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Immunization by Radiotherapy: Enhancing an Immune-mediated Abscopal Effect

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Abstract

Radiotherapy aims to destroy tumors by inducing DNA damage in their cells at the local irradiation site. Its immune-mediated systemic effects, called abscopal effect, has shown to enhance anti-tumor immunity when combined with immune therapies and thus, have gained attention by researchers and clinicians to investigate. Below is a summary of the fundamentals of cancer and the immune system's response to it, as well as the changes in the phenotype and microenvironment of tumor cells after exposure to radiation. Impacts of the abscopal effect and the induction of effective antitumor immunity with various immune therapy strategies will be outlined. The emphasis is set on combination strategies of local radiation therapy with immune therapies such as growth factor inhibitors and immune-checkpoint inhibitors. Limitations Finding the most effective immune therapy in conjunction with radiation and optimizing these therapies to be more patient-centered, offers the potential to improve anti-cancer treatments in the future.

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Introduction

Radiotherapy plays a vital role in cancer treatment. It not only has the ability to affect the DNA of the local tumor, but it also produces systemic and immune-mediated antitumor immunity (Deloch et al., 2016). These effects are enhanced when radiotherapy is used in combination with other therapies that activate the immune system (Frey & Gaipl, 2015). This review focuses on the induction of local and systemic antitumor immune responses by radiotherapy and combined immunotherapies.

The number of cancer incidences is expected to increase by over 70% over the next 20 years, according to the World Health Organization (Steward & Kleihues, 2017), which presents the need for researchers and clinicians alike to find effective antitumor treatments.

Cancer and the immune system

The basics of cancer

Cancer is a condition that is manifested by the presence of one or another type of neoplastic growth—a malignant tumor (Weinberg, 2014). The cells that make up these neoplastic tissues have manipulated the versatile and autonomous nature of normal cells. These individual cells also gain normally denied access to their genomic information and adopt roles that are deemed inappropriate for maintenance and function of normal tissues (Chen & Mellman, 2013). Additionally, with increased genomic instability, the cell becomes progressively susceptible to mutations which can further influence an abnormal phenotype. Simply put, normal cells function to collaborate with each other in order to maintain organismic survival. On the other hand, cancer cells care little about function and have a more focused agenda—growing as much as possible and creating more copies of themselves (Weinberg, 2014).

Cancer is a genetic disease that is an accumulation of exposure to environmental carcinogens and random DNA mutations (Chen & Mellman, 2013). Mutations lead to a cascade of events that start with a change in DNA. Mutated DNA can cause changes in the mRNA product which influence changes in the amino acid sequences and further changes in protein structure and function.

There are two types of mutations, somatic and germ-line. Germ-line mutations are inherited mutations, passed down to offspring that ultimately increase their susceptibility to acquire that type of cancer. Somatic mutations are not inherited but changes in the DNA sequence caused by random mutations accumulated over time (Weingberg, 2014). They include two categories: loss of function and gain of function mutations. Loss of function mutations occur in tumor suppressor genes, which are specific genes that encode a protein that functions to inhibit survival and proliferation signals in normal cells. Tumor suppressor genes are recessive in nature. These recessive genes require two mutates copes (one maternal and one paternal) to yield a loss of function in the protein, leading to the survival and proliferation of cancer cells. Gain of function mutations occur in proto-oncogenes, which are genes that encode a protein that promotes cellular growth and survival. Proto-oncogenes are dominant in nature and can be activated to an

oncogene by a gain of function mutation in only one copy of the gene. This leads to constitutive activity of the protein and continuous downstream signaling of various mechanisms of cellular growth (Chen & Mellman, 2013). However, even in proto-oncogenes and tumor-suppressor gene the mutations are random events, thus, it is possible to speculate that cancer is just a combination of mutated susceptible genes and unfortunate luck.

Tumors with the ability to metastasize can move around, unrestricted within the confines of the body through blood and lymphatic vessels and may establish novel colonies of cancer cells in distant tissues. The newly established colonies, or metastases, can be directly traced back to the site where the cancer began, termed the primary tumor (Formenti & Demaria, 2013). For unknown reasons, tumors in certain tissues have a high probability of metastasizing and tumors from different tissues almost never do. The colonization of sites distant to the primary tumor is a complex seven-step process sometimes referred to as the invasion-metastasis cascade that relies heavily on signals from the surrounding environment (Gajewski et al., 2006).

The tumor microenvironment (TME) houses many different types of cells alongside cancer cells such as fibroblastic cells, lymphocytes, bone marrow-derived inflammatory cells, and an extracellular matrix (ECM) composed of proteoglycans and collagen (Gajewski et al., 2013). The discovery that the stromal microenvironment of tumors has closely related characteristics to normal wounded tissues that do not heal has been recognized for over a century (Weinberg, 2014). However, the more recent discovery concerning its role in stimulating an immune response to attach the cancer cells, known as adaptive immunity, was discovered in 1960 (Schumacher & Schreiber, 2015). The former discovery still has relevance in that chronic inflammation plays a necessary role in promoting tumor formation. It has been thought that macrophages, a type of inflammatory cell, have traditionally been the first line defense against invaders where they consume them and aid in the cascade of immune cell activations through antigen presentation (Gajewski et al., 2013). Other than in the invasion-metastasis cascade, there is evidence that macrophages sent to eradicate the invader also function as sources of tumor promotion. Their role in tumor promotion stems from their production of mitogenic growth factors, liberation of angiogenic factors, and remodeling of the ECM (Gajewski et al., 2006). More and more cells of the immune system that are primarily released to protect the body from infection as well as cancer, are found to be major components in the development of the latter. Paradoxically, further experiments in mice subjected to germ-line reengineering, where one or another type of cell form the immune system was deleted, resulted in the organism being less capable of supporting tumorigenesis, and thus was more immunogenic (Weinberg, 2014).

Immune response to cancer

To be effective in killing cancer cells, the immune response mounted against cancer cells must initiate a series of events an eventually produce the expansion of specifically adapted immune cells to their target (Frey et al., 2014). In the first step of this cycle, neoantigens produced by oncogenesis are seized after cancer cell death by nonspecific dendritic cells (DCs) and

processed. Unless peripheral tolerance to these neoantigens is induced, additional signals must be present at this step for an antitumor T-cell response to be mounted. These signals can include various pro-inflammatory cytokines and factors released from dying cancer cells (Schumacher & Schreiber, 2015). After processing, The DCs present the antigen on their MHCI and MHCII molecules to the T-cell receptors (TCRs) of helper and cytotoxic T-cells. This binding, along with other expressed complimentary costimulatory molecules, enables the priming of effector T-cells and subsequent activation. This results in effector T-cell responses against cancer-specific antigens viewed as non-self or against those from incomplete central tolerance (Reits et al., 2006). The activated T-cells that complete central tolerance travel from the lymph node through lymph and blood vessels to infiltrate the tumor bed, where it will specifically recognize and bind it's TCR MHCI of the target cancer cell and kill it. The killing of cancer cells releases more tumor antigens that then stimulates an increased immune response that ultimately promotes a faster and more specific response in subsequent response cycles (Weinberg, 2014). However, this cycle does not always yield effective T-cell killing machines. For example, tumor antigens may not be detected, and/or they might be recognized as self rather than non-self, resulting in T regulatory cell (Treg) responses rather than effector T-cell responses (Chen & Mellman, 2013).

The derivation of the antigens that permit the immune system to differentiate malignant cells from nonmalignant cells has been unknown for a long time (Tureci et al., 2016). However, we do know that the body's T-cell repertoire can recognize peptide epitopes on the surface of malignant cells via the highly specific major histocompatibility complexes (MHCs). The theoretical explanations for the origin on these cancer rejection epitopes involve the derivation from two classes of antigens (Schumacher & Schreiber, 2015). The first class of possible antigens is made by non-mutation proteins to which incomplete T-cell tolerance occurs due to their limited tissue expression pattern. The second class of possible cancer rejection antigens are made from peptides that are completely absent from the normal human genome—neoantigens. The large group of human tumors without a viral etiology are exclusively created by tumor-specific alterations in DNA that produce new protein sequences (Schumacher & Schreiber, 2015).

The repertoire of neoantigens expressed in cancer at the point it is clinically apparent may be influenced substantially by the interaction of the developing tumor with the immune system (Schumacher & Schreiber, 2015). Cancer progresses and develops in the body in three phases. During the first phase, the immune system senses tissue changes during neoplastic transformation and releases chemicals, such as cytokines, to alert other immune cells to the area. Here, the innate and adaptive immune responses recognize the transformed tissue and attempt to eradicate it before it become clinically detectable This is the elimination phase (Whiteside, Demaria, Rodriguez-Ruiz, Zarour, & Melero, 2016). If elimination is incomplete, the cells that survived the initial immune response have generated escape mutants due to genomic instability. However, tumor proliferation is equal to tumor killing and, is thus, called the equilibrium phase. To tip the scales in favor of tumor survival and proliferation, the tumor's neoantigens and associated epitopes, recognized by T-cells, are constantly

generated. This leads to the escape phase, where a loss of more immunogenic antigens causes the immune system to no longer mount an effective killing response (Derer et al., 2015). Thus, the tumor acquires resistance to immune rejection and now can be clinically detectable Therefore, strategies such as radiotherapy, which mediates the immunogenic release of tumor antigens, along with ways to upset the body's natural immunosuppressive dominance, provide the environment to recover the efficacy of immunotherapies (Whiteside et al., 2016)

Abscopal Effect

Local radiation promotes systemic antitumor vaccine

The application of local radiotherapy, in combination with targeted immunotherapies, where the radiation acts as an antitumor vaccine on tumors outside the radiation field, is a phenomenon known as the abscopal effect (Reynders, Illidge, Siva, Chang, & De Ruysscher, 2015). First proposed by Mole in 1953, the term "abscopal effect" was derived from the Latin "ab" (away from) and "scopos" (target), referring to the systemic effects of local radiation on distant nonirradiated tumor sites in animals after treatment. (Mozdarani, 2012). This rare but well-documented event represents a paradigm shift in cancer therapy—some effects of radiation are seen as beneficial and contribute to the regression of the local primary tumor as well as its metastases (Formenti & Demaria, 2013).

The generalized objective of radiotherapy is to deposit maximum dose of ionizing radiation in the tumor while sparing healthy tissue (Demaria, Golden, & Formenti, 2015). Radiation uses localized beams of intense energy to cause catastrophic DNA damage which produces highly radical oxygen species (ROS) that further damage DNA. The DNA damage checkpoint then arrests the tumor cell from advancing through the cell cycle and employs repair pathways (Deng et al., 2014). If the various DNA damage response mechanisms are unable to compensate the damage, tumor cell death occurs. The apoptotic tumor remnants are phagocytized by nonspecific, antigen-presenting cells (APCs) in the area and presented on their MHC class I molecules to await adaptive immune cells (Derer et al., 2015).

Other non-apoptotic tumor cells at the irradiated site still undergo DNA damage but are either unable to sense the damage or are unable send the signal due to prior mutations that inhibit these steps. Therefore, when the tumor cell machinery senses this damage, instead of signaling apoptosis, it releases signals like damage-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs), and chemokines to enlist the help of inflammatory cells to the TME (McBride et al., 2004). The proinflammatory modifiers responsible for recruiting effector cytotoxic and helper T-cells are the chemokines CXCL9, CXCL10 and CXCL16 and the cytokines interleukin 1β tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ) type 1 and 2 (Lugade et al., 2008). TNF-α and IFN-γ also promote the maturation and cross-presentation of DC's. As these help signals are being sent, tumor cells undergo changes in their phenotype that augment their susceptibility to and recognition by recruited immune effectors. Some of these phenotypic changes include the increased expression of death receptors, MHC class I molecules, costimulatory molecules, adhesion molecule and stressed induced ligands (Reits et al.,

2006). primes the tumor cells which allows them, and the already primed APCs to communicate with the recruited B- and T-lymphocytes to activate their effector functions.

Various mechanisms have been proposed to explain the abscopal effects of radiotherapy. While we know that radiation causes inflammation that induces the activation of antigen-presenting DCs (Gupta et al., 2012), the integral events leading up to the final effect, are poorly understood. Patient specific tumor heterogeneity and unpredictability as well as the individualized immune response to the tumor, further complicate our understanding of the necessities for induction (Tureci et al., 2016). Although, recent studies have shown that an adaptor protein known as the stimulator of interferon genes (STING), a main contributor in innate immunity, is one requirement for the antitumor effect of radiation and the introduction of type I IFNs (Deng et al., 2014). Adaptor proteins are responsible in intracellular signaling pathways where they regulate gene transcription. In regards to cancer, the STING pathway operates by using its cytoplasmic pattern recognition receptor to sense radiation-induced, tumor-released DNA (Rodriguez-Ruiz et al., 2016). The importance of type I IFNs has been elucidated by the prior research of Burnette et al. (2011) testing mice either lacking or not, the type I IFN receptor ($IFNAR^{-/-}$). They found that cytotoxic T-cell function depends on their radiation-induced presence in the TME. As mentioned before, IFNs are a class of proinflammatory proteins that enhance cross-presentation to activate the specific adaptive immune response. The STING pathway bridges that gap between innate and adaptive immunity and in response to radiation, is crucial for the spontaneous generation of antitumor T-cell responses against immunogenic tumors (Woo et al., 2015). However, the diverse range of stimuli needed to generate type I IFN production, as seen in the various nucleic acid-sensing pathways, together with the unknown identity of immune cells that carry out type I IFN responses after radiation, exemplify the difficulties of inducing the abscopal effect. Recent data suggest that radiotherapy increases these responses, thereby providing a potential explanation to this rather elusive event (Deng et al., 2014).

The uncommon occurrence of abscopal effects observed in cancer patients reflects only one barrier to effective tumor rejection. For cytotoxic T-cells to reject a tumor, a set of sequential steps must be followed. First, T-cells must be able to home correctly to the tumor site by extravasating from vessels to access the tumor microenvironment. Secondly, it is imperative for T-cells to retain their effector functions once they arrive at the tumor site. Lastly, stable immunological synapses must be established between the tumor cell and the effector T-cell (Gajewski et al., 2006). In each of these steps, there are multiple obstacles to overcome due to the complex nature of signaling pathways and their messengers.

Another critical barrier to priming of T-cell responses to various tumor antigens, induced by radiotherapy, is the protein known as transforming growth factor beta (TGFβ) (Kang, Demaria, & Formenti, 2016). This cytokine is important in promoting the differentiation of immunosuppressive T-cell subsets, like regulatory T-cells (Tregs) which mediate immune responses from becoming harmful to the body. Therefore, when ROS are produced as a consequence of

radiotherapy, they convert latent TGFβ to its active form (Vanpouille-Box et al., 2015).

While radiation promotes the release of pro-immunogenic signals to the tumor site, it also promotes immunosuppressive mechanisms. Therefore, its ability to induce an immune-mediated abscopal effect most likely depends on altering the pre-existing tumor microenvironment to shift the balance to favor an immunostimulatory one (Gajewski et al., 2013). Despite how the positive effects of radiation generally outweigh the negative effects, without targeted immunotherapy, radiation alone is not insufficient to shift the balance to accomplish tumor rejection and control metastatic progression (Formenti & Demaria, 2013). The therapeutic applications of radiotherapy as well as chemotherapy, whether stand-alone or in conjunction with targeted immunotherapies, should stimulate local and systemic tumor control by the promotion of immunogenic cell death, which can induce persistent antitumor responses by the immune system. (Gaipl et al., 2014).

Effects enhanced by combination strategies

The application of ionizing radiation to cancer therapeutics has long been established due to the combination of its cytocidal influence and selectivity in targeting tumors (Demaria et al., 2015). However, in the past two decades, the concept was proposed to combine local radiation treatment with immunotherapy to induce an abscopal effect focused on inhibiting metastatic growth (Shiraishi et al., 2008).

Combination strategies from these clinical trials utilized targeted immunotherapies to enhance the effects of radiation, such as influencing either the priming or effector phases of antitumor immune responses (Formenti & Demaria, 2013). In one study, cross-priming of antitumor T cells was enhanced by the amplification of DC number and function. This was demonstrated in mice by the administration of DC growth factors such as Flt3-ligand near the irradiated tumor and by the injection of exogenously prepared syngeneic DCs into the irradiated tumor for which inhibition of spontaneous metastases was observed in a lung carcinoma and breast cancer, respectively (Chakravarty et al., 1999). In a phase I trial, patients with hepato-cellular carcinoma were intratumorally injected with autologous DCs 2 days after single fraction radiotherapy and a partial abscopal response was seen in two out of 14 patients (Chi et al., 2005). In another study, DCs injected into sarcomas during fractionated radiotherapy, showed infiltration of T-cells in the tumor at the time of surgery with tumor-specific immune responses from nine out of the 17 patients. A year later, 12 out of the 17 patients were progression-free of their cancer (Finkelstein et al., 2012).

According to Hildner et al., cross-presentation to cytotoxic T-cells are mainly regulated by a specialized subset of DCs that depend on Baft-3 transcription factor and Flt3-ligand as a growth factor for their development (2008). Experiments to test Baft-3-dependent DCs on the induction of antitumor cytotoxic T-cells in Baft-3-deficient mice revealed a loss of the abscopal effect and a diminished control of the local tumor. This suggests, to some extent the critical role of IFNα/β on the therapeutic effects from radiotherapy. Similarly, $BATF^{-/-}$ and $IFNAR^{-/-}$ mice lost the radiation-induced abscopal effects when treated with the combination of anti-PD1 and anti-CD137 monoclonal antibodies, or mAbs (Rodriguez-Ruiz et al., 2016). In addition, strategies focused on the enhancing the local induction

of IFNα/β have potential to elicit more immunogenic tumor cell deaths induced by combination treatments with radiotherapy. The immunogenic cell death induced by radiotherapy, however, only offers temporary systemic control and may be due to the weak immunization effects or because of factors and mechanisms such as TGFβ that work in tandem to suppress the immune system (Vanpouille-Box et al., 2015). For example, strategies tested in preclinical and clinical trials to block TGFβ combined with radiotherapy, have the potential to counteract its immunosuppressive effects and to also thwart DNA damage repair, angiogenesis, and metastasis (Bouquet et al., 2011).

Strategies to promote the effector phase of antitumor immunity have coupled T-cell activation with an antibody that targets the inhibitory checkpoint receptor CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) after radiation therapy (Kang et al., 2016). Currently being developed in the clinic, mAbs that target receptors on immune cells where they either remove coinhibitory signals or supply costimulatory signals to improve antitumor immunity. The combination of ipilimumab (an anti-CTLA-4 mAb) with radiotherapy currently has limited clinical experience as there are only results from two clinical trials that are now available for prostate cancer and metastatic melanoma (Twyman-Saint Victor et al., 2015). In a preclinical study using anti-CTLA-4 mAbs, it was shown that simultaneous positive costimulation with inhibition of negative costimulatory signals increased the avidity by 10-fold. Wherein, avidity is the concentration of antigen required to elicit a T-cell response after target loading (Poleszczuk et al., 2016). Results from prospective clinical trials combining radiotherapy with anti-CTLA has shown success in non-small cell lung carcinoma (NSCLC). According to Golden et al. (2015), since NSCLC is a tumor type that is unresponsive when give anti-CTLA-4 alone, this gives hope that multiple tumor types might also benefit from the radiotherapy combinations.

Conclusion

Radiotherapy induces an immune-mediated abscopal effect which is further enhanced with the combination of immunotherapy to produce a systemic antitumoral vaccine. Targeted immunotherapies using growth factor inhibitors and antibodies such as TGFβ and anti-CTLA-4, respectively, have reported effective antitumor induction (Derer et al., 2015). Overall, these studies show combination strategies are more effective than any one strategy alone and may be applicable to multiple cancer types in the future.

Limitations

As mentioned above, one of the challenges that researchers face is finding treatments that promote tumor immunogenicity and simultaneously hinder immunosuppression. It is also crucial to gain knowledge on the specific mechanisms and mediators involved in the induction of an immune-mediated abscopal effect, such as understanding STING pathway, as well as information on the identify and function of the specialized subset of DCs responsible for T-cell cross-presentation (Kang et al., 2016). Additionally, a more patient-specific treatment could prove more effective when considering their specific tumor type, stage of progression, and immune cell repertoire. Further investigation is needed to find the optimal timing, dose and fractionization of radiotherapy with combination strategies and

how they depend the type of immunotherapy used (Rodriguez-Ruiz et al., 2016).

Future Research

In general, strategies using radiotherapy to induce systemic antitumor immunity, are being tested in various preclinical trials, in combination with immunomodulatory interventions that either block immunosuppressive mechanisms or enhance immune response activation. However, there is much to be known about the effects of targeting key activators or suppressors of the immune system after radiation. Thus, prospective research should focus on combining multiple immune mediators with radiation to augment the primer and effector phases of antitumor immunity. These include likely targets such as Baft-3, Flt3-ligand, IFNα/β, TGFβ, anti-PD1, and anti-CTLA-4 (Deloch et al., 2016).

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Appendix A

American Psychological Associated (APA) 6th edition was the format style used for this literature review. According to the *APA Publication Manual*, the Title page should include a running head, a title, the author's name and the institutional affiliation. Following the Title page is the Abstract, that is written as a single paragraph of 150-250 words. Next, the Table of Contents page, where the first level is bold, centered, including upper and lowercase letters. The second level headings are bold, left-aligned and capitalized first letter. The body contains the introduction, discussion and conclusion that uses 12-point, Times New Roman font with the paragraphs aligned to the left. A shortened version of the title of the paper was used as a header for all subsequent pages after the title page. The References page appears at the end of the document and includes references with a hanging indent. The in-text citations include the author and the year of publication.

Appendix B

In the process of editing my original version, I decided to restart my literature review paper with a more focused topic and thus, documentation of feedback from my external reviewer was unattainable. However, peer-reviewed suggestions were taken into consideration and most were accepted.