四川大学学报（医学版） 2024，55（2）：375－382
J Sichuan Univ（Med Sci） doi: 10.12182/20240360603

在线全文

结直肠癌根治术后患者Tim-3、galectin-9表达水平与其临床病理特征及预后的关系*

张怡然[1,2]，邓 丹[2]，尹 万[2]，罗 俊[3]，刘金星[2]，谢臣健[2]，季星利[2]，马 立[2]，张 莉[2]，夏仙根[2]，程胜君[2]，黄安亮[2]，杨 帆[2△]

1. 成都中医药大学医学与生命科学学院（成都 611137）；
2. 成都市第五人民医院（成都中医药大学附属第五人民医院/第二临床医学院）病理科，成都市肿瘤防治所（成都 611137）；
3. 成都市第二人民医院 检验科（成都 610017）

【摘要】 目的 结直肠癌经根治术治疗后仍有部分患者易复发且预后较差，因此，寻找结直肠癌根治术患者预后判断的潜在生化标志物和药物靶点，对于优化此类群体临床结局具有积极意义。近年来新发现T细胞免疫球蛋白及黏蛋白结构域分子3（T cell immunoglobulin and mucin domain protein 3, Tim-3）及其配体半乳糖凝集素9（galactose lectin 9, galectin-9）在包括结直肠癌等多种肿瘤引起的免疫功能障碍中扮演着关键角色，但是其在结直肠癌中的表达、生物学作用与预后的价值尚不明确，故而本文旨在探究结直肠癌根治术患者Tim-3、galectin-9表达水平与其临床病理特征及预后的关系。**方法** 选择2018年2月–2019年3月于成都市第五人民医院进行结直肠癌根治术治疗患者171例，采用免疫组化测定患者癌组织样本及癌旁组织中Tim-3、galectin-9的表达情况，并分析其与患者基线参数的关系。利用Kaplan-Meier法分析Tim-3、galectin-9与结直肠癌患者无复发生存期（relapse-free survival, RFS）和总生存期（overall survival, OS）的关系，Cox回归分析影响患者预后不良因素。**结果** 免疫组化结果显示，结直肠癌组织中Tim-3、galectin-9的高表达率分别为70.18%（120/171）、32.16%（55/171），而低表达率分别为29.82%（51/171）、67.84%（116/171）。同时，结直肠癌组织Tim-3的表达评分相较于配对癌旁组织偏高，而galectin-9的表达评分则偏低（P<0.05）；进一步分析发现，Tim-3、galectin-9表达与浸润深度、脉管浸润、临床分期均有关（P<0.05）。随访14～63个月，171例结直肠癌患者中有7例失访，剩余患者组织中Tim-3、galectin-9表达呈现异常低表达的患者分别为49、112例，高表达患者分别为115、52例，Kaplan-Meier生存分析显示，结直肠癌组织中Tim-3高表达者的RFS和OS均低于低表达者，差异有统计学意义（RFS：log-rank=22.66，P<0.001；OS：log-rank=19.71，P<0.001），而galectin-9低表达者的RFS和OS则低于高表达者，差异有统计学意义（RFS：log-rank=19.45，P<0.001；OS：log-rank=22.24，P<0.001）。Cox多因素分析表明，TNM分期Ⅲ期〔风险比（hazards ratio, HR）=2.26，95%置信区间（confidence interval, CI）：1.20～5.68〕以及Tim-3高表达（HR=0.80，95%CI：0.33～0.91）、galectin-9低表达（HR=1.80，95%CI：1.33～4.70）均为影响患者RFS和OS的独立危险因素（P<0.05）。**结论** 结直肠癌组织中Tim-3、galectin-9呈现异常表达，其中高表达Tim-3、低表达galectin-9与不良临床病理特征及预后关系紧密，是患者不良预后事件的独立影响因素，有望成为新的治疗靶点和临床指标。

【关键词】 结直肠癌 T细胞免疫球蛋白及黏蛋白结构域分子3 半乳糖凝集素9 临床病理特征 预后判断

Relationship Between Tim-3 and Galectin-9 Expression Levels, Clinical Pathological Characteristics, and Prognosis in Patients After Radical Resection of Colorectal Cancer

ZHANG Yiran[1,2], *DENG Dan*[2], *YIN Wan*[2], *LUO Jun*[3], *LIU Jinxing*[2], *XIE Chenjian*[2], *JI Xingli*[2], *MA Li*[2], *ZHANG Li*[2], *XIA Xiangen*[2], *CHENG Shengjun*[2], *HUANG Anliang*[2], *YANG Fan*[2△]. 1. *School of Medical and Life Sciences, Chengdu University of Traditional Chinese Medicine*, Chengdu 611137, China; 2. *Cancer Prevention and Treatment Institute of Chengdu, Department of Pathology, Chengdu Fifth People's Hospital* (*The Second Clinical Medical College, Affiliated Fifth People's Hospital of Chengdu University of Traditional Chinese Medicine*), Chengdu 611137, China; 3. *Department of Laboratory, Chengdu Second People's Hospital*, Chengdu 610017, China

△ Corresponding author, E-mail: fanyang@cdutcm.edu.cn

【Abstract】 Objective Some colorectal cancer patients still face high recurrence rates and poor prognoses even after they have undergone the surgical treatment of radical resection. Identifying potential biochemical markers and therapeutic targets for the prognostic evaluation of patients undergoing radical resection of colorectal cancer is crucial for improving their clinical outcomes. Recently, it has been reported that the T cell immunoglobulin and mucin domain

* 成都高新医学会“2023年度结直肠癌专项科研基金”（No. 202304）、成都市科技局重点研发支撑计划技术创新研发项目（No. 2022-YF05-01725-SN）和成都市卫生健康委员会“2023年成都市医学科研课题”（No. 2023355）资助

△ 通信作者，E-mail：fanyang@cdutcm.edu.cn

出版日期：2024-03-20

protein 3 (Tim-3) and its ligand galactose lectin 9 (galectin-9) play crucial roles in immune dysfunction caused by various tumors, such as colorectal cancer. However, their expressions, biological functions, and prognostic value in colorectal cancer are still unclear. This study aims to investigate the relationship between Tim-3 and galectin-9 expression levels and the clinicopathological characteristics and prognosis of patients undergoing radical resection of colorectal cancer. **Methods** A total of 171 patients who underwent radical resection of colorectal cancer at Chengdu Fifth People's Hospital between February 2018 and March 2019 were selected. Immunohistochemistry was performed to assess the expression levels of Tim-3 and galectin-9 in the cancer tissue samples and the paracancerous tissue samples of the patients. The relationship between Tim-3 and galectin-9 expression levels and the baseline clinical parameters of the patients was analyzed accordingly. Kaplan-Meier analysis was performed to assess the association between Tim-3 and galectin-9 expression levels and the relapse-free survival (RFS) and the overall survival (OS) of colorectal cancer patients. Cox regression analysis was conducted to identify factors associated with adverse prognosis in the patients. **Results** The immunohistochemical results showed that the high expression levels of Tim-3 and galectin-9 were observed in 70.18% (120/171) and 32.16% (55/171), respectively, of the colorectal cancer tissues, whereas the low expression levels were 29.82% (51/171) and 67.84% (116/171), respectively. Furthermore, the expression score of Tim-3 was significantly higher in colorectal cancer tissues than that in the paracancerous tissues, while the expression score of galectin-9 was lower than that in the paracancerous tissues (P<0.05). Further analysis revealed that the expression of Tim-3 and galectin-9 was associated with the depth of tumor infiltration, vascular infiltration, and clinical staging (P<0.05). During the follow-up period of 14-63 months, 7 out of 171 patients were lost to follow-up. Among the remaining patients, 49 and 112 cases presented abnormally low expression of Tim-3 and galectin-9, respectively, whereas 115 and 52 cases presented high expression of Tim-3 and galectin-9, respectively. Kaplan-Meier survival analysis demonstrated that patients with high Tim-3 expression in colorectal cancer tissues had significantly lower RFS and OS than those with low expression did (RFS: log-rank=22.66, P<0.001; OS: log-rank=19.71, P<0.001). Conversely, patients with low galectin-9 expression had significantly lower RFS and OS than those with high expression did (RFS: log-rank=19.45, P<0.001; OS: log-rank=22.24, P<0.001). Cox multivariate analysis indicated that TNM stage Ⅲ (*HR*=2.26, 95% *CI*: 1.20-5.68), high expression of Tim-3 (*HR*=0.80, 95% *CI*: 0.33-0.91), and low expression of galectin-9 (*HR*=1.80, 95% *CI*: 1.33-4.70) were independent risk factors affecting RFS and OS in patients (P<0.05). **Conclusion** Aberrant expression of Tim-3 and galectin-9 is observed in colorectal cancer tissues. High expression of Tim-3 and low expression of galectin-9 are closely associated with adverse clinico-pathological characteristics and prognosis. They are identified as independent influencing factors that may trigger adverse prognostic events in patients. These findings suggest that Tim-3 and galectin-9 have potential as new therapeutic targets and clinical indicators.

【Key words】 Colorectal cancer T-cell immunoglobulin and mucin domain protein 3 Galactose lectin 9 Clinicopathological characteristics Prognosis assessment

结直肠癌是全球范围内较为常见的癌症之一，流行病学研究结果显示，约有20%的患者在临床诊断时已出现转移，有近30%的患者会发生复发，且随着近年来饮食及生活环境的改变，该病的全球罹患率及死亡率也呈上升趋势，罹患率仅次于肺癌和乳腺癌，死亡率位于第2位[1]。由此可见，寻找结直肠癌预后判断的潜在生化标志物和药物靶点，对于优化此类群体临床结局具备积极意义[2]。该病发生、发展涉及到多因素、多阶段、多分子，而免疫应答则在这一过程中扮演着关键性角色，其中有学者[3-4]指出T细胞免疫球蛋白及黏蛋白结构域分子3(T cell immunoglobulin and mucin domain protein 3, Tim-3)可致肿瘤细胞逃避免疫清除，造成肿瘤免疫逃逸并加速肿瘤的形成与发展，而其配体半乳糖凝集素9(galactose lectin 9, galectin-9)则可促进免疫活性细胞增殖和活化，在阻滞癌细胞的增殖、侵袭以及转移中扮演着关键性角色，故而肿瘤患者Tim-3/galectin-9平衡的改变亦是当下学者关注焦点之一。然而，现阶段临床上对于Tim-3、galectin-9在结直肠癌中的表达、生物学作用与预后的价值尚不明确，两者是否亦存在类似的特性仍缺少令人信服的实验数据。鉴于该背景，本研究以成都市第五人民医院进行结直肠癌根治术治疗的171例患者为研究对象，并测定此类病患癌组织及癌旁组织样本中Tim-3、galectin-9的表达情况，并收集其与基线数据及无复发生存期(relapse-free survival, RFS)和总生存期(overall survival, OS)的相关数据，旨在揭示其潜在的临床意义，为医学实践提供有价值的参考和指导。

1 资料与方法

1.1 一般资料

本研究已通过成都市第五人民医院医学伦理委员会

批准, 批准文号: 2022-010(科)-01, 以2018年2月–2019年3月于成都市第五人民医院进行结直肠癌根治术治疗的171例患者作为研究对象。纳入标准: ①所有受试者均通过病理及免疫组化检测确诊为结直肠癌, 符合《中国结直肠癌诊疗规范》标准[5]; ②符合手术指证, 且均实施规范性根治手术[6]; ③未合并肠道炎性疾病、肠梗阻、肠功能紊乱等并发症; ④留存肿瘤组织及正常组织(距肿瘤边缘5.0 cm)的蜡块标本; ⑤预期生存期> 3个月。排除标准: ①无法进行本研究所设计的长期规律随访, 且病理资料和随访信息不完整; ②5年内有其他恶性肿瘤既往史者; ③合并自身免疫系统疾病, 各项辅助检查不完善; ④合并大脑机能活动发生紊乱而不具备自主行为能力者; ⑤术前已转移、接受姑息性切除; ⑥妊娠或哺乳期妇女。全部受试者对研究知情, 并签署知情同意书, 同时术后均按医嘱进行后续治疗, 即行规范性化疗方案治疗。

1.2　方法

1.2.1　临床资料收集

采用联合自制问卷调查表和病历, 在确保遵循伦理原则和保护患者隐私基础上, 对患者年龄、性别、肿瘤位置、肿瘤大小、分化程度、浸润深度、淋巴结转移、脉管浸润、临床分期等基线信息进行详细记录。

1.2.2　HE染色和免疫组织化学染色

标本均经体积分数为10%中性甲醛溶液固定, 常规脱水后进行石蜡包埋, 选取典型癌组织和相邻癌旁正常组织均进行4 μm厚切片, 进行HE染色和EnVision两步法免疫组化染色, 具体操作步骤严格遵循试剂盒说明书进行。Tim-3、galectin-9抗体均购自上海户实医药科技有限公司(稀释比1∶100)。

1.2.3　免疫组化结果判定

采用双盲法由两位从事病理科工作多年资深病理学医师进行阅片并评分, 有意见分歧的病例, 由第3位医师进行综合判断。采用奥林巴斯的Bx43F显微镜观察染色结果, 在Tim-3、galectin-9阳性颗粒密集部位随机选取5个200倍视野, 计数染色情况, 取均值。结果判定: ①染色强度: 无色、浅黄色、棕黄色和棕褐色分别得0、1、2和3分; ②阳性细胞比例: 阳性表达率< 5%、5% ~ 25%、26% ~ 50%、51% ~ 75%、> 75%分别得分0、1、2、3和4分; 两者乘积为最终评分, 总分范围0 ~ 12分, 其中0 ~ 4分为低表达和5 ~ 12分为高表达。

1.2.4　随访

患者出院后采用电话或复诊等方式进行随访, 并于2023年4月30日截止, 具体频次遵循术后2年内每3个月随访1次, 2年以上每6个月随访1次, 随访时间14 ~ 63个月, 中位时间51个月, 在此期间收集到95.91%(164/171)的患者有完整信息。RFS和OS为研究终点, 其中RFS定义为患者根治术后至复发或末次随访时间, OS定义为患者根治术后至死亡或末次随访时间[7]。

1.3　统计学方法

用SPSS19.0进行统计分析, 经K-S检验服从正态分布的计量资料采取$\bar{x} \pm s$的形式作统计描述, 组间比较采取两独立样本t检验, 不满足正态分布采用中位数(四分位间距)表示, 采取Kruskal-Wallis H秩和检验。计数资料采用率(%)表示, 应用χ^2检验。利用Kaplan-Meier法分析Tim-3、galectin-9与结直肠癌患者终点事件的关系, 组间差异用对数秩检验。采用Cox比例风险模型探究影响结直肠癌病患RFS和OS的独立危险因素。P< 0.05为差异有统计学意义。

2　结果

2.1　不同病理切片中Tim-3、galectin-9蛋白的表达强度及分布

采用免疫组化测定Tim-3、galectin-9在171例患者肿瘤组织和配对癌旁组织中的蛋白表达, 结果显示: Tim-3蛋白主要在结肠癌组织及癌周炎症细胞中表达, 阳性信号定位于细胞质, 见图1A、1B; 癌周肠黏膜组织则不表达该蛋白, 见图1D、1E。Galectin-9蛋白罕见表达于癌周的炎症细胞, 多数结肠癌细胞会在胞质低表达该蛋白, 见图1A、1C; 而癌周肠黏膜组织中galectin-9蛋白同样会在胞质呈现较低表达, 见图1D、1F。

2.2　不同病理切片中Tim-3、galectin-9免疫组化评分比较

根据免疫组化结果判定标准, 结直肠癌组织中Tim-3、galectin-9的高表达率分别为70.18%(120/171)、32.16%(55/171), 而低表达率分别为29.82%(51/171)、67.84%(116/171)。同时, 结直肠癌组织Tim-3的表达评分相较于配对癌旁组织偏高, 而galectin-9的表达评分则偏低(P< 0.001)。见表1。

2.3　结直肠癌组织中Tim-3、galectin-9表达与基线参数的关系

结直肠癌组织中Tim-3、galectin-9表达与患者性别、年龄、原发部位、分化程度、肿瘤大小及淋巴结转移等均无关(P> 0.05); 而高表达Tim-3、低表达galectin-9表达与浸润深度、脉管浸润、临床分期均有关(P< 0.05)。见表2。

2.4　生存分析

171例结直肠癌患者中有7例失访, 剩余患者组织中Tim-3、galectin-9表达呈现异常低表达的患者分别为49、

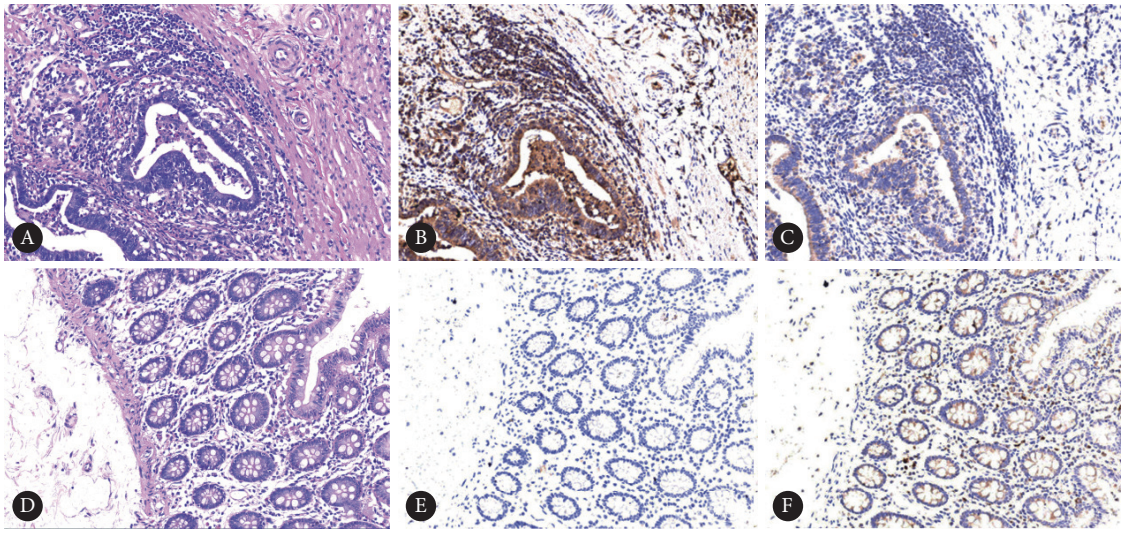

图 1 不同病理切片中Tim-3、galectin-9蛋白的表达强度及分布。×200

Fig 1 The expression intensity and distribution of Tim-3 and galectin-9 proteins in different pathological sections. ×200

A, Colorectal cancer tissue (HE staining); B, tumor cells and inflammatory cells around the tumor cells displaying Tim-3 cytoplasmic positivity (EnVision); C, inflammatory cells around the tumor cells showing galectin-9 negativity (EnVision); D, normal intestinal mucosa adjacent to cancer (HE staining); E, both intestinal mucosa and interstitial inflammatory cells showing Tim-3 negativity (EnVision); F, galectin-9 cytoplasmic positivity in a few inflammatory cells in the interstitium and cytoplasmic positivity in the intestinal mucosa (EnVision).

表 1 不同病理切片中Tim-3、galectin-9免疫组化评分比较

Table 1 Comparison of Tim-3 and galectin-9 immunohistochemical scores in different pathological sections

Group	Tim-3 (median [P_{25}, P_{75}])	Galectin-9 (median [P_{25}, P_{75}])
Colorectal cancer tissue (n=171)	7.49 (4, 12)	2.70 (0, 6)
Paired paracancerous tissue (n=171)	1.93 (0, 4)	7.82 (5, 12)
Z	32.908	28.016
P	<0.001	<0.001

112例, RFS分别为71.43%(35/49)、48.21%(54/112), OS分别为81.63%(40/49)、57.14%(64/112); 高表达患者分别为115、52例, RFS分别为47.83%(55/115)、69.23%(36/52), OS分别为58.26%(67/115)、82.69%(43/52)。Kaplan-Meier生存分析显示, 结直肠癌组织中Tim-3高表达者的RFS和OS均低于低表达者(RFS: log-rank= 22.66, P< 0.001; OS: log-rank= 19.71, P< 0.001), 而galectin-9低表达者的RFS和OS则低于高表达者(RFS: log-rank= 19.45, P< 0.001; OS: log-rank= 22.24, P< 0.001)。见图2、图3。

2.5 影响结直肠癌患者RFS、OS的Cox回归分析结果

将患者基线参数及生化指标纳入单因素分析, 可知, 此类对象淋巴结转移阳性、浸润深度T_3+T_4、有脉管浸润、临床分期Ⅲ期以及Tim-3高表达、galectin-9低表达是影响RFS和OS的危险因素(P< 0.05), 见表3; 另外, 多因素回归分析结果显示, TNM分期Ⅲ期以及Tim-3高表达、galectin-9低表达均为影响患者RFS和OS的独立危险因素(P< 0.05), 见表4。

3 讨论

免疫调节异常一直是结直肠癌发生的重要环节, 从免疫学角度探讨肿瘤的发病机制, 有利于治疗及预后的评估, 而T细胞免疫则在介导肿瘤免疫反应中扮演着关键角色[8]。Tim-3是一种抑制性共刺激分子, 广泛表达于多种免疫细胞表面, 其不仅参与免疫应答的适应性调节外, 还参与调节身体的自然免疫反应, 临床上亦认为Tim-3与程序性死亡受体-1(programmed cell death protein 1, PD-1)具有同等地位[9]。FERRIS等[10]的研究发现, 结直肠癌患者肿瘤浸润淋巴细胞的Tim-3表达相较于癌旁组织T细胞明显更高, 且其体外实验也证实, Tim-3可通过提高T细胞抗原受体信号转导引起T细胞衰竭, 此类证据均证实消化道肿瘤微环境可诱导T细胞表达Tim-3。XU等[11]研究证实, 结直肠癌患者根治术后的Tim-3$^+$ PD-1$^+$ CD8$^+$ T细胞明显升高, 而这类细胞代表的是一群功能障碍的T细胞, 其所分泌的干扰素IFN-γ显著低于Tim-3$^-$ PD-1$^-$ CD8$^+$ T细胞, 提示Tim-3和PD-1可用于评价患者治疗后的康复进程。既往研究[12]证实, galectin-9是一种β-半乳糖苷酶家族的蛋白质, 其与Tim-3结合是导致T细胞凋亡和T细胞免疫负调节的关键因素。Galectin-9最初被鉴定为嗜酸性粒细胞趋化和活化因子, 随后发现其可调节免疫活性细胞增殖和活化, 进而诱导Th1介导的免疫效应[13]。在许

表 2　结直肠癌组织中Tim-3、galectin-9表达与临床病理参数的关系

Table 2　Relationship between Tim-3 and galectin-9 expression and clinicopathologic parameters in colorectal cancer tissues

Baseline parameter	*n*	Tim-3		χ^2	*P*	Galectin-9		χ^2	*P*
		Low expression/ case, *n*=51	High expression/ case, *n*=120			Low expression/ case, *n*=116	High expression/ case, *n*=55		
Age/yr.									
<60	75	18	57	2.165	0.141	55	20	1.850	0.174
⩾60	96	33	63			61	35		
Sex									
Male	99	29	70	0.032	0.859	67	32	0.003	0.958
Female	72	22	50			49	23		
Primary tumor location									
Colon	91	32	59	2.650	0.104	56	35	3.536	0.060
Rectum	80	19	61			60	20		
Differentiation degree									
High+Medium	94	23	71	2.862	0.091	68	26	1.941	0.164
Low	77	28	49			48	29		
Tumor size/cm									
<5	81	28	53	1.654	0.198	50	31	2.631	0.105
⩾5	90	23	67			66	24		
Lymph node metastasis									
Negative	78	26	52	0.844	0.358	51	27	0.395	0.530
Positive	93	25	68			65	28		
Infiltration depth									
T_1+T_2	64	30	34	14.207	<0.001	33	31	12.415	<0.001
T_3+T_4	107	21	86			83	24		
Clinical staging									
Ⅰ+Ⅱ	65	28	37	8.799	0.003	35	30	9.406	0.002
Ⅲ	106	23	83			81	25		
Vascular infiltration									
No	69	30	39	10.304	0.001	38	31	8.637	0.003
Yes	102	21	81			78	24		

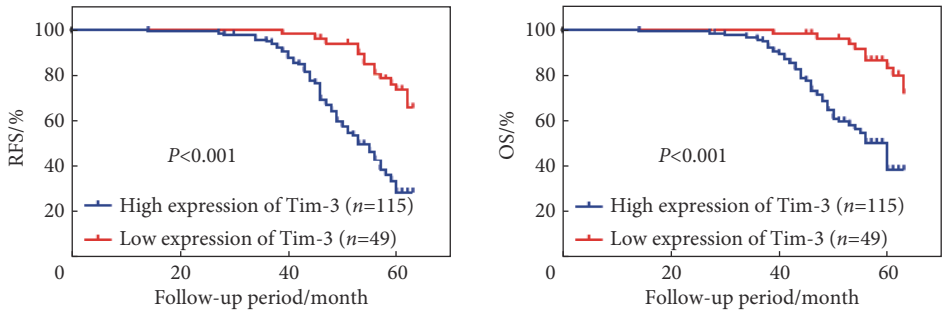

图 2　Tim-3不同表达状态下患者的RFS（左）和OS（右）

Fig 2　RFS (left) and OS (right) of patients with different expression status of Tim-3

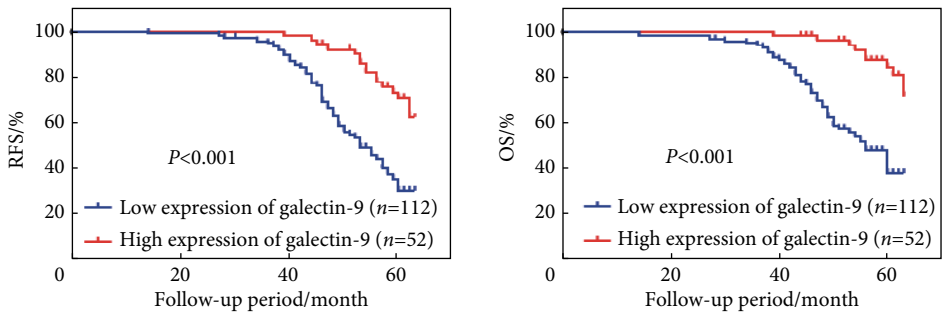

图 3　Galectin-9不同表达状态下患者的RFS（左）和OS（右）

Fig 3　RFS (left) and OS (right) of patients with different expression status of galectin-9

表 3 影响结直肠癌患者预后的单因素分析

Table 3 Univariate analysis of factors affecting the prognosis of patients with colorectal cancer

Variable	RFS		OS	
	HR (95% *CI*)	*P*	*HR* (95% *CI*)	*P*
Age (≥60 yr. vs. <60 yr.)	1.25 (0.61-1.83)	0.406	1.48 (0.70-2.02)	0.339
Sex (male vs. female)	1.28 (0.64-1.94)	0.397	1.30 (0.59-1.94)	0.366
Primary tumor location (rctum vs. colon)	1.34 (0.70-2.08)	0.392	1.26 (0.53-1.85)	0.364
Differentiation degree (high+medium vs. low)	1.27 (0.67-1.85)	0.323	1.51 (0.72-2.23)	0.312
Tumor size (≥5 cm vs. <5 cm)	1.45 (0.84-2.25)	0.386	1.81 (0.65-2.69)	0.254
Lymph node metastasis (positive vs. negative)	1.90 (1.23-3.36)	0.007	2.33 (1.20-5.28)	0.005
Infiltration depth (T_3+T_4 vs. T_1+T_2)	1.82 (1.17-3.04)	0.011	2.11 (1.28-4.75)	0.008
Clinical staging (Ⅲ vs. Ⅰ-Ⅱ)	2.80 (1.68-6.37)	0.001	3.66 (1.80-9.72)	<0.001
Vascular infiltration (yes vs. no)	1.72 (1.11-2.69)	0.018	2.08 (1.44-5.06)	0.013
Tim-3 immunohistochemical score (≤7 vs. >7)	0.77 (0.22-0.94)	0.005	0.60 (0.11-0.87)	0.002
Galectin-9 immunohistochemical score (≤8 vs. >8)	2.64 (1.38-5.77)	0.002	3.06 (1.59-6.89)	0.001

RFS: relapse-free survival; OS: overall survival; *HR*: hazards ratio; *CI*: confidence interval.

表 4 影响结直肠癌患者预后的多因素分析

Table 4 Multivariate analysis of factors affecting the prognosis of patients with colorectal cancer

Variable	RFS		OS	
	HR (95% *CI*)	*P*	*HR* (95% *CI*)	*P*
Lymph node metastasis (positive vs. negative)	1.09 (0.58-1.69)	0.138	1.16 (0.50-1.98)	0.097
Infiltration depth (T_3+T_4 vs. T_1+T_2)	1.12 (0.76-1.85)	0.110	1.18 (0.54-2.09)	0.086
Clinical staging (Ⅲ vs. Ⅰ-Ⅱ)	2.26 (1.20-5.68)	0.006	2.86 (1.31-6.44)	0.002
Vascular infiltration (yes vs. no)	1.22 (0.83-2.09)	0.093	1.32 (0.85-2.27)	0.075
Tim-3 immunohistochemical score (≤7 vs. >7)	0.80 (0.33-0.91)	0.019	0.74 (0.26-0.88)	0.014
Galectin-9 immunohistochemical score (≤8 vs. >8)	1.80 (1.33-4.70)	0.017	2.29 (1.33-5.80)	0.011

The abbreviations are explained in the note to Table 1.

多肿瘤中，如肝细胞癌、乳腺癌和结肠腺癌，galectin-9均呈现异常低表达，LANGHANS等[14]通过体外实验对肿瘤细胞增殖的影响发现，galectin-9能够通过JNK和p38 MAP等激酶途径诱导癌细胞凋亡。一些研究[15]发现，经galectin-9治疗的荷瘤小鼠脾细胞可分化为Tim-3$^+$ CD8$^+$细胞毒性T淋巴细胞，可见galectin-9可诱导Tim-3$^+$ CD8$^+$ T细胞的成熟。但上述观点多停留在研究Tim-3/galectin-9通过相互作用增强Treg在肿瘤微环境中的免疫抑制活性，以调节结直肠组织癌变的相关领域，而其在已发展至结直肠癌患者病情发展及预后评估中的可能作用却少有研究，且Tim-3、galectin-9在上述过程中的具体分子机制亦未完全阐明。

本研究中的免疫组化结果显示，结直肠癌组织Tim-3的表达评分相较于配对癌旁组织明显偏高，而galectin-9的表达评分则偏低，表明Tim-3高表达、galectin-9低表达可为辅助诊断结直肠癌提供指导。同时，本研究分析171例肿瘤组织蜡块标本中Tim-3、galectin-9表达与病理特征的关联，发现浸润深度、脉管浸润、临床分期等临床病理学特征与高表达Tim-3、低表达galectin-9均有关，而上述病理学特征都反映恶性肿瘤的侵袭发展，可见Tim-3、galectin-9可能在结直肠癌病情恶化中发挥重要作用。但是，回顾以往临床上有关Tim-3、galectin-9与消化道肿瘤患者临床特征之间关系的研究结果也存在一定差异。部分临床报道[16-17]称Tim-3高表达、galectin-9低表达与消化道肿瘤患者肿瘤直径、浸润深度、淋巴结转移及分化程度等均具有显著相关性。而YANG等[18]报道称Tim-3、galectin-9基因表达水平与消化道肿瘤患者临床病理特征间的相关性并不显著。考虑造成这些研究结论差异的原因可能与所纳入的研究群体和检测技术不同有关。另外，肿瘤微环境中的其他因素亦可能造成结直肠癌病情进展中Tim-3/galectin-9途径的变化，但是仍需针对性实验加以证实。同时，本研究免疫组化结果显示，Tim-3及galectin-9不仅在癌周炎症细胞中表达，同样在肿瘤细胞或癌旁正常肠黏膜细胞的胞质阳性表达，这对本课题组更深入研究Tim-3及其配体galectin-9在结直肠癌发生发展中作用及机制有提示作用，提示后续可以进一步在细

胞分子水平进行研究。

越来越多研究证实, 组织中Tim-3、galectin-9在不同恶性肿瘤中的表达和作用可能具有癌种特异性, 不同类型疾病中的Tim-3、galectin-9的表达水平、临床意义及分子功能不尽相同。王扬扬等[19]通过一项胃食管结合部腺癌病例回顾性研究发现, 不同部位的肿瘤组织Tim-3的表达水平有所不同, 主要在肿瘤组织浸润的免疫细胞中表达, 但是其在临床分期、原发部位、肿瘤大小、侵犯血管神经比例上表达的差异均无统计学意义。侯楠[20]进行体外细胞实验发现, galectin-9表达水平的下调, 可能与肿瘤的迁移、侵袭相关, 但是临床实践表明, 其水平与食管鳞状细胞癌患者的临床分期、有无淋巴结转移无相关性。而JIANG等[21]却提出低Tim-3与高galectin-9表达的胃癌患者生存率高。WANG等[22]同样指出低Galectin-9表达与结肠癌患者不良预后结局相关。可见, 现阶段有关Tim-3、galectin-9的表达情况与预后评估的研究尚不够深入, 甚至具有矛盾之处。本研究对具有完整随访资料患者的生存分析结果显示, 结直肠癌组织中Tim-3高表达者的RFS和OS均明显低于低表达者, 而galectin-9低表达者的RFS和OS则明显低于高表达者, 可见, Tim-3/galectin-9途径参与了结直肠肿瘤细胞的恶性生物学行为过程。分析原因可能是Tim-3与其配体galectin-9结合介导T细胞的凋亡和免疫耐受, 主要表现为促进Treg细胞抑制Th1细胞和$CD8^+$ T细胞的细胞毒性功能, 进一步在肿瘤微环境中介导机体免疫衰竭, 而这种肿瘤微环境下可诱导肿瘤相关中性粒细胞(tumor-associated neutrophile, TAN)发挥促进肿瘤细胞传播扩散的效应, 越来越多的研究[23-24]表明TAN的存在与不良预后之间存在显著相关性。进一步的Cox分析亦提示, Tim-3高表达、galectin-9低表达是影响结直肠癌患者RFS和OS的独立预后因素, 这也进一步佐证了通过分析结直肠癌患者Tim-3、galectin-9的表达情况, 能够对复发转移及预后预测等方面提供一定指导。

综上所述, 结直肠癌组织中Tim-3、galectin-9呈现异常表达。高表达Tim-3、低表达galectin-9与不良临床病理特征及预后关系紧密, 是诱发患者不良预后事件的独立影响因素, 有望成为新的治疗靶点和临床指标。但是本研究亦存在一定不足, 如仅局限于临床样本分析, 且随访时间相对较短, 再加之实验操作测量误差等也可能造成结论偏移; 同时, 未对Tim-3/galectin-9信号通路介导结直肠癌患者不良临床病理特征及预后的分子机制进行进一步的探索和验证; 尽管如此, 本研究所获得的数据成果也为临床探索Tim-3、galectin-9等相关因子在此类患者复发转移及预后预测的分子功能及潜在机制奠定了一定基础。在今后, 本课题组将在细胞及动物水平, 对Tim-3/galectin-9信号通路相关因子促进该疾病的潜在分子机制进行探究。

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作者贡献声明　张怡然负责正式分析、调查研究、研究方法、初稿写作和审读与编辑写作, 邓丹负责论文构思、正式分析和调查研究, 尹万和罗俊负责正式分析、提供资源和监督指导, 刘金星和谢臣健负责调查研究和研究方法, 季星利、马立和张莉负责调查研究和验证, 夏仙根和程胜君负责数据审编和研究项目管理, 黄安亮负责审读与编辑写作, 杨帆负责论文构思、经费获取、研究项目管理和监督指导。所有作者已经同意将文章提交给本刊, 且对将要发表的版本进行最终定稿, 并同意对工作的所有方面负责。

Author Contribution　ZHANG Yiran is responsible for formal analysis, investigation, methodology, writing--original draft, and writing--review and editing. DENG Dan is responsible for conceptualization, formal analysis, and investigation. YIN Wan and LUO Jun are responsible for formal analysis, resources, and supervision. LIU Jinxing and XIE Chenjian are responsible for investigation and methodology. JI Xingli, MA Li, and ZHANG Li are responsible for investigation and validation. XIA Xiangen and CHENG Shengjun are responsible for data curation and project administration. HUANG Anliang is responsible for writing--review and editing. YANG Fan is responsible for conceptualization, funding acquisition, project administration, and supervision. All authors consented to the submission of the article to the Journal. All authors approved the final version to be published and agreed to take responsibility for all aspects of the work.

利益冲突　所有作者均声明不存在利益冲突

Declaration of Conflicting Interests　All authors declare no competing interests.

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(2023 – 08 – 07收稿, 2024 – 01 – 24修回)

编辑 刘 华