 抗体(ab84903)抗体:Abcam 公司。SP 检测试剂盒、DAB 显色液:北京中杉金桥生物技术有限公司。

1.3 免疫组化检测

90 例胃癌组织及癌旁正常组织蜡块连续切片,采用免疫组化 SP 法,根据下列步骤进行:将厚石蜡包埋标本切片,切片厚度为 4 mm,然后将其置于烘烤箱中烘烤过夜,温度为 60℃。烤片结束后予水化,然后用与二抗同源的正常血清封闭非特异性抗原,在 37℃下孵育 30 分钟后,用滤纸吸去血清,然后滴入 1:1000 比例稀释的 SLC1A5 一抗,在 4℃下孵育过夜。孵育结束后,用 PBS 漂洗三次,每次约 3 分钟。接着加入与一抗属性对应的标记过辣根过氧化物酶的二抗工作液,在湿盒中 37℃孵育 30 分钟。最后用 DAB 显色 5 分钟后,苏木素室温下衬染芯片,最后将切片进行酒精分化,脱水,封片。

1.4 免疫组化结果判读标准

由资深病理科医师进行显微镜下观察,并对免疫组化结果进行评级。表达等级由细胞显色强度和阳性细胞比例决定,分为 4 个等级:无细胞着色为“-”;1%-30%细胞显示强度为弱、中等阳性反应者为“+”;30%细胞显示强度为强阳性或 30%-60%细胞显示强度为中等阳性反应者为“++”;大于 60%细胞显示强度为强阳性反应者为“+++”。“-”和“+”为低表达组,“++”和“+++”为高表达组。

1.5 统计学分析

数据用 GraphPad Prism 5 进行统计分析,SLC1A5 表达强度与临床病理特征之间的关系应用 Chi-square test,采用 Kaplan-Meier 法对 SLC1A5 表达进行累积生存时间分析,以 $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 SLC1A5 在进展期胃癌组织和癌旁组织中的表达情况

图 1A 显示 SLC1A5 在胃癌组织与癌旁组织中的表达情况,统计分析显示胃癌组织中 SLC1A5 表达较癌旁组织显著上调($P < 0.0001$)(图 1B,1C)。进一步通过 GSE 数据库进一步分析 SLC1A5 在胃癌组织和癌旁组织中的表达差异情况,结果显示:与癌旁组织相比,胃癌组织中 SLC1A5 表达显著上调(GSE 65801, $P = 0.0046$; GSE 63809, $P < 0.0001$; GSE 27342, $P = 0.0147$)(图 2)。

2.2 胃癌组织中 SLC1A5 的表达与患者临床病理参数的关系

胃癌组织中 SLC1A5 表达强度与患者的年龄、性别、肿瘤位置、肿瘤分化程度均无显著相关性($P > 0.05$);SLC1A5 的表达强度与肿瘤大小($P < 0.05$)、肿瘤浸润深度($P < 0.05$)、淋巴结转移($P < 0.05$)、TNM 分期($P < 0.05$)和 Ki-67 指数($P < 0.05$)均呈显著相关性,见表 1。

2.3 胃癌组织中 SLC1A5 表达与患者预后的关系

所有患者随访 22~60 个月,失访 17 例,失访率 18.9%。采用 Kaplan-Meier 法对 SLC1A5 表达进行累积生存时间分析,结果显示 SLC1A5 高表达与胃癌患者预后不良有关,可降低胃癌患者的无复发生存率($P = 0.0293$)(图 3A),同时缩短患者总生存率($P = 0.0131$)(图 3B)。我们进一步通过数据库分析了 SLC1A5 表达与胃癌患者预后的关系,统计数据来源于 NCBI, GEO 数据库 GSE 14210 (N=119),GSE 22377 (N=43)。通过 Kaplan-Meier 法对 SLC1A5 表达进行累积生存时间分析,结果见图 4;SLC1A5 表达强度与胃癌患者预后相关。SLC1A5 高表达可明显降低患者的总体生存期 (GSE 14210, $P = 0.011$; GSE 22377, $P = 0.0015$),同时降低患者的无进展生存期(GSE 14210, $P = 0.0095$; GSE 22377, $P = 0.0012$)。

3 讨论

在我国,胃癌的发病率常年居高不下,处于所有恶性肿瘤的第二位,而在全球范围内,其肿瘤致死率居所有恶性肿瘤第二位^[24]。进展期胃癌患者肿瘤转移比例及死亡率较高,临床预

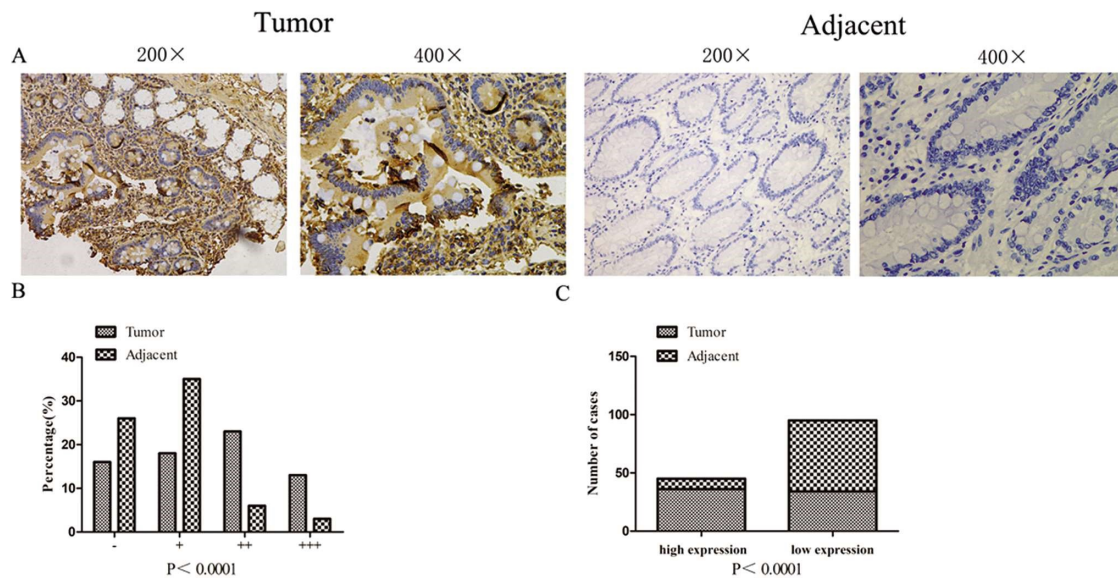


图 1(A)SLC1A5 在肿瘤组织(实验组)、癌旁组织(对照组)中表达水平(200×, 400×); (B,C)SLC1A5 在肿瘤组织和癌旁组织中的表达差异情况

Fig. 1 (A) The expression level of SLC1A5 in tumor tissues (experimental group) and paracancerous tissues (control group) (200×, 400×);

(B, C) The difference of SLC1A5 expression in tumor tissues and paracancerous tissues

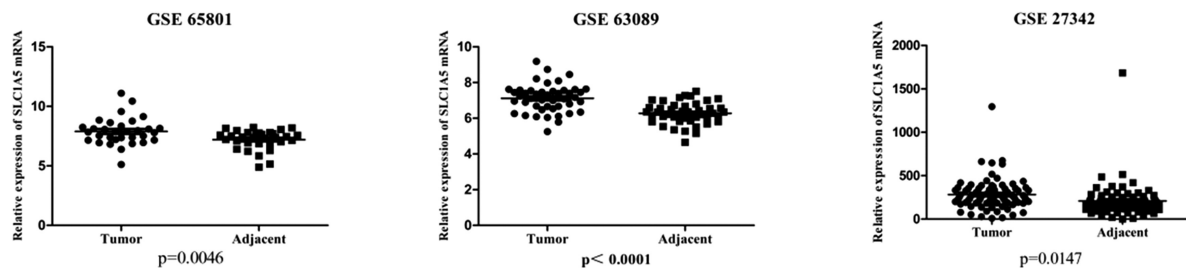


图 2 SLC1A5 在肿瘤组织和癌旁组织中的 mRNA 表达情况。数据来源于 NCBI GEO 数据库

Fig. 2 SLC1A5 gene expression in gastric cancer tissues(Tumor) and adjacent noncancerous tissues (Adjacent) as determined by gene expression array.

Data from NCBI, GEO database(<https://www.ncbi.nlm.nih.gov/geo/>) GSE65801 (N=32), GSE63089 (N=45), GSE27342 (N=80)

后较差。因此,我们需要进一步研究胃癌的发生发展机制,并从胃癌组织和细胞中找寻新的诊断和治疗靶点,进一步阐述胃癌的发病机制,并通过对相关靶点的分析研究,指导胃癌的临床诊断和治疗。

恶性肿瘤由于其快速增殖方式,需要摄取更多的能量,其中谷氨酰胺是肿瘤快速生长的重要能量来源之一^[25-30]。细胞外的谷氨酰胺必须借助于细胞膜上的一系列氨基酸转运载体才能进入细胞内,而 SLC1A5 作为谷氨酰胺最主要的转运载体可能通过适应性反应上调其在肿瘤组织中的表达。Honjo H^[14]等通过免疫组化的方法发现 SLC1A5 在 48% 的食管鳞癌组织中表达上调,其表达越高,提示肿瘤浸润深度越深,越容易出现淋巴结转移和血管侵袭。同时,高表达 SLC1A5 的食管鳞癌患者的 5 年生存率和低表达 SLC1A5 的食管鳞癌患者相比有所降低,提示高表达 SLC1A5 与食管鳞癌的预后不良有关。Yazawa T 等^[20]也通过免疫组化的方法发现在 40% 肺腺癌组织中存在 SLC1A5 高表达,且其高表达与肿瘤分期、淋巴结转移及血管侵袭有关,并且 SLC1A5 的表达还与反应肿瘤增殖活跃度的 ki67 指数呈正相关。同样,高表达 SLC1A5 的肺腺癌患者的预后更差,5 年生存率明显下降。Toyoda M 等^[11]的研究证实舌癌组织中,SLC1A5 也存在高表达(59%, 50/85),同时其高表达也与肿瘤分期、淋巴结转移及 ki67 指数有关。在预后方面,

SLC1A5 高表达患者的 5 年生存率仅为 39.4%,而低表达患者却高达 82.3%,提示 SLC1A5 高表达与舌癌患者的预后不良有关。Liu 等^[17]通过组织芯片检测了 187 名透明细胞性肾细胞癌组织中 SLC1A5 的表达情况,发现 SLC1A5 高表达与肿瘤 TNM 分期相关,并且导致患者 5 年生存率下降。作者进一步进行多因素分析,证实 SLC1A5 为透明细胞性肾细胞癌患者总生存率的独立预后指标,同时研究还表明 SLC1A5 具有比 TNM 分期系统更好的预测价值,提示 SLC1A5 可作为预估肿瘤预后的重要指标。

本研究通过免疫组化实验发现 SLC1A5 在胃癌组织中表达与癌旁组织相比显著上调,NCBI-GEO 数据库数据统计分析结果也证实这一点。此外,胃癌组织中 SLC1A5 高表达与肿瘤大小、肿瘤浸润深度、淋巴结转移、TNM 分期和 Ki-67 指数有关,这与 SLC1A5 在其他恶性肿瘤中的情况相类似。而 SLC1A5 高表达与一些反映肿瘤增殖能力的指标有相关性,如肿瘤大小和 Ki-67;同时其高表达也与预示胃癌患者预后不良的指标相关性较大,如肿瘤浸润深度、淋巴结转移、TNM 分期,提示 SLC1A5 和肿瘤增殖和预后不良有关。我们进一步采用 Kaplan-Meier 法对 SLC1A5 表达进行累积生存时间分析,结果表明 SLC1A5 高表达与胃癌患者预后不良有关,可降低胃癌患者的无复发生存率和总生存率。同时数据库分析也证实这一

表 1 胃癌组织中 SLC1A5 表达与患者临床病理特征的关系

Table 1 Relationship between SLC1A5 expression and clinicopathological features of gastric cancer

Clinicopathologic parameters	SLC1A5 expression		Relative Risk(RR)	P*
	High (n = 48)	Low (n = 42)		
Age (years)				
≤ 60	22	18	1.058	0.7768
>60	26	24		
Age (years)				
Gender				
Male	34	33	0.8337	0.4011
Female	14	9		
Diameter (cm)				
≤ 5	18	26	0.6273	0.0209
>5	30	16		
Location				
Distal third	36	29	1.154	0.5294
Middle or proximal third	12	13		
1				
Differentiation				
Middle/well differential	10	10	0.9211	0.7347
Poorly differential	38	32		
Location invasion				
T1, T2	13	24	0.5320	0.0038
T3, T4	35	18		
Lymph node metastasis				
No	10	20	0.5263	0.0072
Yes	38	22		
TNM stage				
I,II	10	19	0.5535	0.0135
III,IV	38	23		
Ki-67				
Low	13	23	0.5571	0.0075
High	35	19		

点:SLC1A5 表达强度与患者预后相关,SLC1A5 高表达的患者,其总体生存期明显缩短,同时其无进展生存期也显著缩短。

综上所述,SLC1A5 蛋白表达在胃癌组织中明显上调,且

与肿瘤大小、肿瘤浸润深度、淋巴结转移及 TNM 分期有关。SLC1A5 高表达与胃癌患者预后不良密切相关。SLC1A5 在胃癌的发生和发展中起到重要作用,阻断该靶点可能成为胃癌治

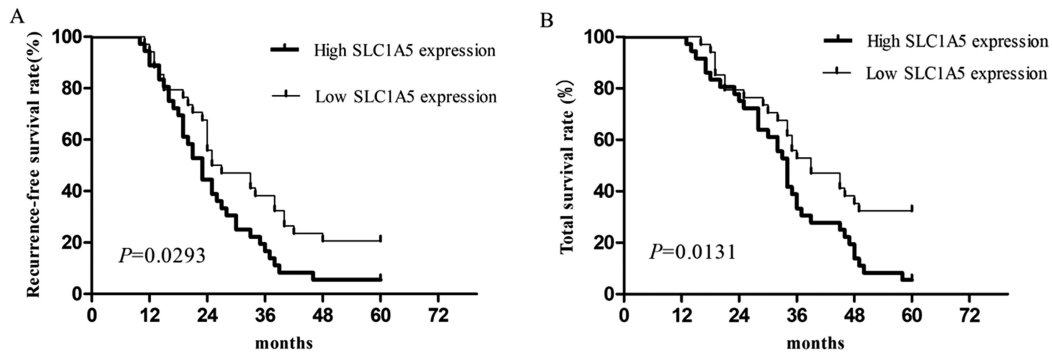


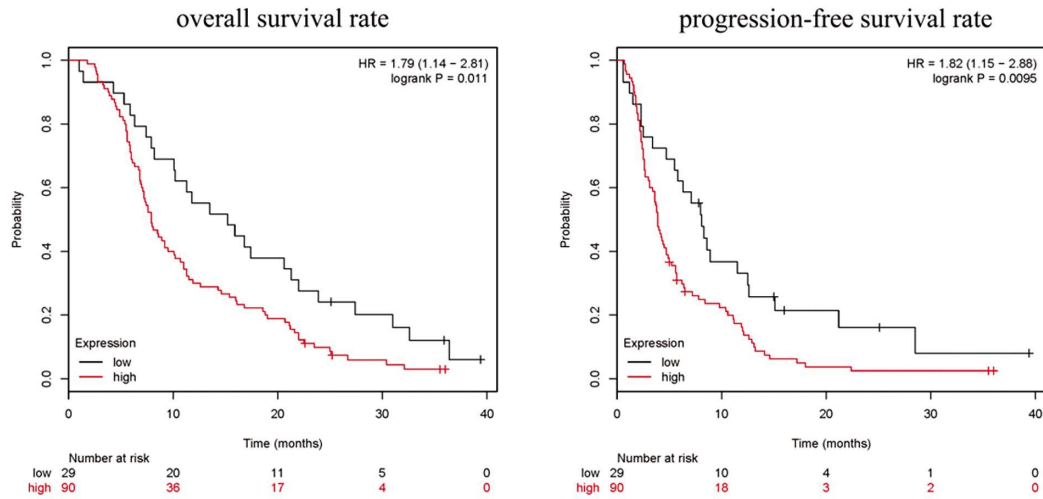
图 3 胃癌组织 SLC1A5 表达强度与患者预后的关系。(A)SLC1A5 表达强度越高,患者无复发生存期越短($P=0.0293$);
(B)SLC1A5 表达强度越高,患者总生存期越短($P=0.0131$)

Fig. 3 The relationship between the expression level of SLC1A5 in gastric cancer and the prognosis of patients.

(A) High expression of SLC1A5 is associated with a shorter recurrence-free survival rate ($P=0.0293$);

(B) High expression of SLC1A5 is associated with a shorter overall survival rate ($P=0.0131$).

GSE14210



GSE22377

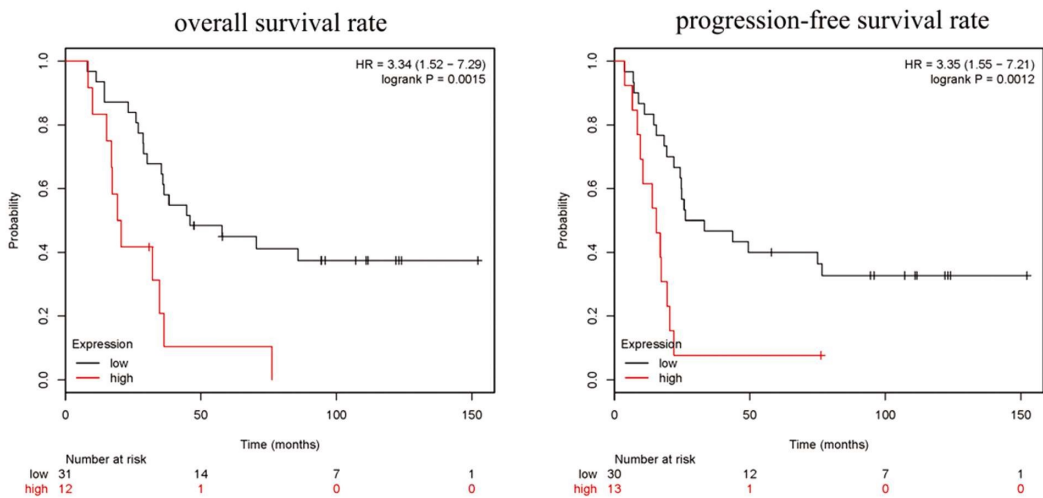


图 4 胃癌组织中 SLC1A5 表达强度与患者预后的关系。SLC1A5 表达强度越高,患者预后越差。

总体生存期(GSE 14210, $P=0.011$;GSE 22377, $P=0.0015$),无进展生存期(GSE 14210, $P=0.0095$;GSE 22377, $P=0.0012$)

Fig. 4 The relationship between the expression level of SLC1A5 in gastric cancer and the prognosis of patients. High expression of SLC1A5 is associated with worse prognosis. Overall survival (GSE 14210, $P=0.011$; GSE 22377, $P=0.0015$), progression-free survival (GSE 14210, $P=0.0095$; GSE 22377, $P=0.0012$)

疗的新方法。SLC1A5 可能成为进展期胃癌患者诊断、治疗和预后预测的参考指标。

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