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• 临床研究 •

宫颈癌组织中 PD-L1 的表达及临床意义分析*

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摘要 目的:探讨宫颈癌组织中程序性死亡因子配体(Programmed death ligand 1, PD-L1)的表达及临床意义,为宫颈癌免疫治疗提供理论和实验基础。**方法:**采用免疫组织化学染色的方法检测 10 例正常宫颈组织、71 例宫颈上皮内瘤变(Cervical intraepithelial neoplasias, CIN)组织和 89 例宫颈癌组织中 PD-L1 蛋白的表达,分析 PD-L1 的表达与宫颈癌患者临床病理参数(临床分期、病理类型、年龄、淋巴转移)的相关性。**结果:**正常宫颈组织中无 PD-L1 表达, CIN 中 PD-L1 表达的免疫评分低,呈弱阳性;宫颈癌中 PD-L1 表达的免疫评分高,呈强阳性。CIN 和宫颈癌组织中 PD-L1 的阳性表达率分别为 42%(30/41)和 75%(66/89),其差异有统计学意义($P<0.05$)。PD-L1 的表达与宫颈癌患者年龄、病理分级、淋巴转移无关($P>0.05$),而与患者临床分期显著相关($P=0.0021$)。**结论:**PD-L1 的表达上调可能参与了宫颈癌的发生和发展。

关键词:程序性死亡因子 1; 程序性死亡因子配体 1; 宫颈癌; 临床意义

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Expression and Clinical Significance of PD-L1 in the Cervical Cancer*

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ABSTRACT Objective: To investigate the expression and clinical significance of PD-L1 in the cervical cancer, thus provide theoretical and experimental basis for the immunotherapy of cervical cancer. **Methods:** The protein expression of PD-L1 was determined in 10 cases of normal cervical tissue, 71 cases of CIN and 89 cases of cervical cancer by immunohistochemical staining, then the relationship between PD-L1 expression level and clinical pathological parameters (FIGO stage, histology type, age, lymph node) were analyzed. **Results:** There was no PD-L1 expression in the normal cervical tissues. The immunological score of PD-L1 expression in CIN was low and weakly positive. The immunological score of PD-L1 expression in cervical cancer was high and strongly positive. The positive expression rates of PD-L1 in CIN and cervical cancer tissues were 42% (30/41) and 75% (66/89), respectively ($P<0.05$). The expression of PD-L1 was not correlated with age, pathological grade and lymphatic metastasis($P>0.05$), but was closely related to the clinical stage($P=0.0021$). **Conclusions:** The up-regulation of PD-L1 expression may be involved in the development and progression of cervical cancer.

Key words: PD-1; PD-L1; Cervical cancer; Clinical significance

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前言

近年来,随着人们社会生活方式的改变,人乳头瘤状病毒(HPV)感染人数增多,宫颈癌的发病率有所增加^[1]。2018 年发布的最新全球癌症图谱中,宫颈癌的发病率和死亡率位居女性癌症的第四位。国际癌症研究机构预估 2018 年全球将有 570,000

例病例和 311,000 例死亡^[2]。随着对肿瘤免疫逃逸机制的深入研究,免疫治疗被认为是肿瘤防治的新方法^[3,4]。

在机体正常免疫反应中,可通过激活程序性死亡受体 1 (PD-1)/ 程序性死亡因子配体 1(PD-L1)信号通路,诱导免疫耐受以确保正常组织免受损伤。但是在肿瘤微环境的作用下,PD-1/PD-L1 的表达异常上调,可抑制 T 细胞的免疫应答,介导

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肿瘤的免疫逃逸,从而参与了肿瘤的发生、发展。因此 PD-1/PD-L1 的抑制剂在肿瘤的免疫治疗中备受关注^[5]。PD-1/PD-L1 抑制剂可阻断 PD-1/PD-L1 通路,恢复 T 细胞活性,促进正性免疫应答杀伤肿瘤细胞。临床上经 FDA 批准的 PD-1/PD-L1 抑制剂已应用于转移性黑色素瘤、非小细胞肺癌(NSCLC)、肾细胞癌(RCC)和膀胱癌等^[6],在其他恶性肿瘤中的应用仍处研究阶段。本研究主要检测了 PD-L1 在宫颈癌组织中的表达情况,并分析其与宫颈癌临床病理特征的相关性,以期为宫颈癌的免疫治疗提供参考依据。

1 资料与方法

1.1 临床资料

标本取自 2016 年 5 月~2018 年 5 月上海市第一人民医院,包括正常宫颈组织 10 例、CIN 组织 71 例和宫颈癌组织 89 例(鳞癌 75 例,腺癌 14 例)。宫颈癌组织标本按国际妇产科联盟(FIGO)临床分期:I、II 期 50 例,III、IV 期 39 例;病理分型:鳞癌 75 例,腺癌 14 例;年龄:<48 岁 67 例,≥48 岁 22 例;淋巴结转

移:阴性 71 例,阳性 18 例。所有标本经甲醛固定,石蜡包埋制片。PD-L1(ab213524)兔单克隆抗体购于英国 Abcam 公司,二甲苯、无水乙醇、EDTA 抗原修复液、PBS 缓冲液、DBA 显色剂等均购自北京康为生物制剂有限公司。

1.2 免疫组化染色及结果判断

免疫组化染色:切片常规二甲苯脱蜡,梯度乙醇水化,PBS (pH7.4) 洗涤 5 min × 3 次,EDTA 抗原抗体修复;3%过氧化氢溶液(双氧水:纯水=1:9)温室避光孵育 25 min,9 PBS 洗涤 5 min × 3 次;正常山羊血清 37℃ 封闭 10 min;弃血清,滴加 PD-L1 一抗(稀释比 1:100),4℃ 孵育过夜;PBS 洗涤 5 min × 3 次,滴加 DAB 显色,Harris 苏木素复染,梯度乙醇脱水,二甲苯透明,中性树胶封片。显微镜镜检,图像采集分析。PD-L1 阳性表达定位于细胞膜和细胞浆中,呈浅黄色至棕褐色颗粒状。免疫组化结果判定:每张切片均选取 10 个不同的染色区域,在倒置显微镜下观察,使用免疫反应积分 (Immune Response Scores,IRS)打分法对免疫染色的切片进行评分。染色强度及染色评分的标准为见表 1。

表 1 ISH 评分标准

Table 1 ISH Grading

Score	0	1	2	3	4
Extent	无	1%-10%	10%-50%	50%-80%	>80%
Degree	Negative	Yellow	Hazel	Brown	

IRS 得分=染色强度分数*阳性染色细胞百分比分数。总分 0-3 分为低表达或阴性表达;总分为 4-12 分为高表达或阳性表达。所有的免疫组织化学结果都是由两个训练有素的观察人员在不知道患者临床和病理资料的情况下独立评估的。

1.3 统计学分析

采用 SPSS20.0 统计软件进行数据分析,计数资料比较采用 χ^2 检验,以 $P<0.05$ 为差异具有统计学意义。

2 结果

2.1 正常宫颈组织、CIN 组织与宫颈癌组织中 PD-L1 的表达

表 2 PD-L1 在正常组织、CIN 组织、宫颈癌组织中的表达情况

Table 2 The expression of PD-L1 in the normal cervical tissue, CIN tissue and cervical cancer tissue

Type	Total	Expression of PD-L1	
		Negative	Positive
Normal Cervical Tissue	10	9	1
CIN Tissue	71	41	30
Cervical Cancer Tissue	89	23	66

2.2 宫颈癌组织中 PD-L1 阳性表达与患者病理分型的关系

从图表中可以看出宫颈癌组织 PD-L1 的阳性表达与患者的病理分型无显著相关性(P 均>0.05),见图 3、4

2.3 宫颈癌中 PD-L1 的表达与临床分期的关系

PD-L1 在 I-II 期宫颈癌中弱阳性表达,在 III-IV 期宫颈癌中强阳性表达。I/II、III/IV 期宫颈癌组织中 PD-L1 的阳性表达率比较差异有统计学意义($P<0.05$),见图 5、6,浸润越深,转移越严重的宫颈癌中,PD-L1 的表达强度越高。这些结果提示

PD-L1 不仅参与了宫颈癌的发生,还在宫颈癌的侵袭和转移中发挥了作用。

2.4 宫颈癌组织中 PD-L1 的表达与患者临床病理特征的关系

PD-1/PD-L1 参与了肿瘤的免疫逃逸,主要在 T 细胞、B 细胞、炎症反应细胞和恶性肿瘤中表达,在淋巴结细胞中呈阴性表达(见图 7、图 8)。宫颈癌组织中 PD-L1 的表达与病理分型、年龄、淋巴结转移均无显著相关性(P 均>0.05),但与宫颈癌 FIGO 分期有关($P<0.05$),见图 3。

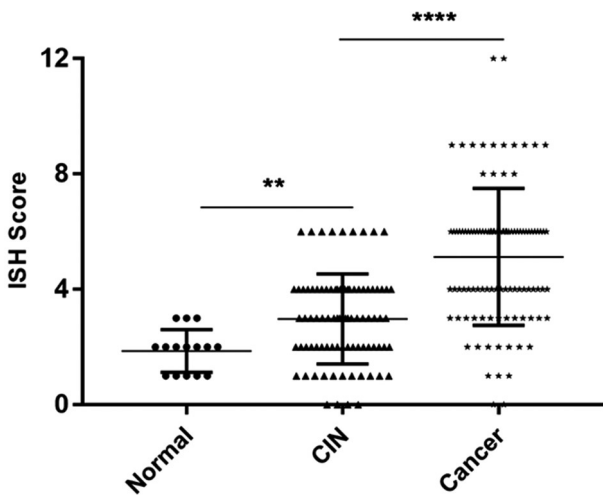


图1 PD-L1 在正常宫颈组织、CIN 组织、宫颈癌组织中的 ISH 评分情况

Fig.1 The IRS of PD-L1 in normal cervical tissue, CIN tissue, and cervical cancer tissue

**** Statistically significant

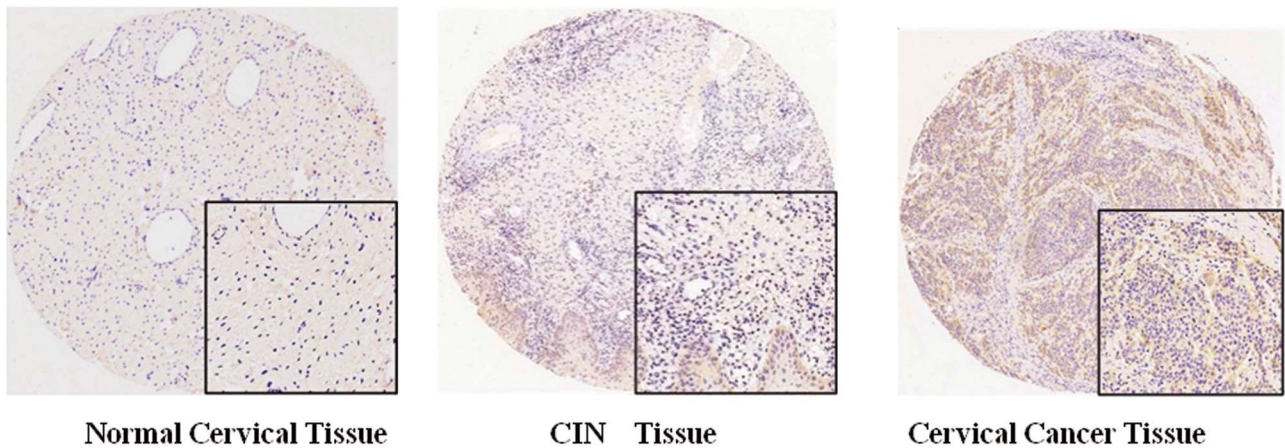


图2 PD-L1 在正常宫颈组织、CIN 组织、宫颈癌组织中的表达情况(sp× 400)

Fig.2 The Expression of PD-L1 in normal cervical tissue, CIN tissue and cervical cancer tissue by immunohistochemical staining(sp× 400)

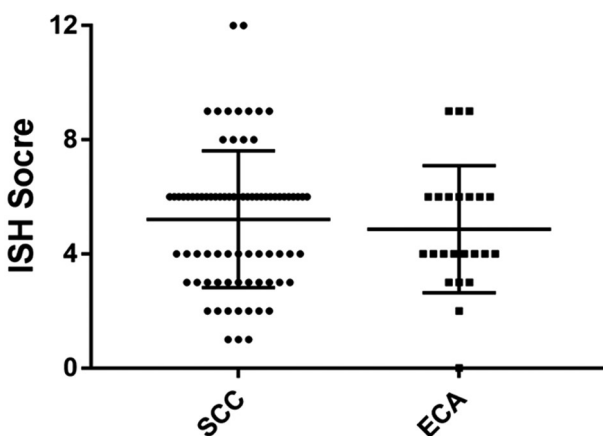


图3 PD-L1 在宫颈鳞癌、宫颈腺癌中 ISH 的评分情况

Fig.3 The IRS of PD-L1 in SCC and ECA

肿瘤组织不表达或极少量表达 MHC II 类分子,同时许多肿瘤细胞高表达的 PD-L1 分子与肿瘤浸润淋巴细胞表达的 PD-1、肿瘤特异性 CD8⁺T 细胞表达的 PD-1 结合后^[10]抑制了周围淋巴细胞的活性,从而使肿瘤细胞逃避了免疫系统的监视与攻击^[11]。

3 讨论

在宫颈癌的治疗中,手术、放疗、化疗仍是三大主要方法,但由于各种治疗方法的创伤性大、靶向性低、易产生耐药性等不足,寻找新的治疗方法仍具有十分重要的临床意义。随着对肿瘤免疫抑制研究的深入,肿瘤的免疫治疗在 2013 年被 Science 杂志评为当年十大科技突破之首^[7]。Burnet 提出的 "免疫监视"学说认为机体的免疫系统可以发挥监视作用,认识并消灭任何表达新抗原的 "异己"成分或突变细胞,以保持机体内环境的稳定^[8]。当机体微环境免疫监视功能低下时,"异己"成分或突变细胞无法及时被清除时,从而导致肿瘤发生。

研究表明癌细胞可以表达高水平的免疫抑制信号蛋白来逃避肿瘤微环境中的宿主免疫。肿瘤浸润淋巴细胞(tumnor infiltrating lymphocyte,TIL)被认为是机体对肿瘤细胞特异性免疫反应的效应细胞^[9],临床研究表明 TIL 在肿瘤中发挥关键作用并具有预后意义。由于抗肿瘤特异性免疫主要为 CD8⁺T 细胞,

因此,由程序性细胞死亡蛋白 1(PD-1)及程序性死亡因子配体 1(PD-L1)介导的肿瘤诱导的免疫抑制(免疫检查点)是该系统中最关键的检查点途径之一。

PD-1 由 288 个氨基酸残基组成,分子量为 50~55KD,为 I 型跨膜糖蛋白,由胞外区、胞内区和跨膜区三个部分组成,属于免疫球蛋白超家族成员。PD-1 细胞外由 IgV 区域跨膜段和胞内段组成,主要由一个免疫受体酪氨酸转换基序(Immunoreceptor tyrosine-based switch motif, ITSM)和一个免疫受体酪氨酸抑制基序(Immunoreceptor tyrosine-based inhibitory motif, ITIM)组成细胞的胞浆区域^[12,13],其中 ITSM 的酪氨酸序列是 T 细胞、B 细胞表面 PD-1 发挥作用所必需的。PD-1 作为免疫抑制受体,主要表达在不同种类的免疫细胞表面,比如 T 淋巴细胞、B 淋巴细胞、自然杀伤细胞及树突细胞等。另外,PD-1 也表达于调节性 T 细胞(Regulatory T cell, Treg),并能促进 Treg 细胞的增殖,从而抑制免疫应答。

PD-1 有两个结合配体,即 PD-L1 及 PD-L2 均为 B7 家族成员。PD-L1 可编码 290 个氨基酸蛋白,位于人染色体 9q24,包括胞外区 IgC 和 IgV 样结构,疏水的中间结构区以及含有蛋白激

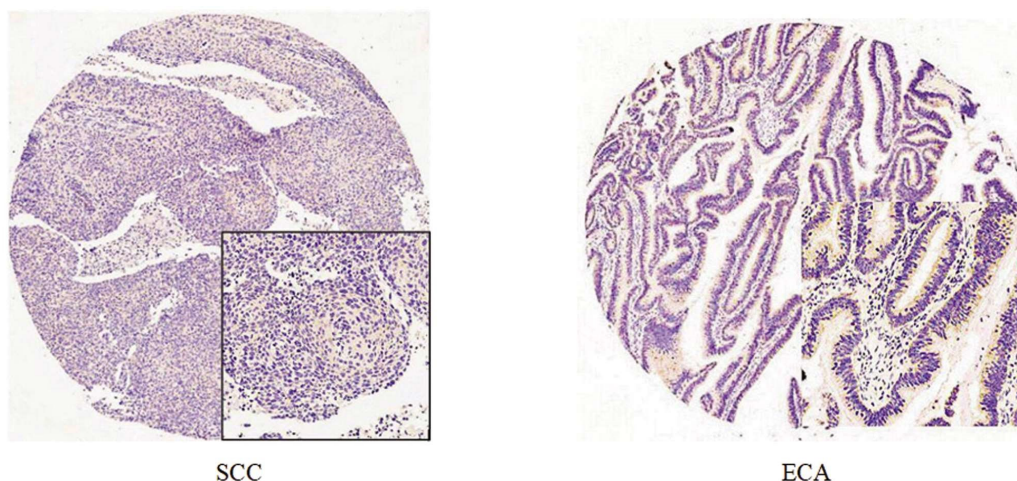


图 4 PD-L1 在宫颈鳞癌、宫颈腺癌中的表达情况(sp× 400)

Fig.4 The expression of PD-L1 in different clinical pathological parameters of cervical cancer detected by immunohistochemical staining(sp× 400)

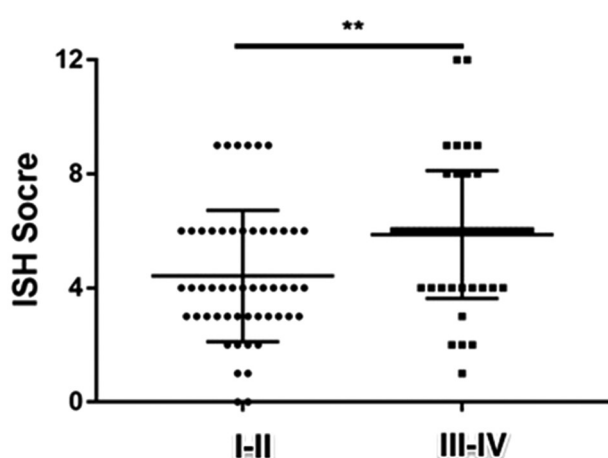


图 5 PD-L1 在 I/II、III/IV 期宫颈癌组织中的 ISH 评分情况

Fig.5 The IRS of PD-L1 in cervical cancer by different clinical stage

** Statistically significant

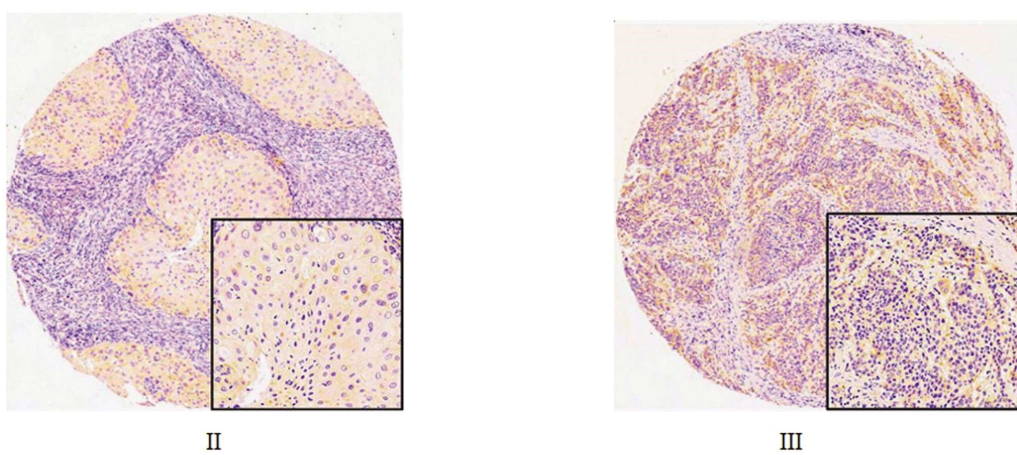


图 6 PD-L1 在不同 FIGO 分期宫颈癌组织中的表达情况(sp× 400)

Fig.6 The expression of PD-L1 in different clinical stages by Immunohistochemical staining in cervical cancer (sp× 400)

与 PD-L1 结合后可能抑制了淋巴细胞的活性,通过免疫抑制参与了肿瘤免疫,也参与了正常宫颈组织癌变的过程。当 PD-1 的胞外区与其配体 PD-L1 的 IgV 结构域结合后,PD-L1 的胞浆区的免疫受体酪氨酸抑制基序(ITLM)ITSM 磷酸化^[20],招募 SIP-2 磷酸酶,使下游的效应分子 PI3K(磷脂酰肌醇 3 激酶)发生去磷酸化,从而传递抑制性信号,抑制淋巴细胞的增殖和活化,最终

酶 C 磷酸化位点的胞内区^[14]。PD-L1 的表达较广泛,在人体的免疫细胞及部分组织细胞均有表达,在一些肿瘤细胞中也有明显表达,主要表达在抗原提呈细胞、活化的 T 淋巴细胞及 B 淋巴细胞,在心肌内皮细胞及胸腺皮质上皮细胞也有表达^[15]。PD-L2 主要表达在活化的树突状细胞、巨噬细胞及少数肿瘤细胞上。PD-L1 在人体内的分布及作用较 PD-L2 广泛^[16,17],其在许多肿瘤组织中高表达,包括 NSCLC、黑色素瘤、乳腺癌、胶质瘤、淋巴瘤、白血病、各种泌尿系统肿瘤、消化道肿瘤、生殖系统肿瘤等^[18,19]。

本研究结果显示正常宫颈组织、CIN 组织与宫颈癌组织中 PD-L1 的阳性表达逐渐上调,提示 PD-L1 的表达与宫颈癌的发生和发展均密切相关。PD-L1 在宫颈癌组织中的阳性表达率高达 74%,远远高于 CIN 组织及正常宫颈组织中的表达。PD-1 在

促进 T 细胞凋亡。同时,PD-1 与 PD-L1 的结合还可抑制炎症细胞因子的释放,起到免疫负调控的作用^[21-24]。此外,本研究结果显示 PD-L1 的阳性表达情况与宫颈癌的 FIGO 分期相关。PD-L1 在 I-II 期宫颈癌中呈弱阳性表达,在 III-IV 期宫颈癌中强阳性表达,提示浸润越深、转移程度越严重的宫颈病变中 PD-L1 的阳性表达越强。有研究表明肾细胞癌患者中 PD-L1 高

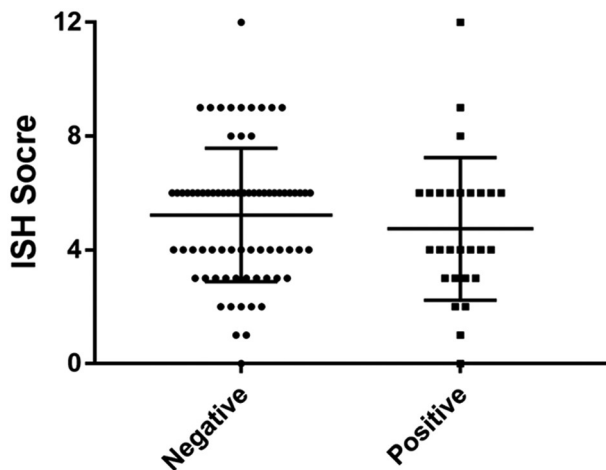


图7 PD-L1 在宫颈癌中不同淋巴结转移情况下的免疫反应积分情况
Fig.7 The IRS of PD-L1 in cervical cancer by different lymphatic metastasis

表达者出现转移和死亡的机率是 PD-L1 阴性者的 4 倍,这说明 PD-L1 在肿瘤的浸润、转移中亦发挥重要作用。术后肿瘤的浸润和转移情况是影响复发率和生存率的重要影响因素。上皮细胞间质化(Epithelial-mesenchymal transition, EMT)是指上皮细胞失去极性获得了移行能力,变成了具有间质细胞形态和功能的细胞^[25],这是癌症浸润转移的重要机制之一。PD-L1 的高表达可抑制上皮性钙粘蛋白的形成,促进 EMT 发生,进而增强肿瘤的侵袭、转移能力。Cao^[26]等在小鼠皮肤肿瘤模型中也证实了这一说法。

目前, FDA 批准用于临床的两类免疫疗法是: (1) PD-1/PD-L1 的抑制剂; (2) 细胞毒性 T 细胞淋巴细胞相关的抑制剂蛋白质 4(CTLA-4)^[27]。Nivolumab 是一种可与 PD-1 高亲和力结合的人源化的抗 PD-1 单克隆抗体,获得 FDA 批准用于晚期黑色素瘤的第一线治疗, 以及用于转移性非小细胞肺癌 (NSCLC), 转移性肾细胞癌(RCC)的二线治疗。Pembrolizumab

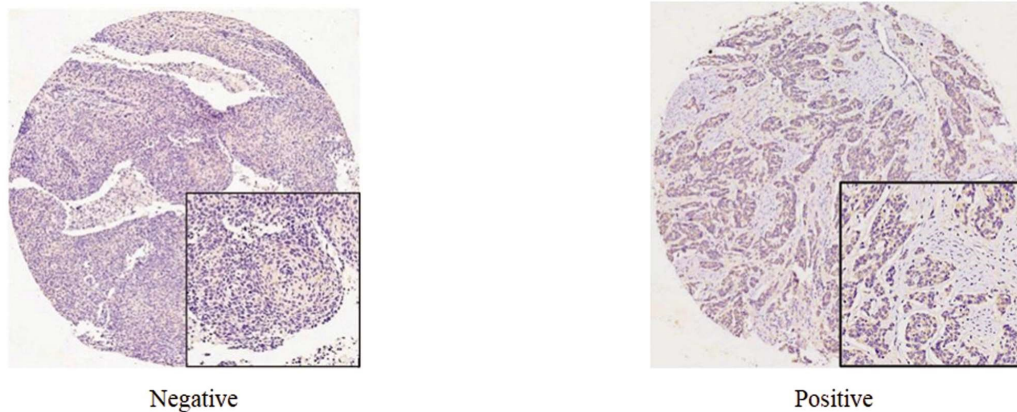


图8 PD-L1 在宫颈癌中不同淋巴结转移情况下的表达(sp× 400)
Fig.8 The expression of PD-L1 in different lymphatic metastasis by Immunohistochemical staining in cervical cancer(sp× 400)

表 3 PD-L1 表达与宫颈癌临床病理特征的关系

Table 3 The relationship between PD-L1 expression level and clinical pathological parameters

Variables	N(=89)	PD-L1		P
		Negative(N=23)	Positive(N=66)	
FIGO Stage				
I, II	50	18	32	0.0021**
III, IV	39	5	34	
Histology type				
SCC	75	19	56	0.6012
ECA	14	4	10	
Age				
<48	67	17	50	0.8911
≥ 48	22	6	16	
Lymph nodes				
Negative	71	18	53	0.8912
Positive	18	5	13	

** Statistically significant

与其作用机制相似,是转移性黑色素瘤、NSCLC^[28]的第一线治疗选择。Atezolizumab 是 FDA 批准的第一种 PD-L1 阻断剂,已被

用于转移性尿路上皮癌、NSCLC 的第一线治疗药物^[29]。其他的 PD-L1 抗体如 BMS-936559, Avelumab, MEDI4736, 目前仍在

I/II 期的临床实验中。Ipilimumab 是 CTLA-4 抗体,可拮抗 CTLA-4 免疫检查点,促进抗肿瘤免疫^[30]。与 Nivolumab 联合应用于晚期黑色素瘤可以延长患者的生存期^[31],但亦可以诱发免疫不良反应,因此仍在人体试验阶段。

近年来,关于阻断 PD-1/PD-L1 通路的免疫疗法发展迅速,也逐渐从实验室走向临床应用,为癌症患者带来曙光和希望。但是目前仍局限应用在黑色素瘤、非小细胞肺癌、肾母细胞瘤、膀胱癌、胃癌等中^[32],在其他恶性肿瘤的作用仍有待研究。本研究结果表明PD-L1 的表达上调可能促进了宫颈癌的发生、发展^[33]。PD-L1 可能成为宫颈癌免疫治疗的新靶点,但这仍需要我们进一步的研究以明确。

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