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Review Article

A translational concept of immuno-radiobiology

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ABSTRACT

Background: Traditional concepts of radiobiology model the direct radiation-induced cellular cytotoxicity but are not focused on late and sustained effects of radiation. Recent experimental data show the close involvement of immunological processes.
Methods: Based on systematic PubMed searches, experimental data on immunological radiation effects are summarized and analyzed in a non-quantitative descriptive manner to provide a translational perspective on the immuno-modulatory impact of radiation in cancer.
Results: Novel experimental findings document that sustained radiation effects are ultimately mediated through systemic factors such as cytotoxic CD8+ T cells and involve a local immuno-stimulation. Increased tumor infiltration of CD8+ T cell is a prerequisite for long-term radiation effects. CD8+ T cell depletion induces radio-resistance in experimental tumors. The proposed sequence of events involves radiation-damaged cells that release HMGB1, which activates macrophages via TLR4 to a local immuno-stimulation via TNF, which contributes to maturation of DCs. The mature DCs migrate to lymph nodes where they trigger effective CD8+ T cell responses. Radiation effects are boosted, when the physiological self-terminating negative feedback of immune reactions is antagonised via blocking of TGF-β or via checkpoint inhibition with involvement of CD8+ T cells as common denominator.
Conclusion: The concept of immuno-radiobiology emphasizes the necessity for a functional integrity of APCs and T cells for the long-term effects of radiotherapy. Local irradiation at higher doses induces tumor infiltration of CD8+ T cells, which can be boosted by immunotherapy. More systematic research is warranted to better understand the immunological effects of escalating radiation doses.

Classical models of radiobiology focus on direct radiation-induced cytotoxicity at a cellular level but are not designed to provide a comprehensive view of other functional systems that are secondarily affected during radiotherapy. Sustained and late effects of focal high dose radiation as applied during stereotactic radiosurgery (SRS) [1] or hypo-fractionated stereotactic body radiation therapy (SBRT) remain ultimately unexplained. While the events after tumor irradiation including the clinical side effects of radiotherapy intimately involve immunological networks, the radiation-induced immune-modulation still remains to be analyzed and summarized in detail.

Active but dysfunctional immune responses are a general feature in cancer patients [2] as malignant tumors significantly interfere with the patient's immune system. The high inherent radiosensitivity of lymphocytes led to the traditional assumption that radiation is generally immunosuppressive but antigen presenting cells (APCs) are generally radio-resistant [3] and at specific doses radiation even activates immunostimulatory processes. Local immuno-modulation provides essential explanations for clinical long-term effects of radiotherapy [4–7].

The objective of this manuscript is to summarize the available experimental data on the immunological processes following irradiation of malignant tumors. The focus is to describe the novel concept of radiation-induced immune effector cells and the chain of events that lead to their stimulation and to the radiation-induced immuno-modulation.

Onco-immunology

While malignant tumors promote an inflammatory microenvironment [2,8–10], tumor cells appear to be protected from immunological eradication by an environment of cytokine-mediated local immunosuppression [2,8,9]. A common cytokine pattern evolves in patients in advanced cancer stages with concomitant increase in both immunostimulatory and immunosuppressive cytokines [2]. In cancer patients Antigen Presenting Cells (APCs) such as dendritic cells (DCs) and macrophages are

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active but dysfunctional [11–13] in their ability to stimulate T cells [11,12], which ultimately results in impaired responses to antigenic stimuli and T cell exhaustion [13–16].

Current radiobiological concepts

Traditional radiobiology is focused on cytotoxic effects at a cellular level and on the initial hours after irradiation. The processes of radiation-induced DNA damage and cell destruction are well defined and unquestioned. Some cells are killed via induction of apoptosis and other cells lose their reproductive integrity [17].

While *in vitro* data analyzed the most basic effects of radiation at a cellular level, the evolving concept of radiobiology does not define the ensuing immunological processes in the tissue surrounding the irradiated field and does not provide a comprehensive and detailed biological concept for delayed adverse radiation effects such as pneumonitis or cerebral leukoencephalopathy. Another radiobiological concept is focused on the vascular impact of radiation, as high dose irradiation causes tumor necrosis through vascular damage [18,19]. The two radiobiological concepts have not yet been integrated into one comprehensive theory.

Radiation and CD8+ T cells

In stark contrast with these traditional radiobiological concepts, recent experimental data reveal that the clinical effects of focal radiotherapy largely depend on tumor infiltrating cytotoxic CD8+ T cells. In 2009 Lee and colleagues demonstrated that the radiation effect is effectively T cell-mediated [20] with cytotoxic CD8+ T cells being instrumental since the tumors became radioresistant following CD8+ cell depletion [20]. Single radiation doses of 20 Gy in wild-type mice achieved significant tumor regression and an increase in infiltrating T cells in the tumor microenvironment, while in T cell-deficient nude mice the tumors became radioresistant and grew progressively [20]. This effect was reproduced by Takeshima and colleagues who reported that depletion of CD8 + T cells resulted in abolition of radiation-induced tumor growth inhibition [21]. Multiple similar experimental studies in mice support the concept that the therapeutic effect of irradiation relies on the presence of cytotoxic CD8+ T cells as their experimental depletion significantly and often completely abolished the effects of radiotherapy at 2 Gy[21], 15 Gy[21], 20 Gy[20], 30 Gy [22–24], 2×12 Gy[25] or 5×6 Gy[26]. Mice lacking adaptive immune cells and mice depleted of CD4+ or CD8+ T cells fail to develop remissions after radiation treatment [27].

Experimental local tumor irradiation induced significantly increased levels of tumor-specific CD8+ cytotoxic T cells [21,25,28,29] with close association between radiation-induced tumor-specific CD8+ T cells at local tumor sites and radiation-induced tumor growth inhibition [21]. Shortly after a single focal radiation dose of 30 Gy cytotoxic CD8+ T cells entered into subcutaneously implanted murine colon tumors and induced durable remissions [24], which required the presence of CD8+ cross-priming DCs [24], while depletion of either CD8+ T cells or CD4+ T cells significantly reduced survival [24]. Focal high-dose radiation reduced the infiltration of immunosuppressive myeloid-derived suppressor cells (MDSC), immunosuppressive tumor associated macrophages (TAMs) and regulatory T cells, and hence transformed the initially immunosuppressive tumor microenvironment [24,27]. Multiple independent preclinical studies provide the circumstantial evidence that radiation-induced activation of CD8+ T cells follows a sequence of events that is outlined in the following paragraphs.

HMGB1 and radiation

As reviewed by Lauber, radiation can induce necrosis or necroptosis, i.e. necrosis in a programmed fashion, or secondary necrosis when apoptotic or damaged cells are not sufficiently removed [30]. Radiation-induced chromosomal damage and cellular necrosis initiates the release of Damage-Associated Molecular Patterns (DAMPs), among those High Mobility Group Box 1 protein (HMGB1) [31]. HMGB1 is passively released from the nucleus of necrotic [32] or apoptotic cells [33] and even secreted by activated macrophages in response to inflammatory stimuli or cytokines such as TNF or IFN-γ [34,35].

Radiation at doses ranging between 4 and 12 Gy induced a cytoplasmic HMGB1 translocation even in normal cells (skin fibroblasts), and stimulated a time- and dose-dependent HMGB1 release both *in vitro* and *in vivo*. At doses ranging between 4 and 8 Gy, HMGB1 release was induced as early as 6 h post stimulation [31]. Similarly, HMGB1 translocation from the nucleus to the cytoplasm and subsequent release into the extracellular space was seen after irradiation in glioblastoma cells [36]. Even without significantly reducing viability, 10 Gy induced HMGB1 secretion in lung and prostate cancer cell lines [37].

In 2007 Apetoh and colleagues reported that HMGB1 released by dying tumor cells was a requirement for an immune response that was triggered via Toll-like receptors (TLR4) [38], with TLR4 being required for preventing tumor outgrowth after experimental local radiotherapy [39]. Blocking HMGB1 inhibited the inflammatory response and decreased the degree of alveolitis in irradiated lung tissue after application of 15 Gy as a single dose to the whole thorax in mice [40].

Macrophages, TNF and radiation

HMGB1 from necrotic cells contributes to an immunostimulatory environment via dose-dependent upregulation of the expression of the cytokines TNF and IL-1β in macrophages and DCs with involvement of TLR4 [41–44]. Many cell types including most malignant tumors are able to produce TNF [45], but activated macrophages and T cells are the main source [46]. TNF is a mediator of inflammation, stimulates activation [47] and maturation of DCs [48–50]. Immune cells vary in their radiosensitivity [3], but macrophages are relatively radioresistant and remain viable after irradiation of $2 \text{ Gy} \times 5$ [51].

Alternatively or in addition to direct stimulation, macrophage activation could be a secondary effect of radiation, resulting from cellular damage signals [52,53] rather than being a direct effect of radiotherapy, as newly recruited lung alveolar macrophages in mice were activated without previous direct exposure to radiation [54].

TNF (and IL-1b) [55] together with pro-inflammatory macrophage markers (HLA-DR, CD86) [51] were increased in irradiated macrophages [56,57] and monocytes [58], while immunosuppressive IL-10 was down-regulated [51]. Crittenden and colleagues reported that a transient upregulation of pro-inflammatory genes in irradiated macrophages [59] and a rapid and transient increase in pro-inflammatory cytokine mRNA levels (TNF and IL-12p40) occurred one hour after in vitro irradiation of murine bone marrow-derived macrophages at 4 Gy [60]. TNF occurred in macrophages after lung irradiation with 15 Gy (in BALB/c mice) [61].

A variety of normal tissues responds to radiation with increased TNF production such as liver Kupffer cells [62], lung tissue (C57BL/6J mice) [63], murine intestine [64] and (rat/mouse) brain [65]. The irradiation of mouse brains with a single dose of 20 Gy increased TNF mRNA levels 11-fold [66]. In lung cancer cell lines

irradiation induced TNF mRNA expression up to 83 times over normal controls with a maximum at 40 Gy [67].

Tumor regression that had been achieved through combination of irradiation with checkpoint inhibitors, was significantly impaired when TNF was neutralized [68]. Similarly, anti-TNF treatment inhibited radiation-induced both local and abscopal effects in murine brain tissues [69]. TNF inhibitors such as Pentoxifylline and gluco-corticosteroids that suppress the release of TNF in monocytes and macrophages [70,71] are used in the treatment of adverse radiation effects [72].

Dendritic cells and radiation

TNF is an important factor for the maturation of immature DCs in the periphery [48–50], which is a crucial initial step for the specific activation of cytotoxic T cells cascade of signals and feedback circuits. Antigen-loaded mature DCs migrate to the lymph nodes after upregulation of chemokines (CCR7) in response to chemoattractants from afferent lymphatics and lymph nodes (CCL19, CCL21) [73]. In lymph nodes mature DCs undergo further maturation termed "licensing" via interaction between CD40 on DCs and CD40 ligand on antigen stimulated CD4+ T cells [74]. Licensed DCs trigger effective CD8+ T cells responses via antigenic cross-presentation, MHC class II expression, co-stimulation and pro-inflammatory cytokines (IL-12) [74–84].

A range of independent experiments and clinical data provide evidence for the interaction of radiation with this immunological activation cascade at multiple levels. Ablative radiation using single doses of 20 Gy induced activation and maturation of DCs as prerequisite of T-cell priming [20]. An increase in tumor-specific CD8+ T cells that was induced by experimental radiotherapy required DC-proficient mice [85]. In a murine melanoma model the therapeutic effect of experimental radiation significantly depended on the presence and functional integrity of both dendritic cells and CD8+ T cells, as depletion of CD8+ T cells and depletion of DC abolished the therapeutic effect of radiotherapy [85]. Irradiation of established murine tumors with high local doses of 20 Gy generated infiltrating DCs with a significant increase in their T cell stimulating potential [12]. Local irradiation with 10 Gy significantly increased immunogenicity by DC expression of co-stimulatory molecules CD70 and CD86 [85]. Interestingly, DC migration and DC activation markers CD80 and CD86 including secretion of stimulatory cytokines were significantly increased by co-incubation with supernatant of irradiated tumor cells [28,86], hence suggesting a radiation-induced factor for DC migration. Demaria summarized the radiation-induced factors that lead to a stimulation of the immune system against the irradiated tumor with an effect resembling a local vaccination [5].

Mature DCs are considered to be radioresistant as a single dose of 30 Gy does not induce apoptosis [87], but DCs that had been irradiated before maturation were less effective and produced significantly less IL-12 than non-irradiated controls [87]. Similarly, DCs derived from monocytes that had been irradiated with 20 Gy and then stimulated by LPS showed reduced functionality compared to DCs derived from non-irradiated monocytes [88]. A common feature of these experiments was that immature DCs were studied.

MHC expression

CD8+ T cell responses require cellular antigens to be presented in association with MHC class I molecules [89]. MHC expression is a prerequisite for adaptive immune recognition. It has been suggested that the loss or reduced expression of MHC molecules on cancer cells is one of the factors for tumor escape from cytotoxic T cells [90].

Local irradiation, in particular with radiation doses above 10 Gy [91], resulted in increased immunogenicity via increased MHC class I expression on tumor cells in murine experimental models [92–95] with IFN-γ being instrumental [92]. In human resected glioblastoma cultures irradiation increased immunogenicity through increased HLA class I antigen expression in a dose-dependent manner with a maximal effect at 12 Gy [96]. DCs exhibited an increased expression of MHC class II within 48 h after single local radiation doses of 20 Gy (in murine B16 melanoma) [20].

Anti-Inflammatory effects of radiation

Immune reactions are generally biphasic with an early inflammatory phase followed by a reparative phase. Phagocytosis of apoptotic cells induces anti-inflammatory cytokine production in macrophages [30,97]. Homeostasis requires the termination of the immune reaction and the antagonisation of immunostimulatory processes. These mechanisms are hijacked by malignant tumors that produce immuno-suppressive factors such as TGF-β or PD-L1.

Comparable to homeostatic immuno-suppression that physiologically terminates the immune-stimulation, in later phases the irradiated tumor environment secondarily promotes an immunosuppressive development with up-regulation of TGF-β and PD-L1 [6], with influx and reparative differentiation of (M2) macrophages [59], and tumor infiltration of TAMs and MDSCs at a maximum between 12 and 15 days [98]. MDSCs are heterogeneous hematopoietic bone marrow derived stem cells that inhibit immune responses [99]. The involved immuno-suppressive mechanisms were reviewed by Weichselbaum [7] and Frey [6]. These seemingly contradictory effects are most likely part of intricate feedback circuits that contribute to homeostasis with both immuno-stimulating and suppressive phases [59].

Treatment failure after radiotherapy has been attributed to these secondary immuno-suppressive effects [59], while the effect of radiotherapy is significantly boosted when the physiological negative feedback is antagonised via blocking of TGF-β [26] or via checkpoint inhibition [29,68,100–105].

Co-stimulation and negative feedback

The activation of cytotoxic T cells requires secondary co-stimulatory signals from DCs as CD28 and CD2 on T cells bind to their respective ligands B7.1 (CD80) or B7.2 (CD86) on APCs [106]. APC associated T cell activation and hence the physiological immune reaction is self-terminating through a number of negative feedback circuits, among others involving TGF-β [107,108], and *checkpoint proteins* CTLA-4, PD1 and PD-L1. For example, PD-1 is the 'off-switch' on the surface of activated T cells that regulates the amplitude of T cell activation [109], while APCs express the PD-1 ligand PD-L1, which is physiologically expressed upon antigen stimulation via IFN-γ and TNF thus terminating the immune reaction [13]. The details of checkpoint inhibition in clinical practice have been detailed in many publications and are not repeated herein.

Corresponding to negative feedback after immune activation, high-dose local radiation upregulates PD-L1 expression (in murine tumor cells) in an IFN-γ-dependent fashion [68,100,110,111], hence ultimately terminating the inflammatory reaction. Localized radiation with 12 Gy increased expression of PD-L1 on DCs and tumor cells in a murine mammary tumor model and the efficacy of radiation was significantly increased by anti-PD-L1 treatment [68]. In murine hepatocellular and squamous cell carcinoma

models the combination of radiation with 10 Gy in one fraction with anti-PD-L1 treatment significantly suppressed tumor growth and improved survival compared to treatment with radiation alone [29,100]. In particular focal irradiation with single doses of 10 Gy combined with anti-PD1 therapy increased CD8 effector T cells and survival significantly and produced long-term cures in 15–40% of treated mice [112] even resulting in 'immunologic memory' as tumors did not grow in re-implanted previously 'cured' animals [112].

Similarly, it was clinically shown that chemo-radiotherapy in patients induced the expression levels of PD-L1 and the density of CD8+ T cells [113]. The radiation-induced PD-L1 expression on tumor cells however, appears to be dependent on the fractionation scheme [114], as an increase in PD-L1 could specifically be induced by normo- and hypo-fractionation [114]. As the effect could not be reproduced in a corresponding in vivo model, it was suggested that immune cells might secondarily contribute to the up-regulation of PD-L1 on tumor cells [114]. The combined effect of radiation and checkpoint inhibition was reproducibly eliminated by depletion of CD8+ T cells [23,25,68,112].

TGF-β

TGF-β is one of the tumor-induced immuno-suppressive cytokines and creates a local environment of immune tolerance. TGF-β is a strong antagonist of IFN-γ and IL-12, down-regulates both MHC expression and co-stimulatory molecules and upregulates PD-L1 [107,108], thus inhibiting the essential stimulating feedback between DCs and cytotoxic CD8+ T cells. Elevated TGF-β serum levels are a frequent finding in late stage cancer patients [2].

TGF-β is up-regulated after radiation of normal tissue [115–117] and TGF-β serum levels correlate with the development of radiation-induced pneumonitis and fibrosis [118–121] several months after radiotherapy [120]. On the other hand initially increased TGF-β serum levels decreased during radiotherapy in patients with lung cancer [122] and local high-dose irradiation with 10 Gy resulted in reduced TGF-β production by fresh ovarian carcinoma [123] or glioblastoma cells [124].

Vanpouille-Box and colleagues demonstrated that experimental radiotherapy (6 Gy × 5) in combination with TGF-β blockade significantly increased the number of tumor-infiltrating DCs [26] and generated CD8+ T cell responses to tumor antigens [26]. Again the combined therapeutic effect was T cell-dependent as depletion of CD4+ or CD8+ T cells abrogated the regression of irradiated tumors [26].

Abscopal effects

Abscopal effects of radiation occur in remote locations that are not included in the radiation field. Traditional radiobiology offers no explanation for this phenomenon. While these remote effects were previously described as rare case reports [125–132], abscopal effects appear to occur in a more reproducible manner when radiotherapy is combined with checkpoint inhibition. Recent literature reviews identified 16 clinical studies reporting on abscopal effects after combination of radiotherapy with ipilimumab in metastatic melanoma, with a median overall abscopal response rate of 26.5% [133] with the abscopal effect occurring at a median latency of 5 months [134].

While the detailed mechanisms underlying abscopal effects still remain obscure, several experimental studies reproduced abscopal effects by combination of radiation and checkpoint inhibition using anti-PD-L1/anti-CTLA-4 antibodies or PD-1 blockade [95,111,135,136]. Again, a common feature appeared to be an increased number of tumor-infiltrating CD8+ T cells [95,100,137].

As early as in 2005 the team of Demaria and Formenti suggested that the abscopal effect that local radiation (2 × 12 Gy) in combination with CTLA-4 blockade had on un-irradiated remote lung metastases in a murine model, resembled a vaccination that elicited antitumor T-cell responses and required CD8+ cells [138].

Latency

Lung irradiation with 15 Gy (in BALB/c mice) stimulated macrophages to produce TNF starting at 3–24 h after radiation with normalization by day 7 [61] and pro-inflammatory genes appeared in irradiated macrophages immediately following radiation and declined by day 7 post-radiation [59]. Hence, in later phases the number of M2 macrophages increases and the tumor environment may become more suppressive [59].

The experimental radiation-induced tumor infiltration of cytotoxic T cells occurs at a latency of 5–10 days [25,28,85]. After high dose local irradiation with 10 Gy In murine melanoma a striking increase in infiltrating tumor-specific CD8+ T cells was recorded at day 7 [85]. Hettich and colleagues confirmed a similar latency in subcutaneous melanoma in C57BL/6 mice: 5 days post-irradiation (2 × 12Gy) more than 95% of cells in the tumor cell suspension were leukocytes, almost all CD8+ T cells [25]. These changes were transient with gradual normalization within 14 days [25]. Similarly, Frey and colleagues demonstrated that at low hypo-fractionated doses (2 × 5Gy) the tumor infiltration of cytotoxic T cells was transient with a peak at day 8 after radiation [28]. Interestingly, a long lasting increase in TNF-mRNA levels from day 4 until day 100 was detectable after single session irradiation of the pulmonary cavity in mice, while TGF-β2 mRNA occurred late at day 120 and correlated with radiation fibrosis [117].

Dose-dependent effects

In vivo, radiation has an impact on tumor cells, on immune cells within the tumor tissue and on 'healthy' cells in the tumor environment. Single session radiosurgery affects tumor tissue and local resident macrophages, whereas fractionated radiotherapy also has a potentially damaging impact even on immune cells that are secondarily attracted by radiation. It has been argued that fractionated radiation might kill off infiltrating effector cells over time, hence leading to early relapse [20], which may not happen when the dose is applied in a single session or through hypo-fractionation.

In addition, systemic factors may influence radiation-induced anti-tumor immunity: A single dose of 20 Gy induced activation and maturation of DCs [20] and had a significantly higher effect on tumor growth in mice than a fractionated dose of 5 Gy × 4, while in the absence of lymphocytes neither regimen had any therapeutic effect [20]. In a murine model (day 35) the number of tumor infiltrating $CD8^+$ cells was significantly higher after single high doses (1 × 30 Gy) than after fractionated radiotherapy (10 × 3 Gy) [24]. On the other hand, when DCs were co-incubated in vitro with supernatant from tumor cells that had been exposed to normo-fractionated radiotherapy (5 × 2 Gy), the secretion of immune activating cytokines increased significantly in comparison to supernatants of tumor cells that had received a high single radiation dose (1 × 15 Gy) [86]. These in vitro experiments led to the suggestion that normo-fractionation induced superior immunogenic effects in comparison to single high doses [139], which would not be supported by other in vivo results.

As HMGB1 is associated with cellular damage, HMGB1 release both *in vitro* and *in vivo appears to be* dose-dependent [31]. Similarly, both TNF mRNA levels in normal murine brain [66], TNF production of macrophages and TNF gene expression in human

peripheral blood monocytes increased in a radiation dose-dependent manner [58,61]. Particularly at higher doses (10–25 Gy) radiation resulted in a dose-dependent increase in MHC class I expression in melanoma cell lines both *in vitro* and *in vivo* [93]. A single dose of 20 Gy resulted in an 11-fold increase in TNF mRNA levels in mouse brains, while only a 2.3 fold increase occurred after fractionated radiation with cumulative doses of 40 Gy [66].

While several immune effects appear to be dose-dependent, even low cumulative doses of 10 Gy (5×2 Gy) induced a significant increase in pro-inflammatory macrophage markers CD80, CD86 and HLA-DR in human monocyte derived macrophages and induced an immunostimulatory macrophage phenotype [51] and a transiently increased infiltration of macrophages and DCs (at 2×5 Gy) [28].

When combined with a CTLA-4 antagonist, various fractionation regimens caused different abscopal effects, as 8 Gy × 3 was significantly more effective than 6 Gy × 5 in inducing abscopal anti-tumor immunity, while a single dose of 20 Gy did elicit local but no abscopal effects [135]. Similarly, abscopal tumor control occurred when PD-1 mAb was combined with hypo-fractionation (8 Gy × 2), but not in combination with conventional low-dose daily fractionation (2 Gy × 10) [95]. Interestingly, Vanpouille-Box, Demaria and colleagues have recently shown that in various cancer cells immunogenicity may be attenuated by high doses above 12–18 Gy due to induction of DNA exonuclease Trex1 [140]. The consequence was a higher immunogenicity of fractionated doses of 8 Gy × 3 compared to single doses of 30 Gy and the induction of abscopal effects only in the fractionated scheme [140]. Hence the radiation-induced local tissue damage does not predict the radiation induced anti-tumor immunity or its ability to synergize with checkpoint inhibition [135,140].

At very low doses radiation can even cause the opposite effect, as single doses of 0.5–1.0 Gy and total doses of 3.0–6.0 Gy induce local immunosuppression in a variety of inflammatory and degenerative conditions [141]. There is evidence that single session low-dose irradiation with less than 1 Gy induces anti-inflammatory effects on macrophages [142,143].

Systematic dose escalating studies have not yet been carried out to analyze the radiation dose requirements for immune modulation and detailed research is warranted.

Immuno-modulation in clinical studies

There is emerging clinical evidence to support these experimental findings. Treatment with the checkpoint inhibitors ipilimumab, nivolumab or pembrolizumab given within 2 weeks of SRS resulted in a significant survival benefit in a cohort of 260 patients with brain metastases from non-small cell lung cancer, melanoma, and renal cell carcinoma [144]. Similarly, a retrospective analysis of patients treated with radiation and ipilimumab for advanced melanoma showed complete responses in 25.7% and a 12-month-survival probability of 72% for the combined treatment, versus 6.5% complete responses and 35% survival probability for patients treated with ipilimumab alone [101]. The application of the PD-L1 inhibitor durvalumab after chemoradiotherapy significantly increased survival in patients with NSCLC [145].

Interestingly, the timing of the combination of radiotherapy and immune therapy appears to be relevant. In pre-clinical studies the therapeutic effect was reduced when checkpoint inhibition (anti-CTLA-4 mAb 9H10) was delayed until two days after the conclusion of fractionated radiotherapy [135]. In corresponding clinical studies, in 2015 both Kiess et al. [103] and Schoenfeld et al. [104] had reported that SRS during or prior to ipilimumab was associated with superior patient survival compared to SRS after ipilimumab. Similarly, in melanoma brain metastases checkpoint inhibition within 4 weeks either prior to or after SRS resulted in improved tumor control compared to combinations given at longer latencies [102]. The local recurrence-free time-span was significantly longer for patients with brain metastases from malignant melanoma who had received SRS before or during ipilimumab treatment [105].

The potential impact of systemic factors on the effect of radiation is supported by clinical studies showing that patients with lymphopenia had an increased risk of intracranial recurrence [146,147] or significantly worse prognosis [148], while higher circulating lymphocyte counts were associated with improved intracranial disease control after SRS of metastatic melanoma [146]. Boosting of $CD4^+$ and $CD8^+$ T cell responses against tumor-associated antigens was seen in a third of breast cancer patients one month after SBRT (3×10Gy) [149]. HSRT (48 Gy/6–8 fx) increased the proportion of activated CD8+ cells 17-fold in NSCLC patients and decreased the frequency of inhibitory Treg cells together with concomitant increase in immunostimulatory cytokines (IL-2, TNF, IFN-γ) [150]. As experimentally predicted, a dense inflammatory lymphocytic infiltration and predominance of T lymphocytes was the most common histopathological feature in controlled brain metastases that had been resected more than 6 months after Gamma Knife SRS, whereas poorly controlled tumors showed absent or scant lymphoid cell population [151,152].

Clinical impact and future directions

The concept of immuno-radiobiology emphasizes the necessity of a functional integrity of APCs and lymphocytes for the clinical effect of radiotherapy with a potential impact on the clinical monitoring of cancer patients and on concomitantly applied chemotherapies and immunotherapies. Furthermore, the immune-modulating capacity of radiation may have an impact on future fractionation schemes: T-lymphocytes are highly radiosensitive and effectively destroyed by relatively low radiation doses [153,154] and the experimental radiation-induced tumor infiltration of cytotoxic T cells occurs at a latency of 5–10 days [25,28,85]. Hence, fractionation within this timeframe may potentially be counter-productive for an optimal immuno-modulation, which emphasizes the role of hypo-fractionation and SRS. Checkpoint inhibitors boost an existing immuno-stimulation, but may be ineffective when the local immune reaction is sufficiently stifled through a cancer-induced immuno-suppression. The experimental results demonstrate that radiotherapy in the right dose and time frame may ultimately provide the necessary local immunostimulation that can be intensified by checkpoint inhibitors.

The consideration of immunological aspects does not contradict but complements existing radiobiological theories, and allows a conceptual integration of current immunotherapy into radiotherapeutical practice.

Summary

The novel finding is that the sustained radiation effect is not only dependent on the deposited physical energy but is ultimately mediated through systemic factors such as the patient's T cells. Tumor-infiltrating cytotoxic CD8+ T cells are instrumental for the long-term effects of focal radiotherapy, and tumors become radioresistant through CD8+ cell depletion. TLRs on macrophages respond to HMGB1 that is released from necrotic cells, and via TNF-production macrophages contribute to an immunostimulatory environment. Maturation of immature DCs is induced with up-regulation of MHC class II and co-stimulatory molecules and with secretion of pro-inflammatory cytokines, including IL-12

and IFN-γ, hence stimulating T cells toward a pro-inflammatory and cytotoxic phenotype. Physiological immune reactions are self-terminating via negative feedback involving TGF-β, CTLA-4 and PD-L1, which is evident even after experimental radiotherapy. Radiation effects are boosted, when this negative feedback is blocked via checkpoint inhibition or by TGF-β antagonisation. Experimental combination of radiation and checkpoint inhibition reproducibly creates abscopal effects. Further systematic dose escalating studies are required for a more detailed definition of the radiation-induced immune modulation.

Declaration of Competing Interest

None.

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