

Combining radiotherapy with immunotherapy

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ABSTRACT

It has long been known that radiation destroys the tumors by killing cells. However, immunotherapy is developing rapidly, combination of immunotherapy with chemotherapy and radiotherapy seems to be a viable option. Preclinical studies combining immunotherapy with radiotherapy resulted in promising data, where manipulating immune response enhances the effects of radiation. There are many ways of immunotherapy-radiotherapy combination, and the field is open for clinical research. This paper reviews the current situation on this topic.

Keywords: Immunotherapy, Radiotherapy

Introduction

Radiotherapy is an important component of the classical anticancer treatment. The effects of radiation on the irradiated organs and tissues are well known since mid-20th century, which shaped the radiotherapy until recent decade. Radiation response at the cellular level and radiation interactions with subcellular structures and molecules are the current focus of the radiation research which expected to contribute to the science of radiation oncology in the coming decades [1].

Radiation attacks DNA and mediates its effects by killing of cells. We know that cancer cells are more prone to death from unrepaired DNA damage than the normal cells; radiation oncologists to treat the cancer patients without creating serious side effects use this gap. Broken and unrepaired DNA either kills the cell through necrosis or apoptosis, where the final result is the disintegration of the cell and phagocytosis of the cellular elements. Frequently the cell survives DNA damage but loses its replicating capacity and sometimes many of its functions discontinue where the cell is forced to live in a steady state, which is called senescence [2].

In addition to its tumoricidal effects there is strong evidence that radiation also activates the immune system and enhances immune responses. At first sight radiation directed activation of the immune system seems to be contradictory, since radiotherapy – like chemotherapy – has long been considered as immunosuppressive [1, 2]. However, recent studies showed that cytotoxic treatments have immunostimulatory effects on tumor cells and their microenvironment. Although currently we know only a tiny part of the all mechanisms and molecules involved in immunostimulation process, it is worthwhile to research this field [3].

There is a promising experimental data supporting the combination of radiotherapy with various immunotherapy techniques, however further clinical studies are required to translate this data into daily practice. Combining radiotherapy with immunotherapy may open new frontiers in the cancer treatment [4].

In this paper, we summarize, the existing knowledge on radiation and immune response and review preclinical and clinical data on the use of radiotherapy with immunotherapy.

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Tumor immunology

Identification and elimination of tumors is among the important roles of the immune system. Tumors include transformed cells expressing antigens, which are not found on normal cells of an individual. These antigens are considered to be alien by the immune system, which provokes the attack of immune cells to those antigen bearing tumor cells [1].

The antigens expressed by tumors originate from several sources. These may be acquired from oncogenic viruses like human papillomavirus, a common cause of cervix uteri cancers, while others are the individual's own proteins that express at low levels in normal cells but reach high levels in tumor cells. Another source of tumor antigens are oncogenes - proteins playing role in regulating cell growth and survival that commonly mutate into cancer inducing molecules [5].

Once a tumor antigen is identified, the immune system destroys the abnormal cells using killer T cells, sometimes with the assistance of helper T cells. Tumor antigens are presented in a similar way to viral antigens, which allows killer T cells to recognize the tumor cell as abnormal. Natural killer (NK) cells also kill tumor cells in a similar way, especially when the tumor cells have fewer MHC class I molecules on their surface than normal, which is common with tumors. Sometimes antibodies are generated against tumor cells as well allowing for their destruction by the complement system [1-3].

Unfortunately, tumors may overcome the suppression by the immune system and proceed to become cancers, this process is called immune evasion. Many tumor cells have a reduced number of MHC class I molecules on their surface, and avoid detection by killer T cells. Some tumor cells able to suppress the activity of macrophages and lymphocytes through releasing molecules that inhibit the immune response, such as secreting the cytokine TGF-. Finally, immunological tolerance against tumor antigens may develop which results in the silencing of the immune system against the tumor cells [6].

Interaction between radiation and immune system

Ionizing radiation causes immunogenic death of cancer cells, modulates antigen presentation by cancer cells, and most importantly alters the microenvironment within the irradiated field. Cells composing the immune system are usually rapidly dividing and proliferating, thus most of them are extremely radiosensitive. Within the irradiated

field almost all immune system cells are depleted including cytotoxic T Lymphocytes (CTLs) and NK cells that are directed against the tumor, and regulatory T lymphocytes (Tregs) programmed to suppress local anti-tumor immunity. The relative importance of these alterations caused by radiation is still not clear, but we know that immune system senses this damage and triggers immune response pathways resulting in several systemic implications [1].

Once a cell is destroyed by radiation, a number of tumor antigens are released to extracellular matrix. These antigens contact with T lymphocytes and causes differentiation of these lymphocytes to antitumor CTLs through a process called immune priming [7]. Several molecules including HMGB1, uric acid and heat shock proteins (HSPs) are among those released from the cellular fragments, where HSPs serve as a marker of damaged cells for elimination by the immune system and facilitate antigen cross-presentation, dendritic cell (DC) maturation, and NK cell activation [8]. Overall result of these processes is the induction and amplification of antitumor responses. The most important event is the uptake of tumor antigens by macrophages and DCs. This process is mediated by the translocation of calreticulin from the endoplasmic reticulum of tumor cells to the cell surface. Radiation damage causes calreticulin to translocate to the tumor cell surface where it sends a message to macrophages and DCs for identifying the tumor as an enemy, and directs macrophages and DCs to absorb calreticulin expressing tumor cells [9].

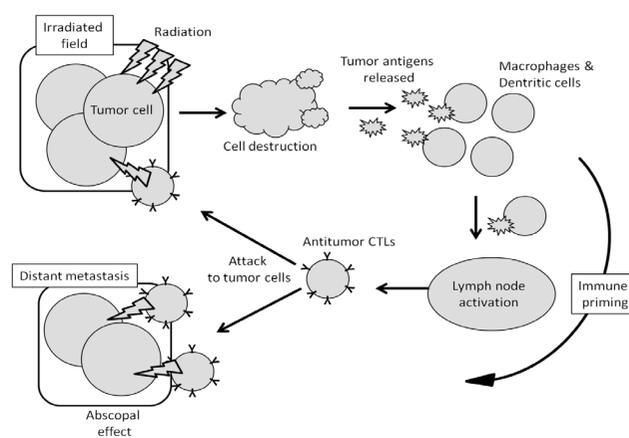


Fig 1: Schematic drawing of the immunologic events after the tumor cell is destroyed by radiation. Antigens released from the tumor cells are considered as foreign by T-lymphocytes and an immune process is started. CTLs directed to tumor attacks not only to tumor bulk in the irradiated area but also to all antigen bearing tumor cells within the organism, a process called abscopal effect.

Tumor eradication through immunologic cell death has also been linked with radiation-induced DNA damage pathways via ataxia telangiectasia mutated (ATM) protein and p53. In the presence of ATM, DNA-damaged tumor cells can up regulate ligands such as NKG2D, making those more efficiently recognized for elimination by NK and activated CD8+ T cells [10].

Systemic antitumor immune response (Abscopal effect)

Radiotherapy directed to a tumor locally may induce regression of metastatic cancers at distant sites, which are away from the irradiation field. This phenomenon is called abscopal effect. Although it is extremely rare, its effect on the cancer can be stunning, leading to the disappearance of malign deposits throughout the entire body. Several cases were reported for a variety of cancers, including melanomas, cutaneous lymphomas, and renal cancers. Abscopal effect appears to be a result of enhanced immune response directed to tumor cells. While there is evidence that radiotherapy can induce cross-priming of CTLs against tumor antigens, this subsequent effect of local irradiation appears to be relatively insignificant. Although radiotherapy presents new antitumor CTLs, these usually cannot overcome the suppressive effect of the tumor microenvironment at distant metastatic sites; this explains why abscopal responses are not seen in daily routine practice.

The destructive effects of the radiation on the immune system cells, along with the unsuccessful results of the early immunotherapies prevented the idea of combining radiotherapy with immunotherapy. However, today's genetic engineering techniques allow us to manipulate DNA and enable to enhance systemic immune response of radiotherapy, which sets the rationale for combining systemic immunotherapies with radiotherapy. Used together, radiotherapy and immunotherapy may have synergistic effects and may shift the tumor immune system balance toward elimination of all systemic tumor cells. Even the "infield" effects of radiation have been shown to be dependent on the immune system, as CD8+T cells and Type-1 interferon are required for tumor regression after radiotherapy, since their depletion impairs tumor control after irradiation [11-13].

Clinical trials involving radiation and immunotherapy

Promising preclinical data from combined approach of RT and immunotherapy has led to clinical trials and a number of phase I-II clinical studies translated the experimental research into clinical setting successfully.

Ipilimumab is the first antibody to modulate tumor immunology approved by FDA for clinical use. This molecule binds to CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) which is a protein receptor that down regulates the immune system through inhibitory signals on CTLs. Ipilimumab silences the inhibitory signal of CTLA-4 on CTLs and allow them to recognize and destroy cancer cells [3]. Apart from preclinical data there is also a case report describing disappearance of malign melanoma deposits in a patient who was under ipilimumab treatment and received paraspinal irradiation to palliate a metastatic mass. This phenomenon was attributed to abscopal effect, and increases the enthusiasm for studying ipilimumab and radiotherapy combination. Ongoing phases I - II trials on malign melanoma are testing the use of ipilimumab with radiotherapy.

Dendritic cell vaccines are also in the focus of radiation researchers. So far radiotherapy and vaccine combinations demonstrated less activity probably related to immunosuppressive effects of the tumor microenvironment. Future combinations of cancer vaccines with immunotherapeutics that enhance T-cell function or modulate the tumor microenvironment may be more effective [6].

The safety of combining radiotherapy with immunotherapy has always been a question. A phase-I demonstrated the safety of combining stereotactic body RT and interleukin-2, an FDA-approved immunotherapy for metastatic renal cell carcinoma and melanoma. Response rates were better in this study when compared to historical data. A similar single- arm phase I-II study also demonstrated safety and feasibility of combining local RT with a TLR9 agonist [14].

One of the most popular questions yet to be answered is the use of immunotherapeutics to modify the immune-suppressive tumor microenvironment prior to radiotherapy. Radiation has its own local effects on the tumor microenvironment, and modulation of the tumor microenvironment has the potential to boost the systemic

anti-tumor-immune response. However currently we have very few data for the dose and fractionation, irradiation site, timing and sequencing of combined modalities. This area requires further research.

Dose and fractionation are important factors in the immunogenicity of RT. Pre-clinical data demonstrated that, 8 Gy in three fractions or 6 Gy in five fractions are superior to standard fractionation or a single dose of 20 Gy when combined with ipilimumab [15]. This is probably related with the difference in immune effect between different dose and fractionation schedules, but these schedules are supported by clinical reports of abscopal effect after palliative radiotherapy to a single metastatic site in malignant melanoma (9.5 Gy x 3) and non-small cell lung cancer (6 Gy x 5) [16, 17].

The target site of radiotherapy may be another important consideration when combining radiation with immunotherapy. Preclinical models are not cleared yet the best way to maximize abscopal effect. Is there a correlation between the amount of the irradiated tumor and the size of the abscopal effect? If so, should we irradiate some of the metastatic deposits besides primary tumor? Or is it good enough to irradiate only a portion of the primary to trigger abscopal phenomenon? At the moment, the only data we

can get from the clinical reports of abscopal effect is that this phenomenon is observed after radiotherapy targeting visceral metastases [18-21].

The timing of radiotherapy - immunotherapy combination is another important question needs to be answered. One possible order may be the manipulation of the immune system by immunotherapy and preparation for irradiation afterwards, but we have no idea yet for the optimum gap between these two modalities. Of course other combination scenarios considering not only radiotherapy and immunotherapy but also chemotherapy and even surgery should be considered as well.

Conclusions

Immunotherapy is developing rapidly. There are numerous preclinical studies involving immunotherapy, where some of them combining with radiation (Table 1). Manipulation of the immune response may enhance the effects of radiation both locally or systemically through abscopal effect. Promising preclinical data on this combination is already accumulated and clinical studies are just starting. Immunotherapy combination may shape the future of radiotherapy.

Table I: Current ongoing trials testing combination of radiotherapy and immunotherapy <https://clinicaltrials.gov>

Study	Tumor Site	Primary endpoint	Radiotherapy schedule
NCT01703507	Metastatic melanoma to brain	-Determine the maximum tolerated dose (MTD) of ipilimumab when combined with whole-brain radiation therapy (WBRT) or stereotactic radiosurgery (SRS)	SRS doses: 24, 21, 18, and 15 Gy. Whole-brain radiation dose: 37.5 Gy
NCT01416831 Phase -II randomized	Metastatic melanoma	-Compare response rate of high dose IL- 2. -Measure the response of SBRT and IL -2 in crossover patients with melanoma who have disease progression after high dose IL- 2 alone.	20 Gy x 1 and 20 Gy x 2
NCT01903083 / phase-I	Locally advanced and borderline resectable pancreatic cancer	-Evaluate the safety of combination gemcitabine, tadalafil, and hypofractionated radiation	8-10 Gy x 3
NIH/NCI11-C- 0247 NCT01496 Phase -I	High- or intermediate risk prostate cancer	-Evaluate the effect of the MUC1-specific vaccine (stimuvax/L-BLP25/ tecemotide) on systemic immune responses when given in combination with standard radiation and androgen-deprivation therapy	Conventional dose and fractionation
NIH/NCI # pending	Metastatic colorectal cancer	-Evaluate the safety of AMP-224—a PD-1 inhibitor—in combination with SBRT in patients with metastatic colorectal cancer.	8 Gy x 1 or 8 Gy x 3

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