ORIGINAL ARTICLE

Radiation-Induced Bystander Effect via GRID Radiotherapy and Medium Transfer in the A-375 Human Melanoma Cancer Cell Line: An In-vitro Study

Marzieh Nabikhani [1], Samideh Khoei [2, 3*], Seied Rabi Mahdavi [3], Jila Rajaee [3], Sakine Shirvalilou [2]

[1] *Medical Physics Department, School of Medicine, International Campus-Iran of Medical Sciences, Tehran, Iran*

[2] *Finetech in Medicine Research Center, Iran University of Medical Sciences, Tehran, Iran*

[3] *Department of Medical Physics, School of Medicine, Iran University of Medical Sciences, Tehran*

*Corresponding Author: Samideh Khoei
Email: skhoei@gmail.com

Received: 16 June 2022 / Accepted: 05 August 2022

Abstract

Purpose: The goal of this research was to investigate the bystander effect in the A-375 cell line under the spatially fractionated radiation therapy (GRID therapy technique). In GRID therapy, due to direct and indirect cell damage after high-dose radiation, evaluation of Radiation-Induced Bystander Effects (RIBE) is of the most importance for investigating the risk of therapy.

Materials and Methods: The potential role of RIBE was evaluated with different doses of 6 MeV electron radiation and different incubation times after irradiation using two methods; GRID therapy and medium transfer. Colony Formation Assay (CFA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) test were used to detect the mentioned effects. Alpha and beta parameters were calculated from the cell survival curve by the quadratic-linear model.

Results: The result showed that the survival fraction significantly decreases by increasing the radiation dose for both bystander and irradiated cells. However, a decrease in the number of colony-forming cells caused by electron radiation greater than 4MeV to target cells was significantly increased compared with bystander cells ($P < 0.05$). While increasing the incubation time after exposure to an electron beam, it had no significant effect on cell survival fraction ($P > 0.05$). Furthermore, the RIBE level in non-target cells increased up to a dose of 4Gy, but decreased significantly at doses higher than 4Gy. This result in high doses confirmed that a negative feedback mechanism was responsible for reducing the RIBE response.

Conclusion: Based on the results, we can state there are classic radiation-induced bystander effects in A-375 monolayer exposed by GRID therapy and medium transfer technique, which can play an important role in pre-clinical and clinical studies.

Keywords: Melanoma; GRID Therapy; Medium Transfer; Radiation-Induced Bystander Effect; Colony Formation Assay.

DOI: https://doi.org/10.18502/fbt.v10i3.13164

Frontiers in BIOMEDICAL TECHNOLOGIES

1. Introduction

Tumor volume has been proposed as an important prognostic indicator of predicting radiotherapy outcome [1]. Treatment of large bulky tumors due to poor perfusion rate and the creation of large hypoxic areas that result in high radio-resistance has been considered a challenging issue in radiotherapy [2-4]. Spatially GRID radiation therapy (SGRT) is often considered an effective and palliative treatment modality in deep-seated bulky tumors therapy [5]. Spatially fractionated GRID radiotherapy (SFGRT) differs from standard conventional radiation because it treats the entire tumor volume with a non-uniform dose [6]. The SFGRT therapy, generally known as GRID therapy, has significantly reduced severe symptoms, above average local control, and minimal toxicity in palliative treatments [7]. In addition to the conventional clinical GRID therapy, in various studies, new ways of treating GRID have been investigated using different types of non-conventional radiation, such as synchrotron kilovoltage X-rays (XGRT), small beam electron GRID and proton beam [8-11]. Researchers like Kijima *et al.* and Meigooni *et al.* have shown that although photon GRID is an excellent treatment, electron GRID is superior to photon for large, superficial tumors where there are radiosensitive structures behind the tumor in the path of the photon beams because electron beam dose decreases rapidly after the beam passes through an environment [9, 12]. In another study, Entezari *et al.* used appropriate grid(s) for the optimum electron beam to treat subcutaneous tumors with 6 and 18 MeV energies. Their results illustrated that this treatment could be developed for a wide range of electron GRID therapies in routine clinical practices [13]. Therefore, electron GRID therapy has recently received attention because it may be able to solve the problem of the lack of protective effect on the skin that occurs in conventional radiation therapy [14].

In GRID therapy a broad field of treatment is considered as multiple smaller fields by means of a perforated screen [15]. In patients with advanced massive tumors, SFRT treatment has shown better local control of the disease [16]. In this method, although malignant tissues situated along the aperture of the grid are directly irradiated and the remaining tumoral regions are blocked, uniform tumor regression is clinically evident [17, 18]. This response cannot be thoroughly attributed to the damages occurred in the cells under the open field of GRID [19]. Furthermore, a non-targeted damage was appeared in the cells that was not directly undergone irradiation but was located close to the irradiated cells. This phenomenon is known as "Radiation-Induced Bystander Effect (RIBE)" and refers to the induction of biological effects in the non-irradiated cells followed by receiving signals from the neighboring cells which have been directly subjected to ionizing radiation [20, 21]. Accordingly, despite the cellular damages that occurred due to the direct incidence of ionizing radiation, the target organ as a complex is systemically affected by the radiation [22, 23]. In this case, the bystander effect may act as a double-edged sword, so that the production of growth factors by non-irradiated cells causes the protective effects of irradiated cells, while the dangerous bystander effects of cells that have not been exposed to radiation may be due to the production of Reactive Oxygen Species (ROS) in the environment under direct radiation [24, 25]. Many studies have reported that RIBE is dependent on several parameters such as dose rate, dose per fraction, and cell or tissue type [26]. However, a clear explanation of how each of these parameters affects RIBE has not yet been provided [27]. Butterworth *et al.* evaluated the survival responses occurring in the blocked and non-blocked cells following radiation therapy using a multi-leaf collimator, as a beam modulator, which shielded 50% of the cell population [28]. There are three possible pathways for transmitting signals from directly irradiated cells to the bystander cells: through direct cell-to-cell contact of membrane structures, through Ggap Junction Intercellular Communication (GJIC), or via medium transfer mediated by the propagation of damaging signals to the medium of directly irradiated cells [29].

From the point of view of Grid therapy, RIBE can be observed in the volume of the tumor under Grid irradiation although not many studies have been conducted on that. Considering the importance of the bystander effect in terms of radiotherapy, in the present study we evaluated the potential role of radiation-induced bystander effect in melanoma cells under 6 MeV electron beam exposure using two methods; 1) Spatially Fractionated Radiation Therapy (FSRT) by a GRID applicator characterized for electron beam and 2) transferring medium of irradiated culture to a separate non-irradiated culture. Since radiobiology experiments to evaluate radiation-induced bystander effect have been made only for photon grade therapy, an electron beam was used for the first time in this study. Finally, in order to evaluate the cytotoxic effects induced by two methods

of A-375 human melanoma cells irradiation direct and non-indirect, Colony Formation Assay (CFA), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) test were used. Also, the values of alpha and beta parameters were determined by the classical LQ model.

2. Materials and Methods

2.1. Cell Culture

A-375 human melanoma cell line was obtained from the Pasteur Institute of Iran. The cells were cultured in RPMI-1640 (GIBCO) supplemented with 10% Fetal Bovine Serum (FBS), penicillin/streptomycin at 37 °C in 5% CO_2. The cells were collected by trypsinizing [30].

2.2. Irradiation Procedure

A-375 cells (3.5 × 105) were plated in 60 mm cell culture Petri dishes. After 48 h, cells were exposed to electron beam using a linear accelerator (Varian medical system, lnc). The cells were exposed to 0 Gy (control group), 2, 4, 6, and 8 Gy of 6 MeV electron beam at a dose rate of 300 monitor units per min. The Source-to-Surface Distance (SSD) was adjusted to 100 cm. After irradiation, the cells were harvested, counted, and assayed for colony formation ability.

2.3. GRID Characteristics

For FSGRT experiments a GRID applicator personalized for electron beam was used (Figure 4a). The grid was made of lead alloys which contain antimony (15-20%). High heat and pressure resistance, low volumetric variation with changes of temperature, inability to dissolve in gases, and low reaction towards oxidation are some prominent advantages of dry lead. This alloy is very cheap and available. The center-to-center distance between the adjacent openings of the designed grid is 1.8 cm and each opening has a diameter of 1.5cm. The dose distribution of isodose curves was assessed with EDR-2 dosimetric film. All calculations were performed at a depth of 1cm. Dosimetric results showed a reduction of approximately 30% in the maximum depth dose in the areas located under the shielded area of the grid compared to the open areas. The results of absolute dosimetry indicated that a GRID factor of 1.25 must be considered for MU calculations.

2.4. Bystander Survival Studies

Two methods: 1) FSRT using GRID and 2) Medium transfer were used to investigate the radiation-induced bystander effects in melanoma cells.

2.5. GRID-Based Bystander Studies

In the first method, A-375 cells were plated in Petri dishes, for 48h. In this experiment, we considered the cells as "in-hole cells" that were exposed to 2, 4, 6, and 8 Gy and "out-hole cells" that were cells adjacent to directly exposed cells. The "out-hole" cells were not directly exposed, but received indirect radiation (i.e., scattering) as "bystander cell" which was also called "out hole" (under shielded areas). These cells were undergone valley doses of approximately 1.4, 2.8, 4.2, and 5.6 Gy. The "in-hole" and "out-hole" cells were identified and separated by a histological marker Pap pen (Ted Pella, Inc. Redding, CA). A-375 cells were collected at different times (0, 24, and 48 hours) after GRID therapy. Clumps were separated by a cell scraper, and after that the cells were cultured in dishes to evaluate colony formation ability after 10 days.

2.6. Non-Targeted Effects Study by Medium Transfer

Non-targeted radiation effects are a term used to describe the effects of radiation on organisms, in which the cell or organism is not directly exposed to ionizing radiation. For this purpose, A-375 cells were seeded in Petri dishes for 48 h after the cells were exposed to an electron beam in various doses (0, 2, 4, 6, and 8 Gy). 4 h after irradiation, all the medium was collected and passed through the 0.22 µm filter to ensure that the irradiated cells were removed. then this medium was transferred to non-irradiated cells. The cells were collected at various times (0, 24, and 48 hours) after medium transfer and cultured in dishes for the CFA test.

2.7. In Vitro Cytotoxicity Assay

The toxicity of the SFGRT therapy (in-hole and out-hole cells) and medium transfer therapy was evaluated by the MTT cell viability assay. The 10,000 cells/well of A-375 cells were cultured in microplates (96-well). After overnight, the cells were treated with different doses of radiation as Grid therapy (0, 2, 4, 6, and 8 Gy of 6 MeV). In another plate, medium transfer was performed 24

hours after the cell was seeded. After 24 hours of all treatment modalities, MTT solution (100 µl/well) was added and plates were placed in an incubator for 4h. After, the MTT dye was removed and 200 µl of Dimethyl sulfoxide (DMSO) was added to each well. The plates were placed on a shaker for 30 min, then the absorbance of wells in 570 nm was read by an Enzyme-Linked Immunosorbent Assay (ELIZA) reader [31].

2.8. Colony Formation Assay

For evaluating the Colony Formation Assay (CFA) of cells after treatment with different modalities, A-375 cells (treated and control cells) were cultured in Petri dishes. After ten days, the medium was removed and the cells were washed with PBS buffer. The cells were then fixed with formaldehyde (2%) and stained with violet crystal [32, 33]. Then, the colonies were counted and the CFA was determined. Plating Efficiency (PE) and Surviving Fraction (SF) were obtained via Equations 1 and 2, respectively:

$$PE\ (\%) = \frac{Number\ of\ colonies}{Number\ of\ cells\ seeded} \times 100 \qquad (1)$$

$$SF\ (\%) = \frac{Colonies\ counted}{Cells\ seeded\ \times \left(\frac{PE}{100}\right)} \qquad (2)$$

The LQ model expresses the surviving fraction of clonogenic cells as a function of radiation dose D (dose–response relationship) with the following Equation.

$$S = exp[-(\alpha D - \beta D^2)] \qquad (3)$$

The parameters α and β represent the intrinsic radiosensitivity of the cells determined from dose-response experiments and show linear and quadratic components of the cell death.

2.9. Statistical Analysis

All the data acquired in this study had a normal distribution; statistical analysis was performed using one-way analysis of variance (ANOVA) using Graph Pad prism. The value of $P < 0.05$ was considered to be significant.

3. Results

3.1. Toxicity of Direct Electron Radiation vs Grid Electron Irradiation

Figure 1 shows the direct/Grid electron radiation and medium transfer response of target and bystander A-375 cells according to the MTT assays. The MTT results of direct irradiation show a continuous decrease in cell viability with increasing dose. The same result applies to in-hole wells irradiated with Grid radiation. The same result applies to in-hole and out-hole wells (relatively less for out-hole wells) that are irradiated with Grid irradiation. While for the medium transfer model, cell viability is reduced to 4 Gy, and then increased at 6 and 8 Gy. However, as can be seen from Figure 1, the cell death rate in the MT method is significantly less than direct or indirect radiation ($P > 0.05$). In general, the highest rate of cell death is in direct radiation for 8 Gy ($P < 0.05$).

3.2. Effects of 6 MeV Electron Radiation on Colony-

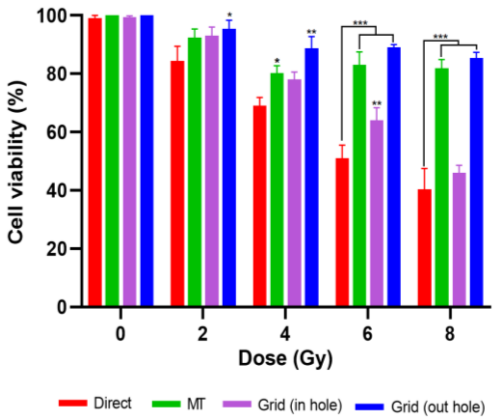

Figure 1. Relative cell viability of target and bystander A-375 melanoma cells, exposed to different doses of electron beams (2, 4, 6 and 8 Gy) by direct radiation, Grid therapy and medium transfer according to the MTT assay. Results reported as mean ± SD of three experiments (*$p < 0.05$)

Forming Ability

The survival curve of the melanoma cells at 0, 24, and 48 hours incubation time after direct exposure electron beam is shown in Figure 2. The survival fraction of the cells was significantly decreased with the increase of radiation dose at all times of incubation ($P < 0.05$). But the increase in incubation time after electron radiation

had no significant effect on the survival fraction of the cells ($P > 0.05$).

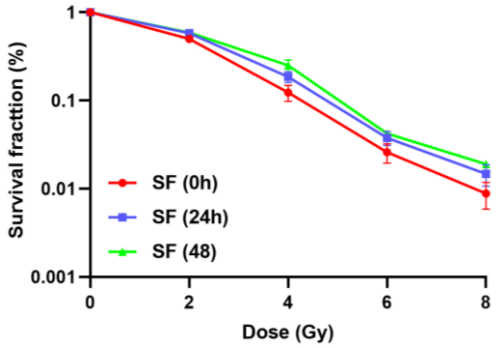

Figure 2. Survival fraction of A-375 cells at 0, 24 and 48 hours' incubation after direct irradiation with 6 MeV electron beams according to the colony formation assay (0, 2, 4, 6 and 8 Gy

3.3. Bystander Effect in the Medium Transfer Method

Four hours after electron radiation, the medium was transferred as described previously. The colony-forming ability was determined for non-irradiated cells at 24 and 48 hours after medium transfer. Figure 3 shows the survival fraction of bystander cells that were not irradiated but rather received medium from directly irradiated cells. When cell culture medium from directly irradiated cells was transferred to non-irradiated cell cultures, bystander killing was observed, which was not dependent on the incubation time with the transferred medium ($P > 0.05$). The survival fraction of the cells that were incubated either 24 h or 48 h after medium transfer was not significantly different ($P > 0.05$). The maximum reduction in cell survival occurred in the cells that had received culture medium from the cells, which were exposed to a 4 Gy electron beam. Figure 3 a and b shows the survival fraction of directly irradiated and bystander cells at 24 and 48 hours after medium transfer. As can be seen, there is no significant difference between the survival fraction of two groups of direct and bystander cells at 2 and 4 Gy ($P > 0.05$), but over 4 Gy at 6 and 8 Gy, there is clearly a significant difference between the survival fraction of these two groups ($P < 0.05$). The results showed that the bystander effect, even in the absence of direct irradiation, could decrease cell viability, although not to the amount of direct irradiation.

3.4. Bystander Effect in GRID Therapy

A-375 cells were plated at specified cell density in Petri dishes. After 48 hours, the cells were exposed to 6 MeV electron beams (0, 2, 4, 6, and 8 Gy) in the presence of the GRID applicator. The cells that were exposed to radiation were named "in-hole" and the adjacent cells which did not receive direct irradiation, as "bystander cell" were called "out-hole" (Figure 4a). A colony forming assay was performed for irradiated and bystander cells after radiation. Figures 4b and 4c showed the cell survival fraction at 0, 24, and 48 h after irradiation for "in-hole" and "out-hole" cells, respectively. The survival fraction declined with increasing the radiation doses for both of the cell populations; directly targeting irradiated and bystander cells. The incubation period has no significant impact on the cell survival ($P > 0.05$). As shown in Figures 4 d-f for different doses individually, for both of the cell populations (in-hole and out-hole), there were significant differences between the survival of the cells which were incubated for various spans and had undergone the same doses ($P < 0.05$). For all incubation periods the survival fraction and clonogenic potential of the directly irradiated cells "in-hole" was

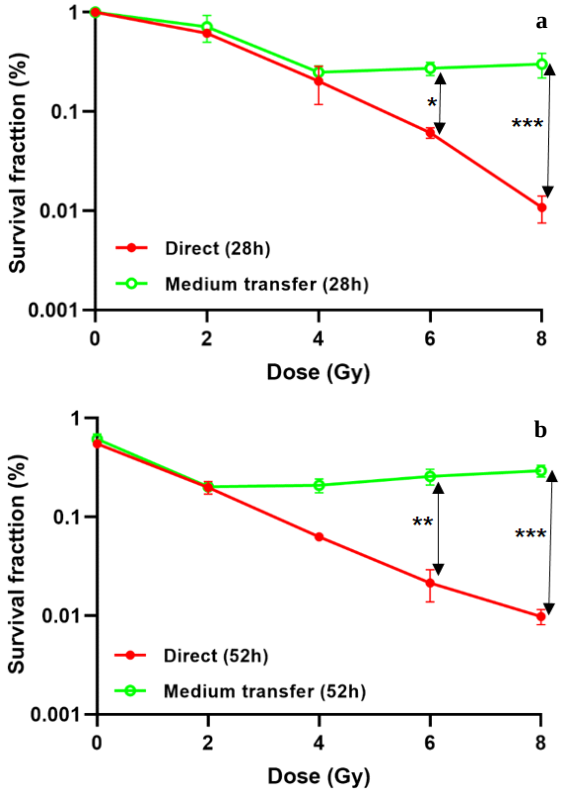

Figure 3. Comparison of the survival curve of directly irradiated A-375 cells and bystander cells (a) 24 and (b) 48 hours after medium transfer (or 28 and 52 hours after radiation), respectively. Results reported as mean ± SD of three experiments (*$p < 0.05$)

significantly lower than that of bystander or "out-hole" cells when the same doses of irradiations were applied ($P < 0.05$). The results clearly showed the maximum lethal effect on cells during direct electron irradiation.

shown in Figure 5a, the LQ diagram was fitted on the survival curve and the alpha and beta parameters were extracted using GraphPad Prism 9 software. As shown in Figure 5b and Table 1, higher values of the α/β ratio

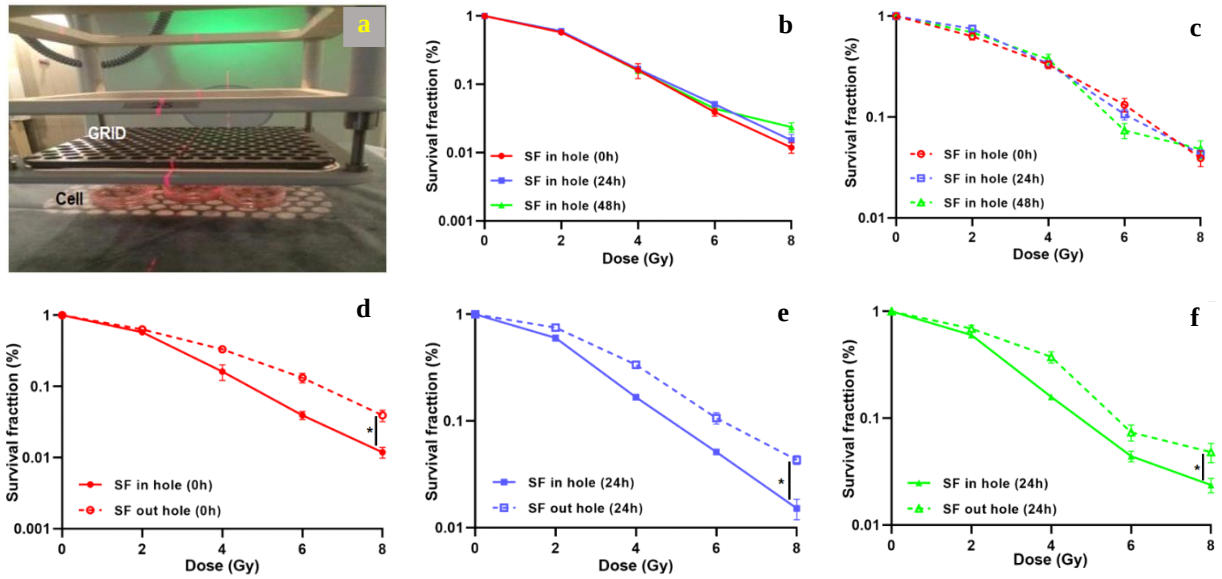

Figure 4. (A) Schematic representation of irradiation of human melanoma cells A-375 by 6 MeV electron beam in the presence of GRID applicator, Survival fraction of (B) "in hole" and (C) "out hole" A-375 cells at 0, 24 and 48 hours' incubation after GRID irradiation with 6 MeV electron beams (0, 2, 4, 6 and 8 Gy), Comparison of the survival fraction of directly irradiated cells "in hole" and bystander cells "out hole", immediately (0) (D), 24 (E) and 48 hours (F) incubation after radiation with 6 MeV electron beams in combination with GRID (0, 2, 4, 6 and 8 Gy). Results reported as mean ± SD of three experiments (*$p < 0.05$)

3.5.Linear-Quadratic (LQ) Model

To quantify the difference between the efficiency of the irradiation method, the parameters of α, β, and α/β were obtained from the survival fraction curves (Figure 5). The α/β ratio was determined as the dose in which the cell killing associated with α (linear parameter) and β (quadratic parameter) was equal. For this purpose, as

indicate that compared to Grid therapy, more cell death occurred under direct radiation. Moreover, the results showed that statistically significant difference ($P < 0.05$) between the α parameter of irradiated and non-irradiated groups of the Grid apertures (in-hole and out-hole, respectively). The non-target cells (bystander cells) had a value of α much smaller than the target cells ($P < 0.05$). Because normal tissue cells with low α/β ratios can

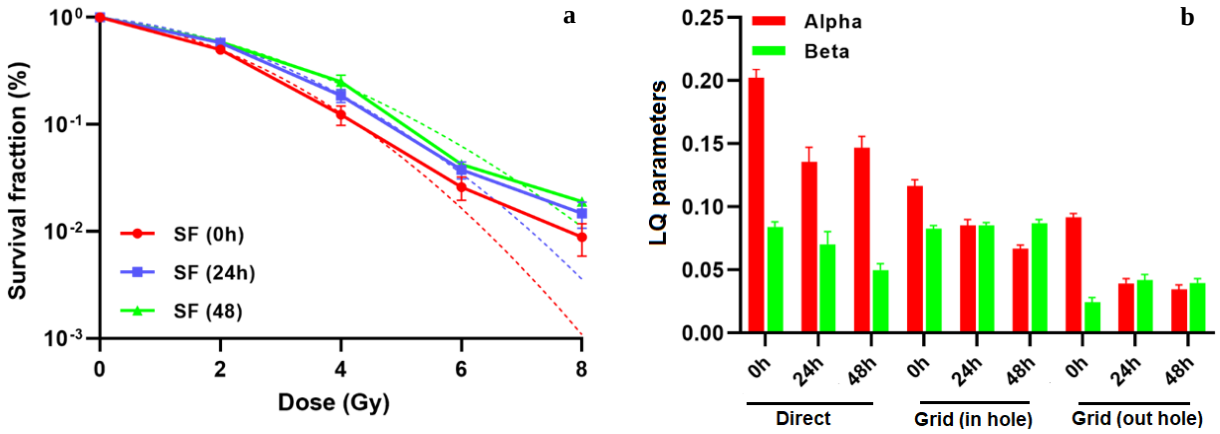

Figure 5. (A) Survival curves were fitted to the linear-quadratic (LQ) model, (B) the values of the linear and quadratic parameters α and β of the LQ model for direct irradiated and Grid therapy of A-375 cells at 0, 24 and 48 h after exposure with 6 MeV electron beams (0, 2, 4, 6 and 8 Gy)

tolerate a higher total dose in lower fraction size or lower dose rate [34], it can be important for heterogeneous doses of Grid therapy in clinical trials.

explain mechanisms involved in the promising clinical outcomes observed so far [36]. In the present study, we evaluated the bystander effect due to the presence

Table 1. Alpha (α) and Beta (β) parameters of A-375 cell line extracted from the survival fraction curves fitted in terms of the linear-quadratic (LQ) model

	α (Gy^{-1})	β (Gy^{-2})	α/β (Gy)
Direct (0h)	0.202 ± 0.005	0.084 ± 0.003	2.404
Direct (24h)	0.138 ± 0.009	0.06 ± 0.008	2.3
Direct (48h)	0.147 ± 0.007	0.05 ± 0.004	2.94
In hole (0h)	0.129 ± 0.004	0.081 ± 0.002	1.592
In hole (24h)	0.088 ± 0.001	0.081 ± 0.004	1.086
In hole (48h)	0.069 ± 0.002	0.082 ± 0.005	0.841
Out hole (0h)	0.0900 ± 0.002	0.094 ± 0.003	0.957
Out hole (24h)	0.048 ± 0.003	0.05 ± 0.003	0.96
Out hole (48h)	0.04 ± 0.002	0.049 ± 0.002	0.816

4. Discussion

In this study, our aim was to evaluate the ability of GRID therapy to induce bystander effects in melanoma cells after exposure to various doses of an electron beam. The results showed that in the cells adjacent to the irradiated areas, bystander killing was significant (0 Gy dose, $P < 0.05$, Figure 4). The reduction in cell survival in adjacent areas was greater than that expected from scattered doses, indicating the presence of true cytotoxic effects after GRID irradiation. Several studies have reported the induction of tumor necrosis factor-α (TNF-α) and decreased regulation of transforming growth factor β1 in the serum of patients after GRID therapy [35]. Generally, and as expected, after a major cytotoxic event, treatment with GRID can lead to increased cytokine production resulting in extensive systemic effects [36, 37]. Given the on/off nature of GRID fields, we have hypothesized that the bystander effect might play a role in killing adjacent non-irradiated cells (Figure 4). The stimulation of a repair mechanism in the viable cells in the "out hole" areas might be responsible for the reduction of cell death in the "in-hole" areas compared to direct irradiated cells. We observed higher cell survival in Grid aperture than in uniform irradiation, which may be related to the effect of bi-directional intercellular communication called reciprocal bystander [38, 39]. To our knowledge, research into the intercellular communication that might exist between adjacent cells in the "in-hole" and "out-hole" regions of GRID therapy has not been performed to date, which may

of scattered rays produced in Grade therapy, through other methods such as medium transfer. Surprisingly, a significant decrease in cell viability (Figure 1) and clonogenic survival (Figure 3) was also shown in the bystander cells after transferring the medium obtained from directly irradiated cells. For instance, more than a 4-fold decrease in survival of the non-irradiated bystander cells which were only received culture medium form directly irradiated cells at 4 Gy has been observed. On the other hand, the results showed that bystander effect levels decreased at doses above 4 Gy (Figure 3). This phenomenon can happen for several reasons. Gow *et al.* attribute this to a negative feedback mechanism that increases survival at high doses. They reported that the cell survival rate decreased in a dose-dependent manner until saturation occurred, then cell survivalincreases with subsequent recovery and repair at high doses [40]. Peng *et al.* and Kishikawa *et al.* indicated that bystander signaling affected cell survival fraction in modulated fields proportional to the local dose, average dose gradient, and also, saturating at higher doses where the effects of direct radiation were predominant [41, 42]. Also, McMahon *et al.* showed that in regions with low-dose exposure, the cell survival fraction is more dependent on intercellular signals than direct radiation damages, which is prevalent in high-dose regions [43]. Our results also confirmed the existence of a negative feedback mechanism that was responsible for reducing the RIBE response at high doses, especially above 4 Gy. It can be important to consider the reduction of RIBE

at high doses and the reciprocal bystander effect at heterogeneous doses used in Grid therapy.

5. Conclusion

Based on clonogenic survival data, we showed that transferring the medium of irradiated culture and GRID therapy induced bystander effects in A-375 monolayer cells. Furthermore, decreased radiation-induced bystander effect level at high dose fractionation (above 4 Gy) could be used to predict normal tissue damage as a result of the bystander effect in radiotherapy procedures such as SFGRT, IMRT, and GRID therapy. Also, according to the obtained results, the bystander effect can affect the adjacent tissues in the new radiotherapy technology, which is delivered in the form of multi-order ultra-high dose rate radiation (FLASH radiotherapy). These results presented here support the interest in performing biological experiments to evaluate these new GRID therapy avenues.

Acknowledgments

Financial support by grant No. 25699 received from the School of Medicine, Iran University of Medical Sciences (IUMS) is acknowledged. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The study protocol manuscript version has been peer-reviewed by the funding body.

All methodology and experiments accomplished in the current study were approved by the Ethics Committee of Iran University of Medical Sciences.

References

1- Sakine Shirvalilou *et al.*, "Enhancement radiation-induced apoptosis in C6 glioma tumor-bearing rats via pH-responsive magnetic graphene oxide nanocarrier." *Journal of Photochemistry and Photobiology B: Biology*, Vol. 205p. 111827, (2020).

2- Slavisa Tubin, Helmut H Popper, and Luka Brcic, "Novel stereotactic body radiation therapy (SBRT)-based partial tumor irradiation targeting hypoxic segment of bulky tumors (SBRT-PATHY): improvement of the radiotherapy outcome by exploiting the bystander and abscopal effects." *Radiation Oncology*, Vol. 14 (No. 1), p. 21, (2019).

3- MA Oghabian, M Jeddi-Tehrani, A Zolfaghari, F Shamsipour, S Khoei, and S Amanpour, "Detectability of Her2 positive tumors using monoclonal antibody conjugated iron oxide nanoparticles in MRI." *Journal of nanoscience and nanotechnology*, Vol. 11 (No. 6), pp. 5340-44, (2011).

4- ANIJDAN SH MOUSAVI *et al.*, "Megavoltage dose enhancement of gold nanoparticles for different geometric set-ups: Measurements and Monte Carlo simulation." (2012).

5- Fatemeh Pakniyat *et al.*, "Enhanced response of radioresistant carcinoma cell line to heterogeneous dose distribution of grid; the role of high-dose bystander effect." *International journal of radiation biology*, Vol. 96 (No. 12), pp. 1585-96, (2020).

6- Ganesh Narayanasamy *et al.*, "Therapeutic benefits in grid irradiation on Tomotherapy for bulky, radiation-resistant tumors." *Acta Oncologica*, Vol. 56 (No. 8), pp. 1043-47, (2017).

7- M. Mohiuddin, M. Fujita, W. F. Regine, A. S. Megooni, G. S. Ibbott, and M. M. Ahmed, "High-dose spatially-fractionated radiation (GRID): a new paradigm in the management of advanced cancers." *Int J Radiat Oncol Biol Phys*, Vol. 45 (No. 3), pp. 721-7, Oct 1 (1999).

8- I Martínez-Rovira, G Fois, and Y Prezado, "Dosimetric evaluation of new approaches in GRID therapy using nonconventional radiation sources." *Medical Physics*, Vol. 42 (No. 2), pp. 685-93, (2015).

9- AS Meigooni, SA Parker, J Zheng, KJ Kalbaugh, WF Regine, and M Mohiuddin, "Dosimetric characteristics with spatial fractionation using electron grid therapy." *Medical Dosimetry*, Vol. 27 (No. 1), pp. 37-42, (2002).

10- Gregory A Szalkowski, "Novel approaches to GRID therapy: Electron GRID and photon minibeams." *Georgia Institute of Technology*, (2019).

11- Thomas Henry, Ana Ureba, Alexander Valdman, and Albert Siegbahn, "Proton grid therapy: a proof-of-concept study." *Technology in Cancer Research & Treatment*, Vol. 16 (No. 6), pp. 749-57, (2017).

12- Kenta Kijima, Anchali Krisanachinda, Mikoto Tamura, Yasumasa Nishimura, and Hajime Monzen, "Feasibility of a tungsten rubber grid collimator for electron grid therapy." *Anticancer Research*, Vol. 39 (No. 6), pp. 2799-804, (2019).

13- Kamran Entezari, Bijan Hashemi, and Seied Rabi Mahdavi, "Dosimetric Evaluation of a Set of Specifically Designed Grids for Treating Subcutaneous Superficial Tumor with 6 and 18 MeV Electron Beam External Radiotherapy." (2021).

14- Kuei-Hua Lin, Chao-Yuan Huang, Jao-Perng Lin, and Tieh-Chi Chu, "Surface dose with grids in electron beam radiation therapy." *Applied radiation and isotopes*, Vol. 56 (No. 3), pp. 477-84, (2002).

15- H. Marks, "A new approach to the roentgen therapy of cancer with the use of a grid." *J Mt Sinai Hosp N Y,* Vol. 17 (No. 1), pp. 46-8, May-Jun (1950).

16- J Isabelle Choi, Janeen Daniels, Dane Cohen, Ying Li, Chul S Ha, and Tony Y Eng, "Clinical outcomes of spatially fractionated GRID radiotherapy in the treatment of bulky tumors of the head and neck." *Cureus,* Vol. 11 (No. 5), (2019).

17- Elisabeth Schültke *et al.*, "Microbeam radiation therapy—grid therapy and beyond: a clinical perspective." *The British Journal of Radiology,* Vol. 90 (No. 1078), p. 20170073, (2017).

18- Samideh Khoei *et al.*, "Enhancement of Radio-Thermo-Sensitivity of 5-Iodo-2-Deoxyuridine-Loaded Polymeric-Coated Magnetic Nanoparticles Triggers Apoptosis in U87MG Human Glioblastoma Cancer Cell Line." *Cellular and Molecular Bioengineering,* pp. 1-13, (2021).

19- VV Petushkova, II Pelevina, IN Kogarko, EA Neifakh, BS Kogarko, and OV Ktitorova, "Some Aspects Related to Transmission of Radiation-Induced Alterations due to the Bystander Effect." *Biology Bulletin,* Vol. 47 (No. 12), pp. 1610-17, (2020).

20- Nasrollah Jabbari, Muhammad Nawaz, and Jafar Rezaie, "Bystander effects of ionizing radiation: conditioned media from X-ray irradiated MCF-7 cells increases the angiogenic ability of endothelial cells." *Cell Communication and Signaling,* Vol. 17 (No. 1), pp. 1-12, (2019).

21- K Kanagaraj, V Rajan, Badri N Pandey, K Thayalan, and P Venkatachalam, "Primary and secondary bystander effect and genomic instability in cells exposed to high and low linear energy transfer radiations." *International journal of radiation biology,* Vol. 95 (No. 12), pp. 1648-58, (2019).

22- A. Kaiser, M.M. Mohiuddin, and Jacksonm J.L., "Dramatic response from neoadjuvant, spatially fractionated GRID radiotherapy (SFGRT) for large, high-grade extremity sarcoma." *Journal of Radiation Oncology,* Vol. 2 (No. 1), pp. 103-06, (2013).

23- Samira Kargar, Samideh Khoei, Sepideh Khoee, Sakine Shirvalilou, and Seied Rabi Mahdavi, "Evaluation of the combined effect of NIR laser and ionizing radiation on cellular damages induced by IUdR-loaded PLGA-coated Nano-graphene oxide." *Photodiagnosis and photodynamic therapy,* Vol. 21pp. 91-97, (2018).

24- Zohreh Eftekhari-Kenzerki, Reza Fardid, and Abbas Behzad-Behbahani, "Impact of silver nanoparticles on the ultraviolet radiation direct and bystander effects on TK6 cell line." *Journal of medical physics,* Vol. 44 (No. 2), p. 118, (2019).

25- Carmel Mothersill and Colin Seymour, "Medium from irradiated human epithelial cells but not human fibroblasts reduces the clonogenic survival of unirradiated cells." *International journal of radiation biology,* Vol. 71 (No. 4), pp. 421-27, (1997).

26- Aisling B Heeran, Helen P Berrigan, and Jacintha O'Sullivan, "The radiation-induced bystander effect (RIBE) and its connections with the hallmarks of cancer." *Radiation research,* Vol. 192 (No. 6), pp. 668-79, (2019).

27- R. S. Asur *et al.*, "Spatially fractionated radiation induces cytotoxicity and changes in gene expression in bystander and radiation adjacent murine carcinoma cells." *Radiat Res,* Vol. 177 (No. 6), pp. 751-65, Jun (2012).

28- K. T. Butterworth, C. K. McGarry, J. M. O'Sullivan, A. R. Hounsell, and K. M. Prise, "A study of the biological effects of modulated 6 MV radiation fields." *Phys Med Biol,* Vol. 55 (No. 6), pp. 1607-18, Mar 21 (2010).

29- Valery Peng *et al.*, "Models for the bystander effect in gradient radiation fields: range and signalling type." *Journal of theoretical biology,* Vol. 455pp. 16-25, (2018).

30- Sara Mohammadi, Samideh Khoei, and Seyed Rabie Mahdavi, "The combination effect of poly (lactic-co-glycolic acid) coated iron oxide nanoparticles as 5-fluorouracil carrier and X-ray on the level of DNA damages in the DU 145 human prostate carcinoma cell line." *Journal of Bionanoscience,* Vol. 6 (No. 1), pp. 23-27, (2012).

31- Sakine Shirvalilou, Samideh Khoei, Sepideh Khoee, Nida Jamali Raoufi, Mohammad Reza Karimi, and Ali Shakeri-Zadeh, "Development of a magnetic nano-graphene oxide carrier for improved glioma-targeted drug delivery and imaging: in vitro and in vivo evaluations." *Chemico-biological interactions,* Vol. 295pp. 97-108, (2018).

32- Zhila Rajaee, Samideh Khoei, Alireza Mahdavian, Sakine Shirvalilou, Seied R Mahdavi, and Marzieh Ebrahimi, "Radio-thermo-sensitivity induced by gold magnetic nanoparticles in the monolayer culture of human prostate carcinoma cell line DU145." *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents),* Vol. 20 (No. 3), pp. 315-24, (2020).

33- Mehrdad Bakhtiary *et al.*, "Comparison of transplantation of bone marrow stromal cells (BMSC) and stem cell mobilization by granulocyte colony stimulating factor after traumatic brain injury in rat." *Iranian biomedical journal,* Vol. 14 (No. 4), p. 142, (2010).

34- MV Williams, J Denekamp, and JF Fowler, "A review of αβ ratios for experimental tumors: implications for clinical studies of altered fractionation." *International Journal of Radiation Oncology* Biology* Physics,* Vol. 11 (No. 1), pp. 87-96, (1985).

35- S. Sathishkumar *et al.*, "Elevated sphingomyelinase activity and ceramide concentration in serum of patients undergoing high dose spatially fractionated radiation treatment: implications for endothelial apoptosis." *Cancer Biol Ther,* Vol. 4 (No. 9), pp. 979-86, Sep (2005).

36- J. A. Penagaricano, E. G. Moros, V. Ratanatharathorn, Y. Yan, and P. Corry, "Evaluation of spatially fractionated radiotherapy (GRID) and definitive chemoradiotherapy

with curative intent for locally advanced squamous cell carcinoma of the head and neck: initial response rates and toxicity." *Int J Radiat Oncol Biol Phys*, Vol. 76 (No. 5), pp. 1369-75, Apr (2010).

37- VA Sergeeva *et al.*, "Low-dose ionizing radiation affects mesenchymal stem cells via extracellular oxidized cell-free DNA: a possible mediator of bystander effect and adaptive response." *Oxidative medicine and cellular longevity*, Vol. 2017(2017).

38- Gabriel Adrian, Crister Ceberg, Ana Carneiro, and Lars Ekblad, "Rescue effect inherited in colony formation assays affects radiation response." *Radiation research*, Vol. 189 (No. 1), pp. 44-52, (2018).

39- Mohammad Taghi Bahreyni Toossi, Sara Khademi, Hosein Azimian, Shokoufeh Mohebbi, and Shokouhozaman Soleymanifard, "Assessment of The Dose-Response Relationship of Radiation-Induced Bystander Effect in Two Cell Lines Exposed to High Doses of Ionizing Radiation (6 and 8 Gy)." *Cell Journal (Yakhteh)*, Vol. 19 (No. 3), p. 434, (2017).

40- MD Gow, CB Seymour, Soo-Hyun Byun, and CE Mothersill, "Effect of dose rate on the radiation-induced bystander response." *Physics in Medicine & Biology*, Vol. 53 (No. 1), p. 119, (2007).

41- Valery Peng, Natalka Suchowerska, Linda Rogers, Elizabeth Claridge Mackonis, Samantha Oakes, and David R McKenzie, "Grid therapy using high definition multileaf collimators: realizing benefits of the bystander effect." *Acta Oncologica*, Vol. 56 (No. 8), pp. 1048-59, (2017).

42- Hiroko Kishikawa, Ketai Wang, S James Adelstein, and Amin I Kassis, "Inhibitory and stimulatory bystander effects are differentially induced by iodine-125 and iodine-123." *Radiation research*, Vol. 165 (No. 6), pp. 688-94, (2006).

43- Stephen J McMahon *et al.*, "A computational model of cellular response to modulated radiation fields." *International Journal of Radiation Oncology* Biology* Physics*, Vol. 84 (No. 1), pp. 250-56, (2012).