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HIF–1和CD24在结肠癌组织中的表达及其意义

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[摘 要] **目的：**检测HIF-1和CD24蛋白在结肠癌组织中表达，探讨HIF-1和CD24蛋白与结肠癌患者预后的关系。**方法：**收集25例明确诊断的结肠癌组织及其相应癌旁组织，提取总蛋白。采用Western-blot方法检测HIF-1和CD24蛋白在结肠癌组织和癌旁组织中的含量。利用Pearson相关性分析评估HIF-1和CD24蛋白表达的相互关联。Kaplan-Meier生存曲线分析HIF和CD24蛋白与结肠癌患者预后的相关性。**结果：**HIF-1和CD24蛋白在结肠癌组织中的含量明显高于癌旁组织(P<0.05)。HIF-1与患者的性别、年龄、肿瘤分化、淋巴侵袭、静脉侵袭、肿瘤大小和病理分期因素无关。CD24与患者的性别、淋巴侵袭、静脉侵袭、肿瘤大小和病理分期因素无关。但与患者年龄和肿瘤分化两个因素相关。Kaplan-Meier生存曲线分析显示同时表达HIF-1和CD24的患者比单独表达HIF-1或CD24的患者预后更差。**结论：**HIF-1和CD24蛋白在结肠癌组织中表达异常升高，并且与结肠癌患者预后相关。

[关键词] 结肠癌；CD24；HIF-1；预后；病理特征

The role of HIF-1 and CD24 in colorectal cancer

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Abstract **Objective:** To determine the HIF-1 and CD24 expression in colorectal cancer and investigate the relationship between the expression of HIF-1 and CD24 and the prognosis of the patients with colorectal cancer. **Methods:** The tissues of cancer center and peritumoral of esophageal carcinoma were collected by surgery. The levels of HIF-1 and CD24 proteins were detected by using Western-blot. The correlation between CD24 protein and HIF-1 protein was evaluated by using Pearson correlation analysis. Kaplan-Meier survival curve analysis was used to analysis the relationship of CD24 and HIF-1 protein with the prognosis of patients with colon cancer. **Results:** The levels of HIF-1 and CD24 expression were higher in cancer tissues than that in normal tissues (P<0.05). HIF-1 expression was not associated with sex, age, differentiation, lymphatic invasion, venous invasion, tumor size and pN category. CD24 expression was not associated with sex, lymphatic invasion, venous invasion, tumor size and pN category, but related with age of patient and tumor differentiation. The survival rate of the patients with both HIF-1 and CD24 expression was lower than the patients with HIF-1 or CD24 expression by using Kaplan-

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Meier survival curve analysis (P<0.05). Conclusion: Abnormal high HIF-1 and CD24 expression was observed in colorectal cancer tissues, which suggest that there was association between HIF-1 or CD24 and the development of colorectal cancer.

Key words colorectal cancer; CD24; HIF-1; prognosis; pathological feature

结肠癌是我国常见的恶性肿瘤，死亡率高居恶性肿瘤第二位[1]。缺氧诱导因子(hypoxia inducible factor，HIF-1)是哺乳动物机体功能在缺氧条件下一个非常重要的转录调节因子。HIF-1可以调节肿瘤的发生和发展、休克及炎症等病理生理过程[2]。CD24在多种恶性肿瘤中不同程度的表达，且与肿瘤的生长、发展、转移和预后密切相关[3-5]。Thomas等[6]的研究证实在肿瘤的生长和转移过程中，CD24是HIF-1的一个效应蛋白。本文利用western-blot检测结肠癌组织和癌旁组织中HIF-1和CD24蛋白含量，并分析二者之间的关联及其与患者预后的关系。

1 资料与方法

1.1 一般临床资料

收集2010年1月至2012年12月在辽宁省肿瘤医院手术的结肠癌组织及相应癌旁组织25例。其中男性17例，女性8例，年龄44~72岁，平均年龄55岁。所有病例通过病理组织细胞形态学加以确诊。

1.2 器材与试剂

抗体CD24(sc-11406)，HIF-1(sc-10790)和β-actin(sc-47778)购自美国Santa Cruz公司。

1.3 方法

HIF-1和CD24蛋白含量检测：将收集的病理标本，加RIPA裂解液(20 mmol/L Tris/HCl PH=7.5，50 mmol/L NaCl，0.1 mmol/L Na_3Vo_4，25 mmol/L NaF，2 mmol/L EDTA，1 mmol/L DTT，1 mg/L 亮肽酶/抑肽酶) 200 μL，冰上放置30 min，匀浆破碎15 sec/次，3~4次。12 000 rpm离心20 min取上清，即为总蛋白。考马斯亮蓝法测定蛋白含量，取40 μg总蛋白上样，经10% SDS-PAGE凝胶电泳分离蛋白后将蛋白电转移到NC膜上。5%脱脂奶粉室温封闭1 h，加入一抗4 ℃过夜，辣根过氧化酶标记的二抗室温作用1 h。每次孵育后均用含1‰ Tween-20的TBS洗脱3次，每次5 min。待NC膜稍干后，在膜上滴加适量的(enhanced chemiluminescence，ECL)显影，并上机扫描测灰度值。

1.4 统计学处理

用SPSS13.0软件进行统计分析，以P<0.05为差异有统计学意义。HIF-1和CD24蛋白在结肠癌组织中的表达差异采取t检验进行分析，二者关系采用Pearson相关性分析。生存时间定义为从初次手术之日起到末次随访或病人死亡的时间。用Kaplan-Meier法建立生存曲线，用Log-rank检验比较两组之间生存率差异。

2 结 果

2.1 结肠癌组织 HIF-1 和 CD24 蛋白含量检测

利用Western-blot检测结肠癌组织与配对癌旁组织中的HIF-1和CD24蛋白水平，并利用gel-pro analyzer软件半定量测定HIF-1和CD24蛋白条带灰度值。实验结果表明结肠癌组织与癌旁组织相比，HIF-1和CD24的蛋白水平显著升高(P<0.05，图1)。

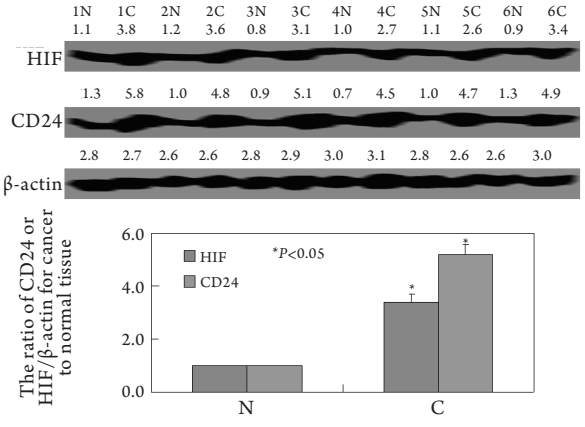

图 1 Western-blot 检测结肠癌组织与配对癌旁正常组织中 HIF-1 和 CD24 的蛋白水平。

Figure 1 Western-blot analysis of the levels of HIF-1 and CD24 in colorectal cancer tissues and matched normal tissues.

N: normal tissue; C: colorectal cancer tissue.

2.2 结肠癌组织 HIF-1 和 CD24 的蛋白相关性

利用Pearson相关性分析，结果提示：在结肠癌组织中，HIF-1和CD24的蛋白表达具有显著的相关性(r=0.925，P<0.05，图2)。

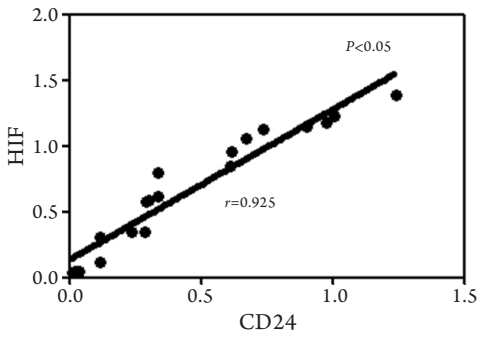

图 2　结肠癌组织中 HIF-1 和 CD24 蛋白的相关性

Figure 2　Correlation between the protein levels of HIF-1 and CD24 in colorectal cancer

2.3　结肠癌组织 HIF-1 和 CD24 的蛋白含量与患者临床资料及生存曲线

如表1所示，HIF-1与患者的性别(P=0.804)、年龄(P=0.592)、肿瘤分化(P=0.443)、淋巴侵袭(P=0.525)、静脉侵袭（P=0.592）、肿瘤大小(P=0.648)和病理分期(P=0.465)因素无关。HIF-1与患者的性别(P=0.560)、淋巴侵袭(P=0.924)、静脉侵袭(P=0.851)、肿瘤大小(P=0.851)和病理分期(P=0.774)因素无关。但与患者年龄(P=0.003)和肿瘤分化(P=0.026)两个因素相关。Kaplan-Meier生存曲线分析显示HIF-1和CD24蛋白与结肠癌患者预后具有显著相关性。同时表达HIF-1和CD24的患者比单独表达HIF-1或CD24的患者预后更差(图3)。

表1　HIF-1和CD24与结肠癌患者临床病理资料相关性分析

Table 1　Relationship between HIF-1 or CD24 and clinicopathological parameters of patients with colorectal cancer

Clinicopathological features	n	HIF-1				CD24			
		low	high	χ^2	P	low	high	χ^2	P
Sex				0.062	0.8039			0.339	0.5604
Female	8	3	5			3	5		
Male	17	4	13			3	14		
Age (years)				0.287	0.5922			8.651	0.0033
<65	7	3	4			5	2		
≥65	18	4	14			1	17		
Differentiation				0.588	0.4432			4.977	0.0257
Differentiated	13	5	8			6	7		
Unidifferentiated	12	2	10			0	12		
Lymphatic invasion				0.405	0.5245			0.009	0.9238
-	10	4	6			3	7		
+	15	3	12			3	12		
Venous invasion				0.287	0.5922			0.035	0.8511
-	18	4	14			5	13		
+	7	3	4			1	6		
Tumor size (cm)				0.208	0.6481			0.035	0.8511
<4	7	2	5			2	5		
≥4	18	5	13			4	14		
pN category				2.560	0.4645			1.112	0.7741
pN0	6	2	4			2	4		
pN1	6	1	5			2	4		
pN2	6	3	3			1	5		
pN3	7	1	6			1	6		

χ^2 value, Chi-square distribution.

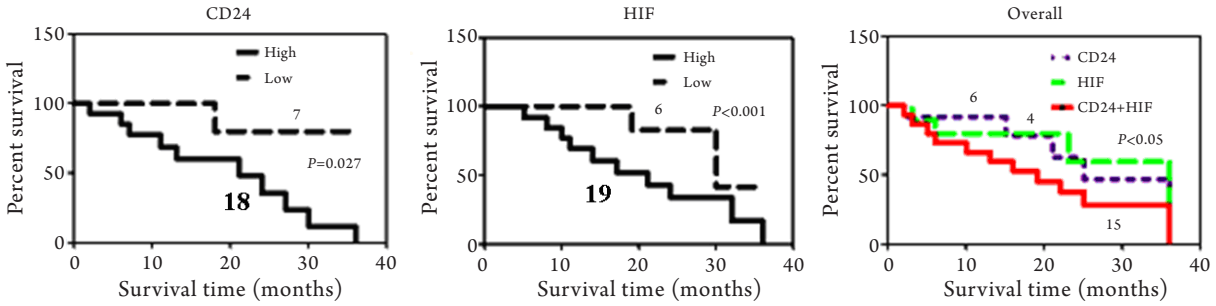

图 3 Kaplan-Meier 生存曲线显示结肠癌 CD24 和 HIF-1 表达与患者生存时间的关系

Figure 3 The relationship between CD24 and HIF-1 protein with the survival time of the patients was showed by Kaplan-Meier survival curve

3 讨 论

HIF-1最先由Semenza和Wang[7]于1992年发现，将其作为被缺氧诱导的连接在(erythropoietin，EPO)基因诱导元件的一个核因子。HIF-1由HIF-lα和β亚基组成的异源二聚体，这两个亚基分子均属于基础螺旋-环-螺旋超家族[8]。目前已经在多种肿瘤组织中发现HIF-l表达，但是机制不尽相同。Shu等[9]研究发现HIF-1表达与肿瘤细胞的增殖、浸润及转移相关，其表达越高，预后越差。Danert等[10]研究表明，HIF-1在神经胶质瘤细胞的VEGF基因缺氧诱导的转录、活化过程中起决定作用。Stoehzing等[11]研究表明，抑制HIF-1能够损害胃部肿瘤生长和瘤内血管发生。近期研究还发现HIF-1能够在CD133阳性的胰腺癌细胞中引起自噬[12]。这为我们今后的研究提供了新的方向。国内学者李振祥等[13]研究表明HIF-1α在细支气管肺泡癌和混合型肺腺癌中的表达增高。龙方懿等[14]研究发现HIF-1在甲状腺滤泡状癌组织中高表达。在陆晔斌等[15]的研究中同样发现HIF-1在胰腺癌组织中高表达并与患者预后相关。和以往的研究结果一致，在我们的研究中发现HIF-1在结肠癌组织中含量增高并导致患者预后不良。CD24同样在多种恶性肿瘤中不同程度的表达，且与肿瘤的生长、发展、转移和预后密切相关[3-5]。在我们的试验中发现，CD24在结肠癌组织中高表达，并且可以作为结肠癌患者预后不良的标志。

尽管以往文献中已经阐明CD24在多种肿瘤中的表达和作用[16-18]，本实验首次在结肠癌组织中同时检测HIF-1和CD24的表达，并且明确了二者之间存在正相关。Wang等[19]在乳腺癌中发现HIF-1在乳腺癌早期CD24阴性的细胞中高表达。我们认为由于组织差异性造成的HIF-1与CD24相关性差异。目前有关二者关系的实验相对较少，我们将收集消化道其他肿瘤样本，以明确二者的关系。Thomas等[6]研究确定CD24是HIF-1导致肿瘤迁移的效应器，但是其具体机理还有待研究。我们将在进一步的试验中检测二者是否存在直接的相互作用。

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