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Role of radiation-induced rescue effect in radiation field size effect

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A B S T R A C T

The present paper reviewed the role of radiation-induced rescue effect (RIRE) in radiation field size effect. "Radiation field size effect" refers to the phenomenon that the radiobiological effects of ionizing radiation depend on the size of the irradiated area, besides depending on the equivalent dose. RIRE refers to the mitigation of detrimental effects in irradiated cells after receiving signals from non-irradiated bystander cells, or after receiving signals from the medium which has previously conditioned the non-irradiated bystander cells. The present paper will first give a brief review on RIRE, including the definition and classification of RIRE, as well as the signalling pathways and chemical messengers which have been identified for RIRE, and will then give a review of selected literature related to radiation field size effects. Discussion as well as some thoughts on future priorities and directions of research in the role of RIRE in radiation field size effect will then be presented.

1. Introduction

"Radiation field size effect" refers to the phenomenon that the radiobiological effects of ionizing radiation depend on the size of the irradiated area, besides depending on the equivalent dose. The radiation field size effect is of fundamental importance in radiological protection issues. Notably, the International Commission on Radiological Protection (ICRP) determines cancer risks from the "average" equivalent doses according to the linear-no-threshold (LNT) model, but without fully taking into account potential influence from different field sizes arising from non-uniform irradiation. This approach inevitably leads to inconsistencies with experimental results in reality, which have widely demonstrated that radiobiological effects of ionizing radiation are not only related to the equivalent doses, but also depend on the size of the irradiated area.

The presence of radiation field size effect has also far-reaching implications in different branches of medical physics. For example, such studies can be important for understanding the effects of modulated fields generated by intensity-modulated radiation therapy (IMRT), including the potential complications caused by the scattered radiation dose as well as contributing doses from multiple beam angles. As another example, spatially-fractionated radiotherapeutic techniques, including e.g., microbeam radiotherapy (MRT), has aroused extensive interest since the techniques appear to be effective destroying tumors while sparing normal tissues (see e.g., Fukunaga et al., 2019, 2020). As such, studies on the radiation field size effect are pertinent.

Almost 40 years ago, Coggle et al. (1984) collaborated with Peel et al. (1984) on investigating the effects of β particles from different sources and with different irradiation field size on mouse skin and pig skin, respectively, and reported different doses required for different skin reactions with the field size of irradiation. About a decade ago, Butterworth et al. (2012) irradiated different cell lines with intensity-modulated X-ray fields, and discovered a significantly larger survival response in human prostate cancer cells when only 25% of the cell population was irradiated. Recently, Ojima et al. (2021) studied in turn the responses of primary normal human lung fibroblasts to an X-ray microbeam with the same dose of 1 Gy but with different field sizes, and found that the number of DNA double strand breaks (DSBs) in the irradiated cells in general increased with the field size.

Matsuya et al. (2019, 2022) studied two different irradiation scenarios, one using a radioactive Cs-bearing particle source to provide non-uniform localized exposures and the other using an external ^{137}Cs source to provide uniform radiation exposures. The different sizes of irradiated areas could be treated as different radiation field sizes. Matsuya et al. (2019) found that above a certain dose, the number of DSBs induced in normal human lung cells by non-uniform irradiation became smaller than that induced by uniform irradiation, and the authors proposed radiation-induced rescue effect (RIRE), a non-targeted radiobiological effect discovered by Chen et al. (2011), as a potential explanation. Matsuya et al. (2019) further performed half-field experiments and demonstrated that the number of DSBs induced in the cells were reduced when compared to uniform irradiation. In a follow-up investigation, Matsuya et al. (2022) succeeded to demonstrate activation of different inflammatory responses under non-uniform localized exposures and uniform radiation exposures.

Interestingly, in 2015, while studying the RIRE, Lam et al. (2015a) noticed that the extent of damages in the irradiated cells depended on the percentage of irradiated cells or equivalently the size of the

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irradiated area. This finding shed light on the mechanism underlying the radiation field size effect. Recently, Ojima et al. (2021) successfully explained their results on radiation field size effect obtained by their microbeam X-ray irradiation of primary normal human lung fibroblasts in terms of RIRE. Matsuya et al. (2019, 2022) also satisfactorily explained their different results obtained for different sizes of irradiated areas (or equivalently different radiation field sizes) in terms of RIRE.

In view of the fundamental importance and far-reaching implications of the radiation field size effect, as well as the link implied or identified between the radiation field size effect and RIRE, it appears timely to explore in greater depth the role of RIRE in radiation field size effect, which forms the objective of the present review paper. Section 2 will first give a brief review on RIRE, including the definition and classification of RIRE, as well as the signalling pathways and chemical messengers which have been identified for RIRE at the time of writing. Section 3 will then give a review of selected literature related to radiation field size effects. Finally, section 4 will give some discussion, as well as some thoughts on future priorities and directions of research in the role of RIRE in radiation field size effect.

2. Brief review on radiation-induced rescue effect (RIRE)

RIRE refers to the mitigation of detrimental effects in irradiated cells after receiving signals from non-irradiated bystander cells, or after receiving signals from the medium which has previously conditioned the non-irradiated bystander cells. RIRE was discovered in 2011 (Chen et al., 2011), and was previously reviewed by Lam et al. (2015c) and Yu (2019). After the discovery of RIRE in 2011, different research groups carried out RIRE research and had their own experimental design. For a better categorization, Yu (2019) defined two types of RIRE, namely, (a) Type 1 RIRE: where detrimental effects in targeted cells were mitigated upon receiving signals from bystander cells, and (b) Type 2 RIRE: where detrimental effects in targeted cells were exacerbated upon receiving signals from bystander cells. The RIRE initially unveiled by Chen et al. (2011) was Type 1 RIRE, while the RIRE subsequently uncovered by Fu et al. (2016a, b) was Type 2 RIRE. Kong et al. (2018) pointed out that the combination of irradiated/non-irradiated cell types in the experiments for Type 2 RIRE was different from those for Type 1 RIRE, and proposed that the mode of metabolic cooperation between generalized "stressed" cells and generalized "bystander" cells could differentiate between Types 1 and 2 RIRE.

Subsequent to the revelation of RIRE using α particles by Chen et al. (2011), various research groups further confirmed the occurrence of Type 1 RIRE through employing photons (Widel et al., 2012; Pereira et al., 2014; Kong et al., 2018), alpha particles (He et al., 2014; Lam et al., 2015a,b) and protons (Desai et al., 2014; Liu et al., 2015; Kobayashi et al., 2017). These works together with the works which uncovered Type 2 RIRE using α particles by Fu et al. (2016a,b) were reviewed by Lam et al. (2015c) and Yu (2019).

As commented by Yu (2019), it was not yet certain whether Types 1 and 2 RIRE were just different manifestations of the same phenomenon, but successful revelation of the respective underlying mechanisms might provide clues. Mechanisms and/or chemical messengers for Type 1 RIRE included (a) being mediated by cyclic adenosine monophosphate (cAMP) through a membrane signaling pathway (He et al., 2014), (b) activation of the nuclear factor κB (NF-κB) response pathway in the

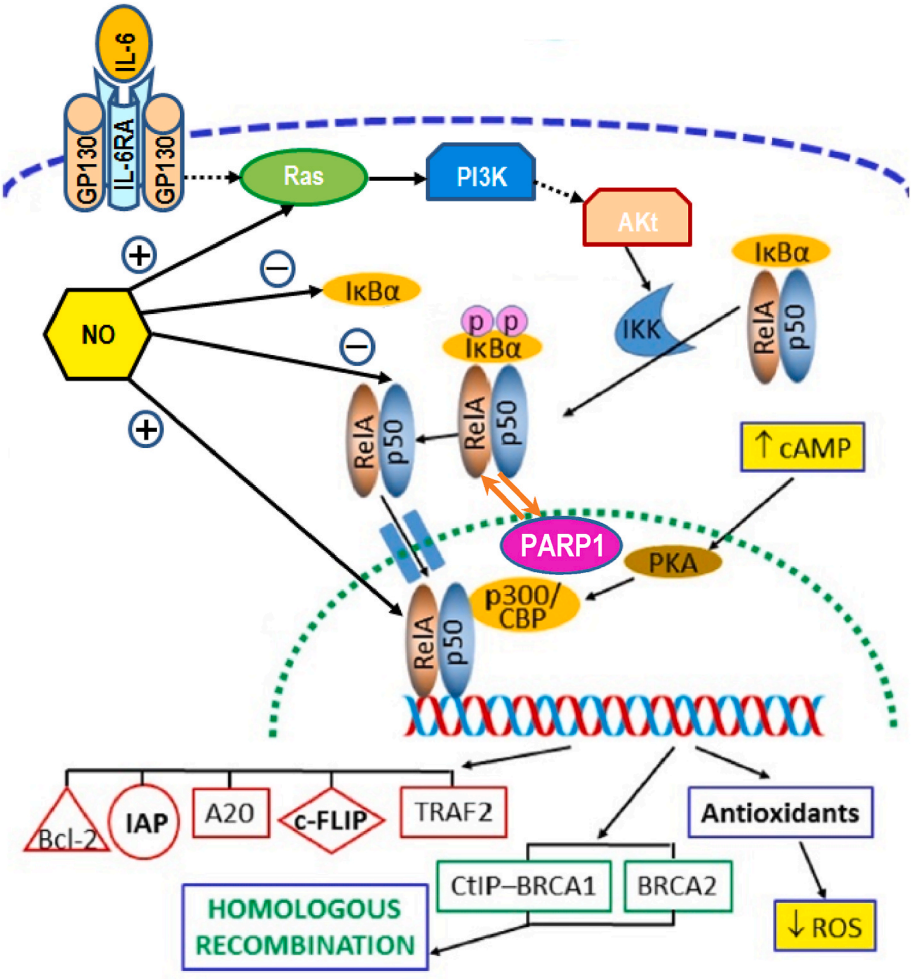

Fig. 1. Summary of mechanisms and chemical messengers involved in RIRE. Blue dashed line: cell membrane; green dotted line: nuclear envelope; dotted arrows: multiple steps involved. IL-6: interleukin 6; IL-6RA: interleukin 6 receptor alpha; RelA (p65) and p50: NF-κB family members (chosen for illustration here); NF-κB: nuclear factor κB; PARP1: poly (ADP-ribose) polymerase 1; cAMP: cyclic adenosine monophosphate; ROS: reactive oxygen species; NO: nitric oxide; GP130: glycoprotein 130; PI3K: phosphatidylinositol 3-kinase; Akt: Protein kinase B (PKB); IKK: IκB-kinase; PKA: protein kinase A; CBP: CREB binding protein; CREB: bcl-2: B-cell lymphoma 2 protein; IAP: inhibitors of apoptosis protein; A20: A20 zinc finger protein; c-FLIP: Cellular FLICE-like inhibitory protein; FLICE: FADD-like interleukin-1β-converting enzyme; FADD: Fas-associated protein with death domain; TRAF2: TNF receptor-associated factor 2; CtIP: C-terminal binding protein (CtBP)-interacting protein; BRCA1: breast cancer type 1 susceptibility protein; BRCA2: breast cancer type 2 susceptibility protein. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

irradiated cells (Lam et al., 2015a,b), being mediated by nitric oxide (NO) (Matsumoto et al., 2001, 2007, 2011; Maeda et al., 2013), (d) activation of the NF-κB pathway in the irradiated cells by interleukin 6 (IL-6) produced in bystander cells as a result of autophagy induction (Kong et al., 2018), and (e) involvement of a poly (ADP-ribose) polymerase 1 (PARP1)–NF-κB positive feedback loop (Pathikonda et al., 2020). Fig. 1 has succinctly summarized these mechanisms and chemical messengers.

On the other hand, mechanisms and/or chemical messengers for Type 2 RIRE included (a) activation of mitogen-activated protein kinases (MAPK) and NF-κB pathways in the bystander cells (Fu et al., 2016a), and (b) upregulation of tumour necrosis factor alpha (TNF-α) and interleukin 8 (IL-8) in the bystander cells, which was relayed on the activated extracellular signal-regulated kinases (ERK) and p38 pathways in the irradiated cells and which was also due to the activated NF-κB pathway in the bystander cells (Fu et al., 2016b).

3. Review of selected literature related to radiation field size effects

In this section, selected literature related to radiation field size effects will be reviewed. For the convenience of discussion, the paper on RIRE and relative abundance between unirradiated cells and irradiated cells (Lam et al., 2015b) will first be reviewed in section 3.1, since this was the first paper that shed light on RIRE being a potential mechanism underlying the radiation field size effect. The papers on non-uniform localized exposures and uniform radiation exposures by Matsuya et al. (2019, 2022), who explained their results in terms of RIRE, will then be reviewed in section 3.2. The paper on exposures of primary normal human lung fibroblasts to X-ray microbeams with different field sizes by Ojima et al. (2021), who also explained their results in terms of RIRE, will then be reviewed in section 3.3. The papers on β-particle irradiation of mouse skin and pig skin (Coggle et al., 1984; Peel et al., 1984), and that on exposures to intensity-modulated radiation fields provided by an X-ray irradiator (Butterworth et al., 2012), which did not make reference to RIRE, will be reviewed in sections 3.4 and 3.5, respectively. A subsection entitled "Supplementary notes" has been added in some sections to provide suggestions on possible links of the reported results to RIRE or related processes (such as radiation-induced bystander effect).

3.1. Radiation-induced rescue effect (RIRE) and relative abundance between unirradiated cells and irradiated cells

Lam et al. (2015b) studied RIRE in HeLa and NIH/3T3 cells irradiated by α particles from an ^{241}Am source, and were the first to prove the presence of a rescue signal in the conditioned medium (CM) which had conditioned the bystander cells previously partnered with irradiated cells. After exposures to 5 cGy of α particles, the 53BP1 foci/cell for both cell lines were significantly decreased in the case where only 2.5% of the cell population was irradiated when compared to the case of 100% irradiation, which confirmed the presence of RIRE in the former case. Through application of an NF-κB activation inhibitor, as well as through staining for phosphorylated NF-κB (p–NF–κB) expression, Lam et al. (2015b) further revealed that RIRE (surrogated by mitigated 53BP1 foci/cell at 12 h post-irradiation) was activated via the NF-κB pathway in the irradiated cells.

Lam et al. (2015b) further investigated the influence on the resulting RIRE from the relative abundance of bystander cells, i.e., the ratio of (number N_U of unirradiated bystander cells)/(number N_I of irradiated cells). Four irradiation scenarios were studied, namely, 0% (or sham irradiation), 2.5%, 75% and 100% of a cell population were irradiated with α particles. For the case of 2.5% irradiation ($N_U/N_I = 39$), the 53BP1 foci/cell in the irradiated cells were significantly reduced when compared to the case of 100% irradiation for both cell lines. For the case of 75% irradiation ($N_U/N_I = 0.33$), the 53BP1 foci/cell in the irradiated NIH/3T3 cells was still significantly reduced but that in the irradiated HeLa cells was not significantly reduced, when compared to the case of 100% irradiation. These results demonstrated considerable dependence of RIRE induction on the N_U/N_I value, and also revealed a saturation (smaller change) in the response (53BP1 foci/cell) for large N_U/N_I values.

3.2. Non-uniform localized exposures and uniform radiation exposures

Matsuya et al. (2019) compared the number of DSBs induced by the absorbed doses in normal human lung cells (WI-38 fibroblasts and HBEC-3KT bronchial epithelial cells) arising from local non-uniform irradiation (by a ^{137}Cs- and ^{134}Cs-bearing microparticle attached to the cell surface) and from uniform irradiation (by γ-rays from ^{137}Cs). The Cs-bearing microparticle (Cs-BMP) provided different doses to the proximal region and the distal region. The doses absorbed by the cells were determined through Monte Carlo simulation with the Particle and Heavy Ion Transport Code System (PHITS). The authors revealed that the number of DSBs increased with the dose arising from uniform irradiation, but was almost independent of the dose arising from local non-uniform irradiation, and these two trends intersected at ~50 mGy and 5–10 mGy for the studied WI-38 cells and HBEC-3KT cells, respectively. In other words, above a certain dose (the intersection point), the number of DSBs induced by non-uniform irradiation became smaller than that induced by uniform irradiation. Matsuya et al. (2019) proposed that the smaller number of DSBs induced by non-uniform irradiation was due to an intercellular feedback signal from non-irradiated cells leading to benefits in the irradiated cells, and suggested the rescue signal from RIRE as one of the possibilities.

In addition, in order to ascertain the initial number of DSBs in the cells upon non-uniform irradiation, Matsuya et al. (2019) further performed half-field experiments to irradiate 50% of WI-38 or HBEC-3KT cells cultured in a dish with 1 Gy X-ray delivered by a 6-MV linac, and demonstrated that the number of DSBs in the cells at 30 min after irradiation were reduced when compared to uniform irradiation.

In a follow-up investigation, Matsuya et al. (2022) further studied the relationship between inflammatory responses and DNA damage induction under non-uniform localized exposures and uniform radiation exposures. As described above, to attain non-uniform localized exposures, the Cs-BMP provided different doses to the proximal region and the distal region. Matsuya et al. (2022) observed significant activation of the inflammatory signaling pathway, viz., nuclear factor κB (NF-κB) p65 and cyclooxygenase 2 (COX-2), in the cells after exposure to Cs-BMP for 24 h. Such inflammatory signaling pathways were related to DSBs, which were surrogated by γ-H2AX foci.

Interestingly, through studying the spatial distribution of inflammation in the cells as a result of exposures to a Cs-BMP, Matsuya et al. (2022) further demonstrated that the NF-κB and COX-2 signaling pathways played important roles in the "rescue effect" on the irradiated proximal cells and "bystander effect" on the non-irradiated distal cells, when compared to responses of the cells to uniform exposures to ^{137}Cs γ-rays. More specifically, Matsuya et al. (2022) revealed that NF-κB was more activated in the proximal cells while both NF-κB p65 and COX-2 were significantly activated in distal cells, when compared to uniform exposures. These observations were important in backing their claim that activation of NF-κB p65 signified the "rescue effect", while significant dual activation of NF-κB p65 and COX-2 signified the "bystander effect". In support of their conjecture, Matsuya et al. (2022) made reference to the earlier work by Lam et al. (2015b) who reported stronger activation of phosphorylated NF-κB when only 2.5% of the cell population was irradiated compared to the situation where 100% of the cell population was irradiated, which led to the onset of RIRE (in terms of a reduction in 53BP1 foci).

To further substantiate their claims on the differential participation of the NF-κB and COX-2 signaling pathways in the "rescue effect" and "bystander effect", Matsuya et al. (2022) examined the cells which had

undergone non-uniform exposures to X-rays, and determined the survival fraction of cells through clonogenic assay as well as activation of NF-κB and COX-2. As a result of non-uniform X-ray exposures, the cell population was divided into out-of-field cells and in-field cells, with an out-of-field dose at 5% of the in-field dose. Matsuya et al. (2022) confirmed significant activation of NF-κB and COX-2 as "bystander effect" in out-of-field cells upon a non-uniform X-ray exposure of 4 Gy. In separate experiments to irradiate different percentages of cells in a culture flask, viz., 25%, 50%, 75% and 100%, Matsuya et al. (2022) observed saturated bystander effect in out-of-field cells for irradiated percentages more than 50% as well as maximal rescue effect in the case of 25% in-field cells. Matsuya et al. (2022) remarked that the tendency of survival data agreed well with the data obtained earlier by Lam et al. (2015b) who reported the induction of "rescue effects" in a 2.5% irradiated cell population (in terms of a reduction in 53BP1 foci).

3.3. Exposures to X-ray microbeams with different field sizes

Ojima et al. (2021) examined the responses of primary normal human lung fibroblasts, MRC-5 (in terms of the number of p53 Binding Protein 1 (53BP1) foci per cell), upon irradiations with the same X-ray microbeam dose of 1 Gy but with different field sizes, viz., 0.02, 0.09, 0.81 and 1.89 mm^2. The 53BP1 foci were employed to surrogate the DSBs.

3.3.1. Key findings

There were a number of key findings.

First, the number of DSBs per cell in general increased with the field size until a saturation at the field size of 0.81 mm^2, which held true for all measurement time points of 1, 4, 24 and 48 h post-irradiation. The authors referred the dependence of radiobiological response of cells on irradiation field size as the radiation-induced field size effect (RIFSE). Ojima et al. (2021) inferred that the response of irradiated cells depended on their percentage within the entire cell population, which however would saturate beyond a certain percentage. The authors explained their findings by means of RIRE where the irradiated cells received a "rescue signal" from bystander non-irradiated cells to enhance the repair of DNA damages in the irradiated cells.

Second, the number of DSBs per cell in the in-field area in contact with the out-of-field area was lower. Ojima et al. (2021) suggested that RIRE occurred in the cells in this in-field area, and proposed to explain the field size effect in terms of the time taken by the bystander signals to diffuse within the medium from irradiated cells to non-irradiated bystander cells (Sokolov et al., 2005). For a small radiation field size, a large proportion of irradiated cells were in contact with non-irradiated cells, so the average time taken by the bystander signals to reach non-irradiated cells to induce RIRE would be shorter. In contrast, for a large radiation field size, a large proportion of irradiated cells were not in contact with non-irradiated cells, so RIRE induction would be delayed.

Third, there were more Ki-67-positive cells in the in-field area in contact with the out-of-field area, regardless of the radiation field size. Ki-67 is a nuclear protein commonly deployed as a marker for cell proliferation. Ojima et al. (2021) speculated that these Ki-67-positive cells had migrated from the out-of-field area and contributed to regeneration of the cell population in the in-field area.

3.3.2. Supplementary notes

Ojima et al. (2021) did not explicitly provide an explanation to the apparently larger number of Ki-67-positive cells just outside the in-field area, which subsequently migrated into the in-field area. This was likely due to the proliferative bystander response which was a radiation-induced bystander effect (RIBE) in the bystander cells (Iyer and Lehnert, 2000; Iyer and Lehnert, 2002; Gerashchenko and Howell, 2003, 2004,2005; Han et al., 2010), and could be mediated by nitric oxide (NO) or transforming growth factor 1 (TGF-β1) (Han et al., 2010), or nucleophosmin 1 (NPM 1) (Gerashchenko et al., 2007). In fact, the enhanced proliferation of bystander cells could be carcinogenic due to the larger probability of mutation from the mis-repaired or un-repaired DSBs (Ames et al., 1993).

3.4. Mouse skin and pig skin irradiated with β particles

Coggle et al. (1984) collaborated with Peel et al. (1984) on researching the effects of β particles with different energies and with different irradiation field size on mouse skin and pig skin, respectively. The studied effects included moist desquamation and ulceration. Moist desquamation was an earlier-stage response due to epithelial cell death, which might be followed by a later-stage ulceration due to dermal blood vessel damages if moist desquamation persisted. Healing from ulceration would lead to tissue scarring. The work of Coggle et al. (1984) would be first reviewed in section 3.4.1, while that of Peel et al. (1984) would be reviewed in section 3.4.2.

3.4.1. Mouse skin irradiated with β particles

Coggle et al. (1984) irradiated mouse skin with β particles emitted from ^{90}Sr (E_{max} = 2.27 MeV) and ^{170}Tm (E_{max} = 0.97 MeV), and established variation of doses required for different skin reactions with (a) energy of the β particles (hereafter referred to as "energy effect"), which determined their penetration depth into the skin, and (b) field size of irradiation (hereafter referred to as "field size effect").

As regards moist desquamation, the doses from β particles from ^{90}Sr that led to moist desquamation in 50% of the irradiated fields (MD-50 doses) were found as 22, 42, 70, and 1000 Gy for field sizes of 400, 95, 20 and 0.8 mm^2, respectively. On the other hand, the MD-50 doses from ^{170}Tm were found as 50, 54, 90 and 170 Gy for field sizes of 860, 64, 20 and 3.1 mm^2, respectively. Generally speaking, the MD-50 doses increased with decreasing field size, or equivalently, the moist desquamation incidence became lower for smaller field size for the same irradiation dose, although the field size effect for ^{170}Tm became less apparent when the field size got larger. Coggle et al. (1984) also concluded that the different energies of β particles from ^{90}Sr and ^{170}Tm did not show significant differences in causing moist desquamation.

As regards ulceration, the MD-50 doses from ^{90}Sr were found as 150, 210 and 3100 Gy for field sizes of 90, 20 and 0.8 mm^2, respectively. On the other hand, the MD-50 doses from ^{170}Tm were found as 260, 550 and 8300 Gy for field sizes of 64, 20 and 3.1 mm^2, respectively. Apparently, the MD-50 doses increased with decreasing field size, or equivalently, the ulceration incidence became lower for smaller field size for the same irradiation dose. Coggle et al. (1984) concluded that the different energies of β particles from ^{90}Sr and ^{170}Tm showed significant differences in causing ulceration.

3.4.2. Pig skin irradiated with β particles

Peel et al. (1984) irradiated pig skin with β particles emitted from ^{90}Sr, ^{170}Tm and ^{147}Pm (E_{max} = 0.225 MeV), and ascertained the "energy effect" and "field size effect". Irradiations with β particles from both ^{90}Sr and ^{170}Tm sources demonstrated field size effects.

As regards moist desquamation, the doses from β particles from ^{90}Sr that led to moist desquamation in 50% of the irradiated fields (ED-50 doses) were found as ~25 Gy for field size of 40 mm–~450 Gy for field size of 1 mm. On the other hand, the ED-50 doses from ^{170}Tm were found as ~80 Gy for field size of 5, 9 and 19 mm, and ~250 Gy for the field size of 2 mm. These data showed that for β particles from ^{90}Sr, the ED-50 doses increased with decreasing field size, or equivalently, the moist desquamation incidence became lower for smaller field size for the same irradiation dose. There was also a glimpse of field size effect for ^{170}Tm, which however appeared to have quickly saturated when the field size got larger (>2 mm). Similar to the conclusion made by Coggle et al. (1984), the data obtained by Peel et al. (1984) also did not seem to show significant differences in causing moist desquamation by β particles with different energies. Explicitly, for field size in the range 15–22.5 mm, the

ED-50 doses from ^{90}Sr, ^{170}Tm and ^{147}Pm were found as 30–45 Gy, ~80 Gy and ~500 Gy, respectively.

3.4.3. Proposed explanations on "energy effect" and the "field size effect"

Coggle et al. (1984) and Peel et al. (1984) proposed that the "energy effect" and the "field size effect" identified in the mouse skin and pig skin experiments, respectively, could be explained in terms of (a) repopulation of cells from edges of the irradiation field, and/or (b) repopulation of cells from basal cells in the epidermis and in the hair follicles, which had evaded killing by the β particles. For the mouse skin, Coggle et al. (1984) determined the doses at the base of the dermis due to β particles from ^{90}Sr (E_{max} = 2.27 MeV) and ^{170}Tm (E_{max} of 0.97 MeV) as 80% and 50% of their corresponding skin-surface doses, respectively. For the pig skin, Peel et al. (1984) determined the doses at the base of the dermis due to β particles from ^{90}Sr and ^{170}Tm as ~50% and <10% of the skin surface doses, respectively. Coggle et al. (1984) and Peel et al. (1984) argued that upon irradiation, the higher-energy β particles from ^{90}Sr could kill both epidermis and follicle cells, in which case repopulation of cells from edges of the irradiation field would dominate, while the lower-energy β particles from ^{170}Tm could leave "islands" of surviving cells at the base of hair follicles, in which case repopulation of cells would be contributed from both the base of hair follicles and edges of the irradiation field. The authors also proposed dominance of repopulation of surviving basal cells over repopulation of cells from edges of the irradiation field to explain their finding on a less apparent field size effect for ^{170}Tm when the field size got larger.

3.4.4. Supplementary notes

As described in section 3.4.3 above, Coggle et al. (1984) and Peel et al. (1984) explained the "energy effect" and the "field size effect" in terms of (a) repopulation of cells from edges of the irradiation field, and/or (b) repopulation of cells from the basal cells in the epidermis and in the hair follicles. The authors also attributed the less apparent field size effect for ^{170}Tm for larger field sizes to their proposed dominance of repopulation of surviving basal cells over repopulation of cells from edges of the irradiation field.

However, as described in section 3.4.2 above, the ED-50 doses from ^{170}Tm were found as ~80 Gy for field size of 5, 9 and 19 mm, and ~250 Gy for the field size of 2 mm. As such, a "field size effect" did occur although it was not apparent for the field size range between 5 and 19 mm. This presented a challenge to the conjecture that repopulation of surviving basal cells would dominate over repopulation of cells from edges of the irradiation field after the pig skin was irradiated with lower-energy β particles from ^{170}Tm. If we have to analyse the two repopulation processes quantitatively, without better information, we might express repopulation of surviving basal cells as $F = j \times n \times d^2$, where j is a proportionality constant, n is the surface density of hair follicles and d is the diameter of radiation field; while repopulation of cells from edges of the irradiation field as $E = k \times d$, where k is another proportionality constant. Since the number (D) of cells killed during irradiation varies with d^2, dominance of repopulation of surviving basal cells will lead to an efficiency of replenishing killed cells given by $\eta \sim F/D$ which is independent of d, i.e., no "field size effect", while dominance of repopulation of cells from edges of the irradiation field will lead to $\eta \sim E/D$ which varies with $(1/d)$. From the experimental data, changes in R were not noticeable when d varied from 19 mm to 9 mm, and from 9 mm to 5 mm, which implied $E \ll F$ for $d \geq 9$ mm, or equivalently $[k/(j \times n)] \ll 9$. On the other hand, for $d = 5$ mm, E becomes larger than (or at least comparable to) F, i.e., $E > F$, or equivalently $[k/(j \times n)] > 5$. It would be indeed challenging to satisfy the conditions $5 < [k/(j \times n)] \ll 9$.

An alternative explanation of the field size effect demonstrated here can be RIRE (Lam et al., 2015b; see also section 3.1 above), which by definition refers to the mitigation of detrimental effects in irradiated cells after receiving signals from non-irradiated bystander cells. In the studies of Coggle et al. (1984) and Peel et al. (1984), the mouse/pig skin cells within and outside the irradiation areas would be the targeted and bystander cells, respectively. An important finding for RIRE was the effect of abundance ratio between targeted cells and bystander cells on the induction of RIRE. Lam et al. (2015b) explored the influence of the ratio of (number N_U of unirradiated bystander cells)/(number N_I of irradiated cells) on the resulting RIRE in irradiated NIH/3T3 and HeLa cells. For NIH/3T3 cells, RIRE was significantly induced when $N_U/N_I = 39$ or $N_U/N_I = 0.33$. For HeLa cells, RIRE was significantly induced only when $N_U/N_I = 39$ (not significantly induced when $N_U/N_I = 0.33$) (see Lam et al. (2015b) and discussion in section 3.1 above). The general increase in the MD-50/ED-50 doses with decreasing field size, or equivalently, the lower moist skin-reaction incidence for smaller field size for the same irradiation dose, could then be explained by the larger (N_U/N_I) values for smaller field size. In particular, the "field size effect" for ^{170}Tm, with ED-50 doses of ~80 Gy for field sizes of 5, 9 and 19 mm, and ~250 Gy for the field size of 2 mm has provided a strong evidence on the influence on RIRE from the relative abundance N_U/N_I. However, the threshold value of N_U/N_I cannot be determined in this case since the in-field and out-of-field area proportions are unknown. The detection of RIRE only for the field size of 2 mm, while not for the field sizes of 5, 9 and 19 mm was in fact somewhat similar to the "field size effect" reported for DU-145 cells by Butterworth et al. (2012), where a significantly larger survival response occurred only for in-field area proportion of 25% compared to that for uniform exposure, but not for in-field area proportions of 50 and 75% (see section 3.5.2 below).

3.5. Exposures to intensity-modulated radiation fields provided by an X-ray irradiator

3.5.1. In-field survival responses vs. in-field area proportions

Butterworth et al. (2012) studied the in-field and out-of-field survival responses (through clonogenic assay) of human prostate cancer cells (DU-145) and primary fibroblast cells (AG0-1522) upon their exposures to intensity-modulated radiation fields provided by an X-ray irradiator. In particular, the authors examined the effects of radiation field size (i.e., in-field areas), dose and dose rate responses. The cells were irradiated with uniform or non-uniform exposures, where the in-field area proportions were varied among 1, 10, 25, 50 and 75% (correspondingly out-of-field area proportions were varied among 99, 90, 75, 50 and 25%). The doses delivered to the in-field areas were 2 and 4 Gy for the AG0-1522 cells, and were 4 and 8 Gy for DU-145 cells. Furthermore, the doses delivered to the out-of-field areas were adjusted to be 1.6, 3, 4.7, 17.4 and 37.2% of the doses delivered to the in-field areas. The survival responses of cells cultured in the in-field areas and out-of-field areas were compared to the survival responses of cells irradiated with uniform exposures.

For the AG0-1522 cells, no significant differences among the in-field survival responses were observed when the in-field area proportions varied among 100% (i.e., uniform exposure), 75%, 50% and 25%. In contrast, for the DU-145 cells, there was a trend showing an increase in the in-field survival response with decreasing in-field area proportions, but with only the survival response for in-field area proportion of 25% significantly larger than that for in-field area proportion of 100% (i.e., uniform exposure).

3.5.2. Supplementary notes

The results which were most relevant to the discussion on the radiation field size effect were the relationships between the in-field survival responses and the radiation field size (i.e., in-field area proportions). For DU-145 cells, the only significantly larger survival response for in-field area proportion of 25% compared to that for uniform exposure had two important implications.

First, the enhanced survival response when compared to that for uniform exposure has provided strong evidence of RIRE which by definition refers to the mitigation of detrimental effects in irradiated cells after receiving signals from non-irradiated bystander cells.

Second, the detection of RIRE only for the in-field area proportion of

25%, while not for in-field area proportions of 50% and 75%, has provided strong evidence on influence on RIRE from the relative abundance of bystander cells, i.e., the ratio of (number N_U of unirradiated bystander cells)/(number N_I of irradiated cells) (see Lam et al. (2015b) and discussion in section 3.1 above). For the in-field area proportions of 25, 50 and 75%, N_U/N_I was 3, 1 and 0.33, respectively. Apparently, RIRE induction in irradiated DU-145 cells was only significant when $N_U/N_I > 1$. As a reference, Lam et al. (2015b) also investigated the influence of the ratio N_U/N_I on the resulting RIRE in irradiated NIH/3T3 and HeLa cells. For NIH/3T3 cells, RIRE was significantly induced when $N_U/N_I = 39$ or $N_U/N_I = 0.33$. For HeLa cells, RIRE was significantly induced only when $N_U/N_I = 39$ (not significantly induced when $N_U/N_I = 0.33$). The observations on RIRE induction in irradiated DU-145 cells were commensurate with those in irradiated HeLa cells. Moreover, the variability in the threshold N_U/N_I values for RIRE induction in NIH/3T3 and HeLa cells (Lam et al., 2015b) hinted that RIRE might also be induced in AG0-1522 cells in the study of Butterworth et al. (2012), but then the threshold N_U/N_I value should be > 3 (corresponding to an in-field area proportion of 25%). Unfortunately, only data down to the field size proportion of 25% were reported by Butterworth et al. (2012), so no conclusions could be made here. The results presented here were also somewhat similar to the "field size effect" reported by Peel et al. (1984) regarding moist desquamation incidence in pig skin irradiated with β particles from ^{170}Tm, viz., ED-50 doses of ~80 Gy for field sizes of 5, 9 and 19 mm, and ~250 Gy for the field size of 2 mm (see sections 3.4.2 to 3.4.4 above).

On another note, the relationships between the in-field survival response and the transmission percentage of irradiation dose to the out-of-field area obtained by Butterworth et al. (2012) also provided some valuable insights into better understanding on RIRE. Butterworth et al. (2012) found that the survival response in general depended on the transmission percentage, and remarked that this implied contribution of intercellular signaling from the out-of-field area to in-field area, which has again provided strong evidence of RIRE in which detrimental effects in irradiated cells are mitigated after receiving feedback signals from non-irradiated bystander cells.

4. Discussion

The current paper reviewed the role of radiation-induced rescue effect (RIRE) in radiation field size effect. "Radiation field size effect" refers to the phenomenon that the radiobiological effects of ionizing radiation depend on the size of the irradiated area, besides depending on the equivalent dose. The radiation field size effect is of fundamental importance and has far-reaching implications. On the other hand, RIRE refers to the mitigation of detrimental effects in irradiated cells after receiving signals from non-irradiated bystander cells, or after receiving signals from the medium which has previously conditioned the non-irradiated bystander cells. In relation, the extent of damages in the irradiated cells will also be determined by the strength of RIRE in the irradiated cells. Notably, Lam et al. (2015b) revealed that the strength of RIRE in the irradiated cells depended on the ratio of (number N_U of unirradiated bystander cells)/(number N_I of irradiated cells). In other words, the strength of RIRE depended on the size of the irradiated area. This finding provided a strong link between RIRE and the radiation field size effect. The current paper first gave a brief review on RIRE, and then a review of selected literature related to radiation field size effects.

Lam et al. (2015b) investigated the influence of N_U/N_I on the resulting RIRE in HeLa and NIH/3T3 cells by irradiating 2.5% and 75% of a cell population with α particles, and compared the results with those for 100% irradiation. For 2.5% irradiation ($N_U/N_I = 39$), significant RIRE was detected in both cell lines. On the other hand, for 75% irradiation ($N_U/N_I = 0.33$), RIRE was significant only for NIH/3T3 cells but not for HeLa cells. These results demonstrated that induction of rescue effect strongly depended on the N_U/N_I value, which led to the connotation between RIRE and the radiation field size effect. The results also showed that induction of rescue effect was cell-line dependent (significant RIRE only for NIH/3T3 cells but not for HeLa cells for $N_U/N_I = 0.33$), revealed the presence of a "threshold" N_U/N_I value for RIRE ($N_U/N_I > 0.33$ for HeLa cells) as well as a saturation in the response for large N_U/N_I values. Remarkably, "threshold" N_U/N_I values for RIRE appeared in the data of Peel et al. (1984) and Butterworth et al. (2012), while saturation in RIRE was also detected in the investigations by Matsuya et al. (2019) and Ojima et al. (2021).

Matsuya et al. (2019) found that above a certain dose, the number of DSBs induced in normal human lung cells by non-uniform irradiation became smaller than that induced by uniform irradiation. The group also further performed half-field experiments and demonstrated that the number of DSBs in the cells were reduced when compared to uniform irradiation. Matsuya et al. (2019) explained their results in terms of an intercellular feedback signal from non-irradiated cells leading to benefits in the irradiated cells, and suggested the rescue signal from RIRE as one of the possibilities. In a follow-up investigation, Matsuya et al. (2022) further studied the relationship between inflammatory responses and DNA damage induction under non-uniform localized exposures and uniform radiation exposures. Interestingly, Matsuya et al. (2022) revealed that activation of NF-κB p65 signified the "rescue effect" while significant dual activation of NF-κB p65 and COX-2 signified the "bystander effect". This was a very important result in that RIRE could then be unambiguously identified in the in-field areas, and the origin of the radiation field size effect could be pinpointed. This would also inspire a research direction to ascertain the signaling pathways and chemical messengers involved in in-field and out-of-field areas, particularly noting that some signalling pathways and chemical messengers have already been identified for RIRE such as cAMP (He et al., 2014), NF-κB response pathway (Lam et al., 2015a,b), NO (Matsumoto et al., 2001, 2007, 2011; Maeda et al., 2013), IL-6 (Kong et al., 2018) and PARP1 (Pathikonda et al., 2020)(see section 2 and Fig. 1). In particular, it would be interesting and informative to monitor changes in the radiation field size effect through applying inhibitors of signaling pathways for RIRE and/or bystander effect, which might further unambiguously confirm the origin of the radiation field size effect. The projects outlined here would be good future priorities in the research on the role of RIRE in radiation field size effect.

Coggle et al. (1984) collaborated with Peel et al. (1984) on researching the effects of β particles with different energies and with different irradiation field size on mouse skin and pig skin, respectively. The studied effects included moist desquamation and ulceration. Coggle et al. (1984) and Peel et al. (1984) explained the "energy effect" and the "field size effect" identified from their data in terms of (a) repopulation of cells from edges of the irradiation field, and/or (b) repopulation of cells from the basal cells in the epidermis and in the hair follicles. The authors also claimed less apparent field size effect for ^{170}Tm for larger field sizes and attribute this observation to the proposed dominance of repopulation of surviving basal cells over repopulation of cells from edges of the irradiation field. However, a relatively unambiguous "field size effect" did show up upon re-examination of ^{170}Tm data for all field sizes, which would then present a challenge to the conjecture put forward by Coggle et al. (1984) and Peel et al. (1984) to explain the "energy effect" and the "field size effect" in terms of repopulation of cells. An alternative explanation of the field size effect demonstrated here can be RIRE, and the detection of RIRE only for the field size of 2 mm, while not for the field sizes of 5, 9 and 19 mm, will then be an evidence of a threshold abundance N_U/N_I for triggering RIRE as revealed by Lam et al. (2015b).

Butterworth et al. (2012) studied the in-field and out-of-field survival responses of DU-145 cells and AG0-1522 cells upon their exposures to intensity-modulated radiation fields provided by an X-ray irradiator. For the AG0-1522 cells, no significant differences among the in-field survival responses were observed when the in-field area proportions varied among 100%, 75%, 50% and 25%. In contrast, for the DU-145 cells, there was a trend showing an increase in the in-field survival response

with decreasing in-field area proportions, but only with the survival response for in-field area proportion of 25% significantly larger than that for in-field area proportion of 100%. The results could be explained in terms of RIRE, and the only significantly larger survival response observed for in-field area proportion of 25% could again be attributed to the threshold abundance ($N_U/N_I > 1$) for triggering RIRE as revealed by Lam et al. (2015b). RIRE might also be induced in AG0-1522 cells, but then the threshold N_U/N_I value should be > 3.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None.

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