Review Article

Role of HIF-1α in the Responses of Tumors to Radiotherapy and Chemotherapy

Chang W Song1,*, Hyunkyung Kim2, Mi-Sook Kim2, Heon J Park3, Sun-Ha Paek4, Stephanie Terezakis1, L Chinsoo Cho1

1Department of Radiation Oncology, University of Minnesota Medical School, Minneapolis, MN, USA, 2Department of Radiation Oncology, Korea Institute of Radiological & Medical Sciences, Seoul, 3Department of Microbiology, College of Medicine, Inha University, Inchon, 4Department of Neurosurgery, Seoul National University College of Medicine, Seoul, Korea

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*Correspondence: Chang W Song

Department of Radiation Oncology, University of Minnesota Medical School, Minneapolis, MN 55812, USA
Tel: 1-952- 994-5755 E-mail: songx001@umn.edu

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Abstract

Tumor microenvironment is intrinsically hypoxic with abundant hypoxia-inducible factors-1α (HIF-1α), a primary regulator of the cellular response to hypoxia and various stresses imposed on the tumor cells. HIF-1α increases radioresistance and chemoresistance by reducing DNA damage, increasing repair of DNA damage, enhancing glycolysis that increases antioxidant capacity of tumors cells, and promoting angiogenesis. In addition, HIF-1α markedly enhances drug efflux, leading to multidrug resistance. Radiotherapy and certain chemotherapy drugs evoke profound anti-tumor immunity by inducing immunologic cell death that release tumor associated antigens together with numerous pro-immunological factors, leading to priming of cytotoxic CD8+ T cells and enhancing the cytotoxicity of macrophages and NK cells. Radiotherapy and chemotherapy of tumors significantly increase HIF-1α activity in tumor cells. Unfortunately, HIF-1α effectively promotes various immune suppressive pathways including secretion of immune suppressive cytokines, activation of myeloid-derived suppressor cells (MIDSCs), activation of regulatory T cells (Tregs), inhibition of T cells priming and activity, and upregulation of immune checkpoints. Consequently, the anti-tumor immunity elevated by radiotherapy and chemotherapy is counterbalanced or masked by the potent immune suppression promoted by HIF-1α. Effective inhibition of HIF-1α may significantly increase the efficacy of radiotherapy and chemotherapy by increasing radiosensitivity and chemosensitivity of tumor cells and also by upregulating anti-tumor immunity.

Keyword

HIF-1α, Immunologic cell death, Radiotherapy, Chemotherapy, Radiosensitivity, Chemosensitivity, Anti-tumor immunity, Immunosuppression.
Introduction

Radiotherapy and chemotherapy are two of the most commonly used treatment modalities for malignant cancers. There have been marked paradigm shifts in radiotherapy in recent years, including the increased use of stereotactic ablative radiotherapy (SABR, also designated as SBRT/SRS) [1,2], particle radiotherapy [3] and development of spatially fractionated radiotherapy (SFRT) and FLASH radiotherapy [4]. In chemotherapeutic oncology, potent new chemical agents and innovatively designed treatment plans for specific cancers have been continuously implemented over recent years [5-8]. The initial response rates of tumors to novel radiotherapy and chemotherapy approaches are impressive. Unfortunately, many of the initially responded tumors either recur or metastasize due, at least in part, to activation of hypoxia-inducible factors 1α (HIF-1α), eventually leading to downfall of patients.

Hypoxia and elevated HIF-1α expression is a common feature of the intratumor microenvironment in most of growing malignant tumors. Hypoxia and HIF-1α effectively protect tumor cells from radiation, leading to an increase in radioresistance [9-16]. Likewise, hypoxia and HIF-1α have been linked to increased chemoresistance [3,14,17-32]. When radiotherapy or chemotherapy drugs causes damage to DNA and other intracellular organelles, a process known as immunological cell death (ICD) is induced, resulting in massive release of tumor associated antigens (TAAs), and various immunologic molecules, chemokines and cytokines [33-36]. Subsequently, innate and adaptive immune systems are activated, leading to elimination of target tumor cells. Unfortunately, however, the elevated anti-tumor immunity by radiotherapy or chemotherapy is counterbalanced or offset by the surge of immune suppressive reactions orchestrated by HIF-1α [33,37-42].

Remarkable responses of tumors to some forms of immunotherapy, particularly that interfering with the immune suppressing pathways, have been reported, and a variety of new
strategies are now being introduced [43-45]. Importantly, combined use of immunotherapy with radiotherapy [33,34,46,47] or chemotherapy [35,48] is more effective than treating with individual modality alone in certain sequences and combination approaches.

Here, we have reviewed the effects of HIF-1α on the radiosensitivity and chemosensitivity of tumor cells. In addition, we described the increasingly recognized role of HIF-1α in the changes of anti-tumor immune profiles evoked by radiotherapy and chemotherapy, and the various likely implications of this highly active transcription factor in the failure of many therapeutic regimes.

**Hypoxia-Inducible Factors (HIFs)**

The tumor microenvironment is hypoxic, since oxygen supply to tumor cells through immature and aberrant tumor vascular networks is insufficient to meet the substantial demands for oxygen by the rapidly expanding tumor cell populations [2,41]. Tumor cells cope with this detrimental situation by upregulating transcription complex HIFs that rapidly facilitate cellular adaptation to hypoxic stress [29-31,50-55]. HIFs play vital roles in glycolysis, angiogenesis, cell survival, proliferation, invasion and metastasis via regulation of hundreds of genes. Virtually all human and experimental tumors show varying degree of HIFs expression [29,55,56]. A growing body of evidence indicates that the expression of HIF-1α in tumors is upregulated by radiotherapy [9,41,56,57] and certain chemotherapeutic drugs [58]. Importantly, HIFs directly or indirectly increase tumor resistance to radiotherapy [9-16], and chemotherapy [17-32], and suppresses the anti-tumor immunity [1,39,41,42,49,50], as discussed in detail later. HIFs are heterodimeric transcription factors consisting of α and β subunits [52]. Three alpha isoforms have been identified to date, denoted HIF-1α, HIF-2α and HIF-3α. HIF-1α is synthesized and constitutively transcribed by a cascade involving a series of growth factors and
signaling [22]. While HIF-1α and HIF-2α are highly homologous and share many functional features, and yet the isoforms differ significantly in terms of tissue-specific expression, and target genes [37,41,51,58-61]. Interestingly, HIF-1α is responsible for cellular adaptation to acute hypoxia (<0.1% O2) whereas HIF-2α is active under chronic hypoxia [62]. In contrast to HIF-1α and HIF-2α, relatively little is known about HIF-3α. Intriguingly, HIF-3α has been reported to act as a negative feedback regulator by inhibiting the functions of HIF-1α and HIF-2α [54,61].

HIF-1α is continuously produced but rapidly disintegrated in the presence of oxygen [52,53,56]. The proline residues of HIF-1α are hydroxylated by prolyl hydroxylase domain proteins (PHDs), a process that requires oxygen. Hydroxylated HIF-1α is subsequently recognized and ubiquitinated by pVHL, a protein product of the von Hippel-Lindau tumor suppressor gene, followed by rapid degradation of ubiquitinated HIF-1α via the proteasome pathways. Under hypoxic condition HIF-1α is not degraded since it is not hydroxylated by PHDs [37,63]. Unlike HIF-1α, HIF-1β is a stable constitutively expressed protein that localizes in the nucleus. In a hypoxic environment, HIF-1α translocate from cytoplasm into the nucleus, where it binds to nuclear HIF-1β to form heterodimers, and functions as a master transcription factor.

Notably, HIF-1α is also activated, stabilized or accumulated in an oxygen-independent manner [37,14,16]. Expression of HIF-1α is increased by physical stress factors such as an exposure to reactive oxygen species (ROSs), that are continuously produced in cells during mitochondrial respiration under both oxic and hypoxic condition. Furthermore, in growing malignant tumors, blood perfusion is sluggish and often intermittent, and thus fractions of tumor cells become intermittently hypoxic and reoxygenate. Reoxygenation of hypoxic cells as a
result of reperfusion of blood in the nearby blood vessels imposes marked stress on mitochondria, resulting in overproduction of ROSs, that promotes upregulation of HIF-1α [42]. In tumors, significant reoxygenation of hypoxic cells also occurs after an exposure of tumors to high-dose radiation, which leads to ROS generation, and consequent elevation of HIF-1α levels [11,12,41]. Additionally, HIF-1α expression is induced by nitric oxide, inflammatory cytokines such as TGF-β, IL-10, TNF-1, and hormone-like growth factors, such as insulin-like growth factor (IGF) [37,64,65]. In this review, we will discuss only the effects of HIF-1α on radiosensitivity, chemosensitivity and immunity because the information on the roles of HIF-2α and HIF-3α in radiotherapy and chemotherapy of cancers is relatively sparse.

Effects of HIF-1α on Radiosensitivity and Chemosensitivity of Tumors

1. Radiotherapy

It is well known that hypoxic environment confers significant radioresistance to a wide range of biological systems: the radiation doses of x-rays or r-rays required to kill hypoxic tumor cells are about three times greater than those required for oxic tumor cells [65]. Ionizing radiation kills cancer cells mainly by damaging the genomic DNA through either by directly ionizing the DNA molecules or indirectly by generating highly reactive ROSs in water, which then ionize and damage the DNA molecules [66]. The fragmented ends of DNA, regardless of formation via direct or indirect effects of radiation, are rapidly oxidized by oxygen, resulting in permanent or “fixed” damage. Single strand breaks (SSBs) may occur simultaneously in both DNA strands, leading to lethal double strand breaks (DSBs). In the hypoxic environment, the oxygenation of the broken ends of DNA would not occur, and thus the DNA damage may be repaired [70]. Importantly, hypoxia not only reduces radiation-induced DNA damage, as mentioned above, but also promotes repair of DNA damage. SSBs are repaired through base
excision repair processes involving PARP1, XPA and XPD, and DSBs repaired through molecular pathways involving ATM, DNA-PK (DNA-PKcs, Ku80, Ku and H2AX [70]. In a hypoxic environment, HIF-1α upregulates the molecules responsible for repair of SSBs and DBSs, thereby increasing the radioresistance of tumor cells [13,14,66]. Moreover, HIF-1α suppresses p53 expression in irradiated tumor cells, leading to a decrease in apoptosis [29].

HIF-1α induces cellular radioresistance also by reprogramming glucose metabolisms [9,13-16,66]. HIF-1α increases the levels of glycolytic enzymes, such as glucose transporter-1 (GLUT1) and hexokinase-2, thereby shifting glucose metabolisms from mitochondrial oxidative phosphorylation to glycolysis [13,14,24,26]. Pyruvates and lactates, formed as products of glycolysis, function as endogenous antioxidants. HIF-1α additionally promotes the activities of enzymes involved in the associated pentose phosphate pathway (PPP), which generates antioxidants NADP(H) and GSH. Overall, by promoting glycolysis, HIF-1α increases the production of NADPH, GSH, pyruvate and lactate [14]. These intracellular redox buffer networks effectively scavenge the radiation-induced intracellular cytotoxic free radicals such as ROSs, thereby decreasing cellular damage resulting in elevation of tumor radioresistance [13,14].

An adequate supply of nutrients including oxygen through tumor blood vessels is essential for tumor growth, progression and metastasis. Tumor blood vessels are mainly formed via angiogenesis mediated by VEGFs, which are secreted from tumor cells and tumor stromal cells, such as tumor-associated fibroblasts. Generation of VEGFs is directly mediated by HIF-1α, implying that HIF-1α increases radioresistance by promoting tumor vascularization and tumor growth [9-11,16].

In summary, hypoxia and HIF-1α confer radioresistance on tumor cells by preventing radiation-induced DNA strand breaks, promoting DNA damage repair, decreasing apoptosis.
through suppression of p53, and by increasing glycolysis, which increases the antioxidant capacity of tumors cells. In addition, HIF-1α increases radioresistance of tumors by enhancing tumor growth through promoting vascularization via upregulation of VEGFs (Fig. 1).

2. Chemotherapy

While chemotherapy remains one of the major therapeutic modalities for cancer, tumors often develop drug resistance, leading to treatment failure. The anti-tumor mechanisms of chemotherapeutic agents are significantly dependent of the drug and tumor types. Similarly, the mechanisms underlying the development of drug resistance vary according to drug and tumor types. In this context, it must be noted that cytotoxicity of chemotherapy drugs in vivo is far less than that in vitro, primarily because the tumor microenvironment is hypoxic, nutritionally deprived, and acidic [29]. For the most of the commonly used drugs, the hypoxic tumor microenvironment rich in HIF-1α plays a major role in the development of drug resistance through multiple pathways [3,18-32]. The high expression of drug efflux pump Multidrug Resistance 1 (MDR1) gene, and the MDR1-encoded P-glycoprotein (P-gp), an ATP-binding cassette transporter, are significantly implicated in the development of multidrug resistance (MDR) [32]. Multidrug Resistance-Associated protein1(MRP1) is another key drug transporter shown to contribute to drug resistance. HIF-1α upregulates both MDR1 and MRP1 through direct binding to HRE sites in their gene promoter regions [17,27,28-30]. In addition, breast cancer resistance protein (BCRP), an ATP-dependent efflux multidrug transporter, is upregulated by HIF-1α. Other than interfering with drug uptake, HIF-1α increases chemoresistance through inducing the anti-apoptotic protein Bcl-2, and inhibiting the pro-apoptotic protein Bax [68].

Autophagy is a highly conserved and genetically defined cellular recycling process, that
is regulated by HIF-1α [14,22]. Autophagy allows cells to escape from apoptosis and thus contributes to the resistance to chemotherapy and radiotherapy.

HIF-1α reduces drug-induced DNA damage, and promotes glycolysis in the drug treated tumor cells [14], such as that occurring after radiotherapy. However, it is worth to note that hypoxia confers tumor cells resistance to some chemotherapy drugs, independent of transcriptional activity of HIF-1α [13].

In summary, HIF-1α enhances chemoresistance by accelerating drug efflux, reducing DNA damage, promoting glycolysis, and inducing autophagy (Fig. 1).

Immune Stimulation Caused by Radiotherapy and Chemotherapy

1. Induction of Immunologic Cell Death by Radiotherapy and Chemotherapy

Radiotherapy causes damage in various cellular organelles, including DNA and endoplasmic reticulum (ER), leading to immunologic cell death (ICD) of tumor cells [34,37,47,48,69-71] (Fig. 2). ICD releases tumor associated antigens (TAAs) and a variety of immunologic cytokines and chemokines, leading to activations of innate and adaptive anti-tumor immunity. Conventional multifractionated radiotherapy involves irradiation of tumors with a small dose (<2.0 Gy) per fraction (each day), 5 times a week, over a total of 30-50 fractions. Although the daily doses of radiation are small in the multifractionated radiotherapy, the tumor cells killed by the radiation may still evoke antitumor immunity. However, it is highly likely that proinflammatory immune cells accumulating in tumors are eliminated by repeated daily radiation exposures. In tumors treatments with high-dose per fraction hypo-fractionated radiotherapy, tumors are precisely irradiated only 1-3 times. Therefore, the immune cells infiltrated into the tumors are less frequently irradiated, and thus the immune cells may be spared.
Chemotherapy drugs usually induce lymphopenia, granulocytopenia and thrombocytopenia leading to immune suppression. On the other hand, many of commonly used chemotherapy drugs have been shown to evoke anti-tumor immune response via inducing ICD [34-36,72-74]. Despite the diverse targets and functions of different drugs, induction of DNA damage, suppression of DNA synthesis and disruption of ER are commonly involved in the tumor cell death caused by the drugs, leading to ICD, and stimulation of anti-tumor immunity (Fig. 2). The chemotherapy drugs that have been demonstrated to induce ICD include the conventional chemotherapy drugs such as anthracyclines (doxorubicin and mitoxantrone) and DNA damaging agents (cyclophosphamide, platinum derivatives but excluding cisplatin, proteosome inhibitors and paclitaxel) [36].

2. Activation of Anti-tumor Immunity following Immunologic Cell Death

The ICD caused by ionizing radiation or chemotherapy drugs triggers a series of transcriptions and molecular responses, evoking anti-tumor immunity [34,35,42,72,75] (Fig. 2). ICD is characterized by changes in the composition of the cell surface membrane and release or secretion of a variety of soluble factors, followed by activation of adaptive immune responses against tumor cells. With progression of ICD, tumor-associated antigens (TAAs) release is accelerated accompanied by release of damage-associated molecular patterns (DAMPs), including calreticulin (CRT), high mobility group box 1 protein (HMGB1), ATP, and HSPs [34-37,48].

CRT is a highly conserved Ca2+ binding chaperon protein residing primarily in the lumen of ER, where it performs various functions, including assembly of major histocompatibility complex class1(MHC-1). After translocation to the cell surface at the beginning of ICD, CRT serves as a potent “eat me” signal, and attracts antigen
presenting cells (APTs) including dendric cells (DCs) to tumor cells. Interestingly, expression of CD47, “do not eat me” signal, on the surface of tumor cells has been shown to decrease with increasing expression of CRT [76]. Simultaneously, MHC-1 is upregulated on the tumor cells, rendering them easily identifiable by DCs for engulfment. The HMGB1 is a nonhistone chromatin-associated protein involved in DNA organization and transcriptional regulation. The protein is released from nuclei to the extracellular space during the late stage of ICD, and serve as a multifunctional alarmin [34-37,48]. Released HMGB1 binds to Toll-like receptor 4 (TLR4) on DCs, promotes DCs maturation, and processing of engulfed TAAs, including mounting of TAAs on MHC-1 molecules [34-37,48]. ATP is another important constituent of DAMP, which is secreted from lysosomes of dying cells and functions as a “find me” signal [41,74]. ATP promotes DC recruitment, and accelerates maturation and differentiation of DCs into antigen presenting cells [34-37,48]. HSPs, such as HSP70 and HSP90, released from dying cells, function as “eat me” signals, and form complexes with TAAs that are engulfed by DCs [74]. Engulfed TAAs subsequently form complexes with MHC-1 in DCs. Type I IFNs are hallmark cytokines produced in immune responses that control many innate and adaptive anti-tumor immune systems including the engulfing of HSP-TAA complexes by DCs [74]. Activated DCs expressing MHC-TAA on the cell membrane subsequently migrate to nearby lymph nodes, where they encounter naïve T cells, and prime them to generate cytotoxic DC8+ T cells (TCLs) specific for TAAs [41,74,75]. In addition to priming T cells with TAAs in the context of MHC-1, matured DCs produce numerous immune cytokines, including IFNs, TNF, IL-12 and IL-1β, which promote the mobilization and activation of natural killer cells (NK cells) [34,37]. NK cells as well as T helper cells have been shown to release Type I IFNs upon stimulation by IL-12 and IL-18 [74]. The IFNs promote the migration of activated DCs to lymph nodes, and upregulates the expression of CD80 and CD86 on DCs, that function as co-stimulators of T-cells.
3. T-cell activation and attack on tumor cells

As mentioned in the previous section, activated DCs migrate to draining lymph nodes. Next DCs cross-present TAAs in the context of MHC-1 to the receptors on naïve T cells (TCR) and prime them to produce cytotoxic CD8+ cytotoxic T cells (CTLs). Primed CTLs are further co-activated via ligations of CD28 on CTLs with CD80 and CD86 on DCs, which is promoted by T helper cells. It has been reported that HIF-1α inhibits the pro-inflammatory DC function, including DC-induced activation and proliferation of CTLs [38,41,77,78]. The fully activated CTLs then migrate to tumors via blood vessels, promoted by IFNs, and other cytokines. Infiltrated CTLs identify target tumor cells mainly via recognizing the MHC-1 expressed on tumor cell surface, which is upregulated by IFNs, and induce apoptosis in tumor cells through two pathways. First, CTLs induce damage in tumor cell membrane by releasing perforin and granzyme, leading to apoptosis. Second, the Fas ligand (FasL) expressed on CTLs interacts with Fas expressed on the tumor cells, thereby triggering apoptotic death cascade. It has been shown that the cells which are treated with radiation or chemotherapeutic drugs but escaped ICD are highly susceptible to attack by NK and CTLs due to elevated expression of death receptors on their surface [79]. The increased antitumor immunity by local irradiation and chemotherapy may induce a systemic immune response, and affects non-irradiated tumors in the same host, which is referred to as “abscopal effect”.

In summary, radiotherapy and chemotherapy induce ICD, releasing TAAs and numerous immune stimulatory molecules, leading to activation of DCs. The activated DCs engulf TAAs, process them, and migrate to lymph nodes, wherein they prime T cells to CTLs. CTLs subsequently migrate and infiltrate tumors and eliminate tumor cells (Fig. 2).
Immunosuppression Caused by Radiotherapy and Chemotherapy via Upregulation of HIF-1α

1. HIF-1α Stimulate Immunosuppressive Responses in Intratumor Microenvironment

As discussed in previous sections, radiotherapy and many chemotherapy agents trigger powerful anti-tumor immunity. Unfortunately, opposing immune suppressive reactions, mediated mainly by HIF-1α, are also evoked in tumors, counterbalancing the anti-tumor immune responses promoted by radiotherapy and chemotherapy [34,37-42,48,70,80,81] (Fig. 2). Following radiotherapy or chemotherapy, the hypoxic tumor microenvironment (TME) becomes further hypoxic due to the destruction of immature tumor vascular networks [2,42,82]. The hypoxic tumor microenvironment stimulates expression of HIF-1α, which facilitates secretion of various immune suppressive cytokines, such as TGF-1β and IL-10 from tumor cells and tumor-associated stromal cells (Fig. 3). TGF-β and IL-10 are multipotent cytokines that promote the differentiation, survival and function of multiple immune suppressive cells including CTLs, macrophages and NK cells [38,66,80,83]. Upon stimulation by HIF-1α and further activated by TGF-β, stromal cell derived factor 1(SDF-1) is secreted from stromal cells, and recruits immature myeloid cells to TME [48,49,84], which subsequently differentiate into tumor-associated macrophages (TAMs) or myeloid-derived suppressor cells (MDSCs) comprising a mixture of pathologically activated monocytes and neutrophils. TAMs are further polarized into either pro-inflammatory M1 and anti-inflammatory M2 macrophages. Polarization of TAMs into immunosuppressive M2 macrophages is promoted by TGF-β and IL-10. In turn, M2 macrophages promote various immune suppressive pathways by secreting TGF-β, IL-10 and VEGFs, which promotes angiogenesis [41,48,66]. These cytokines also promote the immunosuppressive functions of MDSCs, and interfere with the CD-mediated
priming of CD8+ T cells and NK cell functions [80]. Regulatory T cells (Tregs) are generated from naïve T4 cells through the instruction of DCs. Tregs are a specialized subpopulation of T cells that act as immune suppressor by inhibiting CTL proliferation and producing TGF-β and IL-10 [74]. The processes of DC-instructed generation of Tregs and differentiation and functions of Tregs are all directly stimulated by HIF-1α [77,85]. The VEGF, which is transcriptionally elevated by HIF-1α has been shown to provoke the proliferation of Tregs (Fig. 3).

ATP released from cells undergoing ICD after radiotherapy or chemotherapy plays a crucial role in anti-tumor immune stimulating processes, as discussed earlier, while its main metabolite, adenosine plays important roles in immune suppression by inhibiting the activities of antigen presenting cells including DCs, and triggering apoptosis in NK cells and CTLs [86]. Adenosine also indirectly promotes Treg proliferation and plays a role in skewing the polarization of TAMs from M1 to M2 macrophages [87]. HIF1α enhances accumulation of adenosine by inhibiting phosphorylation to adenosine monophosphate.

2. HIF-1α Protects Tumor Cells from CTLs

1) FasL, MHC-1, and CD47

CTLs infiltrating into tumors kills tumor cells by causing apoptosis through the interactions of FasL on surface of CTLs with Fas on tumor cells [41].

HIF-1α suppress FasL expression on CTLs, thereby protecting tumor cells from the attack by CTLs (Figs. 2, 3). Cytotoxic CTLs and NK cells identify tumor cells via recognition of MHC-1 expressed on tumor cell surface, and HIF-1α downregulates the MHC-1 expressed on tumor cells, preventing immunologic attack by CTLs and NK cells [34,37,38,81]. CD47 expressed on the tumor cell surface functions as a “don’t eat me” signal, that facilitate evasion
of phagocytosis by macrophages [87]. Radiotherapy and certain chemotherapy drugs upregulate CD47 expression on tumor cells via elevation of HIF-1α expression [88,89] (Fig. 3).

2) Immune checkpoints

T-lymphocyte-associated antigen-4 (CTLA-4) is a major immune checkpoint receptor expressed on CTLs [33,41,45]. The CTLs primed by DCs in lymph nodes are co-activated through the interaction of CD23 on CTLs with CD80 and CD86 on DCs. However, interaction of CD23 on CTLs with CD80 and CD86 on DCs is interrupted when CTLA-4s on CTLs bind to CD80 and CD86 on DCs. The disruption of the interactions of CD23 with CD80 and CD86 by CTLA-4 leads to anergy, exhaustion and apoptosis of CTLs [33,45]. In addition to CD8, Tregs also express CTLA-4, which interacts with CD80 and CD86 on DCs, thereby interrupting the interactions between D-23 on CTLs with CD80 and CD086 on DCs, leading to inactivation of CTLs. Expression of CTLA-4s on CTLs as well as Tregs is upregulated by HIF-1α (Fig. 3). Another important immune checkpoint is the programmed cell death protein 1 (PD-1) expressed on CTLs. Expression of PD-1 on CTLs has been demonstrated to be upregulated by HIF-1α, and also by VEGF and TGF-β, both are transcriptional targets of HIF-1α [37,38] (Fig. 3). Interaction of PD-1 on CTLs with its ligand PD-L1 expressed on tumor cells, macrophages, DCs and MDSCs leads to exhaustion, anergy and apoptosis of CTLs [33,41,45]. Radiation and many chemotherapy drugs increase PD-L1 expression on tumor cells and immune cells by activating HIF-1α (Fig. 3). It has been shown that HIF-1α upregulates PD-L1 expression by directly binding to a hypoxia-response element (HRE) in the PD-L1 proximal promoter [37,42,90]. Under hypoxic conditions, STAT3 and NF-kB signaling pathways induce PD-L1 expression [80]. In light of the fact that STAT3 not only blocks HIF-1a degradation but also increases synthesis of HIF-1α [90], it is likely that the STAT3-induced upregulation of PD-L1
expression is mediated by an increase in HIF-1α expression.

**Conclusion**

Fig. 4 shows the interrelations among radiotherapy, chemotherapy, HIF-1 α and anti-tumor immunity. Radiotherapy increases the effectiveness of many chemotherapy drugs, and many chemotherapy drugs increases the effectiveness of radiotherapy against tumor cells. Radiotherapy, particularly high-dose radiotherapy, and many chemotherapy drugs induce immunologic cell death (ICD), evoking strong anti-tumor immunity. Radiotherapy and chemotherapy also upregulate HIF-1α, which diminish the effectiveness of radiotherapy and chemotherapy. Furthermore, HIF-1 α stimulates various molecular and cellular immune suppressive responses in tumor microenvironment, counterbalancing or offsetting the anti-tumor immunity, that is elevated by radiotherapy and chemotherapy (Fig. 3). Effective inhibition of HIF-1 α may not only increase the effectiveness of radiotherapy and chemotherapy, but also enhance the anti-tumor immunity.

**Author Contributions**

Conceived and designed the analysis: Song CW, Kim MS, Cho LC.

Collected the data: Song CW, Kim H, Terezakis S, Cho LC.

Contributed data or analysis tools: Kim H, Peak SH.

Performed the analysis: Song CW, Kim MS, Terezakis S, Cho LC.

Wrote the paper: Song CW.

**ORCID iD**

Chang W Song: https://orcid.org/0000-0001-9630-4735
Conflicts of Interest
Contrict of interest relevant to this article was not reported.

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Fig. 1. Effects of HIF-1α on various molecular and physiological factors involved in radioresistance and chemoresistance.
Fig. 2. Radiotherapy and chemotherapy elevate anti-tumor immunity by inducing immunologic cell death (ICD), leading to release of tumor associated antigens (TAA) together with damage associated molecular patterns (DAMPs). DAMP subsequently activates dendritic cells (DCs), which then engulf TAA, migrate to nearby lymph nodes, and prime T cells specific for TAA. The activated cytotoxic T cells (CTLs) subsequently migrate to residual tumors or metastatic tumors, and kill the tumor cells. Intratumor microenvironment is intrinsically hypoxic, which is further increased by radiotherapy and chemotherapy. The hypoxic tumor microenvironment activates HIF-1α, which stimulate various immunosuppressive pathways. Consequently, the elevated anti-tumor immunity caused by radiotherapy or chemotherapy is counterbalanced or offset by the potent immune suppressive reactions mediated by HIF-1α.
Fig. 3. Effects of HIF-1α on the expression or function of various immunological factors.
Fig. 4. The interrelationship among Radiotherapy, Chemotherapy, Anti-tumor immunity and HIF-1α. Radiotherapy enhances the effectiveness of many chemotherapy drugs, and many chemotherapy drugs enhance the effectiveness of radiotherapy. Radiotherapy, particularly high-dose hypo-fractionated radiotherapy, and chemotherapy evoke anti-tumor immunity via induction of immunologic cell death (ICD). However, radiotherapy and chemotherapy also upregulate HIF-1α, which promote various immune suppressive reactions in tumor microenvironment, counterbalancing or offsetting the anti-tumor immunity. HIF-1α also diminishes the effectiveness of radiotherapy and chemotherapy.