Cancer Letters 356 (2015) 105–113

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Contribution of the immune system to bystander and non-targeted effects of ionizing radiation

Franz Rödel [a,*], Benjamin Frey [b], Gabriele Multhoff [c,d], Udo Gaipl [b]

[a] Department of Radiotherapy and Oncology, Goethe-University Frankfurt am Main, Frankfurt am Main, Germany
[b] Department of Radiation Oncology, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[c] Department of Radiation Oncology, Technical University of Munich, Munich, Germany
[d] CCG "Innate Immunity in Tumor Biology", Helmholtz Zentrum München (HMGU), Munich, Germany

ARTICLE INFO

Article history:
Received 9 July 2013
Received in revised form 13 August 2013
Accepted 11 September 2013

Keywords:
Ionizing radiation
Bystander effects
Non-targeted effects
Abscopal effect
Immune system
Heat shock protein 70

ABSTRACT

Considerable progress has recently been achieved in the understanding of molecular mechanisms involved in cellular radiation responses and radiation mediated microenvironmental communication. In line with that, it has become more and more obvious that X-irradiation causes distinct immunological effects ranging from anti-inflammatory activities if applied at low (<1 Gy) doses to harmful inflammatory side effects, radiation-induced immune modulation or induction of anti-tumour immune responses at higher doses. Moreover, experimental and clinical evidences indicate that these effects not only originate from direct nuclear damage but also include non-(DNA) targeted mechanisms including bystander, out of field distant bystander (abscopal) effects and genomic instability. The purpose of the present review is to elucidate immune responses that are initiated or affected by ionizing radiation, with a special emphasis on anti-inflammatory and abscopal effects and the induction of stress-induced anti-tumour immunity.

1. Introduction

Since decades the classical paradigm in radiation biology comprises the concept, that deposition of energy to the nucleus and targeted DNA damages, in particular, DNA double-strand breaks are responsible for the biological consequences of radiation exposure. By contrast, based on more recent findings, there is emerging evidence for non-(DNA) targeted effects. Among these novel findings bystander or distant out of field (abscopal) mechanisms, as well as adaptive responses, low dose hypersensitivity and genomic instability in non-clonal descendants of irradiated cells have been reported [1–3]. In addition, a common hallmark of these effects is that they became dominant at low doses of irradiation and often display non-linear dose–response relationships. These novel concepts further take into consideration a complex intercellular communication [4], and the interaction of irradiated cells with the surrounding tissue and the immune system. Moreover, they appear to be dependent on genetic, epigenetic and environmental factors. Indeed, the demonstration of a bystander effect in three dimensional (3D) human skin tissue [5] and in whole organisms [6] further reveals the relevance of these non-targeted phenomena in human health, in the modulation of stress responses, in carcinogenesis and in anti-tumour immunity. Thus, the purpose of the present review is to outline recent developments in understanding how ionizing irradiation induces non-targeted responses in anti-inflammatory treatment when applied in low doses and how it contributes to the induction of a cellular and tissue stress response and anti-tumour immunity at higher doses.

2. Contribution of non-targeted effects to the anti-inflammatory efficacy of low dose radiation therapy

The interrelationship between ionizing radiation and the immune system is multifactorial and highly depends on the radiation dose/quality and immune cell types investigated. Whereas exposure to higher doses (single doses $\geqslant$2 Gy, total doses $\geqslant$40 Gy) induce pronounced inflammation promoting effects [7], lower doses (single doses $\leqslant$1.0 Gy, total doses $\leqslant$12 Gy) reveal anti-inflammatory effects [8,9]. As a consequence, low dose radiation therapy (LD-RT) has been traditionally used in the clinical settings as early as 1898, when Sokoloff first reported on pain relief in patients with polyarthritis treated with X-rays [10]. Several decades later, a patterns-of-care study performed in Germany was published with 37,410 patients treated by LD-RT for degenerative or hyper proliferative disorders. These include impingement syndrome of the shoulder joint (rotator cuff syndrome), tennis/golfer's elbow, plantar fasciitis (painful heel spur), osteoarthritis or Dupytren's disease [11]. Concerning the most important clinical endpoints such as

* Corresponding author. Address: Department of Radiotherapy and Oncology, Goethe-University of Frankfurt am Main, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany. Tel.: +49 696301 4838; fax: +49 696301 5091.
E-mail address: franz.roedel@kgu.de (F. Rödel).

0304-3835/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.canlet.2013.09.015

pain relief, complete response and long-time analgesic effects, LD-RT in current clinical applications is reported to result in high response rates (>50–100%) without induction of radiogenic acute or chronic side effects (outlined in more detail in [8]). On the contrary, LD-RT is still considered unfashionable in some countries due to reports of harmful late effects and increased mortality from leukaemia and anaemia published in the 1960s [12,13]. Pharmaceutical alternatives such as non-steroidal or steroidal drugs, however, also display numerous severe side effects and a considerable number of patients fail to respond to treatment [14]. Although a carcinogenic risk of low-dose irradiation is still a matter of controversy, improved radiation protection and recent progress in the development of predictive objectives for the response to LD-RT such as pre-treatment sonographic classification of calcifying tendonitis [15] may help to reconsider LD-RT as an effective treatment. In line with that, a recent patterns-of-care study reported 95% referral for radiation therapy in 4500 patients with osteoarthritis of the knee demonstrating an increased acceptance. Finally, Ott and colleagues recently reported that radiation therapy with lower single doses of 0.5 Gy might be as equally effective as single doses of 1.0 in the treatment of painful elbow or shoulder syndrome thus substantially decreasing the potential radiation risk [16,17].

By contrast, underlying radiobiological and immunological mechanisms are far from being fully explored. During the last two decades, however, the modulation of a multitude of inflammatory processes by LD-RT has been reported *in vitro* and *in vivo* [9]. These include modulation of mononuclear/polymorph nuclear leukocyte functions, apoptosis regulation, leukocyte/endothelial cell interaction, as well as surface marker and cytokine/chemokine expression (Fig. 1).

2.1. Non-targeted effects in leukocytes contribute to the anti-inflammatory effects of low dose irradiation

Whereas different lineages of lymphocytes (B and T cells) comprise cellular members of an antigen-specific effector response, polymorph nuclear cells (PMN: neutrophilic, eosinophilic and basophilic granulocytes) and peripheral blood mononuclear leukocytes (PBMC) are major components of the innate immune system representing the first line of host immune defence [18].

Due to their central role in the initiation and the resolution of an inflammatory process, mononuclear leukocytes (macrophages and dendritic cells) derived from peripheral blood precursors are considered as key players in the regulation of inflammation and immune responses [19]. Tissue resident macrophages, for example, support a local inflammatory process by a multitude of functions including phagocytosis, antigen presentation, secretion of cytokines, release of reactive oxygen intermediates (ROIs), and the expression of enzymes like inducible nitric oxide synthase (iNOS) [20]. iNOS processes the synthesis of nitric oxide (NO) that in turn increases vascular permeability and is involved in inflammatory pain [21]. In that context, low dose irradiation ($\leqslant$1.0 Gy) decreases iNOS protein and NO production without affecting iNOS mRNA expression in RAW 264.7 macrophages stimulated with lipopolysaccharide (LPS) and interferon-γ (IFN-γ) [22]. This may indicate a post-translational regulation of the enzyme that is linked to the analgesic properties of LD-RT. Furthermore, low dose X-irradiation significantly reduced oxidative burst capacity in murine RAW 264.7 macrophages after stimulation with tumour necrosis factor-α (TNF-α)/(IFN-γ), Phorbol 12-myristate 13-acetate (PMA) or the yeast product Zymosan, whereas elevated doses had little effect [23]. This further highlights the therapeutic effect and modulation of an anti-inflammatory microenvironment by low dose irradiation.

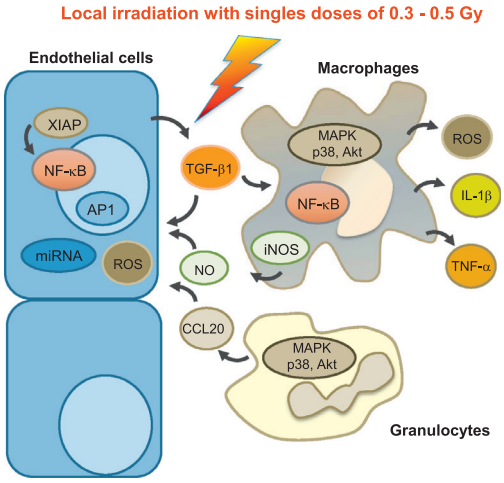

Fig. 1. Current model on cellular compounds and factors involved in a local anti-inflammatory activity of irradiation. Exposure of activated ECs to a dose of 0.3–0.5 Gy resulted in a modulation of miRNA and XIAP expression and as a consequence increased NF-κB activity, increased TGF-β1 expression and a reduced peripheral blood mononuclear cell (PMBC)/endothelial cell (EC) adhesion. In leukocytes an induction of apoptosis, a reduced secretion of the chemokine CCL20, a hampered activity of the iNOS-pathway, a lowered oxidative burst, a decreased secretion of the pro-inflammatory cytokines IL-1β and TNF-α from activated macrophages as well as a lowered expression of MAPK (p38, Akt) may contribute to anti-inflammatory effects.

Further, a pivotal molecular mechanism in the regulation of an irradiation associated inflammatory, stress or bystander response includes the expression and secretion of regulatory peptides namely cytokines, chemokines and growth factors. While inflammation and immune activation promoting factors such as interleukin-1 (IL-1), TNF-α and chemotactic factors (e.g. IL-8 and CCL20) activate the immune system, anti-inflammatory factors such as the isoforms of transforming growth factor (TGF)-β1-3 or IL-10 limit immune responses and inflammatory cascades [24–26].

With regard to cytokine production, a hampered pro-inflammatory TNF-α secretion at doses of 0.5 Gy and 0.7 Gy from human THP-1 derived or RAW 264.7 macrophages stimulated by LPS was reported by Tsukimoto [22] and an additional reduced secretion of the pro-inflammatory cytokine IL-1-β was observed in RAW 264.7 macrophages which have been co-activated with mono sodium urate crystals (MSU) [21]. Mechanistically, the hampered cytokine production correlates with a diminished nuclear translocation of the immune relevant transcription factor nuclear factor-κB (NF-κB) subunit RelA (p65) [21] in line with a decreased expression of NF-κB upstream (p38 mitogen activated protein kinase (MAPK)) and downstream factors like Protein Kinase B (Akt). Additionally, a dephosphorylation of both extracellular-signal-regulated kinases 1/2 (ERK1/2) and p38 MAPK was observed as early as 15 min after a 0.5 Gy X-ray exposure concomitant with a significantly increased expression of MAPK phosphatase-1 (MKP-1) [27]. Recently, Frischholz and colleagues reported that peritoneal macrophages derived from radiation sensitive Balb/c mice respond to a 0.5 or 0.7 Gy exposure with a diminished IL-1β and TNF-α release, whereas macrophages form less radiosensitive C57/BL6 mice did not [28]. This further highlights the complex regulation of low dose irradiation mediated cytokine expression and an involvement of genotype-dependent mechanisms that foster continuative investigations.

Neutrophilic PMN infiltration has been implicated in the pathophysiology of acute and chronic inflammatory diseases, such as rheumatoid arthritis [29], in part by the secretion of chemokines to amplify and direct leukocyte infiltration [30]. Irradiation with doses between 0.5 and 1 Gy resulted in a significant reduction of CCL20 chemokine release from PMN in parallel to a significant