Review Article

Narrative review of emerging roles for AKT-mTOR signaling in cancer radioimmunotherapy

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Objective: To summarize the roles of AKT-mTOR signaling in the regulation of the DNA damage response and PD-L1 expression in cancer cells, and propose a novel strategy of targeting AKT-mTOR signaling in combination with radioimmunotherapy in the era of cancer immunotherapy

Background: Immunotherapy has greatly improved the clinical outcomes of many cancer patients and has changed the landscape of cancer patient management. However, only a small subgroup of cancer patients (~20–30%) benefit from immune checkpoint blockade-based immunotherapy. The current challenge is to find biomarkers to predict the response of patients to immunotherapy and strategies to sensitize patients to immunotherapy.

Methods: Search and review the literature which were published in PUBMED from 2000–2021 with the key words mTOR, AKT, drug resistance, DNA damage response, immunotherapy, PD-L1, DNA repair, radioimmunotherapy.

Conclusions: More than 50% of cancer patients receive radiotherapy during their course of treatment. Radiotherapy has been shown to reduce the growth of locally irradiated tumors as well as metastatic non-irradiated tumors (abscopal effects) by affecting systemic immunity. Consistently, immunotherapy has been demonstrated to enhance radiotherapy with more than one hundred clinical trials of radiation in combination with immunotherapy (radioimmunotherapy) across cancer types. Nevertheless, current available data have shown limited efficacy of trials testing radioimmunotherapy. AKT-mTOR signaling is a major tumor growth-promoting pathway and is upregulated in most cancers. AKT-mTOR signaling is activated by growth factors as well as genotoxic stresses including radiotherapy. Importantly, recent advances have shown that AKT-mTOR is one of the main signaling pathways that regulate DNA damage repair as well as PD-L1 levels in cancers. These recent advances clearly suggest a novel cancer therapy strategy by targeting AKT-mTOR signaling in combination with radioimmunotherapy.

Keywords: Mechanistic target of rapamycin (mTOR); AKT; drug resistance; DNA damage response; immunotherapy; programmed death ligand 1 (PD-L1); DNA repair; radioimmunotherapy

Submitted Aug 06, 2021. Accepted for publication Sep 27, 2021.
doi: 10.21037/atm-21-4544
View this article at: https://dx.doi.org/10.21037/atm-21-4544

Introduction

There are 20 subfamilies of membrane-bound receptor tyrosine kinases (RTKs), including 58 members (1). These RTKs are important regulators of signal transduction pathways that integrate intracellular and extracellular cues to control cell growth, differentiation, proliferation, survival, and metabolism. Genetic and epigenetic alterations in RTKs result in deregulated kinase activity, leading to changes in multiple downstream signaling pathways (2). Alterations in RTK-mediated signaling pathways are one of the main mechanisms of tumorigenesis and anticancer treatment failure, and targeting RTK signaling is the main strategy for the development of targeted cancer therapy as a monotherapy or in combination with other treatment modalities (2,3). AKT (also called protein kinase B)-mechanistic target of rapamycin (mTOR) is one of the most important downstream effectors of RTK signaling (4,5). Deregulation of AKT-mTOR may result from many factors, including, but not limited to, mutations and/or amplification of RTK, overexpression of RTK ligands, mutations and/or amplification of phosphatidylinositol 3-kinase (PI3K) subunits, and mutations of RAS and phosphatase and tensin homolog (PTEN) (6,7). AKT-mTOR signaling is dysregulated in most cancers and is believed to be an important and attractive cancer therapeutic target. In the past decades, extensive efforts have been made to develop inhibitors targeting AKT-mTOR signaling, particularly mTOR kinase inhibitors. However, though very promising in preclinical studies, the results from most clinical trials are disappointing, with poor effects of these inhibitors as a monotherapy (8,9). To understand the underlying mechanisms by which most cancers are insensitive or not responsive to AKT-mTOR targeted cancer therapy under clinical conditions, there is an urgent need to deeply explore the roles of AKT-mTOR signaling in the regulation of autonomous cancer cells as well as the tumor environment. Recent findings on the essential role of AKT-mTOR signaling in regulating cancer immunity (10-13) and the DNA damage response (14-17) may shed light on the distinct discrepancy of the results between preclinical and clinical studies. These recent findings also provide us with new opportunities to rationally combine AKT-mTOR inhibitors with other cancer therapy modalities, particularly immune checkpoint blockade-based immunotherapy. The present review will focus on discussing the mechanisms by which AKT-mTOR signaling regulates programmed death ligand 1 (PD-L1) and the DNA damage response in cancer cells, and proposes the rationale for targeting AKT-mTOR signaling in combination with radiation and immunotherapy. We present the following article in accordance with the Narrative Review reporting checklist (available at https://dx.doi.org/10.21037/atm-21-4544).

AKT-mTOR signaling in cell cycle progression and DNA replication

Accurate and complete duplication and transmission of DNA to daughter cells is essential for the maintenance and survival of an organism, defects of which lead to human diseases including cancer. AKT-mTOR signaling plays a pivotal role in cell cycle progression by coordinating DNA replication and the activity of cyclin dependent kinases (CDKs), the drivers of cell cycle progression (*Figure 1*). p21 and p27 are potent and key inhibitors of CDKs (18), and are the key effectors in the control of cell cycle checkpoints by DNA damage checkpoints. Activated AKT phosphorylates p21 and p27 leading to their cytoplasmic accumulation, thereby release and activation of CDKs, especially cyclin D dependent CDK4/6 and cyclin E dependent CDK2 (19-22). In response to DNA damage or replication stress, ATM and ATR checkpoints will be activated to halt cell cycle progression, providing time to repair DNA damage and resolve replication stress (23-25). These are mainly accomplished by p53 and CHK1, the common downstream effectors of ATM and ATR checkpoints. AKT inhibits the p53-mediated cell cycle checkpoint by phosphorylating and activating MDM2, a direct interactor and inhibitor of the p53 protein. Activation of MDM2 leads to ubiquitination and degradation of p53 (26,27). CHK1 is the key regulator of both DNA damage and the replication stress response (RSR), and its deregulation results in cell death and genome instability (23). AKT phosphorylates CHK1 at Ser280, which leads to cytoplasmic retention of CHK1, thereby preventing the functions of CHK1 in the nucleus (28). Moreover, translesion DNA synthesis (TLS) is the main mechanism accounting for DNA damage tolerance and the high mutator phenotype of cancer cells. TLS is enhanced by RAD6/RAD18-mediated mono-ubiquitination of proliferating cell nuclear antigen (PCNA), which promotes the switch from replicative DNA polymerases δ, θ, and ε to Y-family TLS polymerases η, ι, κ, and Rev 1 (29,30). In response to ultraviolet (UV) radiation, AKT promotes TLS and cell survival by enhancing the mono-ubiquitination of PCNA by RAD6/RAD18 (31). Thus, AKT deregulation may promote cancer cell genome instability and survival

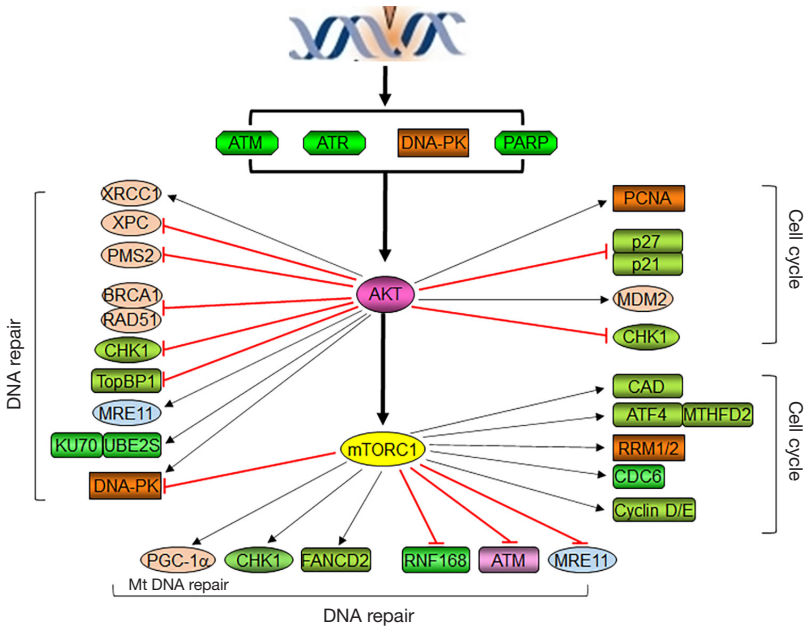

Figure 1 The complex involvement of AKT-mTORC1 signaling in the DNA damage response and cell cycle progression. DNA damaging agent-based chemotherapy and radiotherapy, especially radiotherapy, activate AKT-mTORC1 signaling pathways via ATM, ATR, DNA-PK, or PARP depending on the type of DNA damaging agents. Activated AKT kinase regulates the activity of cyclin dependent kinases (CDKs), DNA replication, and DNA repair via multiple mechanisms. mTORC1 is a downstream target of AKT and is the central node in the regulation of DNA replication and DNA repair in response to genotoxic stress in both nuclear and mitochondrial DNA (via PGC-1α). The specific mechanisms are detailed in the text.

by enhancing DNA replication under both normal and genotoxic conditions.

The more important mechanisms of AKT in the regulation of DNA replication and cell cycle progression are through its downstream target mTORC1, a protein complex formed by mTOR kinase with mLST8, PRAS40, Deptor, and Raptor. One of the conserved functions of mTORC1 is to promote protein translation via activating p70S6K and eIF4E signaling (32). mTORC1 promotes G1 phase progression and G1/S phase transition through enhancing the protein translation of cyclins D and E, which are the partner and activator of CDK4/6 and CDK2, respectively (33-35). Besides promoting CDK activity, mTORC1 increases DNA replication via multiple other mechanisms. A balanced deoxyribonucleotides (dNTP) pool is essential for accurate and efficient DNA synthesis for replication and repair, defects of which lead to cell death and genomic instability (36-38). mTORC1 increases *de novo* pyrimidine synthesis via activating p70S6K to phosphorylate CAD (carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase) at Ser1859 (39,40) and purine production via the ATF4/MTHFD2 axis (41). Ribonucleotide reductase (RNR) catalyzes the rate-limiting step in the production of dNTPs from ribonucleotides, and its expression and activity are tightly controlled in all organisms under normal growth and stressful conditions (36,37,42). The mammalian RNR is composed of two identical RRM1 and two small catalytic subunits of either RRM2 or p53R2. Both RRM1 and RRM2 are dynamically regulated during cell cycle progression, while p53R2 is regulated by p53 in response to genotoxic stress (36-38,42). The activity of RNR is principally controlled by RRM2 levels in mammalian cells (43). mTOR signaling enhances the cap-dependent protein translation and gene transcription of RRM1 and RRM2, and p53 suppresses RRM1 and RRM2 via inhibiting mTORC1 (44). Similarly, mTOR maintains cell survival but at the cost of an increased mutation rate in response to genotoxins by increasing the expression of RNR subunits in budding yeast (45). Similar regulation of RNR subunit expression by mTOR signaling was found in fission yeast (46). Therefore, mTORC1 upregulation may increase cell survival and accelerate genome instability by enhancing the activities of CDKs and the levels of dNTPs, the elevation of which is also required

for TLS polymerases to tolerate DNA damage.

Once and only once per cell cycle for each DNA replication is essential for maintaining the integrity of genetic information. A pre-replicative complex (pre-RC) forms at the origin of replication during late mitosis and early G1 phase (47). In most eukaryotes a pre-RC is composed of six origin recognition complex proteins (ORC1–6), CDC6, CDT1, and a heterohexamer of MCM proteins (MCM2–7). CDC6 and CDT1 are licensing factors of DNA replication and their deregulation results in impaired DNA replication, and therefore, genome instability (48). Insufficient origin licensing during G1 phase, increased and/or ectopic licensing during G1, or re-licensing during the S and G2 phases accounts for oncogene-induced replication stress (48-50). Moreover, aberrant expression of CDT1, CDC6, and ORC or abrogation of their regulation results in re-replication of the genome, leading to genome instability (51-53). mTOR signaling has been shown to promote DNA replication origin licensing through upregulating CDC6 (54,55). CDC6 is essential for the loading of the MCM2-7 complex during DNA replication. Consistently, the dramatic reduction of MCM2-7 components and PCNA on chromatin following mTOR inhibition suggests that mTOR may promote the loading and maintenance of the MCM2–7 complex on chromatin by positively regulating CDC6 (54). In addition to replication licensing, CDC6 also plays multiple other roles in ensuring precise chromosome duplication (56), and is crucial for proper S-phase DNA replication progression (57). Moreover, CDC6 can trigger a checkpoint response, which ensures that all DNA is replicated before mitotic entry (58). Taken together, mTOR signaling promotes DNA replication by positively regulating the activity of RNR and the production of purines and pyrimidines, as well as CDC6 expression.

AKT-mTOR signaling in DNA damage response and repair

All living organisms have developed genome surveillance systems during evolution to cope with constant attacks by physical, chemical, and biological agents (59). A genome surveillance system is a signal transduction cascade composed of signals, sensors, transducers, and effectors (60). ATM-CHK2 and ATR-CHK1 checkpoints surveil the genomic integrity in metazoans. DNA double-stranded breaks (DSBs) produced by ionizing radiation or DNA metabolism-produced reactive oxygen species (ROS) and reactive nitrogen species (RNS) are sensed by the MRE11-RAD50-NBS1 complex (MRN), which recruits ATM to damage sites for phosphorylation and activation (61). Activated ATM in turn phosphorylates the MRN complex and histone H2AX to amplify the signals. Following recruitment to DSBs, a plethora of substrates including CHK2, MDM2, and p53 are phosphorylated by ATM with the help of mediators MDC1, 53BP1, and BRCA1. Single-stranded breaks (SSBs) are common DNA damage intermediates produced by different kinds of genotoxins, and are rapidly coated by replicating protein A (RPA) in response to DNA replication fork stalling or slowing down (replication stress). The ATRIP-ATR complex binds to RPA-coated nucleofilament and phosphorylates CHK1 with the help of TOPBP1, Claspin, RAD9-RAD1-HUS1 (9-1-1 complex), and RAD17-RFC clamp loader (62,63). Activated CHK2 and CHK1 phosphorylate numerous downstream effectors to amplify and relay the signals to induce DNA damage responses (DDRs) such as cell cycle arrest, senescence, or apoptosis (25,59,60). ATR-CHK1 is at the heart of the DDR, especially in the RSR (23,64). ATR orchestrates multiple branches of RSR by signaling to arrest cells at the S and G2/M phases, stabilizing stalled replication forks, inhibiting DNA replication of late origin firing, promoting adjacent dormant origin firing and increasing dNTP biosynthesis (23,25). It was reported that transient inhibition of mTOR kinase leads to CHK1 checkpoint activation (54), while long-term mTOR signaling suppression results in decreased CHK1 levels (65,66). In agreement with the essential role of CHK1 in the stabilization of replication forks, mTOR inhibition results in replication fork collapse under DNA lesions and replication stress in yeast (45). Therefore, mTOR signaling maintains cell survival in part by sustaining the CHK1 checkpoint to stabilize replication forks under replication stress.

The Fanconi anemia (FA) signaling pathway maintains genome integrity and cell survival by promoting DNA damage repair through TLS, nucleotide excision repair (NER), and homologous recombination (HR). FA signaling is activated by different kinds of genotoxins and is important for the activation of the ATM-CHK2 and ATR-CHK1 checkpoints. In response to DNA lesions, activation of the FA core E3 ubiquitin ligase complex leads to mono-ubiquitination of FANCI and FANCD2, which are recruited to DNA damage sites to promote DNA repair (67,68). We discovered that FANCD2 is required for timely ATM-CHK2 activation in the early stages of FA

signaling-mediated repair of interstrand crosslink induced DNA lesions (69). Importantly, we and other labs found that mTOR positively controls FANCD2 expression via multiple mechanisms (69,70). Thus, it is possible that the promotion of FANCD2-dependent activation of the ATM checkpoint in the early response to DNA damage is one of the mechanisms by which AKT-mTOR signaling promotes genome stability under normal growth conditions and cell survival in response to genotoxins. In addition, accumulating evidence has shown an important role for FANCD2 in the maintenance of replication fork stability (71). Under replication stress, mono-ubiquitinated FANCD2 is recruited to stalled replication forks to stabilize forks, restart stalled replication forks, and suppress origin firing (72-74). Taken together, mTOR signaling maintains cell survival and replication fork stability under replication stress through upregulating both CHK1 and FANCD2.

On the other hand, mTORC1 has also been demonstrated to suppress DNA damage response and repair. First, mTORC1 negatively regulates ATM expression in pediatric rhabdomyosarcoma (75). mTORC1 suppresses ATM expression via S6K1/2 signaling by upregulating miR-18a and miR-421, both of which target ATM mRNA. These miRNAs are under the control of the MYCN transcription factor, and one of the mechanisms by which mTORC1 suppresses ATM expression is through sustaining MYCN by S6K1 signaling. Second, mTORC1-S6K signaling can dampen DNA repair via phosphorylation of the E3 ubiquitin ligase RNF168, resulting in its accelerated proteolysis (76). Third, mTORC1 suppresses the activity of DNA-PK through inhibition of PP2A-mediated DNA-PK catalytic subunit (DNA-PKcs) dephosphorylation, which is required for DNA-PK activation (77). Moreover, mTORC1 reduces MRE11 protein levels via p70S6K1-mediated phosphorylation and degradation of MRE11 (78). Regarding the important roles of ATM and MRE11 in the early stages of DDR, mTORC1 seems to prevent ATM checkpoint activation. Furthermore, RNF168 is recruited to DSB sites to ubiquitinate histone H2A at K13/15, which leads to the binding of 53BP1 with H2A-K13/15 to promote NHEJ (79). Therefore, mTORC1 might suppress NHEJ in response to DSBs by inhibiting the functions of DNA-PK and RNF168. However, suppression of the ATM checkpoint and NHEJ by mTORC1 may be cancer type and cell type dependent.

Though upstream of mTORC1 signaling, AKT seems to promote both early DSB repair by regulating DNA-PK and later DSB repair by controlling MRE11. In response to DSBs, AKT binds and promotes the recruitment of DNA-PKcs to DSBs. Moreover, AKT directly phosphorylates DNA-PKcs and increases its activity at DSBs (80,81). Interestingly, AKT physically interacts with and phosphorylates UBE2S at Thr152, leading to the stability and accumulation of UBE2S, which associates with KU70 to enhance NHEJ (82). AKT also increases MRE11 expression after irradiation via the GSK3β-catenin/LEF pathway (83). mTORC1 increases CHK1, while AKT phosphorylates CHK1 leading to CHK1 cytoplasm retention; moreover, AKT phosphorylates TopBP1 (which is required for ATR/CHK1 activation) at S1159 leading to TopBP1 oligomerization, which prevents TopBP1 from interaction with and activation of ATR (28,84). For NER, the role of AKT is controversial, since it can promote the expression of XRCC1 while suppressing the expression of XPC, both of which are the key components of NER machinery (85,86). In addition, dysregulation of DNA mismatch repair (MMR) results in a mutator phenotype and plays an important role in the high tumor mutation burden (TMB) of many cancers. PMS2 is a key component of the MMR machinery and plays an important role in preserving genome stability. It was reported that AKT could modulate PMS2 stability by phosphorylating PMS2, leading to its degradation and impaired nuclear localization (87,88). Moreover, in breast cancer cells, AKT has been shown to inhibit HR by cytoplasmic retention of BRCA1 and RAD51 proteins, resulting in a BRCA1-deficient-like phenotype (89). Overall, AKT seems to promote NHEJ while suppressing HR and MMR, indicating an important role for AKT signaling deregulation in the mutagenesis of cancer cells.

AKT-mTOR signaling in the regulation of PD-L1

Programmed death-1 (PD-1) and its ligand PD-L1 function as an immune checkpoint that plays a key role in immune homeostasis by fine-tuning the adaptive immune response (90). PD-L1 expression on the cell membrane is tightly controlled at different levels by many factors, including intrinsic and extrinsic factors which modulate constitutive and inducible PD-L1 expression, respectively. For survival, during the multiple processes of tumorigenesis and the evolution of cancer cells, tumor cells gain the capacity to hijack the fine tuning of PD-L1 expression to evade immune responses, thereby escaping death by the host adaptive immune system. Accordingly, targeting PD-1 or PD-L1 with specific inhibitors to prevent their

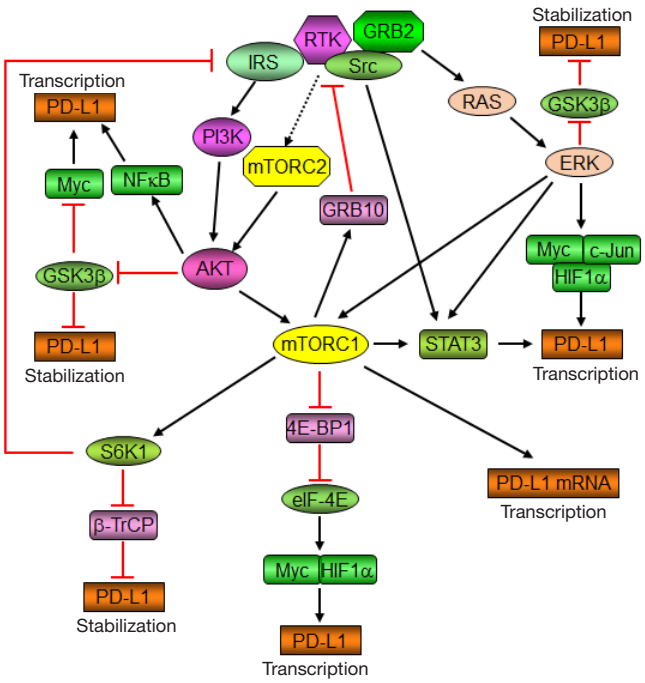

Figure 2 Regulation of PD-L1 by the AKT-mTORC1 signaling pathway. AKT promotes PD-L1 gene transcription via GSK3β-myc and NF-κB, and PD-L1 protein stabilization via GSK3β. mTORC1 increases PD-L1 gene transcription through eIF-4E-mediated translation of PD-L1 gene transcription factors myc and HIF1α. It also increases PD-L1 protein stabilization through S6K1-β-TrCP and PD-L1 protein translation by promoting the association of PD-L1 mRNA with active polyribosomes. mTORC1 also elevates PD-L1 gene transcription through STAT3, which is activated by RTK and RAS-ERK signaling. RAS-ERK signaling promotes PD-L1 gene transcription via PD-L1 gene transcription factors myc, c-Jun, and HIF1α, and PD-L1 protein stabilization via GSK3β. Inhibition of mTORC1 by rapamycin (or rapalogs) results in paradoxical activation of both AKT and ERK signaling via S6K1 and GRB10 negative feedback loops. The specific mechanisms are detailed in the text.

interaction has improved the outcomes of many cancers. Correspondingly, PD-L1 has become a biomarker for cancer immunotherapy, and the detection of PD-L1 by immunohistochemistry using PD-1 or PD-L1 antibodies has become routine in predicting the response of patients to immunotherapy (91-93). Therefore, PD-L1 is both the target and a biomarker for the immunotherapy of many cancers, particularly lung cancers. However, PD-L1 expression on tumor cells is highly variable and is associated with distinct clinicopathological and genomic characteristics in different cancer types, and even the subgroups of the same cancer type (94). Elucidating the mechanisms of PD-L1 deregulation in different caner types with distinct genetic and epigenetic alteration patterns will help to develop effective strategies to delay or overcome the resistance to immunotherapy, which may be achieved by increasing or re-enabling PD-L1 expression on tumor cells or preventing PD-L1 downregulation.

The regulation of PD-L1 expression is wired by complex networks. Extrinsic factors including cytokines, growth factors, hypoxia, chemotherapy, and radiation therapy regulate PD-L1 gene transcription. The main intrinsic factors include genetic alterations of the oncogenic signaling pathways RAS/MAPK, PI3K/AKT/mTOR, and JAK/STAT, tumor suppressors TP53, PTEN, and STK11, DNA damage repair components BRCA1, BRCA2, and ATM, and transcription factors HIF1α, c-Myc, and NF-κB. Moreover, CD274 (the gene encodes PD-L1) promoter methylation, inhibition of histone deacetylase, and regulation of gene translation by miRNAs contribute to PD-LI expression at the epigenetic level. Importantly, phosphorylation, ubiquitination, glycosylation, and palmitoylation play an important role in the regulation of PD-L1 protein stability (12,95). How these complex factors are coordinated to tune PD-L1 levels for cancer cells to evade immunity is largely unknown. Answers to this question may be the key to personalized cancer medicine through the combination of immunotherapy with other cancer treatment modalities including radiotherapy, chemotherapy, hormone therapy, and targeted molecular therapies.

AKT-mTOR signaling is the convergence and center of these complicated modules that regulate PD-L1 at the genetic, transcriptional, translational, and post-translational levels (*Figure 2*). Loss of function mutations of PTEN or gain of function mutations/amplification of PIK3CA lead to activation of AKT-mTOR signaling. Current available data suggests that activated AKT may upregulate PD-L1 via several mechanisms (96-98). First, AKT phosphorylates and activates the transcription factor NF-κB to promote PD-L1 gene transcription (99-101). Second, GSK3β is a key regulator of PD-L1 and directly phosphorylates PD-L1, leading to ubiquitin-proteasome-mediated degradation of PD-L1 (102). GSK3β also phosphorylates and destabilizes c-Myc (103), which is a potent transcription factor for PD-L1 gene transcription (104). AKT may upregulate PD-L1 levels by phosphorylating and inactivating GSK3β, which suppresses PD-L1 gene transcription via destabilizing c-Myc and promotes PD-L1 protein proteolysis. The

third mechanism of AKT in upregulating PD-L1 is through activating its downstream target mTORC1. Both c-Myc and HIF1α are transcription factors of PD-L1 gene expression (104,105). Activation of mTORC1 by AKT leads to enhanced protein translation of c-Myc and HIF1α (106), which in turn can promote PD-L1 gene transcription. Moreover, β-TrCP mediates ubiquitination of PD-L1 to promote its degradation via the proteasome (102), and β-TrCP activity is inhibited by the direct mTORC1 downstream target p70S6K (107). mTORC1 may increase PD-L1 protein accumulation via p70S6K-β-TrCP signaling (107). Moreover, mTORC1 increases the association between PD-L1 mRNA and activated polyribosomes to enhance PD-L1 protein translation (108). In addition, STAT3 is one of the most important transcription factors of PD-L1 gene transcription via IRF1/3 (108,109). mTORC1 phosphorylation and activation of STAT3 may promote its nuclear translocation and activation of PD-L1 gene transcription. Taken together, AKT positively regulates PD-L1 at the transcriptional and post-transcriptional levels in mTORC1 dependent and independent ways.

RAS-ERK1/2 signaling is activated by multiple mechanisms including RAS mutation and RTK activation and has a cross talk with mTORC1 signaling. ERK1/2 kinases directly phosphorylate and activate mTORC1, which in turn may increase PD-L1 levels via STAT3, Myc, and HIF1α-mediated PD-L1 gene transcription, p70S6K-β-TrCP induced PD-L1 stabilization, and polyribosome enhanced PD-L1 protein translation (110). Furthermore, through mTORC1, ERK1/2 may directly phosphorylate and activate STAT3 to promote PD-L1 gene transcription (111). Transcription factors c-Jun, Myc, and HIF1α are important downstream targets of RAS signaling and play key roles in the pathophysiology of human diseases resulting from mutations in RAS-RAF-MEK-ERK signaling (112,113). Independently of mTORC1, activation of ERK1/2 may increase the transcription factors c-Jun, c-Myc, and HIF1α to promote PD-L1 gene expression (114). In addition, similar to AKT, ERK1/2 may stabilize the PD-L1 protein by directly phosphorylating and inactivating GSK3β (102). Therefore, deregulation of RAS-RAF-MEK-ERK signaling increases PD-L1 levels through mTORC1 dependent and independent mechanisms.

Radiation and immunotherapy

Targeting immune checkpoint proteins PD-L1/PD-1 and CTLA-4/CD80/CD86 has greatly improved the clinical outcomes of many cancer patients. However, the response of patients with solid tumors to immunotherapy is modest and depends on individual patients (115). Searching for biomarkers to predict the response of cancer patients to immunotherapy and strategies to sensitize tumors to immunotherapy are urgently needed in the field of immunotherapy. Though cancer cells have the same genome as the host body cells, the success of cancer immunotherapy indicates that cancer cells have been "licensed" for immune response. The origin of the "license" of cancer cells for human immunity has been ascribed to cancer genome instability, which produces neoantigens to be recognized by adaptive immunity (116-118). In agreement with this hypothesis, tumors with a higher mutation burden have a better response to immunotherapy (119). Accumulation and tolerance of DNA damage together with impaired DNA damage response and repair contribute to the genome instability of cancer cells (23,120). Therefore, the induction of DNA damage may enhance cancer immunotherapy (121). Conventional radiotherapy and chemotherapy have been attributed to eliciting DNA damage. Emerging evidence has shown that chemoradiation or radiation "boosts" the cancer immunotherapy response (122,123). Radiotherapy has been shown to enhance cancer immunotherapy, leading to more than 100 clinical trials of radiation and immunotherapy combinations across cancer types (124-126). The main rationale of developing cancer radioimmunotherapy is the "boost" of the immune response by radiation via several mechanisms (122). First, due to the intrinsic defects of cancer cells in cell cycle checkpoints, DNA damage checkpoints, DNA replication checkpoints, and mitotic checkpoints (also called spindle assembly checkpoints), irradiated tumor cells with unrepaired and/or incompletely replicated DNA result in cytoplasmic DNA fragments and micronuclei, which activate cGAS/STING signaling. Activation of cGAS/STING signaling induces type I interferons (IFNα and IFNβ) to activate the innate immune response (126-129). Second, radiation induces DNA damage leading to neoantigen production, which will be presented to antigen presenting cells (APC) including dendritic cells to activate T cells in lymph nodes. Activated T cells will induce the anticancer adaptive immune response both in the locally irradiated tumor cells and non-irradiated metastatic tumors (abscopal effect) (126). Third, radiation treatment of tumor cells with a high mutation burden and microsatellite instability (MSI) increases the production of neoantigens to further enhance the immune response (125,130). In addition, radiation increases PD-L1 production through

the cGAS/STING-IFNα/IFNβ-STAT3 axis as well as activated T cell-mediated IFNγ-STAT1 signaling (130,131). It seems paradoxical that radiation promotes PD-L1 expression to suppress immunity, however, during immune checkpoint blockade-based immunotherapy, PD-L1 will be neutralized by either anti-PD-1 or PD-L1 antibodies. Most importantly, the induction of PD-L1 expression has been shown to enhance the effects of immunotherapy in many cancers, especially lung cancer (12). Therefore, the net effect of radiotherapy is to enhance immunotherapy by the induction of innate and adaptive anticancer immunity.

Targeting AKT-mTOR signaling in combination with radiation and immunotherapy

As discussed above, AKT-mTOR signaling upregulates PD-L1 levels (*Figure 2*) and regulates DNA damage response and repair via multiple mechanisms (*Figure 1*), which is closely correlated with cancer immunity. Most importantly, genotoxic chemotherapy and radiotherapy induce potent and rapid AKT activation through ATM, ATR, DNA-PK, and PARP depending on the types of DNA lesions (76,132-139). In normal cells, DNA damage/replication stress activates p53 signaling pathways to inhibit mTORC1 activity by stimulating the expression of TSC2, Sestrins, REDD1, and AMPKβ, all of which are negative regulators of mTORC1 activity. Nevertheless, in most cancer cells, this inhibition of mTORC1 by DNA damage and replication stress has been abolished mainly by TP53 mutations during tumorigenesis. Thus, cancer cells survive genotoxic stresses including DNA damage and replication stress through the activation of ATM/ATR/DNA-PK as well as AKT-mTORC1 signaling.

AKT-mTORC1 is the convergence of RTK and RAS-ERK signaling, which are upregulated in most cancers (32). AKT-mTORC1 signaling is a key regulating module in cell cycle progression and DNA damage repair (140), which has been supported by mounting evidence that molecular targeting of AKT-mTORC1 signaling enhances chemotherapy and radiotherapy-induced DNA damage and replication stress, and sensitizes cancers to chemotherapy and radiotherapy (64,74,140-142). Therefore, the upregulation of AKT-mTORC1 signaling may dampen radiation-produced cytoplasmic DNA fragments and micronuclei to stimulate anticancer innate immunity and neoantigen production to increase anticancer adaptive immunity. In summary, AKT-mTORC1 signaling deregulation leads to the resistance of cancer cells to genotoxin-based chemoradiation and immunotherapy via several mechanisms (*Figure 3A*). First, AKT-mTORC1 signaling is not only upregulated by genetic and epigenetic alterations but is also activated by chemotherapy and radiotherapy agents. Second, AKT-mTORC1 signaling promotes the survival of autonomous cancer cells by enhancing DNA damage repair. Third, AKT-mTORC1 signaling-mediated DNA damage repair prevents radiation from producing cytoplasmic DNA fragments and micronuclei to stimulate anticancer innate immunity via the cGAS/STING-IFNα/IFNβ axis. Fourth, AKT-mTORC1 signaling-mediated DNA damage repair inhibits radiation-induced neoantigen production to increase anticancer adaptive immunity. Most importantly, AKT-mTORC1 signaling elevates PD-L1 levels to enhance immune checkpoints for cancer cells to escape from immune surveillance. Therefore, targeting AKT-mTORC1 signaling may potently sensitize cancer cells to radioimmunotherapy via several mechanisms (*Figure 3B*). During cancer radioimmunotherapy, inhibition of AKT-mTORC1 signaling may enhance radiation-produced cytoplasmic DNA fragments and micronuclei to stimulate anticancer innate immunity via the cGAS/STING-IFNα/IFNβ axis and neoantigen production to increase anticancer adaptive immunity, while upregulated PD-L1 will be neutralized by antibodies to PD-L1 or PD-1. Thus, the net outcome of AKT-mTORC1 inhibition in combination with radiation and immunotherapy is an enhanced antitumor innate and adaptive immune response.

Intriguingly, though it has been well documented that AKT-mTORC1 signaling positively regulates PD-L1 levels via multiple mechanisms, amounting evidence has demonstrated that targeting AKT-mTORC1 signaling paradoxically leads to PD-L1 upregulation. This is probably due to the complex negative and positive feedback loops of the PI3K-AKT-mTORC1 signaling network (143). For example, inhibition of mTORC1 via either pharmacological inhibition or genetic silencing leads to robust activation of AKT kinase through p70S6K1-IRS1 and GRB10 negative feedback loops (144,145). Moreover, mTORC1 inhibition also induces feedback activation of MAPK/ERK, which is a key positive regulatory node of PD-L1 (146,147). The paradoxical upregulation of PD-L1 after targeting AKT-mTORC1 signaling may be the main reason for the resistance of cancer cells to inhibitors of AKT-mTORC1 signaling in combination with chemotherapy and radiotherapy in various cancer types in clinical trials. This also indicates that the other downstream signaling pathways

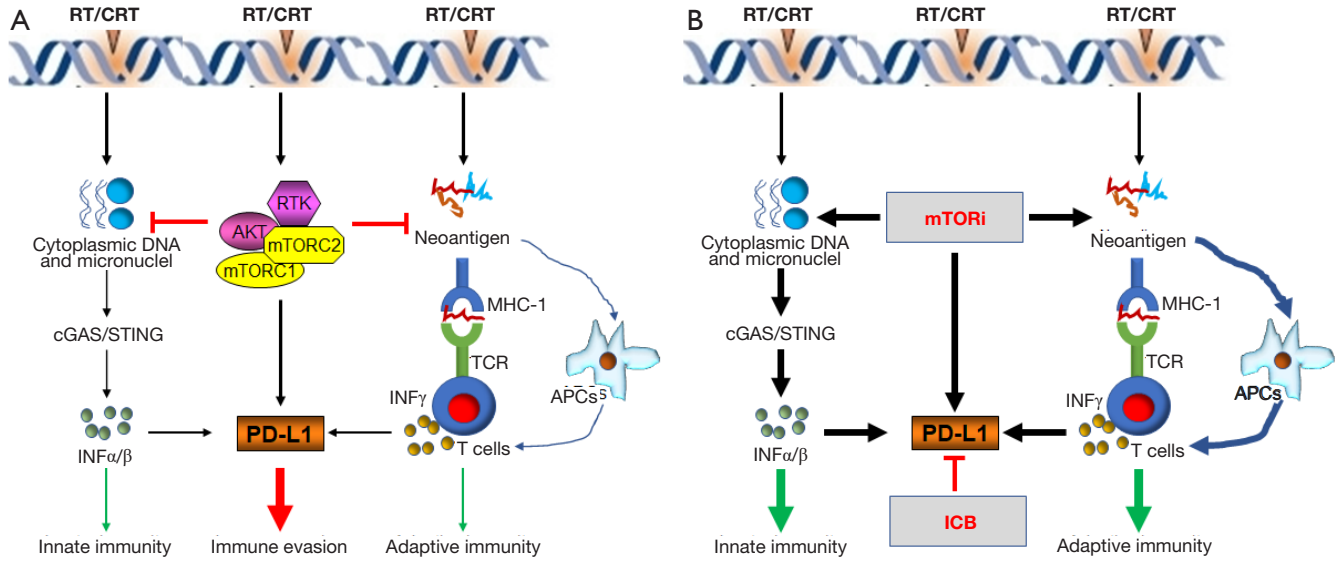

Figure 3 Targeting AKT-mTOR signaling in combination with radioimmunotherapy for cancer treatment. (A) Constitutive activation or radiation therapy (RT)- or chemoradiation therapy (CRT)-induced activation of AKT-mTOR signaling dampens radiation-mediated modulation of innate immunity via preventing the cGAS/STING-IFNα/IFNβ axis and adaptive immunity by suppressing neoantigen production as well as through increasing PD-1 expression to evade the immune system. (B) Targeting RTK-AKT-mTOR signaling (mTORi) sensitizes cancer cells to radioimmunotherapy. Targeting RTK-AKT-mTOR signaling may enhance innate immunity by promoting the accumulation of cytoplasmic DNA and micronuclei to activate the cGAS/STING-IFNα/IFNβ axis and adaptive immunity via elevating neoantigen production to activate T cells. At the same time, both the enhanced innate and adaptive immunity will increase PD-L1 expression via IFNα/IFNβ and IFNγ, respectively. In addition, targeting RTK-AKT-mTOR signaling may increase PD-L1 levels through the paradoxical reactivation of PI3K-AKT and RAS-ERK signaling (depending on cancer types). However, the effects of increased PD-L1 will be neutralized by anti-PD-1 or PD-L1 antibody immune checkpoint blockade-based immunotherapy (ICB).

of AKT kinases are the major regulators of PD-L1 levels for cancer cells to evade the immune surveillance system. The next challenge is how to rationally target the AKT-mTOR signaling pathways during radioimmunotherapy based on the genomic, epigenetic, transcriptional, post-translational, and metabolic profiles of the tumors from individual cancer patients.

Concluding remarks

AKT-mTOR signaling is a central integrator and processor of extracellular and intracellular signals. Emerging evidence has shown that AKT-mTOR signaling modulates anticancer immune responses in many clinical trials of combination therapies of AKT-mTOR inhibitors with immune checkpoint inhibitors. Moreover, AKT-mTOR signaling is a key regulator in the supervision of DNA duplication, DNA damage repair, and the replication stress response in the nucleus as well as in mitochondrial DNA repair via PGC-1α (148). Mounting evidence has demonstrated that immunotherapy enhances radiotherapy, leading to numerous clinical trials of radiation and immunotherapy combinations across cancer types (www.clinicaltrials.gov). Regarding the important roles of AKT-mTOR signaling in the regulation of DNA damage repair and PD-L1 expression, it will be promising and attractive to develop preclinical studies and clinical trials targeting AKT-mTOR signaling in combination with radiation and immunotherapy, especially for hard-to-treat cancers such as lung, pancreatic, and brain cancers. Importantly, mutations of PIK3CA and PTEN, which are among the most important regulators of AKT-mTOR signaling, occur at high frequency across cancer types. It will be intriguing to evaluate whether tumors with PIK3CA and PTEN mutations are more sensitive to AKT-mTOR inhibitors in combination with radioimmunotherapy.

Acknowledgments

Funding: This work was supported by the American

Cancer Society (IRG-67-003-47), the National Institute of Health (R01CA246553), The Major Research Project of Science Technology Department of Zhejiang Province (2021C03124), and Wu Jieping Medical Foundation (320.6750.19092-12).

Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://dx.doi.org/10.21037/atm-21-4544

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-4544). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Shen C, He Y, Chen Q, Feng H, Williams TM, Lu Y, He Z. Narrative review of emerging roles for AKT-mTOR signaling in cancer radioimmunotherapy. Ann Transl Med 2021;9(20):1596. doi: 10.21037/atm-21-4544

