

胃癌组织中PD-L1与肿瘤相关成纤维细胞表达的关系

穆林松, 胡志军, 邵林, 陈建武, 薛达, 王海滨, 刘欢欢, 吕忠船*

青岛大学附属烟台毓璜顶医院普外科, 山东 烟台

收稿日期: 2024年12月22日; 录用日期: 2025年1月15日; 发布日期: 2025年1月24日

摘要

以往的研究主要集中在肿瘤细胞在免疫逃逸中的变化, 而对肿瘤微环境(tumor microenvironment, TME)对免疫逃逸的影响知之甚少。肿瘤相关成纤维细胞(tumor-associated fibroblasts, TAFs)是TME的重要组成部分, 具有特殊的生理生化特性, 但其具体机制尚不清楚。为了研究TAF对胃癌细胞PD-L1表达的影响, 通过transwell将胃癌细胞株MNK45、SGC7901与TAFs非接触共培养1、3、7 d。采用qRT-PCR和流式细胞仪检测PD-L1 mRNA和蛋白表达。然后选择95例胃癌组织, 通过免疫组化检测PD-L1和TAFs的表达。结果显示, 实验组PD-L1 mRNA和蛋白表达量均显著高于对照组。胃癌中PD-L1的表达与大量淋巴细胞浸润、弥漫性/混合性组织学和瘤内TAFs有关。综上所述, TAFs通过提高PD-L1的表达促进胃癌细胞株的生长。

关键词

胃癌, 肿瘤相关成纤维细胞, PD-1/PD-L1, 免疫治疗

Relationship between the Expressions of PD-L1 and Tumour-Associated Fibroblasts in Gastric Cancer

Linsong Mu, Zhijun Hu, Lin Shao, Jianwu Chen, Da Xue, Haibin Wang, Huanhuan Liu, Zhongchuan Lyu*

Department of General Surgery, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai Shandong

Received: Dec. 22nd, 2024; accepted: Jan. 15th, 2025; published: Jan. 24th, 2025

*通讯作者。

文章引用: 穆林松, 胡志军, 邵林, 陈建武, 薛达, 王海滨, 刘欢欢, 吕忠船. 胃癌组织中 PD-L1 与肿瘤相关成纤维细胞表达的关系[J]. 临床医学进展, 2025, 15(1): 1378-1389. DOI: 10.12677/acm.2025.151185

Abstract

Previous studies have focused on the changes of tumour cells in immune escape, and less is known about the effect of the tumour microenvironment (TME) on immune escape. Tumour-associated fibroblasts (TAFs) cells are an important part of the TME and have special physiological and biochemical characteristics, but the specific mechanism has not been clarified. In order to investigate the effect of TAFs on the expression of PD-L1 in gastric cancer cells, gastric cancer cell lines MNK45, SGC7901 were non-contact coculturing with TAFs 1, 3 and 7 d via transwell. PD-L1 mRNA and protein expression were detected using qRT-PCR and FCM. Then, 95 cases of gastric cancer tissues were selected and PD-L1 and TAFs expressions were determined by immunohistochemical examination. The results showed that the mRNA and protein expression of PD-L1 in the experiment group were significantly higher than that in the control group. PD-L1 expression was associated with massive lymphocyte infiltration, diffuse/mixed histology and intratumoral TAFs in gastric cancers. In conclusion, TAFs promoted the growth of gastric cancer cell lines by increasing the PD-L1 expression.

Keywords

Gastric Cancer, TAFs Cells, PD-1/PD-L1, Immune Escape

Copyright © 2025 by author(s) and Hans Publishers Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

1. 引言

胃癌(Gastric cancer, GC)是世界上最常见的消化系统恶性肿瘤之一,尤其是在韩国、日本和中国等东亚国家[1]-[3]。晚期 GC 的预后是严重的,即使使用化疗或其他辅助治疗[4]-[6]。肿瘤细胞可以避免免疫监视。以往的研究主要关注肿瘤细胞在免疫逃逸中的变化,但对肿瘤微环境(tumor microenvironment, TME)对免疫逃逸的影响知之甚少[7]-[9]。

肿瘤相关成纤维细胞(tumor associated fibroblasts, TAFs)是微环境基质中最重要的宿主细胞,参与细胞外基质构建和肿瘤集落发育[10] [11]。与正常组织成纤维细胞相比, TAFs 具有明显不同的形态和蛋白表达模式[12]。例如, TAFs 表达 α -SMA 和 vimentin, 分泌 TGF- β 、PDGF、FGF、VEGF、IL-11、IL-6 和细胞外基质成分,可促进癌细胞生长,诱导肿瘤血管生成,增强转移和侵袭[13]-[15]。现在很清楚,不仅在肿瘤细胞和免疫细胞之间,而且在其他基质细胞(如 TAFs 和免疫细胞)之间也存在持续地串扰。由于它们在肿瘤微环境中的优势, TAFs 最近被研究作为免疫细胞募集和功能调节剂的功能。

程序性死亡因子 1 (Programmed death factor 1, PD-1)及其配体 PD-L1/PD-L2 是负调控因子,在肿瘤逃逸宿主免疫应答中起着非常关键的作用[16]-[20]。PD-L1 在肿瘤患者中的表达与浸润深度、淋巴转移、病理类型及总生存期有关。PD-L1 单克隆抗体在黑色素瘤和非小细胞肺癌等肿瘤中疗效良好,但对消化系统肿瘤疗效较差[21]-[23]。最近,有报道称 PD-L1 在 TAFs [24]中表达。TAFs 可以通过产生生长因子和趋化因子影响肿瘤生长[25] [26]。

为了阐明肿瘤微环境中肿瘤相关成纤维细胞对肿瘤细胞免疫特性的影响,我们通过构建胃癌细胞系与胃癌相关纤维细胞共培养体系,观察免疫抑制分子 PD-L1 的表达,进而观察胃癌临床病理参数与 TAFs 相关 PD-L1 表达的关系。

2. 材料与方法

2.1. 组织样本和细胞系

收取来自毓璜顶术前未接受放疗或化疗的胃癌组织及邻近正常组织 95 例。所有这些组织在手术后立即在液氮中快速冷冻。本研究获得毓璜顶医院伦理委员会批准(2016081206)。

2.1.1. 细胞培养

人胃癌细胞系(SGC7901 和 MNK45)购自中国科学院(中国上海)型培养库, 保存在添加 10%胎牛血清(FBS; Hyclone, USA), 100 U/mL 青霉素和 100 ng/mL 链霉素, 温度 37℃, 湿度 5%, 空气 95%。

人胃 TAFs 来源于 95 例 GC 患者的手术组织。TAFs 来源于肿瘤胃壁, 正常成纤维细胞来源于非肿瘤胃壁。新鲜标本用含有 100 U/mL 青霉素和 100 lg/mL 链霉素的 PBS 洗涤三次, 切成小块, 用胶原酶 i 消化, 离心后用 PBS 冲洗细胞, 将微球重新悬浮在添加 10%FBS 的 DMEM 中, 转移到 100 mm 的培养皿中。这些细胞系在含有 10%胎牛血清(FBS)的 RPMI 1640 或 DMEM 中培养。在共培养实验中, 细胞系在 RPMI 1640 中培养。

2.1.2. 细胞共培养模型

人胃癌细胞(SGC7901 和 MNK45)和 TAFs 通过 transwell (康宁生命科学公司, Acton, MA, USA)进行非接触体外共培养。两个腔室由孔径为 0.4 μm 和 4.5 cm^2 的半透膜(BD Biosciences)隔开。人胃癌细胞(SGC7901 和 MNK45)以 3×10^5 cells/ml 在 transwell 插入物的上腔中 10^5 cells/ml。下腔在 TAFs 细胞中以 5×10^5 cells/ml 或含有正常成纤维细胞, 密度为 5×10^5 cells/ml (对照组)。

2.1.3. 细胞增殖试验

alarBlue 法检测细胞增殖情况。培养 1、3、7 d 后, 更换培养基为培养基 1640 (GIBCO, Carlsbad, CA, USA), 培养基中添加 10% FBS 和 10% alamarBlue。细胞在 5% CO_2 的湿化气氛中 37℃ 孵育 4 h, 用微孔板阅读器(MQX200)在 570 nm 处读取培养基的光密度(OD)。

2.1.4. 逆转录和实时荧光定量 PCR

使用 TRIzolVR 试剂(Invitrogen, CA, USA)根据制造商的说明从细胞中提取总 RNA。使用 Prime Script RT Reagent Kit (TaKaRa, 大连, 中国)进行定量逆转录聚合酶链反应(qRT-PCR)分析。PD-L1 引物为: forward, 5'-AAATGGAACCTGGCGAAAGC-30; 相反, 5'-GATGAGCCCCTCAGGCATTT-30; GAPDH mRNA 引物为: 正向, 5'-acggattttggtcgtatggg-30; 相反, 5'-CGCTCCTGGAAGATGGTGAT-30。

2.1.5. PD-L1 蛋白表达

FCM 分析实验组和对照组 PD-L1 的表达。收集共培养或对照培养的细胞, 用 FCM 缓冲液(含 5% FBS 和 0.1% NaN_3 的 PBS)洗涤两次。用荧光素标记抗体或同型对照抗体在 4℃ 下孵育 30 分钟后, 用 FCM 缓冲液洗涤细胞, 并在 Beckman coulter FC500 上使用 submit5.2 软件(Beckman coulter, USA)进行分析。

2.1.6. 免疫组织化学染色

按照常规方案[27]进行免疫组化。石蜡包埋的标本切片, 厚度为 4 μm , 在 63℃ 的干炉中孵育 1 h, 然后进行脱石蜡和补液。切片用以下一抗孵育: 兔抗人 PD-L1 (克隆 ab82059, abcam, UK; 1:100), 单克隆小鼠抗人 a-sma (克隆 4B12, Dako, 丹麦; 1:80), 室温处理 1 h。阴性对照用小鼠 IgG 同型抗体替代特异性抗体(Sigma, St. Louis, MO, USA)。

2.2. 统计分析

所有统计分析使用 SPSS 18.0 软件进行。所有数值均表示为每组至少三次重复个体实验的平均值 $\pm$

标准差。两组差异采用 t 检验分析, PD-L1 表达与其他临床病理特征的相关性采用 person's 卡方检验和 Fisher's 精确检验。当 $p < 0.05$ 时, 认为差异显著。

3. 结果

3.1. TAFs 和 NFs 原代培养及其特性

免疫荧光染色法测定成纤维细胞标志物、纤维连接蛋白(FN)和 α -平滑肌肌动蛋白(SMA)。结果显示 FN 在 TAFs 和 NFs 细胞中均呈阳性表达。此外, α -sma 在 TAFs 中高表达, 是肌成纤维细胞的生物标志物(图 1)。进一步分析表明, 与 TAFs 共培养的胃癌细胞比对照组生长更快(图 2)。

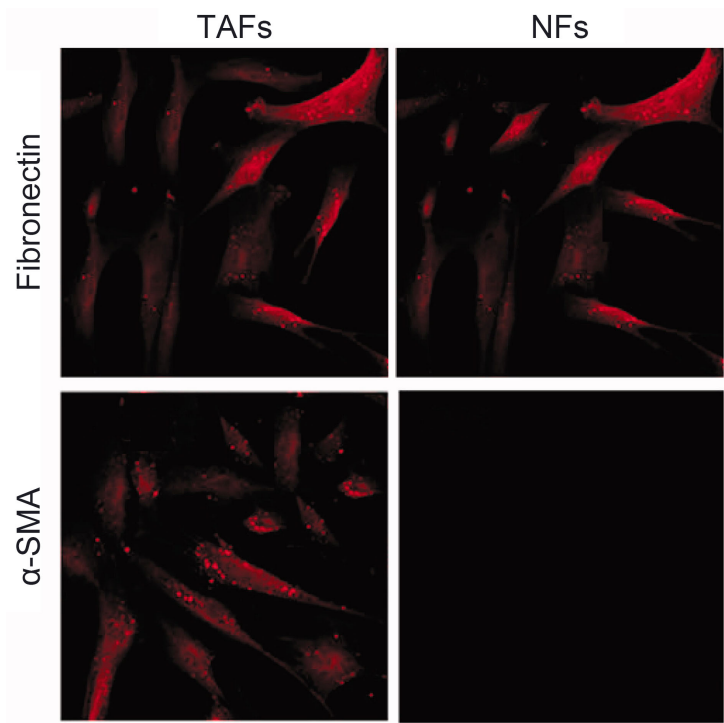


Figure 1. Characterization of tumor-associated fibroblasts (TAFs) and normal fibroblasts (NFs). The primary cultures of TAFs and NFs were isolated from the Tumour tissue and the adjacent normal gastric mucosa. Fibronectin (FN), the fibroblast biomarker, is universally expressed in TAFs and NFs. α -smooth muscle actin (α -SMA), the biomarker of the activated fibroblast, is a high level of expression in TAFs and weak expression in NF.

图 1. 肿瘤相关成纤维细胞(TAFs)和正常成纤维细胞(NFs)的表征。从肿瘤组织和邻近的正常胃黏膜中分离 TAFs 和 NFs 原代培养物。纤维连接蛋白(FN)是成纤维细胞的生物标志物, 在 TAFs 和 NFs 中普遍表达。 α -平滑肌肌动蛋白(α -sma)是激活成纤维细胞的生物标志物, 在 TAFs 中高表达, 在 NF 中表达较弱

3.2. PD-L1 在胃癌细胞中的表达

我们进一步利用 qRT-PCR 和流式细胞术评估 PD-L1 表达对共培养胃癌细胞系的影响。TAFs 组和对照组结果显示, 与 TAFs 共培养的胃癌细胞株中, PD-L1 mRNA 的表达呈时间依赖性显著升高。同样, PD-L1 的蛋白表达也表现出类似的结果(图 3)。

3.3. PD-L1 在胃癌组织中的表达

评价 PD-L1 在胃癌组织中的表达水平, 并分析其与胃癌患者临床及病理的关系。PD-L1 水平与性别 ($p = 0.845$, 表 1)、肿瘤部位($p = 0.123$, 表 1)、肿瘤侵袭($p = 0.634$, 表 1)、转移($p = 0.476$, 表 1)和肿瘤

- 淋巴结 - 转移(TNM)分期($p = 0.675$, 表 1)之间无显著差异。PD-L1 表达与年龄呈正相关($p = 0.023$, 表 1), PD-L1 表达与肿瘤淋巴结状态呈负相关($p = 0.035$, 表 1)。

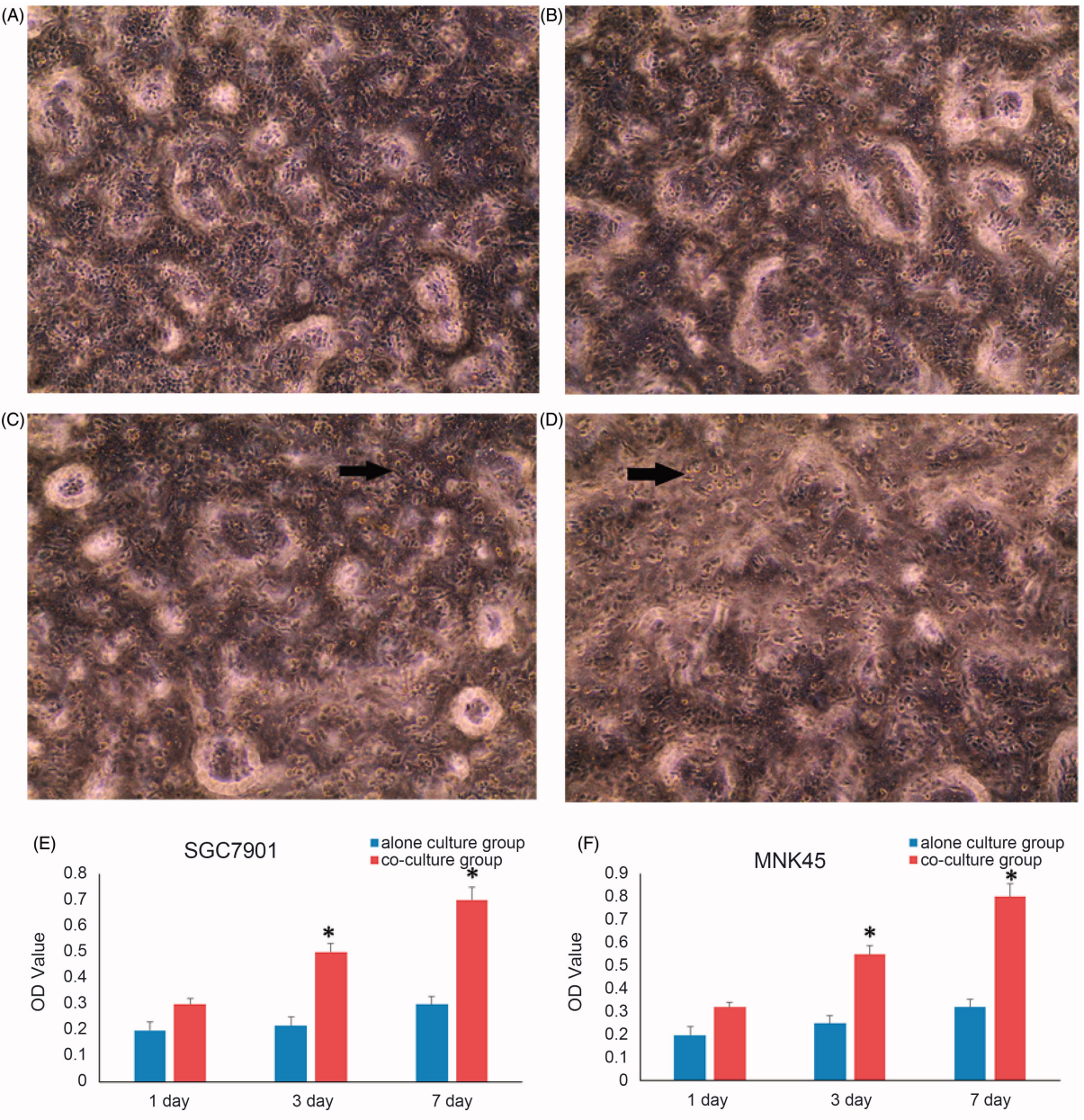


Figure 2. The gastric cancer cells grew faster when co-cultured with TAFs. The proliferation of SGC7901 (A) and MNK45 (B) cells were tested by a lamarBlue assay at 1, 3 and 7 d of culture. There was a significant increase in the viability of gastric cancer cells at 3 and 7 d of co-cultured with TAFs

图 2. 与 TAFs 共培养时, 胃癌细胞生长更快。培养 1、3、7 d 时, 采用 alamarBlue 法检测 SGC7901 (A) 和 MNK45 (B) 细胞的增殖情况。在与 TAFs 共培养的第 3 天和第 7 天, 胃癌细胞的活力显著增加

3.4. PD-L1 表达与 TAFs 密度的关系

53 例(82%) TAFs PD-L1 阳性, 细胞质内呈散点状染色。肿瘤内 TAFs 密度与肿瘤细胞 PD-L1 表达呈显著正相关($p = 0.027$, 表 1), TAFs 与年龄($p = 0.013$, 表 2)、TNM 分期($p = 0.025$, 表 2)也呈正相关。

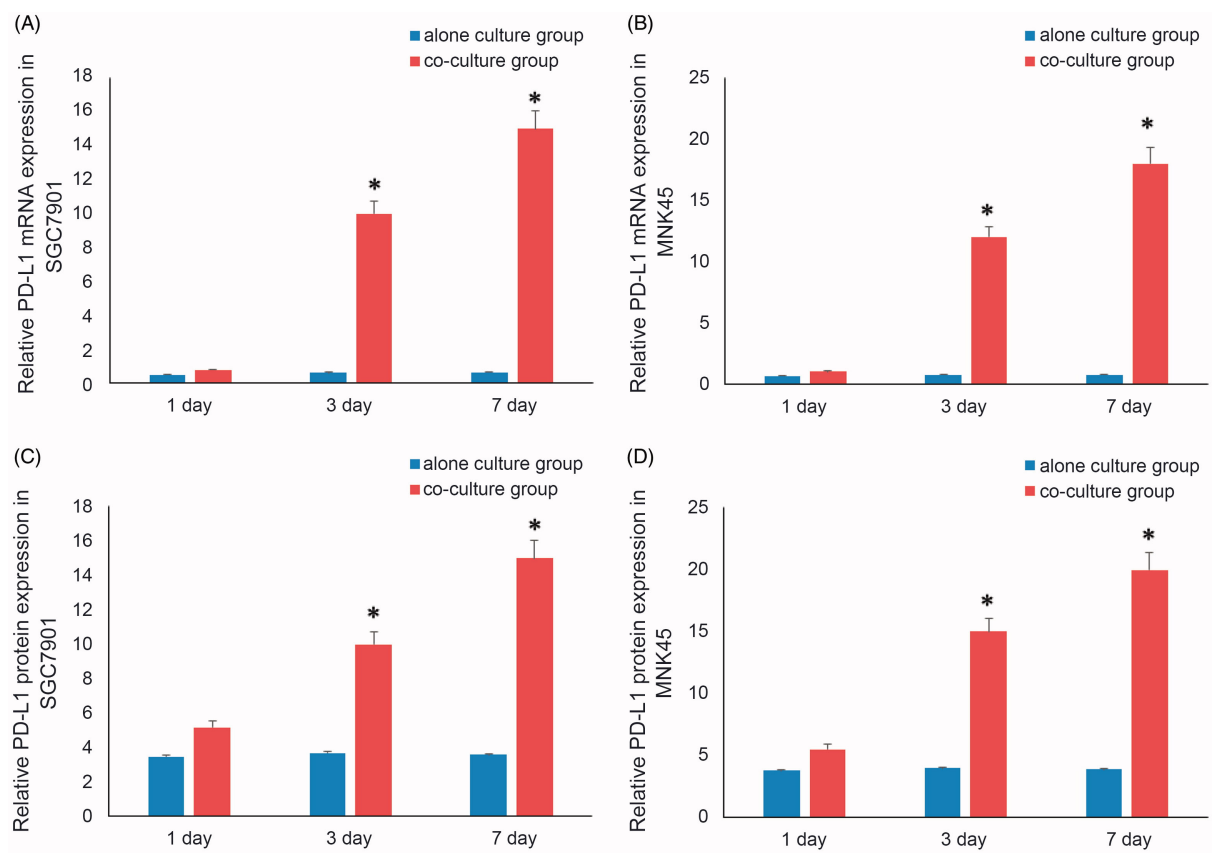


Figure 3. The PD-L 1mRNA and prote in expression in SGC7901cell (A, C) and MNK45 cell (B, D). The expression of PD-L-1 of gastri cancer cells when co-cultured With TAFs were significantly increase dinatime-dependent compared with the control groups

图 3. PD-L1 mRNA 和蛋白在 SGC7901 细胞(A, C)和 MNK45 细胞(B, D)中的表达。与对照组相比，与 TAFs 共培养时胃癌细胞 PD-L1 的表达呈时间依赖性显著增加

Table 1. Correlation between PD-L1 expression and clinicopathological factors.

表 1. PD-L1 表达与临床病理因素

Characteristics	N	PD-L1		p
		PD-L1 (+)	PD-L1 (-)	
Gender				0.845
Male	57	37	20	
Female	38	28	10	
Age				0.023*
≤60	50	40	10	
>60	45	25	20	
Tumour site				0.123
Antral	60	42	18	
Proximal	35	23	12	
Invasion				0.634
T1	25	18	7	

续表

T2	20	17	3
T3	26	13	13
T4	24	17	7
Nodal status			0.035*
N0	42	26	16
N1	13	9	4
N2	21	15	6
N3	19	15	4
Metastasis			0.476
Yes	5	4	1
No	90	61	30
TNM staging			0.675
I	32	25	7
II	26	17	9
III	30	17	13
IV	7	6	1
Lauren classification			0.032*
Intestinal type	40	25	15
Diffuse/mixed type	55	40	15
Tumour-associated fibroblasts			0.027*
Low	30	12	18
High	65	53	12

Table 2. Correlation between PD-L1 expression and density of ATFs

表 2. PD-L1 表达与 ATFs 密度的相关性

Characteristics	N	PD-L1 on tumor-associated fibroblasts		p
		Positive	Negative	
Gender				0.645
Male	37	17	20	
Female	28	18	10	
Age				0.013*
≤60	40	30	10	
>60	25	5	20	
Tumour site				0.323
Antral	42	22	20	
Proximal	23	13	10	

续表

Invasion				0.634
T1	18	10	8	
T2	17	7	10	
T3	13	7	6	
T4	17	11	6	
Nodal status				0.635
N0	26	12	14	
N1	9	7	2	
N2	15	7	8	
N3	15	9	6	
Metastasis				0.476
Yes	4	2	2	
No	61	33	28	
TNM staging				0.025*
I	25	20	5	
II	17	9	8	
III	17	3	14	
IV	6	3	3	
Lauren classification				0.132
Intestinal type	25	10	15	
Diffuse/mixed type	40	25	15	

4. 讨论

胃癌是最常见的消化系统恶性肿瘤之一，特别是在东亚国家比如韩国、日本和中国。肿瘤细胞可以逃脱免疫监视。以往的研究主要关注肿瘤细胞在免疫逃逸中的变化，对肿瘤微环境(tumor microenvironment, TME)对免疫逃逸的影响知之甚少[28]-[30]。

在癌症发展的早期阶段，肿瘤基质中各种类型的细胞阻止肿瘤生长。相反，这些基质细胞中的大多数在晚期阶段促进肿瘤进展[31]。TAFs 通过分泌多种可溶性因子发挥关键作用[32]-[34]。已有研究表明，TAFs 的患病率与胃癌转移的可能性密切相关[35]。此外，成纤维细胞产生的 TGF- β 和 FGF-7 可增加胃癌细胞的增殖和侵袭性[36] [37]。一些报道也表明，TAFs 与癌细胞共培养时表达 MCP-1/CCL2，并诱导细胞存活和免疫抑制[26] [38]。总之，相关结果表明，在肿瘤发生的初始阶段，TAFs 首先由免疫细胞调控，但随着肿瘤的进展，它们获得招募和调节免疫细胞的能力，最终达到与疾病进展相容的免疫抑制。然而，在 TAFs 与癌细胞之间的直接相互作用中，癌症免疫逃逸的重要因素仍然未知。

免疫耐药是多种人类癌症有效肿瘤生物治疗选择的主要障碍之一。PD-L1 是肿瘤微环境中的关键蛋白，可能促进肿瘤细胞的免疫逃逸[39]。目前已知，PD-L1 在癌细胞中的过表达可诱导肿瘤逃避宿主 T 细胞[40] [41]。据报道，阻断 PD-1/PD-L1 相互作用或下调 PD-L1 在癌细胞中的表达可促进抗肿瘤免疫并抑制肿瘤生长[41]。PD-L1 表达的调控在很大程度上仍然未知。在本研究中，我们报道了共培养的 TAFs 能

够以时间依赖性的方式上调人胃癌细胞系中 PD-L1 的表达,并诱导癌细胞增殖。瘤内 TAFs 密度与肿瘤细胞 PD-L1 表达呈显著正相关($p = 0.027$)。结合以往的结果,这表明 TAFs 在癌细胞中诱导 PD-L1 表达是一种常见的效应。

既往研究表明,免疫组化 PD-L1 在 102 例人胃癌中呈强阳性表达,在腺瘤样本中呈弱阳性表达,在正常胃组织[42]中呈完全阴性表达。此外,PD-L1 的表达与更高的淋巴结转移数量、更大的肿瘤大小、更大的浸润深度和更差的总生存期显著相关。同样的结果显示,在研究过程中开发的一种抗 PD-L1 单克隆抗体可显著减少 PD-L1 过表达[43]诱导的 t 细胞凋亡。

多项临床试验表明,用 PD-L1 抗体阻断 PD-1/PD-L1 通路具有抗肿瘤作用[44] [45]。在浸润性导管癌($p = 0.012$) [45]、尿路上皮癌($p < 0.001$) [29]和肝内胆管癌($p < 0.001$) [29]中,肿瘤细胞过表达 PD-L1。在我们的研究中,PD-L1 的表达与年轻($p = 0.023$)和弥漫性/混合型($p = 0.032$)显著相关。PD-L1 表达与肿瘤淋巴结状态呈负相关($p = 0.035$)。然而,PD-L1 的表达与性别、肿瘤部位、肿瘤侵袭转移及 TNM 分期无统计学关系。可能的原因是 PD-L1 阳性病例相对较少。相似结果显示,在非小细胞肺癌中,PD-L1 的表达与临床分期/生存率之间没有统计学关系[46] [47]。

在正常人体中,PD-1 和 CD28 处于动态平衡状态,但 PD-1 及其配体在肿瘤患者[45]中高表达。高表达 PD-L1 的肿瘤细胞抑制 cd8 + T 细胞的增殖和功能。同时,PD-L1 的高表达也抑制 NK 细胞和 B 细胞的增殖和功能[48]。在 PD-L1 刺激下,Treg 细胞增殖能力增强,功能上调[49]。上述所有发现都导致肿瘤逃避免疫监视,促进肿瘤生长。因此,为了充分阐明 PD-L1 与 GC 患者预后的关系,未来需要进一步探索其潜在机制。

总之,我们已经证明,PD-L1 的表达在与 TAFs 共培养的胃癌细胞系中以时间依赖性的方式显着增加。大多数 GC 患者(68%)发现 pd-l1 阳性肿瘤细胞,并且与 TAFs 密度相关(82%)。此外,我们的研究发现 PD-L1 表达与 GC 中大量淋巴细胞浸润、弥漫性/混合性组织学和瘤内 TAFs 有关。综上所述,阻断肿瘤 PD-L1 有望增强免疫治疗的效果。

伦理审批和同意参与

本研究经山东大学(济南,中国)机构动物护理与使用委员会批准。

数据和材料的可获得性

本研究中使用和/或分析的数据集可应通讯作者的合理要求获得。

基金项目

山东省医药卫生科技发展计划项目(202104010626)支持。

参考文献

- [1] Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E. and Forman, D. (2011) Global Cancer Statistics. *CA: A Cancer Journal for Clinicians*, **61**, 69-90. <https://doi.org/10.3322/caac.20107>
- [2] Bhat, S.A., Mir, M.U.R., Majid, S., Hassan, T., Rehman, M.U. and Kuchy, S. (2018) Diagnostic Utility of Glycosyl-transferase mRNA Expression in Gastric Cancer. *Hematology/Oncology and Stem Cell Therapy*, **11**, 158-168. <https://doi.org/10.1016/j.hemonc.2018.03.002>
- [3] Ren, H., Xu, Z., Guo, W., Deng, Z. and Yu, X. (2018) Rab3IP Interacts with SSX2 and Enhances the Invasiveness of Gastric Cancer Cells. *Biochemical and Biophysical Research Communications*, **503**, 2563-2568. <https://doi.org/10.1016/j.bbrc.2018.07.016>
- [4] Kato, Y., Yashiro, M., Noda, S., Tendo, M., Kashiwagi, S., Doi, Y., et al. (2010) Establishment and Characterization of a New Hypoxia-Resistant Cancer Cell Line, OCUM-12/Hypo, Derived from a Scirrhous Gastric Carcinoma. *British*

- Journal of Cancer*, **102**, 898-907. <https://doi.org/10.1038/sj.bjc.6605543>
- [5] Zhang, W. and Song, Y. (2018) LINC00473 Predicts Poor Prognosis and Regulates Cell Migration and Invasion in Gastric Cancer. *Biomedicine & Pharmacotherapy*, **107**, 1-6. <https://doi.org/10.1016/j.biopha.2018.07.061>
 - [6] Rawicz-Pruszyński, K., van Sandick, J.W., Mielko, J., Cisel, B. and Polkowski, W.P. (2018) Current Challenges in Gastric Cancer Surgery: European Perspective. *Surgical Oncology*, **27**, 650-656. <https://doi.org/10.1016/j.suronc.2018.08.004>
 - [7] Liu, J., Chen, Q., Feng, L. and Liu, Z. (2018) Nanomedicine for Tumor Microenvironment Modulation and Cancer Treatment Enhancement. *Nano Today*, **21**, 55-73. <https://doi.org/10.1016/j.nantod.2018.06.008>
 - [8] Meng, W., Xue, S. and Chen, Y. (2018) The Role of CXCL12 in Tumor Microenvironment. *Gene*, **641**, 105-110. <https://doi.org/10.1016/j.gene.2017.10.015>
 - [9] Lee, N., Nikfarjam, M. and He, H. (2018) Functions of the CXC Ligand Family in the Pancreatic Tumor Microenvironment. *Pancreatology*, **18**, 705-716. <https://doi.org/10.1016/j.pan.2018.07.011>
 - [10] Servais, C. and Erez, N. (2012) From Sentinel Cells to Inflammatory Culprits: Cancer-Associated Fibroblasts in Tumour-Related Inflammation. *The Journal of Pathology*, **229**, 198-207. <https://doi.org/10.1002/path.4103>
 - [11] Grimm, S., Jennek, S., Singh, R., Enkelmann, A., Junker, K., Rippaus, N., *et al.* (2015) Malignancy of Bladder Cancer Cells Is Enhanced by Tumor-Associated Fibroblasts through a Multifaceted Cytokine-Chemokine Loop. *Experimental Cell Research*, **335**, 1-11. <https://doi.org/10.1016/j.yexcr.2015.04.001>
 - [12] Tripathi, M., Billet, S. and Bhowmick, N.A. (2012) Understanding the Role of Stromal Fibroblasts in Cancer Progression. *Cell Adhesion & Migration*, **6**, 231-235. <https://doi.org/10.4161/cam.20419>
 - [13] Saigusa, (2011) Cancer-Associated Fibroblasts Correlate with Poor Prognosis in Rectal Cancer after Chemoradiotherapy. *International Journal of Oncology*, **38**, 655-633. <https://doi.org/10.3892/ijo.2011.906>
 - [14] Ernsting, M.J., Hoang, B., Lohse, I., Undzys, E., Cao, P., Do, T., *et al.* (2015) Targeting of Metastasis-Promoting Tumor-Associated Fibroblasts and Modulation of Pancreatic Tumor-Associated Stroma with a Carboxymethylcellulose-Docetaxel Nanoparticle. *Journal of Controlled Release*, **206**, 122-130. <https://doi.org/10.1016/j.jconrel.2015.03.023>
 - [15] Leef, G. and Thomas, S.M. (2013) Molecular Communication between Tumor-Associated Fibroblasts and Head and Neck Squamous Cell Carcinoma. *Oral Oncology*, **49**, 381-386. <https://doi.org/10.1016/j.oraloncology.2012.12.014>
 - [16] Zheng, Z., Bu, Z., Liu, X., *et al.* (2014) Level of Circulating PD-L1 Expression in Patients with Advanced Gastric Cancer and Its Clinical Implications. *Chinese Journal of Cancer Research*, **26**, 104-111.
 - [17] Robert, C., Soria, J. and Eggermont, A.M.M. (2013) Drug of the Year: Programmed Death-1 Receptor/Programmed Death-1 Ligand-1 Receptor Monoclonal Antibodies. *European Journal of Cancer*, **49**, 2968-2971. <https://doi.org/10.1016/j.ejca.2013.07.001>
 - [18] Alaghebandan, R., Stehlik, J., Trpkov, K., Magi-Galluzzi, C., Condom Mundo, E., Pane Foix, M., *et al.* (2017) Programmed Death-1 (PD-1) Receptor/Pd-1 Ligand (PD-L1) Expression in Fumarate Hydratase-Deficient Renal Cell Carcinoma. *Annals of Diagnostic Pathology*, **29**, 17-22. <https://doi.org/10.1016/j.anndiagpath.2017.04.007>
 - [19] Okuma, Y., Wakui, H., Utsumi, H., Sagawa, Y., Hosomi, Y., Kuwano, K., *et al.* (2018) Soluble Programmed Cell Death Ligand 1 as a Novel Biomarker for Nivolumab Therapy for Non-Small-Cell Lung Cancer. *Clinical Lung Cancer*, **19**, 410-417.e1. <https://doi.org/10.1016/j.clc.2018.04.014>
 - [20] Kim, J., Kim, S., Lee, H.S., Yang, W., Cho, H., Chay, D.B., *et al.* (2018) Prognostic Implication of Programmed Cell Death 1 Protein and Its Ligand Expressions in Endometrial Cancer. *Gynecologic Oncology*, **149**, 381-387. <https://doi.org/10.1016/j.ygyno.2018.02.013>
 - [21] Shiraliyeva, N., Friedrichs, J., Buettner, R. and Friedrichs, N. (2017) PD-L1 Expression in HNPCC-Associated Colorectal Cancer. *Pathology-Research and Practice*, **213**, 1552-1555. <https://doi.org/10.1016/j.prp.2017.09.012>
 - [22] Baptista, M.Z., Sarian, L.O., Derchain, S.F.M., Pinto, G.A. and Vassallo, J. (2016) Prognostic Significance of PD-L1 and PD-L2 in Breast Cancer. *Human Pathology*, **47**, 78-84. <https://doi.org/10.1016/j.humpath.2015.09.006>
 - [23] Meng, X., Liu, Y., Zhang, J., Teng, F., Xing, L. and Yu, J. (2017) PD-1/PD-L1 Checkpoint Blockades in Non-Small Cell Lung Cancer: New Development and Challenges. *Cancer Letters*, **405**, 29-37. <https://doi.org/10.1016/j.canlet.2017.06.033>
 - [24] Salgado, R., Denkert, C., Demaria, S., Sirtaine, N., Klauschen, F., Pruneri, G., *et al.* (2015) The Evaluation of Tumor-Infiltrating Lymphocytes (Tils) in Breast Cancer: Recommendations by an International Tils Working Group 2014. *Annals of Oncology*, **26**, 259-271. <https://doi.org/10.1093/annonc/mdu450>
 - [25] Vicent, S., Sayles, L.C., Vaka, D., Khatri, P., Gevaert, O., Chen, R., *et al.* (2012) Cross-Species Functional Analysis of Cancer-Associated Fibroblasts Identifies a Critical Role for CLCF1 and IL-6 in Non-Small Cell Lung Cancer *in Vivo*. *Cancer Research*, **72**, 5744-5756. <https://doi.org/10.1158/0008-5472.can-12-1097>

- [26] Lin, Z., Chuang, Y. and Chuang, W. (2012) Cancer-Associated Fibroblasts Up-Regulate CCL2, CCL26, IL6 and LOXL2 Genes Related to Promotion of Cancer Progression in Hepatocellular Carcinoma Cells. *Biomedicine & Pharmacotherapy*, **66**, 525-529. <https://doi.org/10.1016/j.biopha.2012.02.001>
- [27] Chan, J.A., Blaszkowsky, L.S., Enzinger, P.C., Ryan, D.P., Abrams, T.A., Zhu, A.X., *et al.* (2011) A Multicenter Phase II Trial of Single-Agent Cetuximab in Advanced Esophageal and Gastric Adenocarcinoma. *Annals of Oncology*, **22**, 1367-1373. <https://doi.org/10.1093/annonc/mdq604>
- [28] de Haas, S., Delmar, P., Bansal, A.T., Moisse, M., Miles, D.W., Leighl, N., *et al.* (2014) Genetic Variability of VEGF Pathway Genes in Six Randomized Phase III Trials Assessing the Addition of Bevacizumab to Standard Therapy. *Angiogenesis*, **17**, 909-920. <https://doi.org/10.1007/s10456-014-9438-1>
- [29] Noguchi, E., Saito, N., Kobayashi, M. and Kameoka, S. (2015) Clinical Significance of Hepatocyte Growth Factor/c-Met Expression in the Assessment of Gastric Cancer Progression. *Molecular Medicine Reports*, **11**, 3423-3431. <https://doi.org/10.3892/mmr.2015.3205>
- [30] Lu, Y., Tang, L., Zhang, Q., Zhang, Z. and Wei, W. (2017) Microrna-613 Inhibits the Progression of Gastric Cancer by Targeting CDK9. *Artificial Cells, Nanomedicine, and Biotechnology*, **46**, 980-984. <https://doi.org/10.1080/21691401.2017.1351983>
- [31] Jotzu, C., Alt, E., Welte, G., Li, J., Hennessy, B.T., Devarajan, E., *et al.* (2010) Adipose Tissue-Derived Stem Cells Differentiate into Carcinoma-Associated Fibroblast-Like Cells under the Influence of Tumor-Derived Factors. *Analytical Cellular Pathology*, **33**, 61-79. <https://doi.org/10.1155/2010/695162>
- [32] Orimo, A., Gupta, P.B., Sgroi, D.C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., *et al.* (2005) Stromal Fibroblasts Present in Invasive Human Breast Carcinomas Promote Tumor Growth and Angiogenesis through Elevated SDF-1/CXCL12 Secretion. *Cell*, **121**, 335-348. <https://doi.org/10.1016/j.cell.2005.02.034>
- [33] Yu, Y., Xiao, C., Tan, L., Wang, Q., Li, X. and Feng, Y. (2013) Cancer-Associated Fibroblasts Induce Epithelial-Mesenchymal Transition of Breast Cancer Cells through Paracrine TGF- β Signalling. *British Journal of Cancer*, **110**, 724-732. <https://doi.org/10.1038/bjc.2013.768>
- [34] Nakazawa, K., Yashiro, M. and Hirakawa, K. (2003) Keratinocyte Growth Factor Produced by Gastric Fibroblasts Specifically Stimulates Proliferation of Cancer Cells from Scirrhous Gastric Carcinoma. *Cancer Research*, **63**, 8848-8854.
- [35] Zhi, K., Shen, X., Zhang, H. and Bi, J. (2010) Cancer-Associated Fibroblasts Are Positively Correlated with Metastatic Potential of Human Gastric Cancers. *Journal of Experimental & Clinical Cancer Research*, **29**, Article No. 66. <https://doi.org/10.1186/1756-9966-29-66>
- [36] Jung, D., Che, Z.M., Kim, J., Kim, K., Kim, K., Williams, D., *et al.* (2010) Tumor-Stromal Crosstalk in Invasion of Oral Squamous Cell Carcinoma: A Pivotal Role of CCL7. *International Journal of Cancer*, **127**, 332-344. <https://doi.org/10.1002/ijc.25060>
- [37] Gharaati-Far, N., Tohidkia, M.R., Dehnad, A. and Omid, Y. (2017) Efficiency and Cytotoxicity Analysis of Cationic Lipids-Mediated Gene Transfection into AGS Gastric Cancer Cells. *Artificial Cells, Nanomedicine, and Biotechnology*, **46**, 1001-1008. <https://doi.org/10.1080/21691401.2017.1355311>
- [38] Wu, M., Hong, H., Hong, T., Chiang, W., Jin, Y. and Chen, Y. (2011) Targeting Galectin-1 in Carcinoma-Associated Fibroblasts Inhibits Oral Squamous Cell Carcinoma Metastasis by Downregulating MCP-1/CCL2 Expression. *Clinical Cancer Research*, **17**, 1306-1316. <https://doi.org/10.1158/1078-0432.ccr-10-1824>
- [39] Joyce, J.A. and Pollard, J.W. (2008) Microenvironmental Regulation of Metastasis. *Nature Reviews Cancer*, **9**, 239-252. <https://doi.org/10.1038/nrc2618>
- [40] Dong, H., Strome, S.E., Salomao, D.R., Tamura, H., Hirano, F., Flies, D.B., *et al.* (2002) Tumor-Associated B7-H1 Promotes T-Cell Apoptosis: A Potential Mechanism of Immune Evasion. *Nature Medicine*, **8**, 793-800. <https://doi.org/10.1038/nm730>
- [41] Iwai, Y., Ishida, M., Tanaka, Y., Okazaki, T., Honjo, T. and Minato, N. (2002) Involvement of PD-L1 on Tumor Cells in the Escape from Host Immune System and Tumor Immunotherapy by PD-L1 Blockade. *Proceedings of the National Academy of Sciences*, **99**, 12293-12297. <https://doi.org/10.1073/pnas.192461099>
- [42] Lei, Z., Tan, I.B., Das, K., Deng, N., Zouridis, H., Pattison, S., *et al.* (2013) Identification of Molecular Subtypes of Gastric Cancer with Different Responses to PI3-Kinase Inhibitors and 5-Fluorouracil. *Gastroenterology*, **145**, 554-565. <https://doi.org/10.1053/j.gastro.2013.05.010>
- [43] Scherz-Shouval, R., Santagata, S., Mendillo, M.L., Sholl, L.M., Ben-Aharon, I., Beck, A.H., *et al.* (2014) The Reprogramming of Tumor Stroma by HSF1 Is a Potent Enabler of Malignancy. *Cell*, **158**, 564-578. <https://doi.org/10.1016/j.cell.2014.05.045>
- [44] Herbst, R.S., Soria, J., Kowanetz, M., Fine, G.D., Hamid, O., Gordon, M.S., *et al.* (2014) Predictive Correlates of Response to the Anti-Pd-L1 Antibody MPDL3280A in Cancer Patients. *Nature*, **515**, 563-567. <https://doi.org/10.1038/nature14011>

-
- [45] Ghebeh, H., Barhoush, E., Tulbah, A., Elkum, N., Al-Tweigeri, T. and Dermime, S. (2008) FOXP3+ Tregs and B7-H1+/PD-1+T Lymphocytes Co-Infiltrate the Tumor Tissues of High-Risk Breast Cancer Patients: Implication for Immunotherapy. *BMC Cancer*, **8**, Article No. 57. <https://doi.org/10.1186/1471-2407-8-57>
- [46] Alsuliman, A., Colak, D., Al-Harazi, O., Fitwi, H., Tulbah, A., Al-Tweigeri, T., *et al.* (2015) Bidirectional Crosstalk between PD-L1 Expression and Epithelial to Mesenchymal Transition: Significance in Claudin-Low Breast Cancer Cells. *Molecular Cancer*, **14**, Article No. 149. <https://doi.org/10.1186/s12943-015-0421-2>
- [47] Muro, K., Bang, Y., Shankaran, V., Geva, R., Catenacci, D.V.T., Gupta, S., *et al.* (2014) A Phase 1B Study of Pembrolizumab (PEMBRO; MK-3475) in Patients (PTS) with Advanced Gastric Cancer. *Annals of Oncology*, **25**, v1-v41. <https://doi.org/10.1093/annonc/mdu438.15>
- [48] Ahmadzadeh, M., Johnson, L.A., Heemskerk, B., Wunderlich, J.R., Dudley, M.E., White, D.E., *et al.* (2009) Tumor Antigen-Specific CD8 T Cells Infiltrating the Tumor Express High Levels of PD-1 and Are Functionally Impaired. *Blood*, **114**, 1537-1544. <https://doi.org/10.1182/blood-2008-12-195792>
- [49] Cho, Y., Yoon, H., Lee, J., Hong, S. and Hong, S. (2011) Relationship between the Expressions of PD-L1 and Tumor-Infiltrating Lymphocytes in Oral Squamous Cell Carcinoma. *Oral Oncology*, **47**, 1148-1153. <https://doi.org/10.1016/j.oraloncology.2011.08.007>