Tumor-Intrinsinc PD-L1 Signaling in Cancer Initiation, Development and Treatment: Beyond Immune Evasion

Peixin Dong1*, Ying Xiong2†, Junming Yue3,4, Sharon J. B. Hanley1 and Hidemichi Watari1†

1 Department of Obstetrics and Gynecology, Hokkaido University School of Medicine, Hokkaido University, Sapporo, Japan, 2 Department of Gynecology, State Key Laboratory of Oncology in South China, Sun Yat-Sen University Cancer Center, Guangzhou, China, 3 Department of Pathology and Laboratory Medicine, University of Tennessee Health Science Center, Memphis, TN, United States, 4 Center for Cancer Research, University of Tennessee Health Science Center, Memphis, TN, United States

Although the role of PD-L1 in suppressing the anti-tumor immune response is extensively documented, recent discoveries indicate a distinct tumor-intrinsic role for PD-L1 in modulating epithelial-to-mesenchymal transition (EMT), cancer stem cell (CSC)-like phenotype, metastasis and resistance to therapy. In this review, we will focus on the newly discovered functions of PD-L1 in the regulation of cancer development, describe underlying molecular mechanisms responsible for PD-L1 upregulation and discuss current insights into novel components of PD-L1 signaling. Furthermore, we summarize our current understanding of the link between PD-L1 signaling and the EMT program as well as the CSC state. Tumor cell-intrinsic PD-L1 clearly contributes to cancer stemness, EMT, tumor invasion and chemoresistance in multiple tumor types. Conversely, activation of OCT4 signaling and upregulation of EMT inducer ZEB1 induce PD-L1 expression in cancer cells, thereby suggesting a possible immune evasion mechanism employed by cancer stem cells during metastasis. Our meta-analysis demonstrated that PD-L1 is co-amplified along with MYC, SOX2, N-cadherin and SNAI1 in the TCGA endometrial and ovarian cancer datasets. Further identification of immune-independent PD-L1 functions and characterization of crucial signaling events upstream or downstream of PD-L1 in diverse cancer types and specific cancer subtypes, would provide additional targets and new therapeutic approaches.

Keywords: PD-L1, CD274, metastasis, EMT, cancer stem cells, microRNA

INTRODUCTION

In cancer, the epithelial-to-mesenchymal transition (EMT) is a phenotypic process that promotes the acquisition of a mesenchymal features of epithelial tumor cells, reduces cell polarity and cell-cell adhesion, and enables them to migrate and invade more efficiently, by switching off the expression of epithelial markers, such as E-cadherin, and turning on mesenchymal markers, including N-cadherin and Vimentin (1, 2). Epithelial tumor cells undergoing EMT are shown to contribute to tumorigenesis, invasion, metastasis, and resistance to chemotherapy, radiation and small-molecule-targeted therapy (3).

Cancer stem cells (CSCs) represent a fraction of undifferentiated cancer cells that are the seeds of tumor recurrence, have the ability to self-renew and exhibit significant resistance to conventional
chemo- and radiotherapy (4). Emerging evidence has revealed an association between EMT and the acquisition of CSC-like properties (5). The induction of EMT program is a critical regulator of the CSC phenotype (6, 7). On the other hand, tumors cells that exhibit the CSC phenotype also express genes associated with the EMT features and show enhanced metastatic ability, thus representing a novel mechanism contributing to cancer metastasis (8).

The mutual interactions between tumor cells and the tumor microenvironment are essential for tumorigenesis, tumor progression, metastasis and resistance to drug therapy (9). Tumor microenvironment consists of extracellular matrix and diverse cell populations such as T cells, NK cells, macrophages, dendritic cells, fibroblasts, and endothelial cells (10). Progression of cancer to an advanced or metastatic disease usually suggests a failure or insufficiency of the ongoing immune response. Tumors not only effectively escape immune recognition, they also actively inhibit T-cell-mediated normal anti-tumor activity to promote further tumor growth and metastasis by modulating immune checkpoints, which mediate immune tolerance and inhibit the anti-tumor immune response (11). Multiple checkpoint molecules, such as PD-1/PD-L1, CTLA4, BTLA, B7H3, B7H4, HH LA2, IDO1, Tim-3, CD28, CD40, CD47, CD70, CD137, VISTA, LAG-3, and TIGIT, have been reported (11). Among them, B7H3 has been identified as a critical promoter of tumor cell proliferation, migration, invasion, EMT, cancer stemness, and drug resistance (12).

PD-L1 (also known as CD274 or B7H1) is expressed in tumor cells and plays a crucial role in tumor immune escape and the formation of a permissive immune microenvironment, through at least three mechanisms: (i) tolerizing or anergizing tumor-reactive T cells by binding to its receptor PD-1; (ii) rendering tumor cells resistant to CD8+ T cell and Fas ligand-mediated lysis; and (iii) tolerizing T cells by reverse signaling through T cell-expressed CD80 (13, 14). In addition, PD-L1 is also expressed by tumor-associated myeloid-derived suppressor cells and macrophages, which are the major factors responsible for tumor-associated immune deficiencies (15).

Although PD-L1 is widely implicated in tumor immune evasion, the tumor-intrinsic roles of PD-L1 and the mechanisms by which PD-L1 regulates EMT, the acquisition of tumor-initiating potential and resistance to anti-tumor drugs, as well as the ability to disseminate and metastasize in human cancers are currently less well defined. As will be discussed in more detail below, the identification of tumor-intrinsic PD-L1 signaling may provide critical targets for the development of cancer therapies.

**PD-L1 Dysregulation and Prognosis in Cancer**

An increasing number of studies suggested that PD-L1 is highly expressed in solid tumors, including colorectal cancer (16), lung cancer (17), pancreatic carcinoma (18), hepatocellular carcinoma (19), gastric cancer (20), ovarian cancer (21), endometrial cancer (22, 23), and cervical cancer (24, 25). High expression of PD-L1 was associated with significantly worse overall survival in cervical cancer (25), non-small cell lung cancer (26), gastric cancer (27), esophageal cancer (28), glioma (29), ovarian cancer (30), and other cancers (31). However, the prognostic value of PD-L1 for certain types of cancer is still controversial. Some studies reported that high PD-L1 could predict favorable prognosis (32, 33). In cervical cancer, squamous cell carcinomas tended to express more PD-L1 than adenocarcinomas (34). The possible reasons for these inconsistent results might include cancer type (or subtypes), tumor heterogeneity, sample size, clinical stage, different intervention, the time point of PD-L1 measurement as well as the different methodology used in research (such as detection methods and procedures).

**MECHANISMS OF PD-L1 ACTIVATION IN CANCER**

The tumor-intrinsic PD-L1 signaling pathway is inappropriately activated in many cancers. Mechanisms underlying aberrant PD-L1 activation mainly include genomic alterations (including copy number amplification and 3′-UTR disruption), constitutive oncogenic signaling activation, extrinsic factors and epigenetic mechanisms, such as upregulation of oncogenic microRNAs (miRNAs), downregulation of tumor suppressor miRNAs, aberrant DNA methylation, and histone modifications (Figure 1).

**Copy Number Gain and 3′-UTR Disruption**

Small-cell lung cancer (35), squamous cell carcinoma of the oral cavity (36), cervical cancer (37), ovarian cancer (38), breast cancer (39), melanoma, bladder cancer, head and neck cancer, soft tissue sarcoma and prostate cancer (40) exhibit increased copy number of chromosome 9p24, on which CD274 resides. Here, we investigated the frequency of elevated PD-L1 in ovarian cancer and endometrial cancer in The Cancer Genome Atlas (TCGA) data portal. Analysis of TCGA data by cBioPortal (41) demonstrated that overall, PD-L1 was highly expressed in these two cancers, mainly including gene amplification and mRNA up-regulation (Figure 2A). Moreover, analyses of U133A and U133Plus2 datasets in the GENT (gene expression across normal and tumor tissue) database database (42) revealed that PD-L1 was highly overexpressed in many tumor tissues (Figure 2B). Furthermore, analysis of the TCGA dataset was performed by using the MethHC browser (43). PD-L1 mRNA expression was consistently upregulated across various cancers (Figure 2C). In addition, disruption of the 3′ region of the PD-L1 increases mRNA stability, leading to a marked elevation of aberrant PD-L1 transcripts in multiple cancers (44).

**Constitutive Oncogenic Signaling Activation**

Loss of PTEN expression, activation of PI3K/AKT pathway, activation of RAS/MAPK pathway, inhibition of p53 signaling, upregulation of reprogramming factors (Oct4, Sox2, and c-Myc)
FIGURE 1 | Mechanisms of PD-L1 activation in cancer. The diagram illustrates the diverse mechanisms of PD-L1 activation in cancer, including genetic alterations to PD-L1 (such as gene amplification, 3'-UTR disruption, or dysregulated transcription) and a wide range of epigenetic mechanisms (including upregulation of oncogetic microRNAs, downregulation of tumor suppressor microRNAs, aberrant DNA methylation and histone modifications).

and upregulation of ZEB1 (an inducer of EMT) are clearly linked to the activation of PD-L1 signaling pathway (45, 46) (Figure 1).

PD-L1 expression could be regulated via the PI3K/AKT and/or RAS/MAPK pathways in different tumor cell types (47–49). PD-L1 expression is suppressed by the tumor suppressor gene PTEN. Deletion of PTEN gene results in elevated PD-L1 expression at the translational level by activating the PI3K/AKT signal pathway (50, 51). FOXOs inhibit the expression of PD-L1 through repressing Myc or Wnt/β-catenin signaling pathways in tumor cells (52). MUC1 elevates PD-L1 transcription by recruitment of MYC and NF-κB (a downstream effector of PI3K/AKT pathway (53) to the PD-L1 promoter in breast cancer (54). Also, MUC1 was shown to increase PD-L1 levels via downregulation of miR-34a and miR-200c, two direct suppressors of PD-L1 (55–57).

Abnormal activation of stem cell signaling pathways has been implicated in the regulation of PD-L1. OCT4 is a key regulatory gene that maintains the self-renewal properties of CSC and promotes tumorigenesis of cervical cancer cells by miR-125b/BAK1pathway (58). We recently reported that, OCT4 promotes cervical cancer invasion and proliferation by enhancing PD-L1 expression through a miR-18a-dependent mechanism, by which miR-18a upregulates PD-L1 by targeting PTEN, WNK2 and SOX6 to activate the PI3K/AKT, MEK/ERK and Wnt/β-catenin pathways and inhibit the p53 pathway (25). In addition, SOX2, a transcription factor that controls tumor initiation and cancer stem-cell functions, can directly bind to the PD-L1 promoter and transactivate its expression, contributing to the increased proliferation of hepatocellular carcinoma cells (59). The upstream kinases of the Hippo pathway MST1/2 and LAT51/2 suppress PD-L1 expression, while TAZ and YAP enhance PD-L1 levels in breast and lung cancer cells (60).

Tumor cells undergoing EMT are shown to share a variety of capabilities with experimentally defined CSC (61). In lung cancer, PD-L1 expression was significantly higher in patients with EMT phenotypes (such as increased SNAI1 and Vimentin expression) compared with those with epithelial phenotypes (62). siRNA-mediated ZEB1 knockdown suppressed PD-L1 expression but promoted E-cadherin expression in esophageal squamous cell carcinoma (63). In agreement with these reports, cBioportal analysis of data on somatic copy number variation and mRNA level using TCGA endometrial and ovarian cancer dataset demonstrated that PD-L1 is indeed co-amplified along with MYC, SOX2, N-cadherin and SNAI1 in both cancer types (Figure 2A).

Another study reported that transcription factor NKX2-1 bound to the locus of PD-L1 and induced its expression in mucinous lung cancer cells (64). In non-small cell lung cancer cells, the ubiquitin ligases Cbl-b and c-Cbl inhibit PD-L1 expression by inactivating STAT, AKT, and ERK signaling (65), and overexpression of tumor suppressor gene TUSC2 downregulated PD-L1 expression (66). CDK4 and CDK6 kinase destabilize PD-L1 protein via cullin 3–SPOP, leading to the downregulation of PD-L1 in cancer cells (67).

Regulation of PD-L1 Expression by Epigenetic Mechanisms

The expression of cancer-associated genes can occur by epigenetic mechanisms, including DNA methylation (68),
histone modification (69), chromatin remodeling, and non-coding RNAs (70). The anti-PD-1 therapy could induce PD-L1 promoter methylation and decrease PD-L1 levels in patients with non-small cell lung cancer (71). The class 1 histone deacetylase HDAC8 acts as an epigenetic inhibitor of PD-L1 expression in melanoma cells via modulating HOXA5 and STAT3 (72). Numerous miRNAs, including miR-15a/miR-16 (73), miR-17-5p (74), miR-93/106b (75), miR-138-5p (76), miR-140/miR-340/miR-383 (25), miR-152 (77), miR-155 (78), miR-193 (73), miR-195 (73), miR-324-5p/miR-338 (79) and miR-322/miR-424 (80), have been shown to directly target and inhibit PD-L1 expression in tumor cells. In chemo-resistant non-small-cell lung cancer cells, miR-197 indirectly inhibits PD-L1 expression by regulating the CKS1B/STAT3 axis (81). On the other hand, oncogenic miR-20b and miR-21 inhibited PTEN expression, resulting in PD-L1 overexpression in colorectal cancer (82). Our recent data established that an oncogenic OCT4-miR-18a pathway serves as the key upstream activator of PD-L1 in cervical cancer (27).

Extrinsic Factors Influencing the Expression of PD-L1

The main regulators of PD-L1 are the interferon-γ (83), inflammatory cytokines such as IL-17 (84) and TNF-α (84), TGF-β1 (85), and HIF-1α (86). Of note, overexpressing HPV16E7 oncoprotein increased PD-L1 protein expression, and knockdown of HPV16E7 resulted in a reduction in
PD-L1 protein expression in cancer cells (87). Consistent with this data, PD-L1 protein expression was significantly higher in the normal cervical tissues with HPV infection than those normal cervical tissues without HPV infection (53). Estrogen is a well-known oncogenic driver of endometrial and breast cancer, and it upregulates PD-L1 protein expression in ERα-positive endometrial and breast cancer cells (88).

**THE ROLE OF PD-L1 IN STIMULATING OR INHIBITING CANCER**

A tumor-intrinsic role for PD-L1 in promoting cancer initiation, metastasis, development, and resistance to therapy is emerging (Figure 3). For instance, knockdown of PD-L1 expression in gastric cancer cells could significantly suppress cell proliferation, migration and invasion (89). Also, knockout of PD-L1 expression by CRISPR/Cas9 inhibits the spheroid formation of osteosarcoma cells (90). PD-L1 was shown to promote EMT in esophageal cancer (91). Knockdown of PD-L1 expression significantly suppressed tumor growth in nude mice in gastric cancer (92) and cervical cancer model (27).

Interestingly, a link between PD-L1 expression and EMT/CSC-like phenotypes has been reported. For example, bladder cancer cells with surface expression of PD-L1 exhibited signatures of immune evasion as well as increased stemness (93). PD-L1 has been shown to be preferentially expressed on CD44high CSCs in lung cancer cells (94). Selective expression of PD-L1 was observed on CD44+ head and neck tumor cells compared with CD44− tumor cells (95). CD133+/PD-L1+ colorectal CSC cells showed the characteristic of EMT (96). Tumor cell-intrinsic PD-L1 promotes tumor-initiating cell generation in melanoma and ovarian cancer (97). Similarly, PD-L1 promotes OCT4 and Nanog expression in breast CSCs through the activation of PI3K/AKT pathway (98).

Moreover, PD-L1 overexpression promotes EMT and invasion in glioblastoma multiforme via RAS/ERK/EMT activation (99). RNA-sequencing analysis of glioblastoma multiforme revealed that PD-L1 significantly altered the expression of genes, which were enriched in cell growth/migration/invasion pathways (99). PD-L1 induced EMT via activating SREBP-1c in renal cell carcinoma (100). CRISPR/Cas9 system-mediated PD-L1 disruption increased drug sensitivities for doxorubicin and paclitaxel (90). The interaction of PD-L1 with PD-1 induced phosphorylation of AKT and ERK, resulting in the activation of PI3K/AKT and MAPK/ERK pathways and increased MDR1 expression in breast cancer cells (101).

However, depletion of PD-L1 expression by shRNA in cholangiocarcinoma cells enhances their tumorigenicity and increases ALDH activity, and patients with lower PD-L1 expression shows poorer prognosis when compared with those with higher PD-L1 expression (102), indicating that PD-L1 may also have anti-tumor effects by inhibiting cancer stemness under certain circumstances.

**CONCLUSIONS**

It is becoming clear that, although PD-L1 could serve as a tumor suppressor by inhibiting cancer stem cell properties in cholangiocarcinoma, tumor cell-intrinsic PD-L1 plays a pivotal role in promoting cancer stemness, EMT, tumor invasion, and chemoresistance in several tumor types. Importantly, activation of OCT4 signaling induces PD-L1 expression in cancer cells, thereby suggesting a possible immune evasion mechanism employed by cancer stem cells during metastasis. The continued
characterization of immune-independent PD-L1 functions and identification of crucial signaling events upstream or downstream of PD-L1 in diverse cancer types (or specific cancer subtypes), would provide additional targets and new therapeutic approaches.

**AUTHOR CONTRIBUTIONS**

PD and HW provided direction. PD, YX, and HW wrote the manuscript. JY and SH made significant revisions to the manuscript. All authors read and approved the final manuscript.

**FUNDING**

This work was supported by a grant from JSPS Grant-in-Aid for Scientific Research (C) (16K11123 and 18K09278) and the Science and Technology Planning Project of Guangdong Province, China (2014A020212124).

**REFERENCES**

1. Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nat Rev Clin Oncol. (2017) 14:611–29. doi: 10.1038/nrclinonc.2017.44

2. Dong P, Konno Y, Watari H, Hosaka M, Noguchi M, Sakuragi N. The impact of microRNA-mediated PI3K/AKT signaling on epithelial-mesenchymal transition and cancer stemness in endometrial cancer. J Transl Med. (2014) 12:231. doi: 10.1186/s12976-014-0231-0

3. Nieto MA, Huang RY, Jackson RA, Thiery JP. EMT: 2016. Cell (2016) 166:21–45. doi: 10.1016/j.cell.2016.06.028

4. Ayob AZ, Ramasamy TS. Cancer stem cells as key drivers of tumour progression. J Biomed Sci. (2018) 25:20. doi: 10.1186/s12929-018-0426-4

5. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou YJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell (2008) 133:704–15. doi: 10.1016/j.cell.2008.03.027

6. Ungefroren H, Sebens S, Seidl D, Lehnert H, Hass R. Interaction of tumor cells with the microenvironment. Cell Commun Signal (2011) 9:18. doi: 10.1186/1478-811X-9-18

7. Renner K, Singer K, Koehler GE, Geissler EK, Peter K, Siska PJ, et al. Metabolic hallmarks of tumor and immune cells in the tumor microenvironment. Front Immunol. (2017) 8:248. doi: 10.3389/fimmu.2017.00248

8. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. (2002) 3:991–8. doi: 10.1038/ni1102-991

9. Gajewski TF, Meng Y, Harlin H. Immune suppression in the tumor microenvironment: tumor-induced immunosuppressive pathway: barrier to antitumor immunity. J Immunol (2006) 29:233–40.

10. Marcucci F, Rumio C, Corti A. Tumor cell-associated immune check point factors paralyze T cells. Front Immunother. (2009) 4:e1016700. doi: 10.1080/2162402X.2015.1016700

11. Mezache L, Paniccia B, Nyinawabera A, Nuovo GJ. Enhanced expression of PD-L1 in cervical intraepithelial neoplasia and cervical cancers. Mod Pathol. (2015) 28:1594–602. doi: 10.1038/modpathol.2015.108

12. Saito H, Kono Y, Murakami Y, Shishido Y, Kuroda H, Matsunaga T, et al. Highly activated PD-1/PD-L1 pathway in gastric cancer with PD-L1 expression. Anticancer Res. (2018) 38:107–12. doi: 10.21873/anticancer.12197

13. Yagi T, Baba Y, Ishimoto T, Iwatsuki M, Miyamoto Y, Yoshida N, et al. PD-L1 expression, tumor-infiltrating lymphocytes, and clinical outcome in patients with surgically resected esophageal cancer. Ann Surg. (2017). doi: 10.1097/SLA.0000000000002616. [Epub ahead of print].

14. Xue S, Song G, Yu J. The prognostic significance of PD-L1 expression in patients with glioma: A meta-analysis. Sci Rep. (2017) 7:4231. doi: 10.1038/s41598-017-04023-x

15. Zhao LW, Li C, Zhang RL, Xue HG, Zhang FX, Zhang F, et al. B7-H1 and B7-H4 expression in colorectal cancer: correlation with tumor FOXP3(+) regulatory T-cell infiltration. Acta Histochem. (2014) 116:1163–8. doi: 10.1016/j.acthis.2014.06.003

16. Dong et al. Tumor-Intrinsic PD-L1 Signaling in Cancer

17. Mu CY, Huang JA, Chen Y, Chen C, Zhang XG. High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. Med Oncol. (2011) 28:682–8. doi: 10.1007/s12032-010-9515-2

18. Wang L, Ma Q, Chen X, Guo K, Li J, Zhang M. Clinical significance of B7-H1 and B7-1 expressions in pancreatic carcinoma. World J Surg. (2010) 34:1059–65. doi: 10.1007/s00268-010-0448-x

19. Gao Q, Wang XY, Qu SI, Yamato I, Sho M, Nakajima Y, et al. Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. Clin Cancer Res. (2009) 15:971–9. doi: 10.1158/1078-0432.CCR-08-1608

20. Böger C, Behrens HM, Mathiak M, Krüger S, Kalthoff H, Röcken C. PD-L1 is an independent prognostic predictor in gastric cancer of Western patients. OncoTarget (2016) 7:24269–83. doi: 10.18632/oncotarget.8169

21. Hamanishi J, Mandai M, Iwasaki M, Okazaki T, Tanaka Y, Yamaguchi K, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. Proc Natl Acad Sci USA. (2007) 104:3360–5. doi: 10.1073/pnas.06115331014

22. Liu J, Liu Y, Wang W, Wang C, Che Y. Expression of immune checkpoint molecules in endometrial carcinoma. Exp Ther Med. (2015) 10:1947–52. doi: 10.3848/etm.2015.2714

23. Kharma B, Baba T, Matsumura N, Kang HS, Hamanishi J, Murakami R, et al. TAT1 drives tumor progression in serous papillary endometrial carcinoma. Cancer Res. (2016) 76:6519–30. doi: 10.1158/0008-5472.CAN-14-0847

24. Mezache L, Paniccia B, Nyinawabera A, Nuovo GJ. Enhanced expression of PD-L1 in cervical intraepithelial neoplasia and cervical cancers. Mod Pathol. (2015) 28:1594–602. doi: 10.1038/modpathol.2015.108

25. Dong P, Xiong Y, Yu J, Chen L, Tao T, Yi S, et al. Control of PD-L1 expression by miR-140/340/383 and oncogenic activation of the OCT4-miR-18a pathway in cervical cancer. Oncogene (2018) doi: 10.1038/s41388-018-0347-4

26. Cao L, Wang X, Li S, Zhi Q, Wang Y, Wang L, et al. PD-L1 is a prognostic Biomarker in Resected NSCLC patients with moderate/high smoking history and elevated serum SCCA level. J Cancer (2017) 8:3251–60. doi: 10.7150/jca.21118

27. Saito H, Kono Y, Murakami Y, Shishido Y, Kuroda H, Matsunaga T, et al. Highly activated PD-1/PD-L1 pathway in gastric cancer with PD-L1 expression. Anticancer Res. (2018) 38:107–12. doi: 10.21873/anticancer.12197
32. Wang X, Teng F, Kong L, Yu J. PD-L1 expression in human cancers and its association with clinical outcomes. *Onco Targets Ther.* (2016) 9:5023–39. doi: 10.2147/OTT.S105862

33. Lipson EJ, Vincent JG, Loyo M, Kagohara LT, Luber BS, Wang H, et al. PD-L1 expression in the Merkel cell carcinoma microenvironment: association with inflammation, Merkel cell polyomavirus and overall survival. *Cancer Immunol. Res.* (2013) 1:54–63. doi: 10.1158/2326-0666.CIR-13-0034

34. Heeren AM, Punth S, Bleeker MC, Gaarenstroom KN, van der Velden J, Kenter GG, et al. Prognostic effect of different PD-L1 expression patterns in squamous cell carcinoma and adenosquamous of the cervix. *Mod Pathol.* (2016) 29:753–63. doi: 10.1038/modpathol.2016.64

35. George J, Saito M, Tsuta K, Iwakawa R, Shiraishi K, Scheel AH, et al. Genomic aberrant PD-L1 expression through 3'-UTR disruption in multiple cancers. *Nature* (2016) 534:402–6. doi: 10.1038/nature18294

36. Straub M, Drecoll E, Pfarr N, Weichert W, Langer R, Hapfelmeier A, et al. CD274/PD-L1 gene amplification and PD-L1 protein expression are common events in squamous cell carcinoma of the oral cavity. *Oncoargets* (2017) 6:1204–34. doi: 10.18632/oncotarget.7593

37. Howitt BE, Sun HH, Roemer MG, Kelley A, Chapuy B, Aviki E, et al. Genetic basis for PD-L1 expression in squamous cell carcinomas of the cervix and vulva. *JAMA Oncol.* (2016) 2:518–22. doi: 10.1001/jamaoncol.2015.6326

38. Budczies J, Denkert C, Gyorffy B, Schirrmecher P, Stenzinger A. Chromosome 9p copy number gains involving PD-L1 are associated with a specific proliferation and immune-modulating gene program active across major cancer types. *BMC Med Genomics* (2017) 10:74. doi: 10.1186/s12920-017-0308-8

39. Barrett MT, Anderson KS, Lenkiewicz E, Andreozzi M, Cunliffe HE, Klassen CL, et al. Genomic amplification of 9p24.1 targeting JAK2, PD-L1, and PD-L2 is enriched in high-risk triple negative breast cancer. *Oncoargets* (2015) 6:26483–93. doi: 10.18632/oncotarget.4494

40. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* (2014) 513:202–9. doi: 10.1038/nature13480

41. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer data. *Cancer Discov.* (2012) 2:401–4. doi: 10.1158/2159-8290.CD-11-0640

42. Shin G, Kang TW, Yang S, Baek SJ, Jeong YS, Kim SY. GENT: gene expression knowns and unknowns. *Cell Death Dis.* (2018) 9:462–81. doi: 10.15252/emmm.201606711

43. Coelho MA, de Carné Trécsson S, Rana S, Zechin D, Moore C, Molina-Arcas M, et al. Oncogenic RAS signaling promotes tumor immunoresistance by stabilizing PD-L1 mRNA. *Immunity* (2017) 47:1083–99.e6. doi: 10.1016/j.immuni.2017.11.016

44. Dong P, Jiang Y, Wang X, Zhang W, Yue Z, Wang J, et al. Suppression of iASPP-dependent aggressiveness in cervical cancer through reversal of methylation silencing of microRNA-124. *Sci Rep.* (2016) 6:35480. doi: 10.1038/srep35480

45. Dong P, Ichikawa T, Sato Y, Hamajima M, Nakada T, Takai M, et al. Reactivation of epigenetically silenced miR-124 reverses the epithelial-to-mesenchymal transition and inhibits invasion in endometrial cancer cells via
the direct repression of IQGAP1 expression. Oncotarget (2016) 7:20260–70. doi: 10.18632/oncotarget.7754

71. Zhang Y, Xiang C, Wang Y, Duan Y, Liu C, Zhang Y. PD-L1 promoter methylation mediates the resistance response to anti-PD-1 therapy in NSCLC patients with EGFR-TKI resistance. Oncotarget (2017) 8:101535–44. doi: 10.18632/oncotarget.21328

72. Wang YE, Liu E, Sherwin S, Farrelly M, Yan XG, Croft A, et al. Cooperativity of HOXA5 and STAT3 is critical for HDAC8 inhibition-mediated transcriptional activation of PD-L1 in human melanoma cells. J Invest Dermatol. (2018) 138:922–32. doi: 10.1016/j.jid.2017.11.009

73. Kao SC, Cheng YY, Williams M, Kirschner MB, Madore J, Lum T, et al. Tumor suppressor microRNAs contribute to the regulation of PD-L1 expression in malignant pleural mesothelioma. J Thorac Oncol. (2017) 12:1421–33. doi: 10.1016/j.jtho.2017.05.024

74. Audrito V, Serra S, Stangi A, Oseo F, Gaudino F, Bologna C, et al. PD-L1 up-regulation in melanoma increases disease aggressiveness and is mediated through miR-17-5p. Oncotarget (2017) 8:15894–911. doi: 10.18632/oncotarget.15213

75. Ciolfi M, Trabulo SM, Vallespinos M, Raj D, Kheir TB, Lin ML, et al. PD-L1 and is mediated through miR-17-5p. Mol Med Rep. (2017) 8:30276–87. doi: 10.18632/oncotarget.16326

76. Zhao L, Yu H, Yi S, Peng X, Su P, Xiao Z, et al. The tumor suppressor miR-20b, -21, and is mediated through miR-17-5p. Mol Med Rep. (2016) 8:101535–44. doi: 10.18632/oncotarget.21328

77. Zhu J, Chen L, Zou L, Yang P, Wu R, Mao Y, et al. MiR-20b, -21, and is mediated through miR-17-5p. Mol Med Rep. (2016) 8:101535–44. doi: 10.18632/oncotarget.21328

78. Fujita Y, Yagishita S, Hagiwara K, Yoshioka Y, Kosaka N, Takeshita F, et al. The clinical relevance of the miR-197/CKS1B/STAT3-mediated PD-L1 network in chemoresistant non-small-cell lung cancer. Cancer Med. (2017) 6:24193. doi: 10.1002/cmm.19732

79. Kao SC, Cheng YY, Williams M, Kirschner MB, Madore J, Lum T, et al. Tumor suppressor microRNAs contribute to the regulation of PD-L1 expression in malignant pleural mesothelioma. J Thorac Oncol. (2017) 12:1421–33. doi: 10.1016/j.jtho.2017.05.024

80. Xu S, Tao Z, Hai B, Liang H, Shi Y, Wang T, et al. miR-424(322) regulates immune response via targeting B7-H1 in gastric carcinoma. Oncotarget (2017) 8:28125–34. doi: 10.18632/oncotarget.15924

81. Yee D, Shah KM, Coles MC, Sharp TV, Lagos D. MicroRNA-155 induction via TNF-α and IFN-γ suppresses expression of programmed death ligand-1 (PD-L1) in human primary cells. J Biol Chem. (2017) 292:20683–93. doi: 10.1074/jbc.M117.809053

82. Holla S, Stephen-Victor E, Prakhar P, Sharma M, Saha C, Udupa V, et al. Mycobacteria-responsive sonic hedgehog signaling mediates programmed death ligand-1 and prostaglandin E2 induced regulatory T cell expansion. Sci Rep. (2016) 6:24193. doi: 10.1038/srep24193

83. Xu S, Tao Z, Hai B, Liang H, Shi Y, Wang T, et al. miR-424(322) reverses chemoresistance via T-cell immune response activation by blocking the PD-L1 immune checkpoints. Nat Commun. (2016) 7:1406. doi: 10.1038/ncomms11406

84. Fujita Y, Yagishita S, Hagihara K, Yoshioka Y, Kosaka N, Takeshita F, et al. The clinical relevance of the miR-197/CKS1B/STAT3-mediated PD-L1 network in chemoresistant non-small-cell lung cancer. Mol Ther. (2015) 23:717–27. doi: 10.1038/mt.2015.10

85. Zhou J, Chen L, Zou L, Yang P, Wu R, Mao Y, et al. MiR-20b, -21, and is mediated through miR-17-5p. Mol Med Rep. (2016) 8:101535–44. doi: 10.18632/oncotarget.21328

86. Wang Y, Wang D, Xie G, Yin Y, Zhao E, Tao K, et al. MicroRNA-152 regulates immune response via targeting B7-H1 in gastric carcinoma. Oncotarget (2017) 8:28125–34. doi: 10.18632/oncotarget.15924

87. Fujita Y, Yagishita S, Hagiwara K, Yoshioka Y, Kosaka N, Takeshita F, et al. The clinical relevance of the miR-197/CKS1B/STAT3-mediated PD-L1 network in chemoresistant non-small-cell lung cancer. Mol Ther. (2015) 23:717–27. doi: 10.1038/mt.2015.10

88. Wang Y, Wang D, Xie G, Yin Y, Zhao E, Tao K, et al. MicroRNA-152 regulates immune response via targeting B7-H1 in gastric carcinoma. Oncotarget (2017) 8:28125–34. doi: 10.18632/oncotarget.15924

89. Yee D, Shah KM, Coles MC, Sharp TV, Lagos D. MicroRNA-155 induction via TNF-α and IFN-γ suppresses expression of programmed death ligand-1 (PD-L1) in human primary cells. J Biol Chem. (2017) 292:20683–93. doi: 10.1074/jbc.M117.809053

90. Holla S, Stephen-Victor E, Prakhar P, Sharma M, Saha C, Udupa V, et al. Mycobacteria-responsive sonic hedgehog signaling mediates programmed death ligand-1 and prostaglandin E2 induced regulatory T cell expansion. Sci Rep. (2016) 6:24193. doi: 10.1038/srep24193

91. Xu S, Tao Z, Hai B, Liang H, Shi Y, Wang T, et al. miR-424(322) reverses chemoresistance via T-cell immune response activation by blocking the PD-L1 immune checkpoints. Nat Commun. (2016) 7:1406. doi: 10.1038/ncomms11406

92. Fujita Y, Yagishita S, Hagihara K, Yoshioka Y, Kosaka N, Takeshita F, et al. The clinical relevance of the miR-197/CKS1B/STAT3-mediated PD-L1 network in chemoresistant non-small-cell lung cancer. Mol Ther. (2015) 23:717–27. doi: 10.1038/mt.2015.10

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Dong, Xiong, Yue, Hanley and Watari. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.