Review Article

Radiation-induced PD-L1 expression in tumor and its microenvironment facilitates cancer-immune escape: a narrative review

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Background and Objective: Radiotherapy (RT) is one of the fundamental anti-cancer regimens by means of inducing in situ tumor vaccination and driving a systemic anti-tumor immune response. It can affect the tumor microenvironment (TME) components consisting of blood vessels, immunocytes, fibroblasts, and extracellular matrix (ECM), and might subsequently suppress anti-tumor immunity through expression of molecules such as programmed death ligand-1 (PD-L1). Immune checkpoint inhibitors (ICIs), especially anti-programmed cell death 1 (PD-1)/PD-L1 therapies, have been regarded as effective in the reinvigoration of the immune system and another major cancer treatment. Experimentally, combination of RT and ICIs therapy shows a greater synergistic effect than either therapy alone.

Methods: We performed a narrative review of the literature in the PubMed database. The research string comprised various combinations of "radiotherapy", "programmed death-ligand 1", "microenvironment", "exosome", "myeloid cell", "tumor cell", "tumor immunity". The database was searched independently by two authors. A third reviewer mediated any discordance of the results of the two screeners.

Key Content and Findings: RT upregulates PD-L1 expression in tumor cells, tumor-derived exosomes (TEXs), myeloid-derived suppressor cells (MDSCs), and macrophages. The signaling pathways correlated to PD-L1 expression in tumor cells include the DNA damage signaling pathway, epidermal growth factor receptor (EGFR) pathway, interferon gamma (IFN-γ) pathway, cGAS-STING pathway, and JAK/STATs pathway.

Conclusions: PD-L1 upregulation post-RT is found not only in tumor cells but also in the TME and is one of the mechanisms of tumor evasion. Therefore, further studies are necessary to fully comprehend this biological process. Meanwhile, combination of therapies has been shown to be effective, and novel approaches are to be developed as adjuvant to RT and ICIs therapy.

Keywords: Radiotherapy (RT); tumor microenvironment (TME); PD-L1 expression; immune checkpoint inhibitors

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Introduction

As oncology treatment entered the new era of immunotherapy, it presented a concept shift by overcoming immunosuppression induced by tumor cells so that the immune system was allowed to target and kill tumor cells. Immune checkpoint blockade is an inbuilt mechanism to prevent activated T cell damage to normal surrounding tissues and is achieved by co-inhibitory receptors. However, this mechanism is exploited by tumor cells, which express such receptors [i.e., programmed death ligand-1 (PD-L1)], inhibit T-cell activation, and induce apoptosis. Immune checkpoint inhibitors (ICIs) are developed to block immune checkpoints and result in T-cell reinvigoration, in a process also known as immunotherapy.

Acting as a traditional tumor therapy, radiotherapy (RT) is applied for over 60% of newly diagnosed patients (1,2). Researchers have accepted the concept that RT could not only control lesions *in situ* but also trigger a systemic immune response called immunogenic cell death (ICD) (3). Growing evidence in research on the immune regulatory function of RT mainly contained its influence on the release of pro-inflammatory mediators and immune cells (4). Through this mechanism, RT enhances tumor immunogenicity both inside and outside of the irradiation field (5). After triggering ICD, multiple damage-associated molecular patterns (DAMPs) were released (6) and recognized by dendritic cells (DCs) (7). DCs can further present these antigens to cytotoxic T cells (8) and also activate natural killer (NK) cells thus inducing *in situ* immune modulations (9-11). The cell death that can activate downstream immune responses and stimulate immune surveillance is characterized as immunogenic and this leads to the "in situ" vaccination effect (12,13). Increasing evidence indicates the immunomodulatory effects of RT in systemic antitumor responses (14-16). Strikingly, a phenomenon observed in clinical case reports, termed the radiation-dependent abscopal effect, signifies tumor regression outside the irradiated fields (17,18). Unfortunately, even if the abscopal effect is so well-known, its rarity of occurrence rate indicates that there is seemingly no broad application value (19). The underlying mechanisms of why abscopal effects happen rarely remain unknown, and it should be correlated with the immunosuppression responses that occur after irradiation (20).

The radiation-induced regulation of the immune microenvironment is a double-edged sword that renders the body immune in a very delicate balance between immune activation and immunosuppression (4). In addition to the activation of the innate and adaptive immune systems, RT can also induce immunosuppression responses such as the polarization of macrophages with the M2 phenotype (21,22), neutrophils with the N2 phenotype (23-26), and accumulation of myeloid-derived suppressor cells (MDSCs) (27,28). These cells generally induce immunosuppressive effects via PD-L1 expression and other mechanisms.

PD-L1 is known as a major co-inhibitory checkpoint signaling protein that controls the activation of T cells. Under normal circumstances, the programmed cell death 1 (PD-1)/PD-L1 pathway constrains the hyperactivation of immune cells and inhibits autoimmune diseases (29). However, in tumor microenvironment (TME), tumor cells hijack this axis and induce immune escape (30). The overexpression of PD-L1 on tumor cells binds to PD-1 on tumor-infiltrating lymphocytes and counteracts the TCR-signaling cascade (31,32). Through the binding of PD-1 and PD-L1, signaling transmits negative signals to T cells which leads to T cell apoptosis and reduction of proliferation which effectively reduces immune responses and improves tumor growth. Therefore, the activation of T cells is impaired. Multiple types of human cancer including non-small cell lung cancer (NSCLC) (33,34), melanoma (35), renal cell carcinoma (36), prostate cancer (37), and gastric cancer have been verified to highly express PD-L1 (38). In addition to cancer cells, available data suggest that host cells (39) in the TME and lymph nodes also express PD-L1. These infiltrating cells consist of dendritic cells, macrophages, neutrophils, fibroblasts, and MDSCs and contribute to protumor activities. Moreover, recent studies that focused on extracellular vesicles (EVs) have shown that exosomal PD-L1 possesses the same biological function as cellular PD-L1 and could be the key to systemic immunosuppression (40,41).

Irradiation leads to an immune re-modulation in the TME by influencing almost all steps of the cancer immunity cycle. To be specific, it can increase the expression of PD-L1 on both cancer-associated cells and tumor cells and the level of PD-L1 in EVs. In this article, we detailly review the upregulation of PD-L1 on various types of cells under the delivery of RT. From the perspective of clinical applications in oncologic treatment, a better understanding of biomarker expression between pre- and post-RT provides new insight into the establishment of an optimal combination strategy. We present the following article in accordance with the Narrative Review reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6049/rc).

Table 1 The search strategy summary

Items	Specification
Date of search	22/10/2022
Databases and other sources searched	PubMed
Terms for search	"Radiotherapy", "programmed death-ligand 1", "tumor cell", "microenvironment", "exosome", "myeloid cell", "tumor immunity"
Timeframe	From 1998 to June 2022
Inclusion and exclusion criteria	
Inclusion criteria	Focus on the mechanism and signaling pathways correlated to PD-L1 expression after irradiation, including cell line and animal studies
	Peer-reviewed, published literature, including review papers
	English-language papers
Exclusion criteria	Meta-analysis, systematic review, and clinical research
	Abstracts, editorials, and letters to the editors
	Studies involving human
	Non-English-language papers
Selection process	Two authors searched the database independently, and a third reviewer mediated the disagreements and came to a consensus

Methods

We performed a narrative review of the literature in the PubMed database. The search terms utilized comprised various combinations of "radiotherapy", "programmed death-ligand 1", "microenvironment", "exosome", "myeloid cell", "tumor cell", and "tumor immunity". Two authors searched the database independently, and a third reviewer mediated any disagreements to reach a consensus. To be included, papers had to be focused on the radiation-induced upregulation of PD-L1 and its impact on tumor immunity. All English-language papers published at any time were eligible. Peer-reviewed, published literature, and reviews were considered eligible for inclusion. Meta-analyses, systematic reviews, clinical research, and abstracts were excluded. Reference lists of included papers were hand-searched and included if the inclusion criteria were met. The search strategy is summarized in *Table 1*.

RT increases tumor cell PD-L1 expression

After radiation damages tumor cells and induces direct breakage of DNA, a series of biological events induced by DNA damage will occur in tumor cells which plays an important role in immunomodulatory responses (42-45). On the one hand, the dying tumor cells release damage-associated molecular patterns to interact with cells in TME (46). On the other hand, DNA damage causes changes in the immunogenicity of irradiated tumor cells (47). The upregulation of PD-L1 expression in tumor cells is one of the most representative responses among these changes. RT is a well-documented trigger of PD-L1 expression, and this occurs via 4 primary mechanisms: (I) DNA damage signaling pathway; (II) interferon gamma (IFN-γ) signaling; (III) the cGAS-STING pathway; and (IV) the epidermal growth factor receptor (EGFR) pathway. Of note, all 4 of these mechanisms are involved in the JAK-STAT pathway and form the truth of PD-L1 expression in tumor cells after RT.

DNA damage signaling pathway

RT targets solid tumors and directly induces DNA double-strand breaks (DSBs) which is the most critical type of DNA damage. Sato *et al.* discovered that the expression of PD-L1 upregulates in response to DSBs in living cancer cell lines including osteosarcoma, lung cancer, and prostate cancer (48). Following DSBs is DNA damage repair (DDR). Three central DDR kinases including DNA-dependent protein kinase catalytic subunit (DNA-PKcs), ataxia telangiectasia-mutated (ATM), ataxia telangiectasia

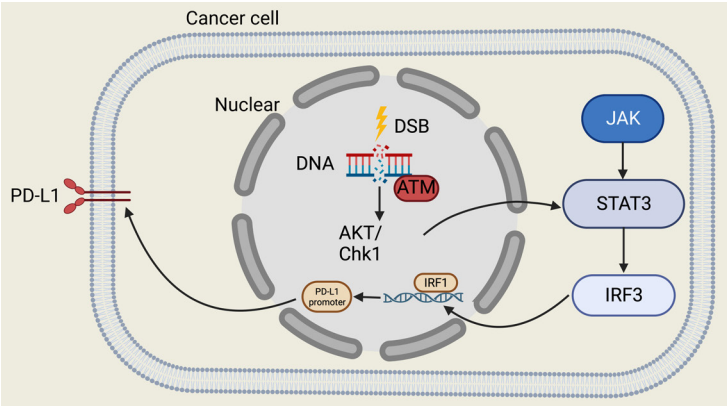

Figure 1 The DNA damage caused by RT activates the downstream ATM/ATK/Chk1 signaling pathway and is correlated with STAT3 to produce IRF3, which promotes PD-L1 expression. Created with Biorender.com. The figure is made in © Biorender – biorender.com (https://app.biorender.com/) and exported under a paid subscription. PD-L1, programmed death ligand-1; DSB, double-strand break; JAK, Janus kinase; STAT, signal transducer and activator of transcription; ATM, ataxia telangiectasia-mutated; RT, radiotherapy.

and Rad3-related protein (ATR), and the phosphoinositol-3-kinase-related kinases are activated to arrest the cell cycle and trigger apoptosis. Among them, ATM is the most important signal transducer to serve as a sensor of DSBs (49). Following ATM, the transient activation switches to ATR, followed by Chk1 activation. This ATM/ATR/Chk 1-dependent manner not only constitutes the DSBs signal axis but also regulates PD-L1 expression after DSBs caused by RT. A recent report further demonstrated that there was a correlation between ATM/Chk signaling and JAKs-STATs-IRF1 pathway phosphorylation since the depletion of ATM or Chk1 significantly reduced interferon regulatory factor 1 (IRF1) expression after RT. Moreover, the depletion of IRF1 deregulated the PD-L1 expression. Therefore, PD-L1 upregulation after DSBs is correlated with the IRF1 pathway (48). Cheon *et al.* indicated that IFN-related DNA damage resistance signature (IRDS) expression, which is upregulated following DNA damage, is also correlated with PD-L1 expression (50). To be specific, the IRDS expression is induced by the cGAS-STING pathway, leading to cancer production of IFN-β that may be responsible for IFN-I responses and IRDS expression (51-53) (*Figure 1*).

IFN-γ signaling

Multiple proinflammatory molecules induce the expression of PD-L1, such as GM-CSF, tumor necrosis factor alpha (TNF-α), lipopolysaccharide (LPS), I and II IFN-γ, and vascular endothelial growth factor (VEGF). Granulocyte-macrophage colony-stimulating factor (GM-CSF) and VEGF are produced by various cancer stromal cells (54-56). IFN-γ and TNF-α are produced by activated type I T cells. Recent studies have revealed that IFN is one of the most critical factors. This is because type I IFN (α and β) and type II IFN (γ) cause PD-L1 upregulation in all cells and it is believed that IFN-γ is the strongest inducer among all 3 IFNs (57). IFN-γ binds to its receptor and stimulates downstream JAKs-STATs-IRF1 signaling, thus inducing PD-L1 expression (58,59). Evidence has concluded the role of STAT1, STAT3, and the downstream transcription factor interferon regulatory factor 1 (*IRF1*) gene in upregulating PD-L1 upon IFN-γ exposure (60,61). Garcia-Diaz *et al.* demonstrated the PD-L1 promoter pathway as the IFN-γ-JAK1/JAK2-STAT1/STAT2/STAT3-IRF1 axis (58). Moreover, the IFN-γ-mediated PD-L1 expression is dependent on mTOR.

Apart from DNA breakage, RT damages cancer cells through the generation of reactive oxygen species (ROS). ROS can provoke inflammation responses *in situ* and play an important role in cell signaling (62). In this way, RT induces secretion of inflammatory mediators including NF-κB and SMAD2/3, cytokines including IL-1, 2, 6, 8, and 33, TNF-α, β, and IFN-γ (63-65). There is a strong relationship between the dose of RT and the duration of inflammatory responses (66). In this situation, RT increases the PD-L1 expression by upregulating IFN-γ (*Figure 2*).

cGAS-STING pathway

The cyclic GMP-AMP synthase-stimulator of interferon

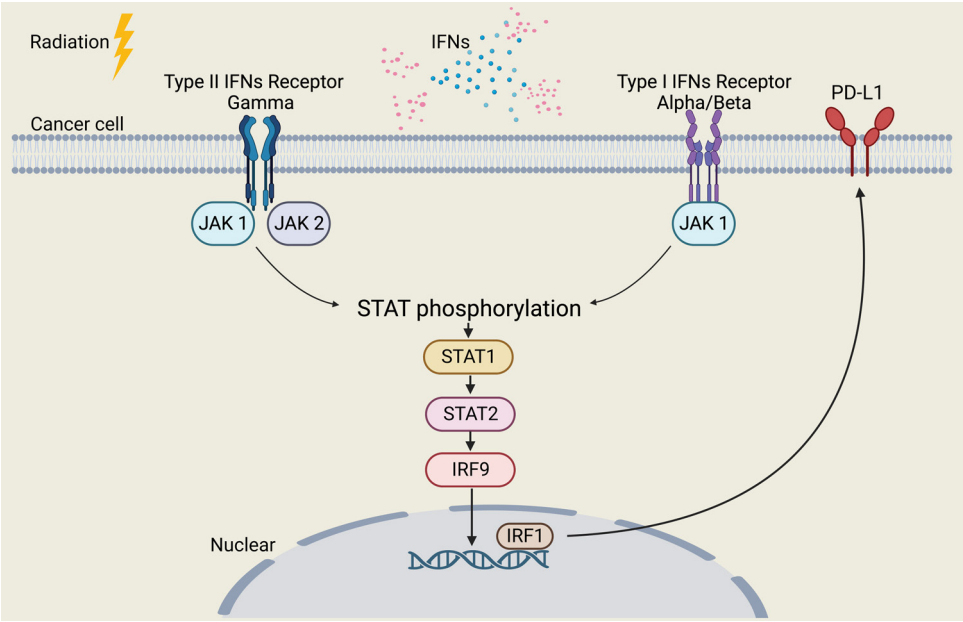

Figure 2 RT stimulates the escalation of IFN-γ that binds with IFNR and further activates the JAKs/STATs pathway to produce IRF1, which promotes PD-L1 expression. Created with Biorender.com. The figure is made in © Biorender – biorender.com (https://app.biorender.com/) and exported under a paid subscription. IFN, interferon; PD-L1, programmed death ligand-1; JAK, Janus kinase; STAT, signal transducer and activator of transcription; IRF, interferon regulatory factor; RT, radiotherapy; IFNR, interferon receptor.

genes (cGAS-STING) pathway is an important cytosolic DNA sensing pathway and is affected by RT (67,68). It acts as a double-edged sword in cancer immunity modulation following RT.

On the one hand, the cGAS-STING pathway can trigger innate immune responses and participate in multiple links of adaptive immune responses (69,70). There is a well-established phenomenon that RT could induce DSB and thus produce micronuclei in cancer cells. cGAS build up a bridge between DDR and STING by surveillance of these micronuclei. The downstream signaling molecular system contains IRF3 and canonical nuclear factor-κB (NF-κB) cooperatively turn on the transcription of type I IFN production signaling and trigger innate immune responses (71).

On the other hand, this pathway still has negative effects such as facilitating immunosuppressive cell infiltration and upregulating immune checkpoint expression (72,73). After DNA damage caused by RT, STING-induced TBK1 upregulation facilitates the non-canonical NF-κB p52/RelB activation (74,75). The RelB can bind to the IFNB gene promoter and inhibit the production of type I IFN. Besides, the activation of the cGAS-STING pathway induced by RT triggers the phosphorylated IRF3 to enter the nucleus and interacts with IRF1. With the augments of IRF1 activation, PD-L1 transcription increased (76). This upregulation of PD-L1 post-RT is confirmed blocked after the knockdown of cGAS, STING, and IRF3 (*Figure 3*).

EGFR pathway

EGFR is one of the ERBB family receptor tyrosine kinases, its major downstream pathways contain RAS/RAF/MAPK, PI3K/AKT/mTOR, and IL-6/JAK/STAT3/5 which regulate cell proliferation, survival, migration, and differentiation. The Atlantic trial reported that patients with $EGFR^+/ALK^+$ NSCLC had higher PD-L1 expression in tumor cells (77-79). Some studies have demonstrated that if the activity of EGFR is inhibited by EGFR-TKIs, the PD-L1 expression in NSCLC with mutant EGFR would be decreased (80,81). Evidence has also proven that the activation of the EGFR pathway could trigger the immune response in murine melanoma models (82). The findings of several studies reported that EGFR regulates PD-L1 expression through the MAPK/Hippo Kinase/yes-associated protein (YAP) signaling pathway in human NSCLC

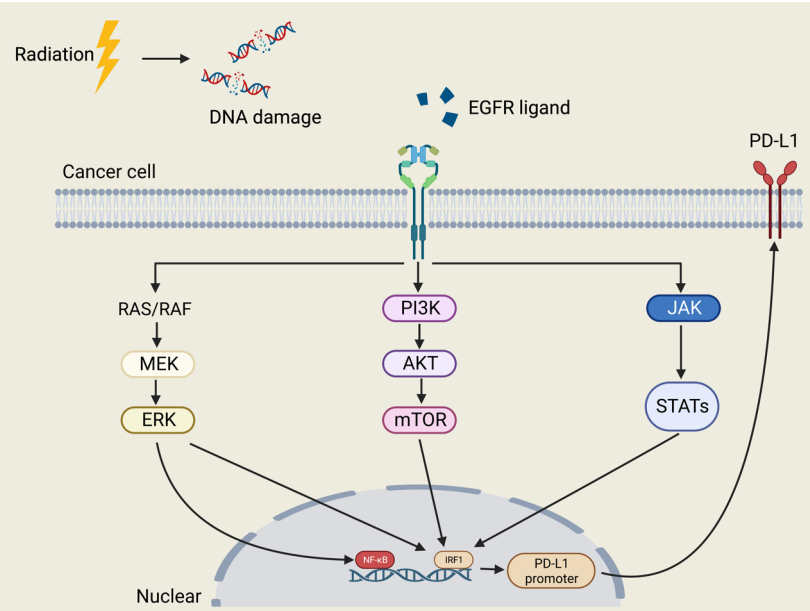

Figure 3 cGAS is activated by JAK/STATs and binds with DNA damage. STING-induced TBK1 upregulation facilitates the NF-κB activation and promotes PD-L1 expression. Created with Biorender.com. The figure is made in © Biorender – biorender.com (https://app.biorender.com/) and exported under a paid subscription. EGFR, epidermal growth factor receptor; PD-L1, programmed death ligand-1; JAK, Janus kinase; STAT, signal transducer and activator of transcription; RAS, rat sarcoma; RAF, rapidly accelerated fibrosarcoma; MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; IRF1, interferon regulatory factor 1; cGAS, cyclic GMP-AMP synthase; NF-κB, nuclear factor kappa-B.

(83-87). The AKT/mTOR pathway has also been validated as responsible for EGFR-mediated PD-L1 expression (88). In addition to these 2 pathways, the AKT/STAT3 pathway might also play a role in the regulation of PD-L1 expression on NSCLC cell lines (89-91). Even though the underlying mechanism is still unclear, there is a strong correlation between the EGFR pathway and PD-L1 expression at the molecular level.

When it comes to discussing the influence that RT made on the EGFR signaling pathway, the RT-induced DSB is of central importance. According to the preceding paragraph, DSB can facilitate the assembly of the DNA-PK complex. Hence, these kinases further facilitate the non-homologous end-joining (NHEJ) process to repair DNA breakage (92). However, irradiated cells show a reduction of the nuclear activity of the DNA-PK complex which is important in NHEJ. To escape cell death, cancer cells enhance the activation of EGFR and further stimulate the AKT to mediate DNA-PKcs phosphorylation (93-95). It can be concluded that radiation directly activates EGFR which in turn facilitates the PI3K-AKT cascade and mediates anti-apoptosis responses by inducing DNA-PK activation and helping the DDR (96-98) (*Figure 4*).

JAK/STAT pathway

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway is a cytokine-stimulated signal transduction pathway and directly regulates the communication from transmembrane receptors to the nucleus (99-101). The JAKs-STATs-IRF1 pathway was shown to regulate PD-L1 expression after treatment (58). The JAK/STAT pathway can be activated by DSBs and ROS accumulation induced by RT (102,103), in fact, all the mechanisms above are correlated with the JAK/STAT pathway. Khashab *et al.* found that JAK inhibition can prevent DNA damage and apoptosis by modulation of the ATM/ATR/Chk pathway in the testicular ischemia-reperfusion injury model (104). This phenomenon indicates the fact that the JAK/STAT pathway is a part of DNA damage-induced downstream signaling, and that JAK/STAT is the main signaling pathway that mediates IFN-induced gene expression and results in the activation of IFN-γ activation sites (GASs).

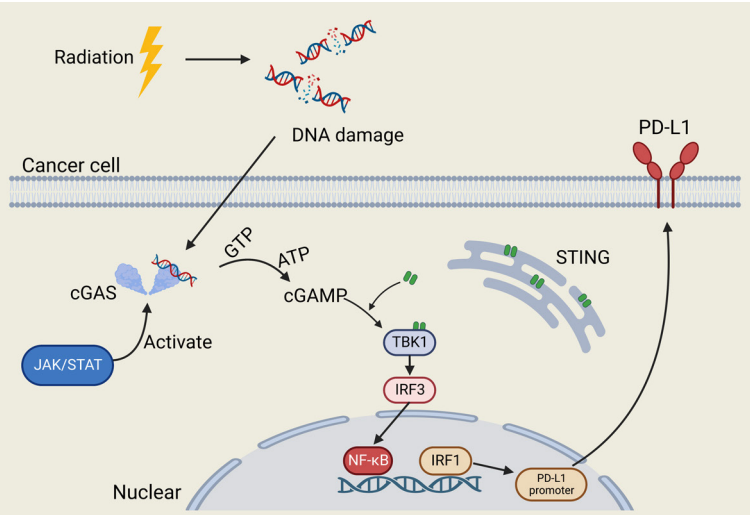

Figure 4 EGFR activates any of RAS/RAF/MAPK, PI3K/AKT/mTOR, or JAK/STATs pathways and further upregulates PD-L1 expression. Created with Biorender.com. The figure is made in © Biorender – biorender.com (https://app.biorender.com/) and exported under a paid subscription. cGAS, cyclic GMP-AMP synthase; JAK, Janus kinase; STAT, signal transducer and activator of transcription; GTP, guanosine triphosphate; ATP, adenosine triphosphate; cGAMP, 2'3'-cyclic-guanosine monophosphate (GMP)-adenosine monophosphate (AMP); TBK1, tank-binding kinase 1; IRF, interferon regulatory factor; PD-L1, programmed death ligand-1; NF-κB, nuclear factor kappa-B; EGFR, epidermal growth factor receptor; RAS/RAF/MAPK, rat sarcoma/rapidly accelerated fibrosarcoma/mitogen-activated protein kinase signaling; PI3K/AKT/mTOR, phosphatidylinositol-3 kinase/protein kinase B/mammalian target of rapamycin signaling.

Increased PD-L1 on EVs derived from irradiated tumor cells induces tumor evasion

PD-L1 generally expresses on tumor membranes and binds with PD-1 on immunocytes ($CD8^+$ T) to achieve a crucial part of tumor immune evasion. Meanwhile, multiple types of research have concluded that PD-L1 level in tumors increases both *in vivo* and *in vitro* post-RT (42,105,106), and the upregulation of PD-L1 induced by cGAS-STING pathway activation after radiation was revealed by Du *et al.* (76) Since the discovery of EVs, numerous studies have reported that PD-L1 is also found on tumor-derived EV(TEX) membranes, especially exosomal PD-L1, which contributes significantly to immunosuppression through $CD8^+$T cell deactivation (41,107,108). Hence, exosomal PD-L1 is determined to be associated with tumor growth, progression, and metastasis (109). Another study revealed that total exosomal PD-L1 increases through either escalated exosome secretion or enhanced PD-L1 synthesis via multiple pathways activation, including the previously mentioned cGAS-STING pathway (107). Several mechanisms involving exosome secretion have been conducted and verified; it is suggested that the MAPK and P53 pathways are responsible for increased secretion after exposure to radiation stimuli (110,111). EV PD-L1-induced immune evasion has been verified in different types of tumors, such as NSCLC, prostate, breast, gastric, head, and neck cancer, among others (112). A study conducted by Timaner *et al.* (113) found that microparticles derived from irradiated breast cancer are associated with immune modulation and are responsible for cytotoxic T-cell inhibition; however, after blocking the PD-1/PD-L1 axis, T-cell inhibition is alleviated. This study also reveals that TEXs are mainly distributed in the spleen and liver instead of lymph nodes, which may be the potential mechanism of systemic immunomodulatory effects post-RT (114). Theodoraki *et al.* (115) demonstrated that tumor PD-L1 level is positively correlated with exosomal PD-L1 level. Therefore, we come to assume that radiation stimulates PD-L1 expression in tumor cells, which further impacts on exosomal PD-L1, and eventually causes tumor evasion. Although it is reported that increased PD-L1 expression on tumor cells is a positive marker for immunotherapy administration (116), evidence shows that exosomal PD-L1 has the potential to counteract with anti-PD-1/PD-L1 therapy due to competitive antagonizing with PD-1 on T-cells resulting in T-cell exhaustion and leading

to failure of ICI treatment (40,41,112). Poggio *et al.* (40) suggested that exosomal PD-L1 blockade rescues anti-tumor immunity and $CD8^+$ T-cell reinvigoration. Furthermore, studies have discovered glioblastoma-derived exosomes impact on not only $CD8^+$ T-cells, but induce macrophage phenotype skewing towards M2 as well as enhance PD-L1 expression on monocytes, which results in local TME immunosuppression (117-119). This phenomenon was also reported in another study, which deduced that TEXs signal through TLR2 and induce macrophage NF-κB activation leading to PD-L1 upregulation and conversion to immunosuppressive phenotype (120). Hence, inhibiting exosome secretion could be a potential target for immuno-RT adjuvant therapy.

RT stimulates PD-L1 expression in tumor-infiltrating cells

MDSCs

MDSCs are a heterogeneous population of immature myeloid cells which are capable of inducing T-cell inactivation and dysfunction. MDSCs are divided mainly into two subsets: polymorphonuclear MDSCs (PMN-MDSCs) and monocytic MDSCs (M-MDSCs), and several pieces of evidence indicate that M-MDSCs are principally responsible for its immunosuppression effect by PD-L1 expression (121). Thus, PD-L1 expression is found highly relative to tumor existence, inferring that TME might impact MDSCs (122). MDSCs alterations are also observed after RT, and PD-L1 expression on MDSCs in TME is detected elevated after single high-dose radiation. Meanwhile, the combination of anti-PD-L1 therapy and RT is shown to induce tumor regression in up-regulated PD-L1 on MDSCs scenarios (25). The effects of ablative hypofractionated radiotherapy, however, are drawing conflicting results that PD-L1 on MDSCs either increased (123) or decreased (124). RT also affects the quantities of circulating and tumor-infiltrating MDSCs. Studies indicate that circulating MDSCs increase after chemo-RT in cervical cancer and human papilloma virus (HPV)-related oropharyngeal cancer patients, whereas a decrease was observed in hepatocellular carcinoma patients who underwent RT. Xu *et al.* (27) illustrated that the CSF1/CSFR signaling pathway is responsible for MDSCs recruitment upon irradiation by recruiting the DNA damage-induced kinase ABL1 which binds with CSF1 gene promoter and eventually results in CSF1 escalation post-RT. In addition, other investigations suggest that the STING/type I interferon pathway (28) and NF-κB (125) also potentiate MDSCs recruiting. Interestingly, Chen *et al.* (126) reported that SBRT combined with sunitinib treatment alleviates immunosuppression by reducing MDSC and Treg infiltration. Similar studies also found that in TUBO (25) and MC38 (127) tumor animal models, concurrent anti-PD-L1 therapy and RT significantly decreased MDSC in TME and reinvigorated $CD8^+$ T-cells. In conclusion, RT can have either immunosuppressive or anti-tumor properties, however, the properties are dose-, scheduled regimen-, and tumor type-related. Hence, further investigations are needed to unveil MDSCs functions in tumor progression or remission and to instruct pharmaceutical and clinical practices.

Macrophages

Macrophages are critical components in the TME. Tumor-associated macrophages (TAMs) are generally categorized into two types, M1 and M2. The M1 phenotype is generally considered tumor-killing and innate immunogenic, M2 on the other hand, is immunosuppressive due to PD-L1 and other checkpoint molecules expression on its membrane, and is associated with tumorigenesis, immune evasion, and T-cell inhibition (128,129). Several studies have confirmed macrophage recruitment after RT through various approaches, including the CCL2 and CSF1 pathways, oxygen deprivation and HIF upregulation, and CXCR-4-signaling pathways (117,130,131). These recruited macrophages are mostly M2 phenotype and therefore, result in tumor progression and treatment failure (21). Genard *et al.* (22) reported that macrophage polarization induced by radiation is dose-related. Commonly, low-dose radiation promotes polarization towards M2 phenotype, whereas high-dose towards M1 phenotype in vitro. In another study, Meng *et al.* (132) concluded that a single large dose at 20 Gy or at 2 Gy in 10 fractions both leads to the M2 phenotype macrophage conversion. Contrarily, Klug *et al.* proposed an opposite view regarding macrophages under low-dose radiation differentiates to iNOS+/M1 phenotype and orchestrates T-cell function (133). Thus, similar to TEXs, M2 TAMs-derived exosomes are found to promote cancer cell migration through the PI3K-AKT signaling pathway (134) and M2 TAMs-derived exosomal miR21 is closely related to the mechanism of glioma immune escape (135). However, contradictory to most TEXs' functions as mentioned above, a study revealed that

microparticles secreted by radiated tumors locate and convert M2 TAMs in the TME to M1 TAMs by JAK-STAT and MAPK pathways activation (136). From the conflicting results, we can only conclude that the effect of macrophages in the TME is complex and still unclear due to various factors (e.g., cancer types and classifications, TME differences, radiation, etc.). Depleting M2 TAMs in the TME, of which the result has been verified, is still an effective approach to eliminate infiltrative macrophage-relevant immunosuppression (129). However, other than macrophage-derived exosomes, cancer-associated fibroblasts-derived exosomes are also found to be immunosuppressive via the miR92/PD-L1 pathway in breast cancer (137).

Conclusions

This article mainly summarized current research on PD-L1 in TME and tumor cells upon radiation and discussed its immunosuppression effects. Some published research suggests that PD-L1 level is widely upregulated after RT, not only on tumor cells, but macrophages, MDSCs, and derived exosomes. We summarized existing PD-L1 synthesis signaling pathways within tumor cells. It is noteworthy that the JAK/STAT pathway is considered the cardinal axis to trigger other signaling pathways involved in PD-L1 expression and to mediate other cancer-associated biological processes. RT is primarily responsible for DNA damage and ROS generation and subsequently activates JAK/STAT pathways.

Recognition of the immunomodulatory properties of RT provided inspiration to combine it with agents such as ICIs to induce synergistic anticancer function. Escalated tumoral PD-L1 has been confirmed with immune suppressive functions, such as $CD8^+$ T cell inhibition, tumor progression, metastasis, and so on, and inhibits synthesis and release of cytokines, and diminishes abscopal effects. Although some patients with high PD-L1 expression are reported to exhibit better outcomes after anti-PD-1/PD-L1 therapies (138,139), new evidence shows that tumor-derived exosomal PD-L1 are positively related to RT and are capable of inducing resistance to ICI treatment (140). Hence, targeting the secretion of EVs might be a potential therapeutic method against the immunosuppression induced by exosomal PD-L1.

When it comes to the issue regarding T cell exhaustion in the TME, PD-L1 is currently considered the main cause of this phenomenon, however, other molecules are also found responsible. Cytotoxic T-cell-associated antigen 4 (CTLA-4), T-cell immunoglobulin mucin-3 (TIM-3), lymphocyte activation gene 3 (Lag-3), T-cell immunoglobulin and ITIM domain (TIGIT) (141) are T-cell receptors that mediate T cell inactivation, and other substances such as IL-2 (142) and cholesterol (143) also contribute to T cell exhaustion. Study found that TIM-3 upregulation and Treg infiltration mediate resistance to RT and PD-L1 blockade in head and neck squamous cell carcinoma. And significant enhanced T-cell cytotoxicity is achieved when treated with anti-TIM-3 concurrently with anti-PD-L1 and RT (144). Similarly, promoted responses and immunity are also observed in melanoma treated with RT, anti-CTLA-4, and anti-PD-L1 combination (145).

In addition to the diversity of immune checkpoint inhibitor selection, delivery technology and imaging improvements in recent decades have promoted the development of different radiotherapy methods. More than one modulation of RT was reported to improve immune regulation in TME. Including the delivery of using numerous fractions of relatively low doses, stereotactic ablative radiotherapy (SABR), and moreover, personalized ultrafractionated stereotactic adaptive radiotherapy (PULSAR). Low dose radiotherapy of murine tumors was reported to promote T cell infiltration and facilitate the efficacy of the combinatorial immunotherapy in an IFN-dependent manner (146). And also, there was evidence to elucidate the safety and efficacy of the SABR-ICI combination (147). Recently reported PULSAR combined with PD-L1 achieved better tumor control than traditional daily fractions (148).

Despite the several mechanisms of tumor evasion induced by RT, they should not be the reason to deny the therapeutic effect of RT, let alone other therapies are proven more effective when combined with RT. Conventional photon RT may be more likely to induce tumor evasion, recent studies show that carbon-ion radiotherapy (CIRT) could trigger immune responses and sensitize tumors to PD-1 therapy (149,150). Even if certain tumors don't respond well to RT, other treatment options can still be taken. Meanwhile, a thorough review conducted by Yap *et al.* (151) demonstrated that combination of immunotherapy with chemotherapy and angiogenesis inhibitors or dual immune-checkpoint blockade benefits non-immunogenic tumors as well as bring up novel immunotherapy combination approaches.

In this article, we tried to elaborate PD-L1-related signaling pathways and their radiation-related expression

cell profiles, nevertheless certain mechanisms remained unveiled. Although some novel molecular studies on PD-L1 have suggested other possible pathways, they are not mentioned in this article because insufficient concrete evidence on the mechanisms has been demonstrated. The functions exosomal PD-L1 induces are mostly experimental conclusions and theories as well, further verifications remain needed. As for the effects of tumor-infiltrating cells, such as TAMs and MDSCs, conflicting results of their functions post-RT are reported possibly due to various factors (e.g., dose and fractionation of RT, tumor state, and type). Hence, more studies on the biological behavior of these cells are needed for greater understanding, in order to treat against or induce these cells' transitions into anti-tumor phenotypes.

Generally, the effect of RT alone has been diminished as more cases of tumor evasion, metastasis, and resistance to therapies are observed and reported. A combination of multiple therapies is shown to be more effective and prolongs patients' overall survival significantly. Meanwhile, as the study of the cancer-immunity cycle extends to molecular aspects, new strategies could be developed to amplify the efficacy of RT.

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Footnote

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