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Response of Liver and Gastric Cancer Cells to Electron and X-ray Radiation

Abstract. Radiotherapy remains a critical pillar of cancer treatment worldwide. This study evaluates the in vitro efficacy of high-energy ionizing radiation, specifically 6 MV electrons and 12 MV X-rays, generated by a Varian Clinac iX linear accelerator (linac), on human HepG2 (liver) and AGS (gastric) cell lines. Cell samples (1 ml) were irradiated with doses ranging from 0.5 Gy to 4 Gy. Cell viability was assessed using the WST assay 4-5 hours post-irradiation. The measured survival rates were critically compared with those predicted using the established linear-quadratic (LQ) model. The results revealed significant and consistent discrepancies between the experimental measurements and the theoretical predictions for both cell lines. For HepG2 cells, the measured survival rate at 4 Gy was higher than the predicted rate. Interestingly, AGS cells irradiated with 12 MV X-rays exhibited minimal cytotoxicity, with a viability rate of 99.0% at 3 Gy versus a predicted rate of 73.6%. These findings suggest a discrepancy between theoretical predictions and the short-term biological responses observed under the shallow in vitro irradiation conditions employed in this study. While the present study was not designed to isolate the underlying mechanisms, the results imply that factors inherent to high-energy beam delivery in thin in vitro geometries, together with the early (four to five hour) post-irradiation assessment window, may have contributed to the limited cytotoxicity observed in both cell lines. Further studies employing extended observation periods or complementary assays would be valuable in clarifying the temporal progression of MV-beam-induced cellular effects.
Keywords: Varian Clinac iX, high-energy radiation, HepG2, AGS, cell viability, WST assay, Monitor Unit (MU).

Introduction

The global increase in cancer cases necessitates continuous improvements in treatment methods [1]. Radiotherapy is a vital component of cancer treatment. Current practices involve using high-energy linear accelerators, such as the Varian Clinac iX linac, to deliver precise doses of X-rays or electrons. These high-energy beams are purposefully designed to penetrate deeply, targeting tumors while minimizing damage to surrounding healthy tissue [2-3].

Previous radiobiological studies, which often used lower-energy gamma sources such as cobalt-60 (^{60}Co), have typically shown that significant cell death occurs at conventional clinical doses [4-5]. However, the high-energy X-rays and electrons used in modern linear accelerators (linacs) have notably different physical properties. Specifically, they produce a deeper dose maximum (D_{max}) and exhibit a lower mass-energy absorption coefficient near the surface. Consequently, the biological effects of these high-energy beams require specific evaluation, particularly when administered at low doses to shallow targets or *in vitro* cell cultures. A key question remains regarding the dose efficacy delivered by these MV-energy beams compared to lower-energy sources when the target resides within the dose build-up region.

The aim of our current study is to explore whether higher-energy beams produce similar radiobiological outcomes in the shallow dose region to those observed in established low-energy studies. To investigate this, we will examine the radiobiological responses of two distinct human carcinoma cell lines:

HepG2 (liver) and AGS (gastric) [6-9]. The cells will be exposed to high-energy beams from a linear accelerator, and the experimental setup will be designed to reflect principles of beam physics. The HepG2 cells were irradiated with 6 MV electron beams to simulate targets close to the body's surface. This approach takes advantage of the beams' rapid energy loss and shallow dose maximum (D_{max} typically <1 cm), thus eliminating the need for a thick phantom. In contrast, AGS cells were irradiated with 12 MV X-ray beams using a 2.5 cm tissue-equivalent water phantom.

Experiment

HepG2 and AGS cancer cell samples were obtained and prepared in the Gene Engineering Laboratory at the National University of Mongolia (NUM).

All cell samples were prepared as 1 ml aliquots in plastic tubes measuring 4.5 cm in length and 1 cm in diameter. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with a nutrient solution containing 1 % penicillin–streptomycin, 10 % fetal bovine serum (FBS), and 1% L-glutamine.

The preparation process is summarized in Fig. 1. Panel (a) shows the initial state of the cancer cell samples prepared at the NUM. Panel (b) illustrates the cell samples secured within a custom-designed holder beneath the Varian Clinac iX linac beam at the NCCM, ready for irradiation.

Irradiation was performed using the Varian Clinac iX linac at the NCCM. The cell samples were placed at a Source-to-Surface Distance (SSD) of 100 cm.

- HepG2 cells were irradiated with 6 MV electron beams at doses of 1 Gy, 2 Gy, and 4 Gy.
- AGS cells were irradiated with 12 MV X-ray beams at doses of 0.5 Gy, 1 Gy, and 3 Gy.

For the AGS irradiation, a tissue-equivalent water phantom was used to position the centre of the cell sample at a depth of 2.5 cm, thereby simulating clinical conditions for superficial tumors.

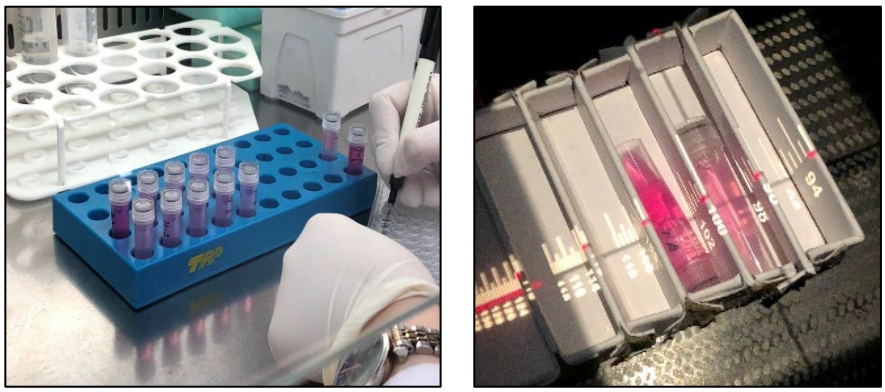

Figure 1 – (a) Prepared cancer cell samples in the Gene Engineering Laboratory, NUM. (b) Ready-to-irradiate cell samples secured within the custom-designed holder (case) beneath the Varian Clinac iX linac beam at the NCCM

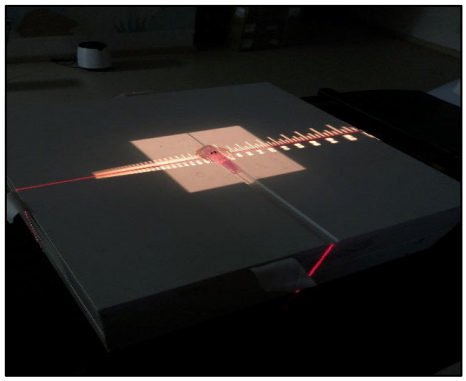

Figure 2 – The Varian Clinac iX setup for AGS cell irradiation used a tissue-equivalent water phantom to simulate clinical conditions for superficial tumors by positioning the gastric cancer sample at a depth of 2.5 cm

High-energy X-ray beams exhibit a dose build-up effect, meaning the D_{max} is deposited several centimeters beneath the surface, not directly at the entrance. For a 12 MV X-ray beam, this D_{max} occurs at approximately 2.5 cm to 3.0 cm (Fig.2).

- The 2.5 cm water phantom layer provides the required tissue-equivalent material for the photon beam to generate a sufficient number of secondary electrons. This ensures that the cell sample, positioned at this depth, receives the maximum and intended prescription dose (e.g., 1 Gy), thereby accurately simulating the dose received by a superficial tumor *in vivo*.

In contrast, the HepG2 cells were irradiated with a 6 MV electron beam, which requires no such deep build-up layer.

- Electron beams are characterized by rapid energy loss and shallow dose deposition. The D_{max} for a 6 MV electron beam occurs very close to the surface (typically less than 1 cm).
- Therefore, the HepG2 cells, placed in thin culture vessels, receive the full and intended dose without the need for a thick water phantom, making the setup appropriate for simulating treatment of targets close to the body surface.

The 12 MV X-ray and 6 MV electron beams used in this study had different dose deposition characteristics. This meant that specific experimental setups were required.

High-energy X-ray beams demonstrate a dose build-up effect, whereby the D_{max} is deposited several centimeters beneath the surface rather than at the point of entry. For the 12 MV X-ray beam, D_{max} occurs at a depth of approximately 2.5–3.0 cm.

Consequently, a 2.5 cm layer of tissue-equivalent water phantom was used for the AGS cell irradiation. This layer of the phantom provided the necessary material for the photon beam to generate a sufficient number of secondary electrons. This ensured that the cell sample, precisely positioned at this depth, received the intended maximum prescription dose (e.g. 1 Gy), thereby accurately simulating the dose received by a superficial tumor *in vivo*.

In contrast, the HepG2 cells were irradiated with a 6 MV electron beam, for which no deep build-up layer is required. Electron beams are characterized by rapid energy loss and shallow dose deposition. The D_{max} for a 6 MV electron beam occurs very close to the surface (typically less than 1 cm). Therefore, when placed in thin culture vessels, the HepG2 cells receive the full intended dose, eliminating the need for a thick water phantom. This makes the setup appropriate for simulating the treatment of targets close to the body's surface.

The general scheme of this experiment is displayed in Fig. 3. Figure 3 shows the process of irradiating cancer cells with electrons and X-rays using a Varian Clinac iX linac.

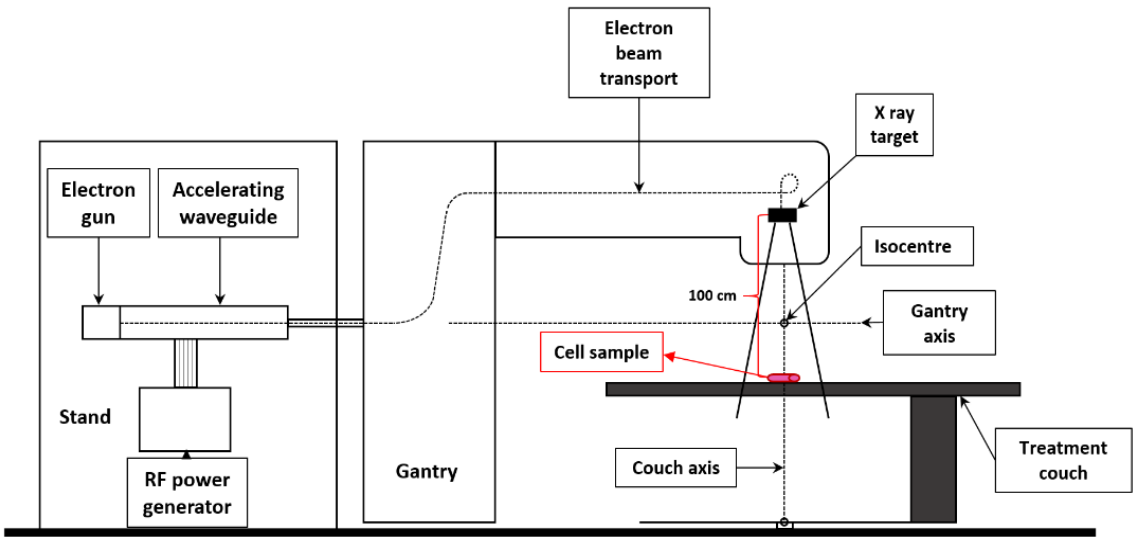

Figure 3 – Scheme of irradiation of cancer cells with electron and X-rays in using a Varian Clinac iX linac

Subsequently, the WST assay was used to determine the effect of ionizing radiation (IR)-induced cell death [10-11]. This colorimetric assay is based on the cleavage of the tetrazolium salt WST to formazan by mitochondrial cellular dehydrogenases. The greater the number of viable cells, the higher the activity of the mitochondrial dehydrogenases, resulting in greater formation of measurable formazan dye.

Results and Discussion

The accuracy of the absorbed dose was validated by comparing the calculated monitor units (MU) with those calculated by the Varian Clinac iX system. The MU required for a given dose were determined using the dosimetry formula for the linac based on the tissue-phantom ratio (TPR) model. The equation for calculating the absorbed dose (D) in a cell sample using the linear accelerator, based on the TPR, is given below:

$$MU = \frac{D}{D_0' \cdot S_c(r_c) \cdot S_p(r_{d0}) \cdot TPR(d, r_d) \cdot WF(d, r, x) \cdot TF \cdot OAR(d, x) \cdot \left(\frac{SSD_0 + d_0}{SPD}\right)^2}. \quad (1)$$

Where: – MU-the measurement of Linac output for a given prescription dose,

- D – the radiation dose to be delivered to the cell sample (in Gy. Prescribed dose).

- D_0' – the output dose rate corresponding to the specific beam energy (often at a standard field size and depth. Reference dose rate),

- $S_c(r_c)$ – the output ratio relative to a reference field size measured in air (Collimator scatter factor),

- $S_p(r_d)$ – the change in scatter contribution from the phantom as the field size changes (Phantom scatter factor),

- $TPR(d, r_d)$ – the ratio of dose at a depth d to the dose at a reference depth d_{ref} in a phantom, measured at a constant Source-to-Surface Distance (SSD). Tissue-Phantom Ratio (TPR).

- $WF(d, r_d, x)$ – a factor accounting for dose reduction caused by a physical wedge filter (Wedge factor), TF-transmission factor (or similar component, often incorporated into TPR or S_p),

- $OAR(d, x)$ – the ratio of the dose rate at a point off the central beam axis to the dose rate on the central axis at the same depth (Off-axis ratio), d_0-the normalized depth (depth of the cell sample),

- SSD_0 – the distance from the X-ray source to the surface of the phantom/sample,

- SPD – the distance from the source to the entrance surface (equivalent to Source-to-Phantom Distance).

The results of the MU comparison are presented in Table 1. This shows a comparison of MU calculated using the analytical TPR model and the internal Varian Clinac iX system calculation. For the 6 MV electron beam (used for HepG2), the analytical calculation for the 1 Gy dose yielded a value of 110.2 MU, showing excellent agreement with the value of 109.2 MU calculated by the Clinac system. Similarly, for the 12 MV X-ray beam (used for AGS), the analytical MU for 1 Gy was 110 MU, compared to 106 MU for the system.

Across all calculated doses for both electron and X-ray beams, the analytical MU values were compared with those calculated by the Clinac system (see Table 1), confirming analytical consistency with a deviation of less than 5%. This validates the accurate delivery of the prescribed dose to the target volume for both irradiation protocols.

Table 1 – Comparison of MU calculated using the analytical TPR model and the internal Varian Clinac iX system calculation

Absorbed Dose to Cell Sample [Gy]	Calculated Monitor Units [MU]	Varian Clinac iX system [MU]
Electron		
1	110.2	109.2
2	220.4	218.4
4	440.8	436.8
X-ray		
0.5	55	53
1	110	106
3	330	318

The viability of HepG2 and AGS cells was evaluated using the WST assay four to five hours after irradiation as illustrated in Figures 4 and 5, respectively. The HepG2 response to 6 MV electron beams.

As shown in Fig. 4, HepG2 liver carcinoma cells irradiated with 6 MV electrons at doses of 1, 2, or 4 Gy exhibited moderate reductions in viability. Viability decreased from approximately 100% (control) to 95% at a dose of 1 Gy. Interestingly, however, viability increased slightly at 2 Gy, before dropping to 91% at 4 Gy. Our results suggest that the HepG2 cell line exhibits a certain degree of radioresistance, given that a notable cytotoxic effect was only observed at a dose of 4 Gy. This was the highest dose within the radiobiologically relevant range, which extends from common curative fractional doses of approximately 2 Gy to higher doses employed in palliative treatment protocols of approximately 4 Gy.

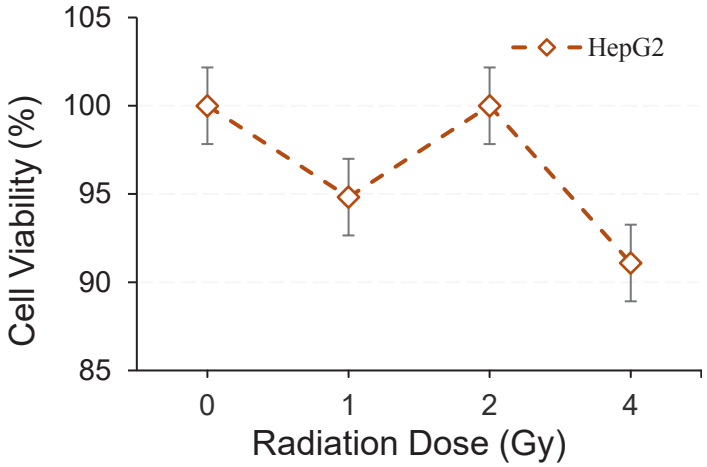

Figure 4 – The WST assay for determining the viability of HepG2 cells

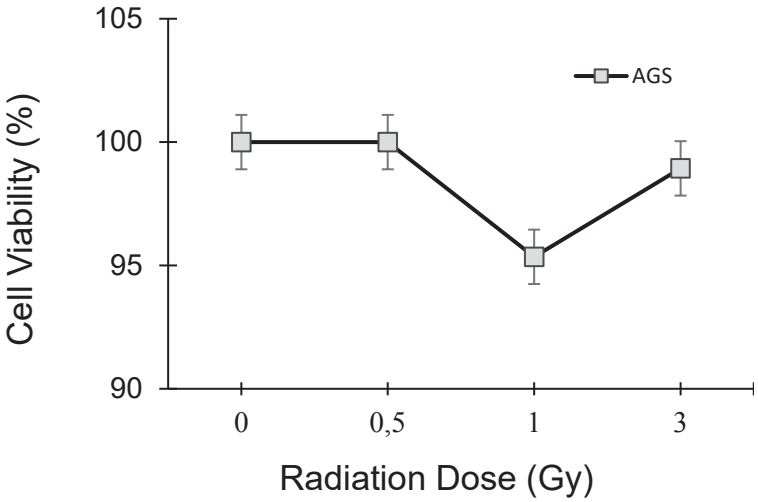

Figure 5 – The WST assay for determining the viability of AGS cells

As shown in Fig. 5, AGS (gastric carcinoma) cells irradiated with 12 MV X-rays at doses of 0.5 Gy, 1 Gy, or 3 Gy exhibited negligible cytotoxicity. Viability remained at 100% at a dose of 0.5 Gy. The lowest recorded viability was 95% at 1 Gy, before increasing again at 3 Gy. Overall, viability remained tightly confined within a range of 95% to 100% across the entire dose spectrum.

This finding is significant, as it suggests a very low probability of biological interaction between the

high-energy 12 MV X-ray beam and the cells, despite their precise positioning within the 2.5 cm water phantom at the theoretical D_{max}.

The survival rate (S) of the cancer cells after irradiation was calculated using the linear-quadratic (LQ) model [12], for which the relevant cell-specific parameters were obtained from references [13-15]. The viability of 1 ml of HepG2 and AGS cancer cell samples was calculated using Eq. (4) from Ref. [4]. The calculated and measured cell viability for the HepG2 and AGS cell lines is summarized in Table 2.

Table 2 – Results of the experimental work performed using the Varian Clinac iX linac and the calculated cell viability using Eq. (4) in Ref. [4]

HepG2 liver cells				
Cell Sample	Radiation Dose [Gy]	Irradiation time [min]	Cell viability [%]	
			Calculated	Measured
H-1 (1st sample)	1.0	13.0	81.6	75.4
H-2 (2nd sample)	2.0	25.0	59.3	79.5
H-3 (3rd sample)	4.0	50.0	25.7	72.4
Monitor cell sample	0.0	0.0	100.0	100.0
AGS gastric cells				
A-1 (1st sample)	0.5	7	98.3	100.0
A-2 (2nd sample)	1	14	87.5	95.0
A-3 (3rd sample)	3	42	73.6	99.0
Monitor cell sample	0	0	100.0	100.0

A comparative analysis reveals significant discrepancies between the analytical prediction and the experimental outcome for the HepG2 electron and AGS X-ray irradiation (see Table 2).

These cells exhibited a higher survival rate than predicted by the LQ model when irradiated with 6 MV electron beams and 12 MV X-rays. The measured viability was substantially higher than the LQ prediction for both cells. The discrepancy between the measured and calculated viability increased at higher doses, showing a different trend to that observed in our previous work [4]. In our previous study, we used low-energy radiation sources, whereas in this study we used high-energy sources. In future studies, we will need to increase the dose range and apply different radiation sources for higher energies.

Summary

This study examined the response of HepG2 and AGS cancer cells to high-energy electron and photon beams delivered by a clinical linac. Across all tested doses, the experimentally observed viability remained consistently higher than the survival predicted by LQ-based models. For HepG2, the measured survival at 4 Gy (72.4%) was substantially greater than the predicted 25.7%, while AGS cells exhibited minimal reduction in viability even at 3 Gy (99.0% measured versus 73.6% predicted). These findings indicate a clear divergence between theoretical expectations and the short-term biological response observed under the shallow in vitro irradiation conditions used here.

Although the present work was not designed to isolate the underlying mechanisms, the results suggest that factors inherent to high-energy beam delivery in thin in vitro geometries—together with the early (4–5 h) post-irradiation assessment window—may have contributed to the limited cytotoxicity detected in both cell lines. Further studies employing extended observation periods or complementary assays would be valuable for clarifying the temporal progression of MV-beam–induced cellular effects.

Overall, the data provide an empirical benchmark for interpreting radiobiological outcomes in shallow in vitro systems exposed to therapeutic beam energies and highlight important considerations for the design of future experiments using clinical Linac platforms.

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