

# 食管鳞状细胞癌(ESCC)中PD-1/PD-L1/PD-L2的表达与临床因素及预后的相关性

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**【摘要】** 目的 检验程序性细胞死亡分子1(programmed cell death-1, PD-1)/PD配体1(PD ligand-1, PD-L1)/PD配体2(PD-L2)在食管鳞状细胞癌(esophageal squamous cell carcinoma, ESCC)患者中的表达,并评估其与临床特征及预后的关系。**方法** 收集2007年1月至12月在复旦大学附属中山医院胸外科接受手术治疗的325例食管肿瘤患者的临床数据,通过免疫组织化学染色分析石蜡包埋的肿瘤样品及部分对应的癌旁组织中PD-1、PD-L1和PD-L2的表达,用免疫反应评分并分析其与临床特征及预后的关系。**结果** PD-1、PD-L1、PD-L2三者阳性表达率分别为12.0%、52.6%、32.9%,PD-L( $P<0.001$ )、PD-L1( $P<0.001$ )、PD-L2( $P=0.023$ )在癌旁组织和癌组织中的表达有差别。PD-L1的阳性表达与T分期( $P<0.001$ )、术后分期( $P=0.006$ )相关,PD-L2的阳性表达与T分期( $P<0.001$ )、术后分期( $P=0.002$ )及淋巴结转移( $P=0.044$ )相关,与其他临床因素的相关性无统计学意义。PD-1( $P=0.500$ )、PD-L1( $P=0.058$ )、PD-L2( $P=0.096$ )单阳性与患者生存状况均无明显关联。但三者表达均阴性的患者预后要显著优于仅其中一个因子表达阳性( $HR=1.669$ )或二个以上因子表达阳性的患者( $HR=1.606$ )。**结论** PD-L1和PD-L2与ESCC患者的多个临床特征有关。虽然PD-1、PD-L1、PD-L2各自与患者预后之间无统计学意义关联,但表达均阴性的患者预后相对较好。

**【关键词】** 程序性细胞死亡分子1(PD-1); 程序性细胞死亡分子配体-1(PD-L1); 程序性细胞死亡分子配体-2(PD-L2); 食管鳞状细胞癌(ESCC)

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## Connection of PD-1/PD-L1/PD-L2 expression with clinical factors and prognosis in esophageal squamous cell carcinoma (ESCC)

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**【Abstract】 Objective** To value the expression of (programmed death-1, PD-1)/(PD ligand-1, PD-L1)/PD-L2 in patients with esophageal squamous cell carcinoma (ESCC) and focus on evaluating its connection with clinical features and prognosis. **Methods** The clinical data of all 325 patients with esophageal tumor who underwent surgery in Zhongshan Hospital, Fudan University from Jan. to Dec. 2007 were recorded. Immunohistochemical staining was used to analyze PD-L1 and PD-L2 in paraffin-embedded tumor samples and paracancerous tissues. The expression was scored using an immune response score and analyzed for its relationship with clinical features and prognosis. **Results** Of the 325 patients, 39 (12.0%) patients had PD-1 positive expression, 171 (52.6%) had PD-L1 positive expression and 107 (32.9%) had PD-L1

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positive expression. The overexpression of PD-1 ( $P<0.001$ ), PD-L1 ( $P<0.001$ ), PD-L2 ( $P=0.023$ ) in cancer tissues and adjacent normal tissues has significant difference. The overexpression of PD-L1 was associated with T stage ( $P<0.001$ ) and postoperative stage ( $P=0.006$ ), while the overexpression of PD-L2 was associated with T stage ( $P<0.001$ ), postoperative stage ( $P=0.002$ ) and lymph node metastasis ( $P=0.044$ ). There was no significant relationship between the overexpression of PD-1, PD-L1, PD-L2 and other clinical factors. Single overexpression of PD-1 ( $P=0.500$ ), PD-L1 ( $P=0.058$ ), and PD-L2 ( $P=0.096$ ) had no significant relationship with the survival status. However, the prognosis of patients without any overexpression was significantly better than those with overexpression of single factor ( $HR=1.669$ ) or multiple factors ( $HR=1.606$ ). **Conclusions** PD-L1 and PD-L2 are associated with multiple clinical features of patients with ESCC. Although there was no statistically significant association between PD-1, PD-L1 or PD-L2 and the prognosis of patients, patients without any overexpression of the three have better postoperative survival.

**【Key words】** programmed death-1 (PD-1); programmed death ligand-1 (PD-L1); programmed death ligand-2 (PD-L2); esophageal squamous cell carcinoma (ESCC)

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食管癌是全球十大最常见和最致命的肿瘤之一<sup>[1]</sup>。在中国,食管癌是与癌症相关的第四大死因,而其中食管鳞状细胞癌(esophageal squamous cell carcinoma, ESCC)占食管癌的90%以上<sup>[2]</sup>。即使随着诊断和治疗的进展,ESCC患者的5年生存率仍然不甚理想<sup>[3]</sup>。

程序性细胞死亡受体1(programmed death-1, PD-1, NCBI Gene Name: PDCD1)基因由Ishida于1992年发现<sup>[4]</sup>, PD-1受体有两种天然配体, PD-L1 (PD ligand 1, PD-1, NCBI Gene Name: CD274)和PD-L2 (PD ligand 2, PD-L2, NCBI Gene Name: PDCD1LG2),三者之间相互作用抑制T细胞活化<sup>[5]</sup>。除免疫细胞外,三者还可以在多种肿瘤细胞中表达<sup>[6]</sup>,其在阻断“癌症免疫周期”中起着至关重要的作用<sup>[7]</sup>。但PD-1等在ESCC中的表达仍然有待明确,在本研究中,我们采用免疫组化的方法,对325例ESCC患者肿瘤组织及部分对应正常组织中PD-1、PD-L1、PD-L2的表达进行研究,以确定其临床意义。

## 资料和方法

**研究对象** 收集2007年1—12月在复旦大学附属中山医院胸外科接受手术治疗的全部325例食管肿瘤患者的临床基本信息和随访信息。临床基本信息包括年龄、性别、吸烟状况、肿瘤组织学类型、

肿瘤术后TNM分期、总分期(以UICC8版分期为参考)、肿瘤标本最大径;临床基本信息来源为我院留存病史。随访信息包括是否生存、死因、生存时间;信息均为出院后通过电话或门诊的方式获得,随访截止时间为术后72个月。详细的临床和病理数据见表1。本研究经复旦大学附属中山医院伦理审查委员会批准(伦理编号:B2017-233R)。

**标本来源** 所有标本均取自患者术后石蜡包埋组织,取材后镜下观察,避开肿瘤坏死区域和炎症区域。取材满意后,将石蜡组织制成组织芯片,以供后续的免疫组化实验。

**免疫组化** 使用基因科技(上海)股份有限公司的GTVision™ III抗兔/鼠通用型免疫组化试剂盒检测PD-1、PD-L1和PD-L2表达。组织切片首先在柠檬酸钠抗原修复液中处理,脱蜡脱水后用山羊血清溶液(美国Thermo Fisher Scientific公司)封闭处理。然后采用针对PD-1(1:200, 86163), PD-L1(1:200, 13684)和PD-L2(1:200, 82723)(美国Cell Signaling Technology公司)的兔抗人单克隆抗体处理,将载玻片在4℃下避光孵育18 h。充分洗涤后用二抗处理组织切片1 h,洗涤后加DAB显色液至覆盖完全。充分显色后终止并采用苏木精复染,中性树胶封片,镜下观察。

二位医师独立按免疫反应评分(immunoreactivity score, IRS)判别染色是否为阳性。IRS的

表1 PD-L1和PD-L2阳性表达比例与各临床因素相关性

Tab 1 Correlation between PD-L1 and PD-L2 positive expression ratio and clinical factors

[n(%)]

Clinical factor	Total samples (n)	PD-1 positive	P	PD-L1 positive	P	PD-L2 positive	P
Total	325	39 (12.0)		171 (52.6)		107 (32.92)	
Age			0.285		0.127		0.803
<65 y	216	29 (13.4)		107 (49.5)		70 (32.4)	
≥65 y	109	10 (9.2)		64 (58.7)		37 (33.9)	
Sex			0.680		0.402		0.477
Male	254	29 (11.4)		137 (53.9)		81 (31.9)	
Female	71	10 (14.1)		34 (47.9)		26 (36.6)	
Smoking			0.086		0.181		0.555
Yes	178	16 (9.0)		100 (56.2)		56 (31.5)	
No	147	23 (15.6)		71 (48.3)		51 (34.7)	
Tumor site			0.141		0.241		0.067
Cervical	35	8 (22.9)		17 (48.6)		8 (22.9)	
Thoracic	223	26 (11.7)		122 (54.7)		79 (35.4)	
Abdominal	67	7 (10.4)		29 (43.3)		15 (22.4)	
Maximum diameter of tumor			0.859		0.250		0.391
<3.5 cm	118	15 (12.7)		57 (48.3)		35 (29.7)	
≥3.5 cm	207	24 (11.9)		114 (56.7)		72 (35.8)	
N stage			0.486		0.061		<b>0.044</b>
N0	186	25 (13.4)		88 (47.3)		53 (28.5)	
N1	80	7 (8.8)		50 (62.5)		30 (37.5)	
N2	48	5 (10.4)		28 (58.3)		19 (39.6)	
N3	11	2 (18.2)		5 (45.5)		5 (45.4)	
T stage			0.983		<b>&lt;0.001</b>		<b>&lt;0.001</b>
T0+T1	36	4 (11.1)		13 (36.1)		8 (22.2)	
T2	87	11 (12.6)		38 (43.7)		21 (24.1)	
T3+T4	192	24 (12.5)		120 (62.5)		78 (40.6)	
Clinical stage			0.393		<b>0.006</b>		<b>0.002</b>
0+I	94	13 (13.8)		40 (42.5)		21 (22.3)	
II	103	13 (12.6)		52 (50.5)		32 (31.1)	
III+IV	128	13 (10.2)		78 (60.9)		54 (42.2)	

计算方法为染色强度分数与阳性细胞比例分数的乘积<sup>[8]</sup>。其中染色强度的判断标准为:无色0分,淡黄色1分,深黄色2分,黄褐色3分;该标准以着色细胞与着色背景相对比。阳性细胞比例的判断标准为:0%为0分,1%~30%为1分,31%~60%为2分,61%~100%为3分。对于每例组织,IRS<4分时判读为免疫组化染色阴性,IRS≥4分时判读为免疫组化染色阳性,PD-1、PD-L1、PD-L2染色均以此为标准<sup>[9]</sup>。

**统计学分析** 采用配对检验分析肿瘤组织和癌旁组织中PD-1等表达差异。用 $\chi^2$ 检验以及秩和检验分析PD-1等阳性表达与ESCC患者各临床因素有无统计学关联;用Kaplan-Meier生存曲线法分析PD-1、PD-L1、PD-L2一个或多个表达与ESCC患者的生存时间之间有无统计学关联;配对检验、 $\chi^2$ 检验、秩和检验用SPSS 25.0软件,Kaplan-Meier生

存曲线用R语言(版本号3.5.2,https://www.r-project.org)进行, $P<0.05$ (双尾)为差异有统计学意义。

## 结 果

**PD-1、PD-L1、PD-L2在ESCC肿瘤组织及癌旁组织中的表达** 染色评分结果显示,102对肿瘤与正常组织中,PD-1在正常组织中均未见表达,13例(12.8%)肿瘤组织中PD-1呈阳性;9例(8.8%)PD-L1为阳性,57例(55.9%)检测到PD-L1在癌组织中表达;21例(20.6%)癌旁组织PD-L2为阳性,34例(33.3%)肿瘤组织PD-L2呈阳性表达。

325例ESCC肿瘤组织中,有39例(12.0%)可见PD-1表达,171例(52.6%)PD-L1阳性,107例(32.9%)PD-L2阳性。PD-1( $P<0.001$ )、PD-L1( $P$

$<0.001$ )、PD-L2 ( $P=0.023$ )在癌旁组织和癌组织中的表达差异有统计学意义。

**PD-1、PD-L1、PD-L2表达与各临床因素** 分析全部325例病例各临床因素与PD-1、PD-L1、PD-L2阳性表达的相关性,纳入分析的临床因素包括年龄、性别、吸烟情况、肿瘤位置、肿瘤最大径、淋巴结转移(N分期)、T分期、术后分期等8项,由于M分期数据较少,因而未纳入分析范畴。

根据统计分析及秩和检验结果,T分期越高,PD-L1、PD-L2的表达率越高,且不同分期组间两者阳性比例有显著差异。与不同T分期PD-L1的阳性表达比例( $P<0.001$ )比较,PD-L2( $P<0.001$ )在不同T分期间的差异更为显著,但PD-1与之无关。

不同术后分期患者PD-L1、PD-L2的阳性表达率之间有显著差异,总体上,术后分期越高的患者,

两者的阳性表达率越高。与不同术后分期的PD-L1阳性表达比例比较( $P=0.006$ ),PD-L2( $P=0.002$ )的差异性更为显著,但PD-1与之无关。

PD-1、PD-L1的表达与患者年龄、性别、吸烟情况、肿瘤大小、淋巴结转移、肿瘤位置之间均未见显著性关联(表1)。而PD-L2的表达与淋巴结转移( $P=0.044$ )相关。

**PD-1、PD-L1、PD-L2单阳性表达对ESCC预后的影响** 在有随访记录的239例样本中,PD-1阳性表达的有32例(13.4%),中位生存时间为42个月;阴性的有207例(86.6%),中位生存时间为36个月。二者的Kaplan-Meier生存曲线如图1A所示。PD-1表达阳性的患者与阴性患者相比,总生存率无显著差异( $P=0.500$ )。

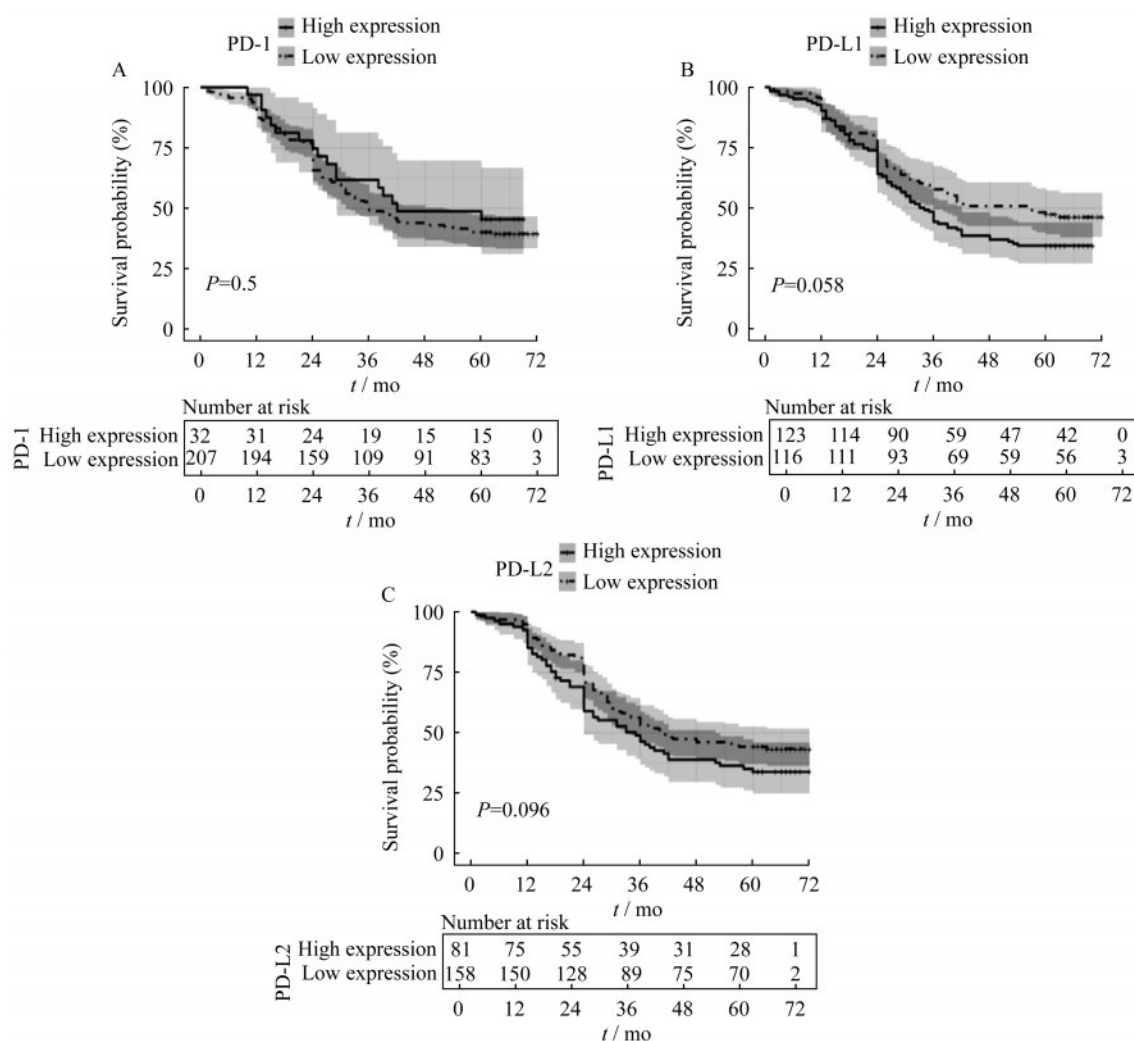


图1 PD-1、PD-L1、PD-L2单阳性表达生存曲线

Fig 1 The survival curves of PD-1, PD-L1 and PD-L2 single positive expression



239例样本中,共有123例(51.5%)患者肿瘤组织中PD-L1呈阳性表达,中位生存时间为34个月;116例呈阴性(48.5%),中位生存时间为57个月;二者的生存曲线如图1B所示,总生存率差异无统计学意义( $P=0.058$ )。

239例样本中,共81例(33.9%)肿瘤组织中PD-L2表达为阳性,中位生存时间为33个月;158例呈阴性(66.1%),中位生存时间为41个月;二者的生存曲线如图1C所示,总生存率差异无统计学意义( $P=0.096$ )。

**PD-1、PD-L1、PD-L2中多个阳性表达对ESCC预后的影响** 239例样本中,82例(34.3%)肿瘤组织中无PD-1、PD-L1、PD-L2表达,92例(38.5%)肿瘤组织有PD-1、PD-L1、PD-L2中的一个阳性表达,65例(27.2%)肿瘤组织中有PD-1、PD-L1、PD-L2中的2~3个阳性表达,不同组间的总生存率差异有统计学意义( $P=0.025$ ),特别是无阳性表达组,其生存状况明显优于有阳性表达组,但单阳性表达组与多个阳性表达组间无明显差异(图2)。

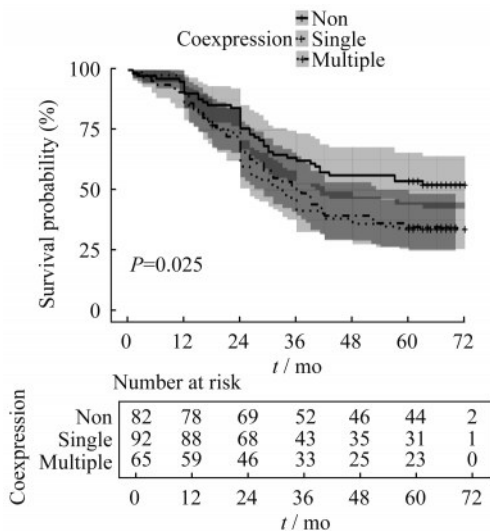


图2 PD-1、PD-L1、PD-L2共表达生存曲线比较

Fig 2 Comparison of survival curves among co-expressing of PD-1, PD-L1 and PD-L2

## 讨 论

**PD-1、PD-L1、PD-L2在食管癌中的表达及意义** PD-1是主要在活化T细胞上表达的阴性共刺激受体,可调节相关细胞的活化与凋亡<sup>[10]</sup>。PD-L1

通常由响应免疫介导的IFN $\gamma$ 等信号驱动,在肿瘤中活跃的T细胞炎症区域中表达<sup>[11]</sup>,其在癌细胞和免疫细胞上的表达可以抑制T细胞抗肿瘤反应,从而允许肿瘤生长。PD-L2在某些B细胞淋巴瘤中高度上调<sup>[12]</sup>。对于PD-L2在肿瘤组织中的表达与预后的相关性及其与PD-1靶向治疗反应相关性的研究并不多。PD-L2似乎在活化的CD4和CD8T细胞亚群上表达,PD-1与T细胞表面的PD-L2结合能够下调细胞因子的产生,若在T细胞上直接靶向PD-L2可阻断T细胞增殖<sup>[13]</sup>。

Leng等<sup>[14]</sup>报道其研究中46.2%的ESCC组织中PD-L1呈阳性,42.5%的ESCC组织中PD-L2呈阳性。在本研究中PD-L1在52.6%的ESCC中表达,PD-L2在32.9%的ESCC中表达,PD-L1的表达明显高于PD-L2。与之相类似,Grosso等<sup>[15]</sup>观察到ESCC中具有更高的PD-L1表达。

Leng等<sup>[14]</sup>发现PD-L1阳性患者的预后明显差于阴性患者,而他们的预后与PD-L2表达无关,并报道PD-1 + TILs(肿瘤浸润淋巴细胞)的数量与PD-L1和PD-L2表达呈负相关。但是,在另一项研究中,食管癌中TILs和PD-L2的存在呈负相关,而PD-L1与TILs无明显相关性。此外,有关于晚期食管癌患者的研究中,通过多变量分析发现PD-L1阳性表达是独立的预后因素<sup>[9]</sup>。

本研究中PD-L1和PD-L2在不同T分期食管鳞癌患者中的阳性表达率差异有统计学意义( $P<0.05$ ),并且PD-L2阳性表达差异率与不同T分期之间的关联比相应PD-L1更为显著,这与先前大部分研究结果相似。而且,PD-L2表达还与术后分期以及淋巴结转移(N分期)显著相关,这些结果提示ESCC的PD-1、PD-L1、PD-L2通路免疫治疗中,针对肿瘤原发灶的标准应以肿瘤浸润程度或T分期为准,而不应以肿瘤最大径为标准。此外,PD-1、PD-L1和PD-L2在癌组织及癌旁组织中的表达差异有统计学意义,而肿瘤位置与PD-L2阳性表达差异率之间似乎有一定关联,PD-L2阳性表达率随肿瘤位置的深入而增大,但差异无统计学意义,未来可以进一步加大样本量探讨两者之间是否确实有关联。

目前认为PD-L1在细胞质中的过表达是食管癌切除术后ESCC患者无病生存期的独立预后因素<sup>[16]</sup>。本研究通过Kaplan-Meier生存曲线分析证

实,PD-1、PD-L1、PD-L2单个表达与食管癌患者存活时间之间无显著相关性,但总体上,存活组PD-L1、PD-L2的阳性表达率低于死亡组,并且PD-L1阳性表达组与阴性表达组间生存曲线的差异接近于显著差异,未来需加大样本量进一步检验PD-1、PD-L1、PD-L2表达与ESCC患者预后的关系。另一方面,本研究针对PD-1、PD-L1及PD-L2中多个阳性表达对ESCC预后的影响进行了评估。结果显示,这三者任意两个阳性表达或是全部呈阳性表达时,患者的预后较单表达组未出现统计学差异,但是其与无PD-1、PD-L1、PD-L2表达组间的生存曲线有明显差异,表明这三者均未出现阳性表达的患者术后存活时间更长,相类似,Hsieh等<sup>[3]</sup>通过研究150例ESCC病例得出结论,PD-L1和PD-L2的表达之间存在相关性,没有过度表达PD-L1和PD-L2的患者似乎具有更好的总体存活率,但差异无统计学意义。这些结果提示,对于患者术后生存状况的判断,应将PD-1、PD-L1、PD-L2三者均纳入考量范围,三者共阴性与良好生存状况相关程度更高,而非仅考虑PD-L1和PD-L2。

而除了ESCC,比较引人注目的是Derks等<sup>[17]</sup>发现在食管腺癌(esophageal adenocarcinoma,EAC)中,51.7%的EAC癌细胞中有PD-L2表达,而上皮PD-L1仅在2%的病例中表达。这一发现可能会引发关于PD-1死亡信号通路的另一个研究方向,如PD-1/PD-L1通路在上皮细胞肿瘤中位于主导地位,而PD-1/PD-L2通路在腺癌中更为重要。

总之,目前主流观点认为PD-L1的表达与ESCC的生存率相关,而PD-L2的表达与ESCC生存率的关联性不如PD-L1显著,PD-1与ESCC生存率无关联,并且不同实验结果差异性较大。总体上,PD-L1、PD-L2均未过表达的患者表现出更好的总体生存率<sup>[18]</sup>。这一点在本研究中也明显表现,其差异具有统计学意义,而其中相关机制还有待进一步的实验证明。

**PD-1、PD-L1、PD-L2通路相关药物在ESCC中的研究进展** 目前,两种抗PD1单克隆抗体纳武单抗和帕姆单抗可用于多种类型癌症的临床治疗<sup>[19]</sup>。

Ib期KEYNOTE-028试验,II期KEYNOTE-059试验和II期KEYNOTE-180试验研究了帕姆单抗在食管癌患者中的抗肿瘤活性和安全性。针对经过大量预处理的PD-L1阳性晚期食管癌患

者(78%ESCC,22%EAC)的Ib期KEYNOTE-028试验,帕姆单抗显示出有希望的抗肿瘤活性(客观缓解率:30%)和可控的安全性<sup>[20]</sup>。KEYNOTE-059研究纳入了259例局部或晚期转移性胃癌(49%)或胃食管交界腺癌(51%)患者<sup>[21]</sup>,其中,55%( $n=143$ )的肿瘤表达PD-L1,客观反映率为13.3%(95%CI:8.2~20.0),1.4%有完整回复<sup>[22]</sup>。在参加II期KEYNOTE-180研究的121名接受过预治疗的晚期转移性ESCC或EGJ晚期Siewert 1型腺癌患者中,接受帕姆单抗治疗的患者客观反应率为9.9%<sup>[23]</sup>。III期KEYNOTE-181(Clinical Trials.gov,NCT02564263)用于比较帕姆单抗单药治疗与紫杉醇、多西紫杉醇或伊立替康等标准疗法治疗晚期EAC或ESCC患者的疗效,试验结果表明,这一免疫疗法使PD-L1联合阳性分数(combined positive score,CPS) $\geq 10$ 的肿瘤患者总生存率有显著的改善<sup>[24]</sup>。然而,一项包含592名胃食管癌患者的临床III期实验<sup>[25]</sup>中表明,与紫杉醇相比,尽管帕姆单抗具有更好的安全性,但帕姆单抗作为PD-L1(CPS $\geq 1$ )晚期胃食管连接癌的二线疗法并未显著改善总生存率。此外,目前,KEYNOTE-590试验正在进行,该III期临床试验旨在研究帕姆单抗作为晚期食管癌(包括晚期不可切除或转移性腺癌、ESCC、食管胃交界的晚期Siewert 1型腺癌)一线治疗的安全性和有效性,并验证帕姆单抗与化疗相结合是否可提供比单独化疗更明显的效果<sup>[26]</sup>。

纳武单抗是一种人单克隆IgG4抗体,Kudo等<sup>[27]</sup>证明纳武单抗在治疗难治性食管癌患者中有一定的活性以及可控的安全性。同样,Kang等<sup>[28]</sup>关于纳武单抗的III期研究中显示,无论PD-L1表达状态如何,使用纳武单抗组患者的总体生存效益优于安慰剂组。ATTRACTION-4研究中评估了纳武单抗联合S-1加奥沙利铂(SOX)或卡培他滨加奥沙利铂(CapeOX)作为不可切除晚期或复发人表皮生长因子受体2阴性胃/胃食管交界癌患者一线治疗方法的安全性和有效性,结果证明,纳武单抗加化疗具有可控的安全性和抗肿瘤活性<sup>[29]</sup>。

目前,针对于PD-1通路的药物均仍在临床试验阶段,并且已有较为显著的效果,但是其与传统化疗效果的差异性以及其与标准疗法相结合的治疗思路是否有更优的疗效等还有待进一步考证,更大规模、更深入的试验及研究可以为日后相关癌症的治疗提供新方向。

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