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Mini-review

Building immunity to cancer with radiation therapy

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ABSTRACT

Over the last decade there has been a dramatic shift in the focus of cancer research toward understanding how the body's immune defenses can be harnessed to promote the effectiveness of cytotoxic anti-cancer therapies. The ability of ionizing radiation to elicit anti-cancer immune responses capable of controlling tumor growth has led to the emergence of promising combination-based radio-immunotherapeutic strategies for the treatment of cancer. Herein we review the immunoadjuvant properties of localized radiation therapy and discuss how technological advances in radio-oncology and developments in the field of tumor-immunotherapy have started to revolutionize the therapeutic application of radiotherapy.

Introduction

Our immune system is our first line of defense against cancer. Much like infectious agents, cancer cells express antigenic determinants that can distinguish them from normal tissue and mark them for elimination by cytotoxic effector cells [1]. The highly dynamic relationship that exists between tumor cells and the immune system is best outlined by the "three E" theory of immunosurveillance [2,3], which posits that both innate and adaptive immune cells can directly eliminate or maintain small tumor masses in a long-term state of equilibrium [4,5]. Tumor escape can occur through the immunological selection and outgrowth of poorly immunogenic tumor cell variants, induction of immune cell exhaustion and/or activation of immune suppressor mechanisms, commonly associated with the accumulation of inhibitory immune cells such as T-regulatory cells, myeloid derived suppressor cells (MDSCs) and M2 macrophages [6–8]. Extensive analysis of the immune contexture of cancers, relating to tumor-associated immune cell distribution, density and functional status, and its impact on the evolution and prognosis of a patient's cancer is now helping to guide the clinical management of a diverse array of cancers [9–11].

Immunotherapeutic approaches have been designed to selectively harness host immune defenses against cancer. However more often than not, their efficacy is dependent on there being a pre-existing anti-cancer immune response, often limiting the clinical success of such approaches to more immunogenic cancers like malignant melanoma [12]. In an effort to increase the breadth and frequency of cancers capable of supporting the therapeutic benefits of immunotherapy, traditional and experimental cytotoxic anti-cancer agents are now being screened for their ability to: (i) alter the immunogenic nature of tumor cells; (ii) change the cellular content of the tumor microenvironment in favor of tumor immunity and (iii) kill tumor cells in a manner that can prime durable anti-tumor immune responses [13].

While it was long believed that the immune system did not contribute to the anti-cancer effects of ionizing radiation, there is a growing body of preclinical and clinical data to suggest that irradiated tumor cells can become a robust source of antigen with adjuvant properties, similar to that of an *in situ* vaccine. Through eliciting anti-cancer immune responses with diverse antigenic repertoires, ionizing radiation has the capacity to impact upon tumor growth both within and external to the site of radiation therapy and in turn promote the systemic anti-cancer activity of immunotherapy.

Abbreviations: Gy, gray; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor; SASP, senescence-associated secretory phenotype; MCP-1, monocyte chemoattractant protein-1; MHC, major histocompatibility complex; DAMP, damage-associated molecular patterns; ICD, immunogenic cell death; CRT, calreticulin; MDSC, myeloid derived suppressor cells; IFN, interferon; DC, dendritic cell; IDO, indoleamine 2,3-dioxygenase; SDF, stromal cell-derived factor; CTLA-4, cytotoxic T lymphocyte antigen-4; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; SABR, stereotactic ablative body radiation therapy; LQ, linear quadratic; MRT, microbeam radiotherapy; RAE-1, retinoic acid early inducible-1.

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Radiation-induced cell death

The potential benefits of ionizing radiation for cancer treatment were realized soon after the discovery of X-rays by Wilhelm Conrad Roentgen in 1895. Since its introduction into clinical practice in 1896 by Emil Grubbé, radiation therapy has been employed for its powerful ability to cause tumor cell death through the induction of irreparable DNA damage and cell cycle arrest [14]. The resultant mechanisms of lethality following radiation therapy can include: mitotic catastrophe, apoptosis, necrosis, autophagy and senescence [15–17].

Mitotic catastrophe

Mitotic catastrophe is a delayed form of cell death resulting from the premature or inappropriate entry of cells into mitosis [18]. This form of cell death results from the combination of deficient cell cycle checkpoints and cellular damage and is commonly triggered in non-hematopoietic tumor cells, particularly those with mutated or inactive p53, as well as stromal and parenchymal cells, in response to ionizing radiation [19]. Radiation-induced death via mitotic catastrophe has been demonstrated in preclinical models of solid cancer [20,21] and is thought to be an important mode of radiation-induced cell death in clinically treated tumors. Radiation-induced mitotic catastrophe may precede other modes of cell death including apoptosis or necrosis.

Apoptosis

Apoptosis is a programmed form of cell death that can be triggered by DNA damage, activation of death receptors from the tumor necrosis factor (TNF) receptor superfamily, production of cytoplasmic ceramide due to hydrolysis of sphingomyelin from the plasma membrane or direct mitrochondrial damage [22–24]. This mode of cell death is common in response to mid-to-high dose radiation treatments (5–10 Gy) [16]. Tumor cells with high basal p53 mRNA expression and cells of hematopoietic origin tend to be more prone to radiation-induced apoptosis [25,26]. While long thought to be a silent form of cell death, recent data would suggest that ionizing radiation and some chemotherapeutic agents can kill via apoptotic mechanisms in a manner that can prime anti-tumor immunity; a phenomenon that will be discussed later in the review.

Necrosis

Necrosis is typically referred to as a passive form of cell death that is characterized by the loss of cell membrane integrity followed by DNA degradation [27]. However, more recently a programmed form of necrotic cell death was described, referred to as necroptosis. Necroptosis shares the same morphological features as primary necrosis but is induced by ligation of the TNF receptor [28]. Radiation-induced necroptosis has been documented in thyroid and adrenocortical carcinoma cell lines [29]. Both necrosis and necroptosis are commonly associated with high/ablative-doses of radiation therapy and can trigger protective anti-tumor immunity through the release of damage-associated molecular patterns (DAMP) [30] and apyrase-sensitive nucleotides that can stimulate monocyte chemokinesis [31]. Paradoxically, however, such inflammatory responses can initiate wound-healing responses that may limit the durability of these anti-cancer immune responses [32].

Autophagy

Autophagy is a catabolic process involving the lysosomal degradation of old, damaged or over-active cytoplasmic components and organelles, which under normal physiological circumstances help to maintain cellular homeostasis and promote cell viability [33]. Prolonged or excessive induction of autophagy, caused by exposure to treatments such as radiation therapy, can lead to cell death via the cell literally "eating itself" in an effort to remove damaged cellular components [17]. Leakage of lysosomal hydrolases into the cytosol due to incomplete fusion of autophagosomes with lysosomes may also contribute to cell death via this mechanism.

Senescence

Senescence is a metabolically active condition of permanent cell cycle arrest, commonly caused by telomere attrition in response to DNA damage [34]. Radiation-induced senescence, however, is more closely linked with the activation of p53 and expression of the cell cycle regulatory protein p21 and/or activation of the p16INK4/pRb family of suppressor proteins [35]. Both ionizing and non-ionizing radiation can trigger stress-induced senescence [36]. Senescent cells have been found to secrete multiple inflammatory factors including interleukin (IL)-1β, IL-6, IL-8 and monocyte chemoattractant protein-1 (MCP-1/CCL2), which make up what is known as the senescence-associated secretory phenotype (SASP) [37]. While these inflammatory factors can support tumorigenic processes they can also activate host-innate immune mechanisms that may contribute to tumor suppression and clearance of senescent tumor cells [38,39].

The extent to which these various death-associated processes contribute independently or collaboratively to the curative outcomes of radiotherapy is likely to be cell, tissue and dose-dependent. Indeed in the context of breast cancer, hormone receptor and p53 status were found to influence the mechanism of cell death induced by radiation treatment [31]. Importantly, localized radiation itself and all described outcomes, as well as the presence of a tumor itself, induce acute or chronic local stress that can lead to genotoxic events in surrounding and distant tissues and organs [40–46]. Immunological responses that may ensue following radiation therapy will ultimately depend on the type of cell death that is induced and the cellular and structural makeup of the surrounding tumor microenvironment.

Immune stimulatory effects of radiation therapy

Radiation-induced changes to tumor immunogenicity

Due the inherent susceptibility of naive immune cells to radiation-induced apoptosis, radiotherapy was long viewed as an immunosuppressive form of cancer therapy [47]. However extensive preclinical analysis of the anti-cancer effects of ionizing radiation has revealed that radiation therapy has the capacity to engage host immune effector mechanisms that may contribute to the control and/or eradication of cancer [48] (Fig. 1). Indeed, irradiated tumor cells have been reported to become a robust source of antigen with adjuvant properties, through the upregulation and diversification of MHC class I expression [49]. This phenomenon has been demonstrated to occur in response to activation of the mTOR pathway, leading to enhanced translation and *de novo* protein production. Radiation-induced modulation of MHC class I expression was shown to increase T cell recognition of irradiated tumor cells, making them vulnerable to cytotoxic T lymphocyte (CTL)-mediated clearance [49]. Notably, by increasing the release of antigens from cancer cells, ionizing radiation may also result in the transient expression of tumor-specific MHC/peptide complexes on stromal cells, thereby exposing the structural matrix of the tumor to the tumoricidal effects of infiltrating CTLs [50]. Additional changes to the immunogenic status of irradiated tumor cells, including the up-regulation of death receptors [e.g. CD95 (Fas)] [51], activating NKG2D ligands [e.g. Retinoic acid early inducible (RAE)-1] [52] and heat shock proteins [e.g. Heat

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