PD-1/PD-L1轴与骨肉瘤治疗研究进展

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[摘要] 骨肉瘤是儿童和青少年最常见的原发性恶性肿瘤，其发生机制目前尚未明确。随着免疫学相关研究的不断深入，肿瘤免疫逐渐成为肿瘤领域研究的热点。部分研究证实，程序性死亡蛋白1(PD-1)及其配体1(PD-L1)可介导免疫抑制，减弱免疫细胞对肿瘤细胞的杀伤作用，从而导致肿瘤细胞发生免疫逃逸，进而促进肿瘤的发生、发展。该文阐述了PD-1/PD-L1介导骨肉瘤发生免疫逃亡的机制及其在骨肉瘤治疗中的研究进展，旨在为了解PD-1/PD-L1轴在骨肉瘤治疗中的作用及开发治疗骨肉瘤的新药物提供参考。

[关键词] 程序性死亡蛋白1；骨肉瘤；肿瘤免疫

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Research progress of PD-1/PD-L1 axis in pathogenesis and treatment of osteosarcoma

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[**Abstract**] Osteosarcoma is one of the most common primary malignant tumors in children and adolescents, and its development mechanism in human body has not been clearly explained by scholars. With the in-depth study of immunology, tumor immunity has gradually become a hot spot in the field of tumor research. A large number of studies have shown that programmed death protein-1 (PD-1) and its ligand 1 (PD-L1) can mediate immunosuppression, weaken the killing effect of immune cells on tumor cells, and lead to immune escape of tumor cells, thus promoting the development of tumor. This paper reviews the immune escape mechanism of PD-1/PD-L1 in osteosarcoma and the latest progress of PD-1/PD-L1 axis in the treatment of osteosarcoma, aiming to provide theoretical reference for understanding the role of PD-1/PD-L1 in osteosarcoma and developing new drugs for the treatment of osteosarcoma.

[**Key words**] programmed death protein-1; osteosarcoma; tumor immunity

骨肉瘤是最为常见的骨恶性肿瘤，其特点为好发于长骨干骺端，进展迅速且预后不良，在青少年和儿童中病死率很高[1-2]，突出症状为肿瘤部位疼痛，且存在高度转移性，如脑转移、肺转移[3-4]，5年总生存率为71%[5]。尽管有证据表明，骨肉瘤起源于间充质干细胞(mesenchymal stem cells，MSCs)或更具可塑性的成骨细胞前体[6-7]，但具体机制目前尚未明确。近年来多项研究发现，作为抗肿瘤免疫检查点，程序性死亡蛋白1(PD-1)及其配体1(PD-L1)在骨肉瘤中高表达[8-10]，PD-1/PD-L1轴在介导骨肉瘤发生免疫逃逸并导致其进展中发挥着重要作用[11]。本文综述了PD-1/PD-L1轴在骨肉瘤中最新研究进展，以期为认识和治疗骨肉瘤提供新的理论依据。

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1 PD-1/PD-L1轴研究

1.1 主要特点 PD-1又称分化簇279(CD279)，是一个由288个氨基酸构成的膜蛋白，也是一种重要的免疫抑制分子，在活化的T淋巴细胞、B细胞、巨噬细胞和自然杀伤(NK)细胞等免疫细胞中表达。与PD-1结合的配体PD-L1又称表面抗原分化簇274(CD274)或B7同源体(B7-H1)，通常在肿瘤微环境中的肿瘤细胞和肿瘤浸润淋巴细胞(TIL)中表达，并通过PD-1/PD-L1途径调控肿瘤微环境中肿瘤细胞的免疫耐受进程[12-13]。PD-1负调节T细胞受体(TCR)信号，可上调E_3-泛素连接酶CBL-b和c-CBL，触发T细胞受体下调，抑制T细胞的活化及细胞因子的释放，PD-1/PD-L1可广泛地负调节免疫反应。随着肿瘤免疫相关研究的深入，越来越多

的证据表明，实体肿瘤可逃避自身免疫系统的监视，进而促进肿瘤进展，而相关抗肿瘤免疫治疗取得了良好的效果，逐渐引起了研究者的关注[14-17]。作为肿瘤免疫检查位点，PD-1/PD-L1在骨肉瘤治疗中的研究也已深入开展。

1.2 PD-1/PD-L1与骨肉瘤微环境中免疫细胞的变化 肿瘤微环境在肿瘤的发生、发展过程中发挥了重要作用。除肿瘤细胞外，骨肉瘤中还存在免疫细胞浸润，当出现肿瘤时，机体可通过自身的细胞免疫或体液免疫消除肿瘤细胞或抑制其增长，从而起到免疫监视作用。当骨肉瘤细胞表现出较高的免疫原性时，滤泡辅助性T细胞高表达PD-1，低表达抗肿瘤物质白细胞介素-21(IL-21)，从而减弱$CD8^+$ T细胞释放干扰素γ(IFN-γ)及脱颗粒的能力，进而降低T细胞对骨肉瘤细胞的毒性[18]。IFN-γ是由有丝分裂原刺激T淋巴细胞产生的一种高效的抗病毒生物活性物质，在肿瘤免疫中发挥一定作用[19]。研究发现，IFN-γ可诱导血管肉瘤中PD-L1的表达进而产生免疫耐受[20]，Yoshida等[21]在骨肉瘤中也观察到了此现象，并发现抗PD-L1抗体可明显减少$CD4^+$ T细胞中$Foxp3^+$调节性T细胞(regulatory cells，Tregs)的数量，增加$CD8^+$ T细胞浸润[22]，进而发挥抗肿瘤作用。Markel等[23]的研究发现，抗PD-L1抗体能减缓T细胞的耗竭，提示T细胞在PD-L1介导骨肉瘤产生免疫耐受的过程中发挥重要作用。据报道，在MG-63型骨肉瘤细胞系中PD-L1呈高表达，且该细胞系能耐受NK细胞的杀伤作用，当阻断PD-1/PD-L1时，NK细胞的细胞毒性增强，并通过颗粒酶B(GZMB)发挥对骨肉瘤细胞的杀伤作用[24]。Yoshida等[25]研究19例骨肉瘤临床标本发现，IFN-γ和GZMB的表达与PD-L1呈明显正相关。骨肉瘤原发部位存在较多的$CD4^+$ T细胞和$CD8^+$ T细胞浸润，相应地PD-L1表达也明显增强，且IFN-γ刺激可增加骨肉瘤细胞系中PD-L1的表达[26]，提示骨肉瘤可适应免疫抗性，产生PD-L1，进而逃避免疫细胞的杀伤作用。综上，PD-1/PD-L1可通过弱化$CD4^+$ T细胞、$CD8^+$ T细胞、NK细胞的功能而导致骨肉瘤细胞的免疫逃逸，因此，上述免疫细胞可作为拮抗PD-1/PD-L1对骨肉瘤作用的靶向免疫细胞，从而为骨肉瘤的治疗提供策略。

1.3 PD-1/PD-L1经信号通路介导骨肉瘤免疫逃逸 肿瘤发生发展涉及的相关信号转导机制备受研究者关注，深入研究和理解骨肉瘤相关通路对认识骨肉瘤以及探寻新的干预手段有一定指导意义。JAK/STAT信号通路是参与肿瘤发生的常见信号通路，信号转导子和转录激活子3(STAT3)是其重要组成部分，阻断STAT3可增加肿瘤组织中细胞因子和趋化因子的表达，激活先天性免疫激活的树突细胞，引起肿瘤T细胞反应[27-28]，从而介导机体免疫反应。研究发现，STAT3可调节PD-L1的表达[29]。Dhupkar等[30]在人体原发和转移的骨肉瘤中均观察到PD-L1的表达，并发现抗PD-L1抗体能阻滞p-STAT-3/p-ERK1/2信号传导，激活M1巨噬细胞，减少M2巨噬细胞的数量，导致骨肉瘤肺转移灶的数量显著减少。作为抗肿瘤靶向药物，舒尼替尼(sunitinib)和阿帕替尼(apatinib)可通过特异性阻断相关信号转导途径发挥抗肿瘤效应，表明舒尼替尼和阿帕替尼可使STAT3失活，从而降低骨肉瘤细胞中PD-L1的表达，减弱体内骨肉瘤的肺转移潜力[22,31]。有研究发现，组蛋白脱乙酰基酶6(HDAC6)能促进骨肉瘤细胞中PD-L1的表达，其调节PD-L1的机制主要由STAT3介导，而选择性HDAC6抑制剂能明显抑制骨肉瘤的生长[32]。由此可见，PD-L1经STAT3转导通路在致骨肉瘤免疫逃逸中扮演着重要角色，选择性阻断该信号通路可能在骨肉瘤治疗中发挥更有效的作用。

磷脂酰肌醇3激酶(PI3K)在维持细胞的生长、代谢中具有重要作用。研究发现，PI3K/AKT信号传导途径参与了骨肉瘤的发展，可调节骨肉瘤细胞的增殖、细胞周期、凋亡、迁移、侵袭和化疗耐药性[33]。既往有研究报道，PD-L1由PI3K/AKT信号途径传导[34]，使用p110γ特异性抑制剂能抑制髓样细胞肿瘤浸润，使肿瘤对PD-1阻断治疗更敏感[35]，因此PD-1/PD-L1经PI3K信号通路可能参与了骨肉瘤的发展。磷酸酶和张力同系物(PTEN)基因位于人类10号染色体，编码含有403个氨基酸的蛋白质，作为PI3K/AKT信号通路负调节剂，PTEN蛋白能抑制PD-L1在细胞中的表达[36]，与骨肉瘤的侵袭性密切相关[37]。最近Wu等[38]研究发现，FYVE、RhoGEF和含PH结构域的蛋白1(FGD1)可结合PTEN的N端区域，从而抑制PTEN的功能，进而促进PI3K/AKT活化、PD-L1上调以及骨肉瘤的进展。此外，骨肉瘤中髓源性抑制细胞(MDSCs)高表达，可重塑肿瘤微环境以促进肿瘤的发展，而PI3Kδ/γ在MDSCs中高表达，应用PI3Kδ/γ抑制剂能诱导骨肉瘤细胞$CD8^+$ T细胞浸润，上调PD-L1的表达[39-40]。

1.4 MicroRNA可经PD-L1致骨肉瘤进展 MicroRNA(miRNA)是一类由内源基因编码的长度约为22个核苷酸的非编码单链RNA，参与转录后基因的表达调控，且与PD-L1的表达密切相关[41]。Ji等[42]发现，miR-140在骨肉瘤中低表达，可与下游靶基因mRNA的3'UTR结合，直接下调PD-L1的表达，增加细胞毒性$CD8^+$ T细胞浸润，降低Tregs的表达，进而抑制骨肉瘤细胞的生长，而这种作用

可能是通过抑制mTOR/S6Ks信号通路实现的。最近Liu等[43]发现，microRNA-200a参与了骨肉瘤的进展，其在骨肉瘤中高表达，与骨肉瘤预后不良明显相关，且在体内、体外均可通过PTEN上调PD-L1蛋白水平，减少$CD8^+$ T细胞、$CD4^+$ T细胞数量，增加$Foxp3^+$ Tregs比例，抑制细胞毒性T淋巴细胞(CTL)分泌IFN-γ，从而促进骨肉瘤的生长。据报道，miR-106a与长链非编码RNA(LINC00657)相互作用，可上调PD-L1的表达，进而导致骨肉瘤的侵袭[44]。由此可见，miRNA可经PD-L1调节骨肉瘤的发展，因此基于敲除相关miRNA及阻断PD-L1或许能加强干预骨肉瘤的效果，但仍需进一步研究加以证实。图1为PD-1/PD-L1轴介导骨肉瘤细胞免疫逃逸的机制。

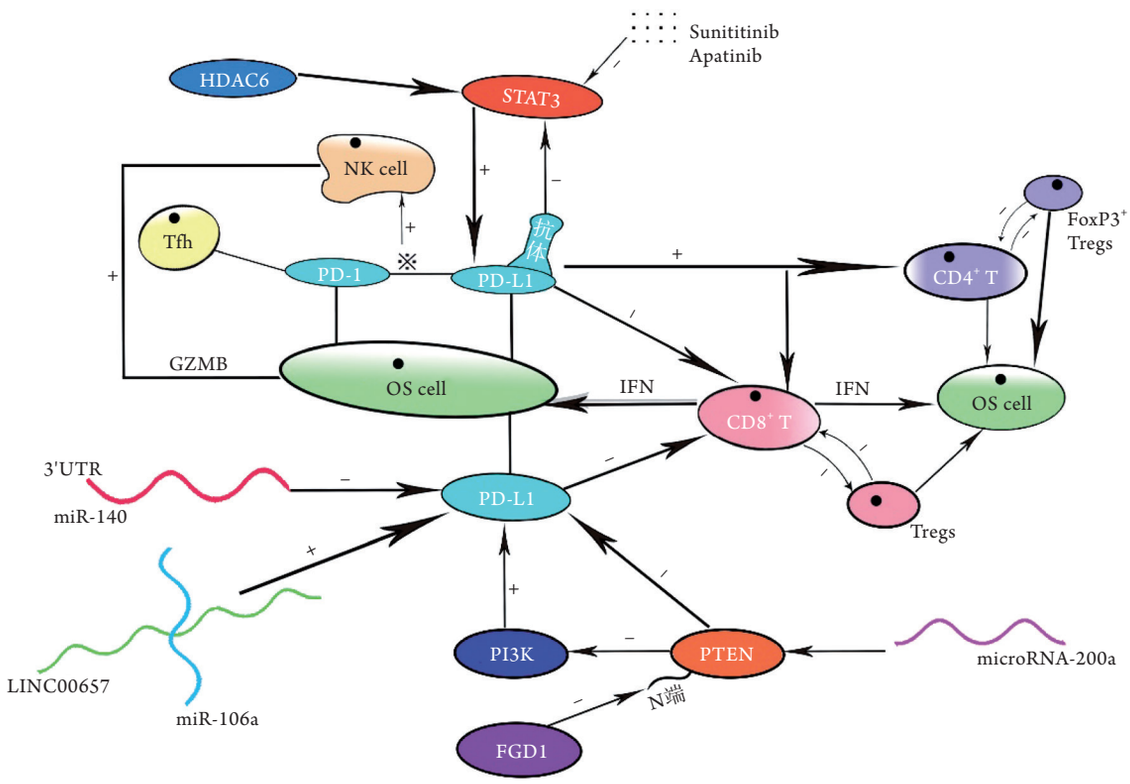

图1 PD-1/PD-L1轴介导骨肉瘤细胞免疫逃逸的机制

Fig.1 Mechanism of immune escape mediated by PD-1/PD-L1 axis in osteosarcoma

HDAC6. 组蛋白脱乙酰基酶6；STAT3. 信号转导子和转录激活子3；Sunitinib. 舒尼替尼；Apatinib. 阿帕替尼；Tfh. 滤泡辅助性T细胞；PD-1. 程序性死亡蛋白1；PD-L1. 程序性死亡受体配体1；GZMB. 颗粒酶B；OS cell. 骨肉瘤细胞；IFN. 干扰素；Tregs. 调节性T细胞；PTEN. 磷酸酶和张力同系物；PI3K. 磷脂酰肌醇3激酶；FGD1. FYVE、RhoGEF和含PH结构域的蛋白1；+. 正反馈、促进；—. 负反馈、抑制；※. 阻断

2 骨肉瘤治疗研究

2.1 动物实验 手术切除后再进行局部放疗和全身化疗是目前肿瘤治疗的标准方法，但仍存在全身性进展或远距离转移以及对化疗耐药等现象，进而导致肿瘤预后不良，因此寻找新的治疗措施尤为重要。两项Meta分析显示，PD-1/PD-L1在骨肉瘤中高表达，与骨肉瘤的转移密切相关，常提示预后较差[45-46]，因此阻断PD-1/PD-L1可能为抑制骨肉瘤进展的有效措施。研究发现，将抗PD-1抗体用于LM8系骨肉瘤大鼠，骨肉瘤生长不仅得到了较好的控制，且明显延长了大鼠的生存时间[(35.2 ± 3.7) d *vs.* (25.4 ± 1.6) d，P=0.047][21]。Zheng等[47]研究发现，PD-1、PD-L1在骨肉瘤组织中阳性表达率为27.4%、35.5%；骨肉瘤大鼠腹腔注射10 mg/(kg·5 d)基于抗PD-1抗体的纳武单抗(nivolumab)，可明显抑制骨肉瘤的转移潜能。Shimizu等[48]将200 μg抗PD-1/PD-L1抗体注射于实验大鼠腹腔中，发现其可明显延长了大鼠的生存时间(P=0.002)，50%的大鼠在肿瘤移植后存活超过16周，并无肺部转移。由此可见，抗PD-1/PD-L1抗体能有效治疗骨肉瘤并抑制其进展。

基于当前对肿瘤的早期手术以及放化疗治疗方式，肿瘤治疗效果仍然不理想，研究人员尝试常规治疗手段结合PD-1/PD-L1靶向治疗，并取得了一定成果。Shimizu等[49]将早期减瘤手术与抗PD-L1抗体联合应用发现，该治疗方式能明显延长实验大鼠的生存期，且约50%的实验大鼠存活超过16周

(P=0.001)。也有研究证实，放疗(辐射剂量5 Gy和8 Gy)尽管在早期能抑制骨肉瘤的生长，但后期却出现肿瘤逆生长，分析原因为放疗反应性增加了骨肉瘤PD-L1的表达，而放疗联合抗PD-1抗体可明显改善实验大鼠外周血$CD4^+$ T细胞、$CD8^+$ T细胞比例，减少MDSCs，增强抗肿瘤效应，降低脑转移风险[50-51]。顺铂、阿霉素为恶性肿瘤一线用药，研究发现，阿霉素联合抗PD-L1抗体治疗实验大鼠，能逆转骨肉瘤免疫抑制微环境状态，使$CD4^+$ T细胞、$CD8^+$ T细胞比例增加，并降低Tregs细胞比例，进而产生更好的抗肿瘤效果[52]。此外，联合应用抗PD-L1抗体亦可增强顺铂的抗肿瘤效果(表1)[53]。因此，在手术、放化疗的基础上联合应用PD-1/PD-L1靶向药为延缓骨肉瘤进展提供了新策略。

2.2　临床试验　目前基于PD-1/PD-L1轴的临床试验逐渐开展，纳入临床研究的药物有基于抗PD-1/PD-L1抗体的哌姆单抗(pembrolizumab)和纳武单抗。一项两队列、单臂、开放性2期临床研究共纳入86例晚期肉瘤患者，其中40例为骨肉瘤患者，每3周静脉输注200 mg哌姆单抗(输注时间30 min)进行治疗，中位随访时间17.8个月，发现2例(5%)部分缓解(partial response，PR)，9例(23%)疾病稳定(stable disease，SD)，29例(73%)疾病进展(progressive disease，PD)，中位总生存期(median survival time，MST)为52周[54]，由此可见，骨肉瘤患者对治疗的不良反应很少。Le Cesne等[55]的一项开放性、多中心、2期临床研究中，晚期骨肉瘤患者采用口服环磷酰胺(50 mg/次，2次/d)和静脉注射200 mg哌姆单抗(每3周1次)方式治疗，中位随访时间18.9个月，发现2例6个月内无进展(progression-free survival，FPS)，无进展率为13.3%(95%CI 1.7~40.5)，1例部分缓解(PR)，且骨肉瘤PD-L1表达呈阴性，4例肿瘤体积缩小，表明哌姆单抗联合低剂量环磷酰胺无不良反应，对晚期骨肉瘤的治疗显示出中等活性，因此有必要进一步研究PD-1抑制剂与药物联合使用对骨肉瘤的影响。另一项多中心、开放标签、单臂1–2期针对儿童实体肿瘤的临床试验中，儿童每14 d服用3 mg/kg纳武单抗治疗，中位随访时间30 d，发现患儿对纳武单抗治疗耐受性良好，但在扩大的2期队列中并未观察到骨肉瘤对单药纳武单抗治疗的客观反应(表1)[56]。

表1　基于PD-1/PD-L1轴治疗骨肉瘤的研究

Tab.1　Researches on the treatment of osteosarcoma based on PD-1/PD-L1 axis

研究对象	治疗方式	治疗效果	参考文献
LM8系骨肉瘤大鼠	抗PD-1抗体	生存时间(35.2 ± 3.7) d (P=0.047)	[21]
大鼠	纳武单抗[10 mg/(kg·5 d)]	抑制肿瘤转移	[46]
大鼠	抗PD-1抗体+抗PD-L1抗体(200 μg)	生存时间>16周(P=0.002)，无肺转移	[47]
大鼠	减瘤手术+抗PD-L1抗体	生存时间>16周(P=0.001)	[48]
大鼠	放疗+抗PD-1抗体	减少脑转移	[49]、[50]
大鼠	抗PD-L1抗体+顺铂 抗PD-L1抗体+阿霉素	抗骨肉瘤效应增强	[51]、[52]
40例骨肉瘤患者	哌姆单抗(200 mg/3周)	MST 52周，PR(2例，5%)，SD(9例，23%)，PD(29例，73%)	[53]
15例晚期骨肉瘤患者	哌姆单抗(1次/3周)+环磷酰胺(50 mg/次，2次/d)	FPS(2例，13.3%)，PR(1例，6.7%)，肿瘤体积缩小(4例，26.7%)	[54]
儿童骨肉瘤	纳武单抗[3 mg/(kg·2周)]	无反应	[55]

MST. 中位生存期；PR. 部分缓解；SD. 疾病稳定；PD. 疾病进展；FPS. 无进展生存期

3　总结与展望

PD-1/PD-L1轴已经在多数研究中被证实可介导肿瘤免疫逃逸，与骨肉瘤的进展密切相关，基于PD-1/PD-L1轴治疗骨肉瘤的动物实验也取得了良好的效果，为研究和治疗骨肉瘤提供了新的思路与策略。基于PD-1/PD-L1轴开发的药物可作为治疗骨肉瘤的新兴药物，然而目前的临床试验研究发现，虽然PD-1/PD-L1轴阻断药治疗骨肉瘤效果较好，但由于骨肉瘤存在肿瘤异质性，加之该类药物昂贵，限制了纳入临床研究的样本量，因此扩大招募研究对象，延展基础研究与临床试验对深入认识PD-1/PD-L1靶向药治疗骨肉瘤尤其重要。另外，联合应用放化疗与PD-1/PD-L1轴阻断药可能是应对骨肉瘤恶化的有效手段，但仍需进一步的试验验证。

【参考文献】

[1]　Brown HK, Tellez-Gabriel M, Heymann D. Cancer stem cells in osteosarcoma[J]. Cancer Lett, 2017, 386: 189-195.

[2]　Isakoff MS, Bielack SS, Meltzer P, *et al*. Osteosarcoma: current treatment and a collaborative pathway to success[J]. J Clin Oncol, 2015, 33(27): 3029-3035.

[3]　Gill J, Ahluwalia MK, Geller D, *et al*. New targets and approaches in osteosarcoma[J]. Pharmacol Ther, 2013, 137(1): 89-99.

[4]　Ahmad O, Chan M, Savage P, *et al*. Biology and treatment of

metastasis of sarcoma to the brain[J]. Front Biosci (Elite Ed), 2016, 8: 233-244.

[5] Smeland S, Bielack SS, Whelan J, *et al*. Survival and prognosis with osteosarcoma: outcomes in more than 2000 patients in the EURAMOS-1 (European and American Osteosarcoma Study) cohort[J]. Eur J Cancer, 2019, 109: 36-50.

[6] Gaebler M, Silvestri A, Haybaeck J, *et al*. Three-dimensional patient-derived *in vitro* sarcoma models: promising tools for improving clinical tumor management[J]. Front Oncol, 2017, 7: 203.

[7] Mutsaers AJ, Walkley CR. Cells of origin in osteosarcoma: mesenchymal stem cells or osteoblast committed cells?[J]. Bone, 2014, 62: 56-63.

[8] Torabi A, Amaya CN, Wians FH Jr, *et al*. PD-1 and PD-L1 expression in bone and soft tissue sarcomas[J]. Pathology, 2017, 49(5): 506-513.

[9] Sundara YT, Kostine M, Cleven AH, *et al*. Increased PD-L1 and T-cell infiltration in the presence of HLA class I expression in metastatic high-grade osteosarcoma: a rationale for T-cell-based immunotherapy[J]. Cancer Immunol Immunother, 2017, 66(1): 119-128.

[10] Koirala P, Roth ME, Gill J, *et al*. Immune infiltration and PD-L1 expression in the tumor microenvironment are prognostic in osteosarcoma[J]. Sci Rep, 2016, 6: 30093.

[11] Miwa S, Shirai T, Yamamoto N, *et al*. Current and emerging targets in immunotherapy for osteosarcoma[J]. J Oncol, 2019, 2019: 7035045.

[12] LaFleur MW, Muroyama Y, Drake CG, *et al*. Inhibitors of the PD-1 pathway in tumor therapy[J]. J Immunol, 2018, 200(2): 375-383.

[13] Wang SD, Li HY, Li BH, *et al*. The role of CTLA-4 and PD-1 in anti-tumor immune response and their potential efficacy against osteosarcoma[J]. Int Immunopharmacol, 2016, 38: 81-89.

[14] Wolchok JD, Chiarion-Sileni V, Gonzalez R, *et al*. Overall survival with combined nivolumab and ipilimumab in advanced melanoma[J]. N Engl J Med, 2017, 377(14): 1345-1356.

[15] Herbst RS, Baas P, Kim DW, *et al*. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial[J]. Lancet, 2016, 387(10027): 1540-1550.

[16] Motzer RJ, Tannir NM, McDermott DF, *et al*. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma[J]. N Engl J Med, 2018, 378(14): 1277-1290.

[17] Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade[J]. Science, 2018, 359(6382): 1350-1355.

[18] Gao W, Zhou J, Ji B. Evidence of interleukin 21 reduction in osteosarcoma patients due to PD-1/PD-L1-mediated suppression of follicular helper T cell functionality[J]. DNA Cell Biol, 2017, 36(9): 794-800.

[19] Castro F, Cardoso AP, Goncalves RM, *et al*. Interferon-gamma at the crossroads of tumor immune surveillance or evasion[J]. Front Immunol, 2018, 9: 847.

[20] Honda Y, Otsuka A, Ono S, *et al*. Infiltration of PD-1-positive cells in combination with tumor site PD-L1 expression is a positive prognostic factor in cutaneous angiosarcoma[J]. Oncoimmunology, 2017, 6(1): e1253657.

[21] Yoshida K, Okamoto M, Sasaki J, *et al*. Anti-PD-1 antibody decreases tumour-infiltrating regulatory T cells[J]. BMC Cancer, 2020, 20(1): 25.

[22] Duan XL, Guo JP, Li F, *et al*. Sunitinib inhibits PD-L1 expression in osteosarcoma by targeting STAT3 and remodels the immune system in tumor-bearing mice[J]. Future Oncol, 2020, 16(24): 1815-1824.

[23] Markel JE, Noore J, Emery EJ, *et al*. Using the spleen as an *in vivo* systemic immune barometer alongside osteosarcoma disease progression and immunotherapy with alpha-PD-L1[J]. Sarcoma, 2018, 2018: 8694397.

[24] Zhang ML, Chen L, Li YJ, *et al*. PDL1/PD1 axis serves an important role in natural killer cell induced cytotoxicity in osteosarcoma[J]. Oncol Rep, 2019, 42(5): 2049-2056.

[25] Yoshida K, Okamoto M, Sasaki J, *et al*. Clinical outcome of osteosarcoma and its correlation with programmed death-ligand 1 and T cell activation markers[J]. Onco Targets Ther, 2019, 12: 2513-2518.

[26] Toda Y, Kohashi K, Yamada Y, *et al*. PD-L1 and IDO1 expression and tumor-infiltrating lymphocytes in osteosarcoma patients: comparative study of primary and metastatic lesions[J]. J Cancer Res Clin Oncol, 2020, 146(10): 2607-2620.

[27] Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment[J]. Nat Rev Immunol, 2007, 7(1): 41-51.

[28] Germain D, Frank DA. Targeting the cytoplasmic and nuclear functions of signal transducers and activators of transcription 3 for cancer therapy[J]. Clin Cancer Res, 2007, 13(19): 5665-5669.

[29] Seki N, Kan OK, Matsumoto K, *et al*. Interleukin-22 attenuates double-stranded RNA-induced upregulation of PD-L1 in airway epithelial cells *via* a STAT3-dependent mechanism[J]. Biochem Biophys Res Commun, 2017, 494(1-2): 242-248.

[30] Dhupkar P, Gordon N, Stewart J, *et al*. Anti-PD-1 therapy redirects macrophages from an M2 to an M1 phenotype inducing regression of OS lung metastases[J]. Cancer Med, 2018, 7(6): 2654-2664.

[31] Zheng B, Ren T, Huang Y, *et al*. Apatinib inhibits migration and invasion as well as PD-L1 expression in osteosarcoma by targeting STAT3[J]. Biochem Biophys Res Commun, 2018, 495(2): 1695-1701.

[32] Keremu A, Aimaiti A, Liang Z, *et al*. Role of the HDAC6/STAT3 pathway in regulating PD-L1 expression in osteosarcoma cell lines[J]. Cancer Chemother Pharmacol, 2019, 83(2): 255-264.

[33] Zhang J, Yu XH, Yan YG, *et al*. PI3K/Akt signaling in osteosarcoma[J]. Clin Chim Acta, 2015, 444: 182-192.

[34] Lastwika KJ, Wilson W 3rd, Li QK, *et al*. Control of PD-L1 expression by oncogenic activation of the AKT-mTOR pathway in non-small cell lung cancer[J]. Cancer Res, 2016, 76(2): 227-238.

[35] De Henau O, Rausch M, Winkler D, *et al*. Overcoming resistance to checkpoint blockade therapy by targeting PI3Kgamma in myeloid cells[J]. Nature, 2016, 539(7629): 443-447.

[36] Parsa AT, Waldron JS, Panner A, *et al*. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma[J]. Nat Med, 2007, 13(1): 84-88.

[37] Xi Y, Chen Y. PTEN plays dual roles as a tumor suppressor in osteosarcoma cells[J]. J Cell Biochem, 2017, 118(9): 2684-2692.

[38] Wu W, Jing D, Meng Z, *et al*. FGD1 promotes tumor progression

and regulates tumor immune response in osteosarcoma *via* inhibiting PTEN activity[J]. Theranostics, 2020, 10(6): 2859-2871.

[39] Kumar V, Patel S, Tcyganov E, *et al*. The nature of myeloid-derived suppressor cells in the tumor microenvironment[J]. Trends Immunol, 2016, 37(3): 208-220.

[40] Shi X, Li X, Wang H, *et al*. Specific inhibition of PI3Kdelta/gamma enhances the efficacy of anti-PD1 against osteosarcoma cancer[J]. J Bone Oncol, 2019, 16: 100206.

[41] Li WN, He F. Research progress on the role of miR-140 expression in the development of tumor[J]. Med J Chin PLA, 2018, 43(6): 523-529. [李维娜, 何飞. miR-140表达与肿瘤发生发展关系的研究进展[J]. 解放军医学杂志, 2018, 43(6): 523-529.]

[42] Ji X, Wang E, Tian F. MicroRNA-140 suppresses osteosarcoma tumor growth by enhancing anti-tumor immune response and blocking mTOR signaling[J]. Biochem Biophys Res Commun, 2018, 495(1): 1342-1348.

[43] Liu Z, Wen J, Wu C, *et al*. MicroRNA-200a induces immunosuppression by promoting PTEN-mediated PD-L1 upregulation in osteosarcoma[J]. Aging (Albany NY), 2020, 12(2): 1213-1236.

[44] Zhang J, Chou X, Zhuang M, *et al*. LINC00657 activates PD-L1 to promote osteosarcoma metastasis *via* miR-106a[J]. J Cell Biochem, 2020, 121(10): 4188-4195.

[45] Huang X, Zhang W, Zhang Z, *et al*. Prognostic value of programmed cell death 1 ligand-1 (PD-L1) or PD-1 expression in patients with osteosarcoma: a meta-analysis[J]. J Cancer, 2018, 9(14): 2525-2531.

[46] Zhu Z, Jin Z, Zhang M, *et al*. Prognostic value of programmed death-ligand 1 in sarcoma: a meta-analysis[J]. Oncotarget, 2017, 8(35): 59570-59580.

[47] Zheng B, Ren T, Huang Y, *et al*. PD-1 axis expression in musculoskeletal tumors and antitumor effect of nivolumab in osteosarcoma model of humanized mouse[J]. J Hematol Oncol, 2018, 11(1): 16.

[48] Shimizu T, Fuchimoto Y, Fukuda K, *et al*. The effect of immune checkpoint inhibitors on lung metastases of osteosarcoma[J]. J Pediatr Surg, 2017, 52(12): 2047-2050.

[49] Shimizu T, Fuchimoto Y, Okita H, *et al*. A curative treatment strategy using tumor debulking surgery combined with immune checkpoint inhibitors for advanced pediatric solid tumors: An *in vivo* study using a murine model of osteosarcoma[J]. J Pediatr Surg, 2018, 53(12): 2460-2464.

[50] Takahashi Y, Yasui T, Minami K, *et al*. Carbon ion irradiation enhances the antitumor efficacy of dual immune checkpoint blockade therapy both for local and distant sites in murine osteosarcoma[J]. Oncotarget, 2019, 10(6): 633-646.

[51] Xia L, Wu H, Qian W. Irradiation enhanced the effects of PD-1 blockade in brain metastatic osteosarcoma[J]. J Bone Oncol, 2018, 12: 61-64.

[52] Wang J, Hu C, Wang J, *et al*. Checkpoint blockade in combination with doxorubicin augments tumor cell apoptosis in osteosarcoma[J]. J Immunother, 2019, 42(9): 321-330.

[53] Liu X, He S, Wu H, *et al*. Blocking the PD-1/PD-L1 axis enhanced cisplatin chemotherapy in osteosarcoma *in vitro* and *in vivo*[J]. Environ Health Prev Med, 2019, 24(1): 79.

[54] Tawbi HA, Burgess M, Bolejack V, *et al*. Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial[J]. Lancet Oncol, 2017, 18(11): 1493-1501.

[55] Le Cesne A, Marec-Berard P, Blay JY, *et al*. Programmed cell death 1 (PD-1) targeting in patients with advanced osteosarcomas: results from the PEMBROSARC study[J]. Eur J Cancer, 2019, 119: 151-157.

[56] Davis KL, Fox E, Merchant MS, *et al*. Nivolumab in children and young adults with relapsed or refractory solid tumours or lymphoma (ADVL1412): a multicentre, open-label, single-arm, phase 1-2 trial[J]. Lancet Oncol, 2020, 21(4): 541-550.

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