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REVIEW

Adapting conventional cancer treatment for immunotherapy

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Abstract The efficacy of directly killing tumors by conventional cancer therapies, such as chemotherapy and radiotherapy, has been for several decades well established. But, a suppressed immune response might become a lethal side effect after repeated cycles of intensive treatment. Recently, achievements in immune checkpoint inhibitors and adoptive T cell-mediated immunotherapies have resulted in changes in frontline management of advanced cancer diseases. However, accumulated evidence indicates that immunotherapeutic and conventional strategies alone are often ineffective to eradicate big tumors or metastasis. To improve the outcomes of treatment for advanced cancer diseases, the combination of conventional cancer treatment with various immunotherapeutic approaches has been attempted and has shown potential synergistic effects. Recent studies have unexpectedly demonstrated that some strategies of conventional cancer treatment can regulate the immune response positively, thus the understanding of how to adapt conventional treatment for immunotherapy is crucial to the design of effective combination therapy of conventional treatment with immunotherapy. Here, we review both experimental and clinical studies on the therapeutic effect and its mechanisms of combining conventional therapy with immunotherapy in treatment of cancer.

Keywords Chemotherapy · Radiotherapy · Combination of conventional therapy and immunotherapy · Immunological mechanisms

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Introduction

Conventional chemotherapy or radiotherapy itself is not sufficient to eradicate all tumor cells in advanced cancer, resulting in recurrence with multiple disadvantages, such as non-specifically targeting normal cells including effective immune cells, promoting a generation of drug- and radiotherapy-resistant cancer cells, and inducing systemic and local toxicity during treatment. To overcome these problems, most studies have focused on the understanding of intrinsic mechanisms to develop targeted therapies, such as small molecules targeting oncogenic signaling or genes related to oncogenic pathways that contribute to unsuccessful chemotherapy and radiotherapy treatment [1–10]. Chemotherapy and radiotherapy have been generally designed to aim at inducing direct tumor cell death to control local tumor growth. However, tremendous advances in understanding the molecular mechanisms of tumor immunity have allowed the studies of conventional chemotherapy- and radiotherapy-mediated immunomodulatory changes that could potentially influence their therapeutic effects. Recently emerging clinical success of immunotherapies, particularly immune checkpoint blockade treatment, confirm that the efficacy of cancer treatment can be achieved through acting on immune cells/molecules, instead of only through direct cytotoxic effects on tumor cells. Building on recent studies that either chemotherapy or radiotherapy can induce an immune response by promoting immunogenic tumor cell death, or by subverting the tumor microenvironment including inhibition of immunosuppressive cells, we will review whether and how harnessing conventional therapy along

with immunotherapy can become a potent strategy for developing novel immune-based combinatory therapies.

Immune effects of chemotherapy

Rationales of adapting chemotherapy for immunotherapy

In most clinical studies, conventional chemotherapy is usually used at the maximum-tolerated dose (MTD) to massively kill tumor cells. Although such a dose regimen could cause lymphopenia and immunosuppression of host responses, it has been shown that induction of lymphopenia by chemotherapy increases the efficacy of adoptive effector immune cell transfer in cancer patients [11]. This is due to newly transferred T cells responding to T cell-reactive cytokines for their homeostasis or antigen-driven proliferation. While in the clinical routine, chemotherapy is administered at lower doses than MTD, and it has been shown that chemotherapeutic treatment at this dose is compatible in tumor vaccine studies, eliciting an immune response against tumors in patients [12]. Moreover, a combination of carboplatin/paclitaxel-based chemotherapy with ipilimumab (anti-cytotoxic T lymphocyte-associated protein 4 (CTLA4) antibodies) has demonstrated improved efficacy in the treatment of lung cancer patients [13, 14]. However, the impact of conventional chemotherapy on the host immune response has not been well defined.

Immunological effects and mechanisms of chemotherapy with or without combining immunotherapeutic modalities

Although it was stated 50 years ago by Mihich that chemotherapy could lead to curative effects through induction of the immune response against tumor cells in a murine leukemia model [15–17], the immune-based molecular mechanisms in the context of chemotherapy have been comprehensively investigated only in the last decade. Accumulating preclinical and clinical evidence demonstrate that both innate and adaptive immune systems of the host could make crucial contributions to the outcomes of conventional chemotherapy in the treatment of cancer. Moreover, the molecular and cellular mechanisms of chemotherapy are various, depending on the type and dose scheme of therapeutic drugs used in the treatment. For example, cyclophosphamide (CPA) treatment in a metronomic regimen (frequent administration of a low dose of chemotherapy drugs with minimal or no drug-free breaks over prolonged periods) has shown to stimulate natural killer (NK) activity against the tumor, increase dendritic cell (DC) recruitment to tumor sites, and promote the skewing of immunosuppressive M2 macrophages into stimulatory M1 type of macrophages [18–20]. Also, the combination of cyclophosphamide, doxorubicin, and vincristine is able to repolarize tumor-associated M2 type of macrophages (TAMs) into M1 type upon concomitant anti-CD40 plus CpG-ODN immunotherapy [21]. In addition, the paclitaxel can induce the activation of DCs, NK cells, and cytotoxic T lymphocytes (CTLs) through stimulating TAMs to produce interleukin-12 (IL-12) and tumor necrosis factor (TNF) [22]. Furthermore, the effects of chemotherapy on the adaptive immune system have also shown therapeutic benefits. For example, the combination of cisplatin and paclitaxel at low dosage induces a strong tumor-specific $CD8^+$ T cell response in both mice and patients. Single 5-fluorouracil (5-FU) treatment of tumor-bearing mice showed selective killing of tumor-associated myeloid-derived suppressor cells (MDSCs), boosting T cell-dependent antitumor immunity [23]. Further, combining 5-FU with cisplatin could increase both $CD4^+$ and $CD8^+$ T lymphocytes in the tumor microenvironment in esophageal squamous cell carcinoma patients [24, 25]. Additionally, single CPA treatment enhances adoptive T cell therapy, which is also dosage and tumor model dependent [26–28]. This is more likely due to the fact that the immune effects induced by low-dose CPA depend on tumor immunogenicity when CPA is combined with adoptive immune cell therapy [27, 29]. However, whether interferon-α/β (IFN-α/β) also plays a pivotal role in the combination of CPA and adoptive immune cell therapy is controversial, as results were mixed. Such a discrepancy might be explained by the use of different experimental settings to assess the role of IFN-α/β: in one study, anti-IFN-α/β antibody was used to deplete cytokines in the context of combination therapy [27], while in another study, recombinant IFN-α/β was combined with adoptive T cell therapy [28]. Moreover, the different sources of type I IFNs—namely, chemotherapy-induced endogenous type I IFNs generated by tumor cells or stromal cells [27] and added exogenous type I IFNs [28] to the host—might also be responsible for the effects of CPA on potentiating adoptive immune cell therapy. Further, the combination therapy of CPA treatment and anti-4-1BB (CD137) antibody demonstrated synergistic CD8-mediated anticancer effects in a mouse model [30]. In addition to the effects on innate and adaptive immune cells, some chemotherapeutics can also inhibit tumor-induced immune suppression. For example, cyclophosphamide can downregulate the activity of T regulatory cells (Tregs) at a low dose [31–34]. Gemcitabine can reduce circulating MDSCs and promote TAM toward stimulatory M1 type of macrophages [35, 36].

Recent advances in the immune-based molecular mechanisms of chemotherapy

The molecular mechanisms by which chemotherapeutic drugs regulate tumor immunogenicity, triggering host immunity, have not been defined until recently. Several studies have demonstrated that some chemotherapeutic drugs induce immunogenic cell death (ICD), rendering tumor cells to be recognized by the host immune system and eliciting the immune response against the tumor (Fig. 1). The results showed that dying tumor cells release the danger signal high-mobility group

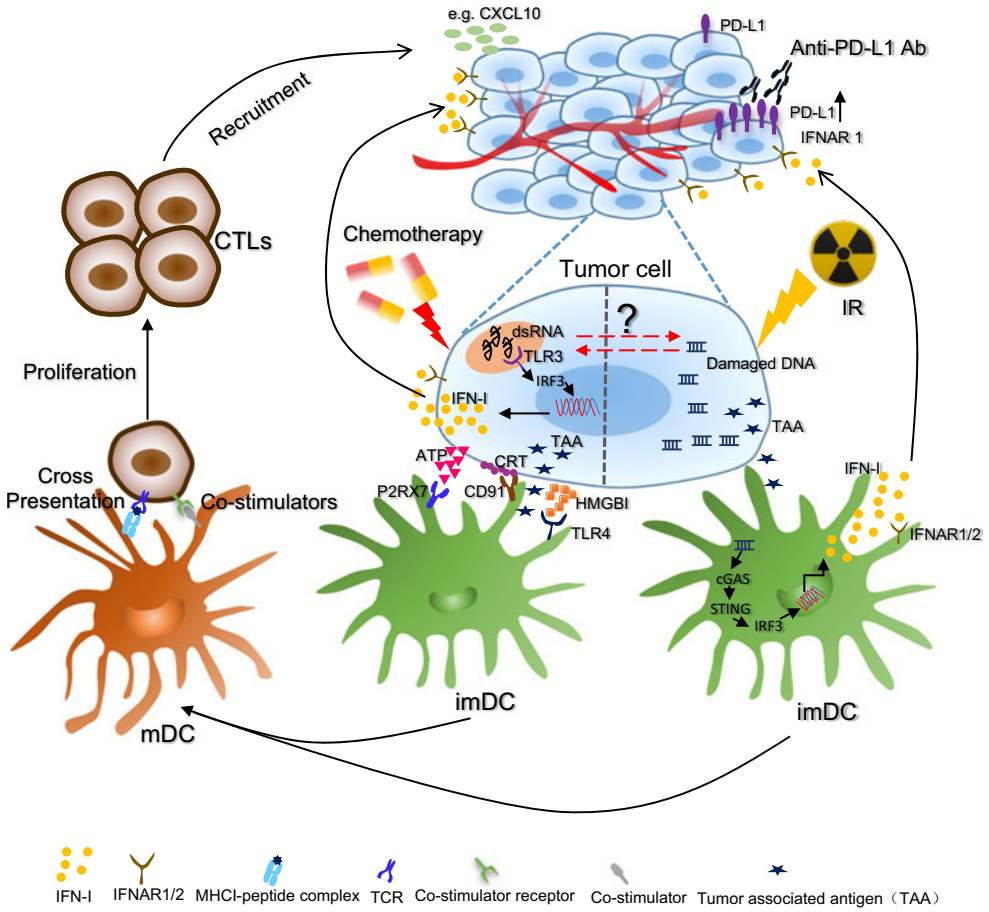

Fig. 1 Immune-based mechanisms of conventional chemotherapy and radiotherapy. Chemotherapy and radiotherapy can induce the anti-tumor immune response through several different pathways. Some chemotherapeutic drugs can promote CRT exposure and the release of HMGB1 and ATP, as well as the expression of type I IFN and CXCL10 inside tumor microenvironment, which would enhance tumor associated antigen (*TAA*) cross-presentation by DCs and the recruitment of CTLs into the tumor microenvironment. Local irradiation can induce massive DNA damage within the tumor tissues, triggering the innate DNA sensing-cGAS/STING pathway to generate abundant type I IFN. This further increases the cross-priming and maturation of DCs, resulting in the activation of CTLs. However, type I IFN can also upregulate PD-L1 expression, which indicates that the combination of radiotherapy and anti-PD-L1 antibody may improve clinical outcomes for tumor patients. As described above, chemotherapy and radiotherapy can induce immunological effects through distinct mechanisms. Whether these two conventional treatment modalities share some common pathway remains to be further investigated

box 1 (HMGB1), which can promote DC maturation and activation through its binding to Toll-like receptor 4 (TLR4) [37]. Also, chemotherapeutic drugs such as taxanes (e.g., docetaxel, paclitaxel) and vinca alkaloids (e.g., vinorelbine, vinblastine) have been shown to increase calreticulin (CALR) exposure to facilitate tumor cell recognition by the immune system [38]. In addition, after chemotherapy, adenosine triphosphate (ATP) is released by tumor cells in an autophagy-dependent manner, at which point it can bind to both P2RY2 (purinergic receptor P2Y, G-protein coupled, 2) and P2RX7 (purinergic receptor P2X, ligand-gated ion channel, 7) to recruit myeloid cells into the tumor bed and stimulate them to differentiate into inflammatory DC-like cells for tumor antigen presentation [39–41]. Moreover, after chemotherapy, the expression of type I IFN can be significantly upregulated in cancer cells, which can both activate DC for cross-priming and recruit T cells through the CXCL10 pathway [42]. Consistent with this preclinical study, a type I IFN-related signature was reported as predicting a clinical response to anthracycline-based chemotherapy in several independent cohorts of patients with breast carcinoma [42].

Taken together, both preclinical and clinical studies suggest that adapting chemotherapy to immunotherapy during the combinatory therapy of these two treatment modalities is a promising strategy to enhance antitumor effects and improve clinical outcome of cancer treatment.

Immune effects of local radiotherapy

Rationales of adapting radiotherapy for immunotherapy

Radiotherapy has conventionally been used for patients with localized disease. Despite recent improvements in radiotherapy

through increasing biologically effective doses of larger radiotherapy fractions to kill as many tumor cells as possible, a newly emerging paradigm is the use of radiotherapy to stimulate the immune system to treat metastatic tumors [43]. It has been increasingly observed that the use of local radiotherapy may stimulate an antitumor immune response by increasing both apoptosis and necrosis of tumor cells and subsequently increasing antigen presentation and expression of immunomodulatory genes [44].

Immunological effects and mechanisms of radiotherapy with or without combining immunotherapeutic modalities

Most studies have focused on the immune-modulating effects directly induced on tumor cells. Radiation can modulate the peptide repertoire and enhance major histocompatibility complex (MHC) class I expression on tumor cells, which boosts the efficacy of adoptive CTL immunotherapy [45]. Other reports have illustrated that local radiation of tumors alters the phenotype of tumor cells, rendering them more susceptible to vaccine-mediated killing of T cells [46, 47]. Local radiation may also work by altering the tumor microenvironment to promote greater infiltration of immune effector cells [48–50]. For example, it can induce the expression of certain chemokines, including CXCL9 (chemokine (C-X-C motif) ligand 9), CXCL10, and CXCL16, which promote the recruitment of T cells into the tumor microenvironment [49, 50]. It is acknowledged that radiation can trigger host immunity against tumors, however, the extent of tumor reduction by this process is poorly defined.

Recent advance in the immune-based molecular mechanisms of radiotherapy

Our lab and other groups have unexpectedly observed that rapid reduction of tumor burden after a short course of ablative radiation largely depends on the T cell response [51, 52]. We have demonstrated that ablative radiation-initiated immune response and tumor reduction are sometimes abrogated by conventional fractionated radiation or certain adjuvant chemotherapies but are greatly amplified by local immunotherapy [51]. Although the mechanisms of local irradiation (IR)-mediated tumor regression are likely to be multiple, our study further demonstrated that the effect of type I IFN on host cells is essential, as interferon-alpha/beta receptor alpha chain (IFNAR)-deficient mice fail to control tumor growth by an otherwise ablative dose of IR [53]. Further, the mice that are lacking in IFNAR only in $CD11c^+$ cells also fail to control tumor growth by ablative IR. Together, it suggests that host cells, especially dendritic cells (DCs), have to respond to type I IFN in order to generate an effective IR-mediated immune response against the tumor. The source of IFN after IR has not been well defined. It appears that $CD45^+$ and $CD11c^+$ cells produce more IFN type I than other cells, but a relative contribution is not easy to calculate due to the lack of the specific deletion of type I IFN on various types of cell and the involvement of more than one type of cell.

There are several pathways that control IFN production. To trace how IR induces type I IFN, we first tested the most recognized pathway, MyD88 (myeloid differentiation primary response gene 88) pathway, and observed no impact of MyD88 deficiency on IR-mediated tumor regression. We then tested another pathway that controls IFN, TRIF (TIR domain-containing adapter-inducing interferon-β), and also observed no impact [53]. Considering massive DNA damage by ablative IR inside tumor tissues, we evaluated the role of IR for IFN production by testing a key DNA sensing pathway, the STING (stimulator of interferon genes) pathway. We observed that the STING pathway is essential for IR-mediated IFN production, cross-priming of DC, and most importantly, tumor regression (Fig. 1) [53]. Recent studies showed that cGAS (cyclic GMP-AMP synthase) is a key enzyme that processes DNA into dinucleotide in cytosol, activating innate immune signaling [54–56]. Indeed, cGAS-deficient DCs fail to produce IFN in the presence of an irradiated tumor cell line while normal DCs can. Therefore, local IR can trigger innate sensing through the DNA sensing pathway to produce type I IFN, which then increases cross-priming and maturation of DCs for reactivating newly arrived CTL [53]. These data support the rationale for the synergy between radiotherapy and immunotherapy, emphasizing the need for proper radiotherapy that not only reduces tumor burden but also enhances immune activation. Therefore, subsequent immunotherapy can sustain or amplify the IR-initiated immune response.

To date, one primary focus has been on targeting immune checkpoints, CTLA4 and PD-L1/PD-1 pathways with blocking antibodies. Our lab discovered that radiotherapy can upregulate PD-L1 expression through increased type I IFN production in the tumor microenvironment, triggering a tumor escape mechanism from infiltrating effector T cells [57, 58]. Moreover, we showed that the combination of radiotherapy with anti-PD-L1 antibody therapy synergized in the treatment of murine breast cancer and colon tumor models. Furthermore, the combination treatment not only led to prolonged antitumor immunity upon tumor rechallenge but also induced an abscopal effect, thereby controlling secondary tumors distant from the irradiated primary tumor in both tumor models. More importantly, we confirmed the pivotal contribution of $CD8^+$ T cell effector functions. Thus, our results reveal not only that $CD8^+$ T cells are essential for the synergy of irradiation and anti-PD-L1 antibody therapy but also that the effector functions of replenished CTLs in the tumor microenvironment following irradiation are restored by PD-L1 blockade [57]. In further support of our data, a study from another group demonstrated that anti-PD-L1 antibody treatment can also reverse the T cell exhaustion that is associated

with high expression of PD-L1 in the treatment of radiotherapy and anti-CTLA4 therapy in the murine melanoma model [59]. Moreover, our results indicate that the combination of irradiation and anti-PD-L1 antibody therapies synergistically achieved effective tumor control by enhancing CTL effector functions, which in turn negatively regulates the accumulation of MDSCs through TNF signaling [57, 58]. Although previous studies showed that the combination of radiotherapy and anti-CTLA4 antibodies resulted in a successful T cell-mediated immune response and inhibition of metastases in several murine tumor models [60, 61], a recent clinical study has shown that melanoma patients with high PD-L1 tumor expression did not respond to radiation and anti-CTLA4 therapy [59]. Taken together, it suggests that the combination of radiotherapy, CTLA4, and PD-L1 blockade may be a potent strategy for cancer treatment, although the potential toxicity induced by such a combination therapy remains to be further investigated.

It is now known that radiation creates stress for tumor cells, causing them to release danger signals that are recognized by patrolling DCs [53]. Additionally, tumor-derived DNA is released to the cytosol of DCs and, in turn, activates the cGAS-STING-IRF3-IFN-β axis [53, 62]. The mechanisms by how tumor DNA gets into the cytosol of DCs remain to be determined. Natural dinucleotides fail to trigger innate sensing, while additional local IR can enhance its effect, suggesting the additional effect of IR for DNA sensing inside the cytosol. However, it is unclear whether local IR allows DNA to enter into the cytosol or if additional signaling is required for processing of cytosol DNA. Radiation therapy can enhance the activation significantly in a more quantitative manner compared to the natural immunity of the tumor. This recently redefined mechanism has bridged the tumor DNA damage response and host cell cytosolic DNA sensing pathways in the context of radiation therapy. Despite remaining uncertainties, the evidence at minimum indicates that cytosolic DNA sensing in immune cells potentially plays an important role during the antitumor immune response, and supports the notion that the nucleic acid sensing pathways are responsible for the induction of type I IFN and are essential for an effective adaptive immune response after radiation. These results demonstrate that the combination of irradiation with various immunotherapeutic approaches can potentially control both local and distal tumors.

Conclusions and perspectives

In conclusion, it is a promising and practical strategy to combine conventional chemotherapy and radiotherapy with various immunotherapeutic approaches to achieve improved antitumor effects. Understanding the mechanisms of combination therapy is necessary for the clinical development of novel effective conventional treatment and immune mechanism-based cancer treatment. Key details addressing chemotherapy and radiotherapy regimens—including timing, dosage, frequency, fractionation, and treatment sequences—need to be defined in both preclinical and clinical settings. The biomarkers to predict the immune response against tumors during or after conventional treatment alone or in combination with immunotherapy are also urgently needed. Taken together, the combination of conventional treatment with immunotherapy has great potential for treatment of advanced cancer patients and needs to be further investigated in larger controlled and randomized phase trials.

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