

# Radiobiological evaluation of radiation cells survival based on linear quadratic and multi-target model

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**Abstract:** Radiobiological model such as linear quadratic is widely used in clinical radiotherapy to predict the biophysical response of the tumour cells toward radiation. This study investigates the radiation cell survival using Linear Quadratic and Multi-Target models together with their radiobiological parameters. The experimental works were conducted *in-vitro* using HeLa cells that were irradiated using photon and electron beams at different energy. Cells irradiation were performed in full scatter condition and exposed to radiation doses ranges from 1 to 10 Gy. Clonogenic assay is used as an endpoint to obtain the cell survival curves which later be fitted with linear quadratic and multi-target model. The results demonstrate that Multi-target model produce the fitting curves that are closed to the experimental data compare to linear quadratic model especially at high doses. Parameter analysis from both models indicates more biological damage inflicted by high energy electron beams. Correlation between the experimental cell survival data and radiobiological model analysis suggesting that radiobiological model could be applied in analyzing cells' radiation survival and damage

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**Keywords:** *linear quadratic model, multi-target model, radiobiology, radiation cell survival*

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## 1. Introduction

The cell culture assay technique was widely used in radiobiology research in order to elucidate the radiation induced damage to the biological materials. The cell survival curves can be obtained from numbers of cell colony form after irradiation. Puck and Marcus (1956) used cell survival curves to describe the effects of high energy irradiation on the reproductive capacity of single HeLa cells by plotting the graph of number of cells colony versus dose.<sup>1</sup> The graph defines the relationship between the radiation dose and the proportion of cells that survive. Cell survival curves are often described by radiobiological models. There are several models that have been used to analyze the cell survival curve such as linear quadratic (LQ) and multi-target (MT) models. Classical MT model presents a straight line at high doses, which is not supported by the mechanism of the underlying radiobiological processes but it is still valuable because MT model fits the empirical data well, especially in the high-dose range. Iwata et al. (2012) states that conversion with the MT model is not easy in clinical practice since there are many parameters which generally cannot be determined.<sup>2</sup> Later, LQ model was proposed by Chadwick and Leenhouts in (1981) in order to correct the deficiencies of the MT model.<sup>3</sup> Fertil and Malaise (1985) found that human cell survival curves could be better described by the LQ model compare to MT model.<sup>4</sup> LQ model describe low  $\alpha$ -value indicate resistance in the low dose range. The exponential survival curve shows the high radiosensitivity of cells while shouldered survival curve represents the poor radiosensitivity. There are several studies that compare different models to analyze the cell survival curves. One of the studies comparing the different model was one by Iwata et al.(2012).<sup>2</sup> From the study, it shows that MT models seem to be more reliable than the LQ model at 6 Gy or higher single doses. This result might be caused by the characteristics of the two models where at the high-dose range the data can be approximated by

linear regression. The LQ model might only applicable to fractional doses of 5 Gy or less. Despite MT model is a basic and classical linear model, it still fits the empirical data well at high doses compared to the LQ model.<sup>2</sup> Besides that, Fertil and Masaile (1985)<sup>4</sup> found that parameters used by MT model that are  $n$  and  $D_0$  do not accurately describe the differences in radiosensitivity that are a feature of initial of the survival curves. In LQ model, the use of the parameter  $\alpha$  not only allows the differentiation between different cell lines, but also leads to consistency between different studies of a single cell line, as in the case of HeLa cells.<sup>4</sup> In this study, the applicability of the MT and LQ model to analyse survival curves was investigated and the model parameters were analysed to find correlation with experimental data.

## 2. Materials and methods

### 2.1. HeLa cell lines preparation

HeLa (ATCC® CCL-2™) cell lines were prepared in Dulbecco's Modified Eagle's Medium (DMEM) which was supplemented with 10% FBS and a 100 unit/mL penicillin-streptomycin (Gibco, Life Technologies, CA, U.S.A). The cells were grown in 37°C and 5% CO<sub>2</sub> humidified atmosphere until confluence and harvested using 0.25% Trypsin-EDTA (Gibco, Life Technologies, CA, U.S.A).

### 2.2. HeLa cells irradiation setup

The cell samples were prepared in suspension in 0.5 ml PCR tube (Greiner Bio-One, Austria) which contains approximately 1000 cells. Irradiation was carried out using clinical photon and electron beams of different energies using Primus Linear Accelerator (Siemens Healthcare, USA). The cell samples were set up on water phantom at source to surface distance (SSD) of 100 cm with 10 x 10 cm<sup>2</sup> field size. Bolus was placed on top of cell samples as a build up so that the maximum prescribed dose was delivered to the cells. Irradiations were done in single fraction with constant dose rate of 100 MU/min with radiation doses ranging from 0 to 10 Gy. Irradiated cells were incubated for 10 days to observe the colony.

### 2.3. Clonogenic assay

After 10 days of incubation, the irradiated and control cell samples were washed using 0.5 ml of PBS. Then the cells were fixed using 0.5 ml ice cold methanol for 15 minutes. The fixed cells were stained with crystal violet for 30 minutes and were rinsed gently using tap water. After rinsing, the stained cells were left it dry at room temperature. The visible cell colonies were counted using microscope and analyzed in form of cell survival fraction data using OriginPro 7.5 software (OriginLab Corporation, Northampton, MA, USA).

### 2.4. Radiobiological analysis

The linear quadratic (LQ) and multi-target (MT) model were fitted to the experimental data point using OriginPro 9.0 software. The models equations are shown in equation 1 and 2. Analysis of the parameter's value from each model was also done. Linear quadratic model:

$$S = \exp^{-(\alpha D + \beta D^2)} \quad (1)$$

where  $S$  is the cell survival fraction,  $\alpha$  and  $\beta$  are the linear and quadratic parameters of this model, respectively and  $D$  is the absorbed dose. The LQ model assumes that  $S$  is made of two terms: a linear term  $\alpha D$  and a quadratic term,  $\beta D^2$ . The modern molecular and cellular justifications are as follows:

- A lethal lesion can be thought of as an unrepaired DNA double strand break (DSB) that leads to chromosome breaks, which in turn lead to cell death.
- A lethal lesion can be produced either by single radiation track (which is proportional to dose,  $\alpha D$ ) or by interactions of two or more less severe DNA lesions (sublethal lesions) generated by two separate radiation tracks (which is proportional to the dose square,  $\beta D^2$ ).<sup>5</sup>

The second radiobiological model used is multi-target model. Data were fitted according to the model equation expression that also describes the relationship between the cell survival and irradiation dose: Multi-Target Model :

$$S = 1 - (1 - \exp^{-D/D_0})^n \quad (2)$$

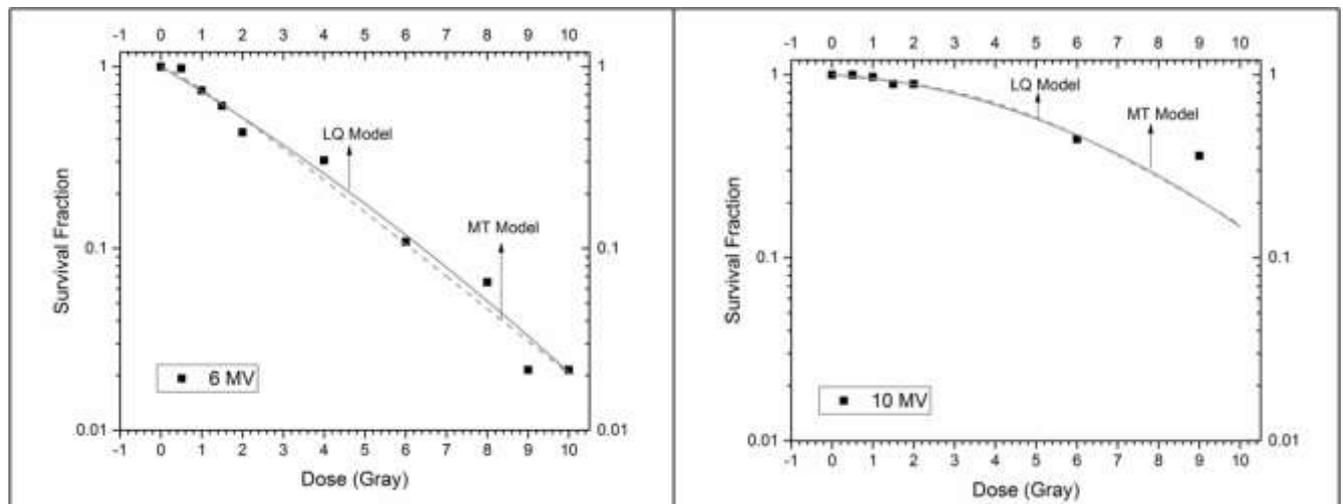
$D$  is the dose in Gray,  $D_0$  is parameter that determine the final slope of the survival curve, and  $n$  is the y-intercept of the asymptote. The MT model is a basic and classical linear model which fits the empirical data well at high dose compared to the LQ model.<sup>2</sup>

### 3. Results and discussion

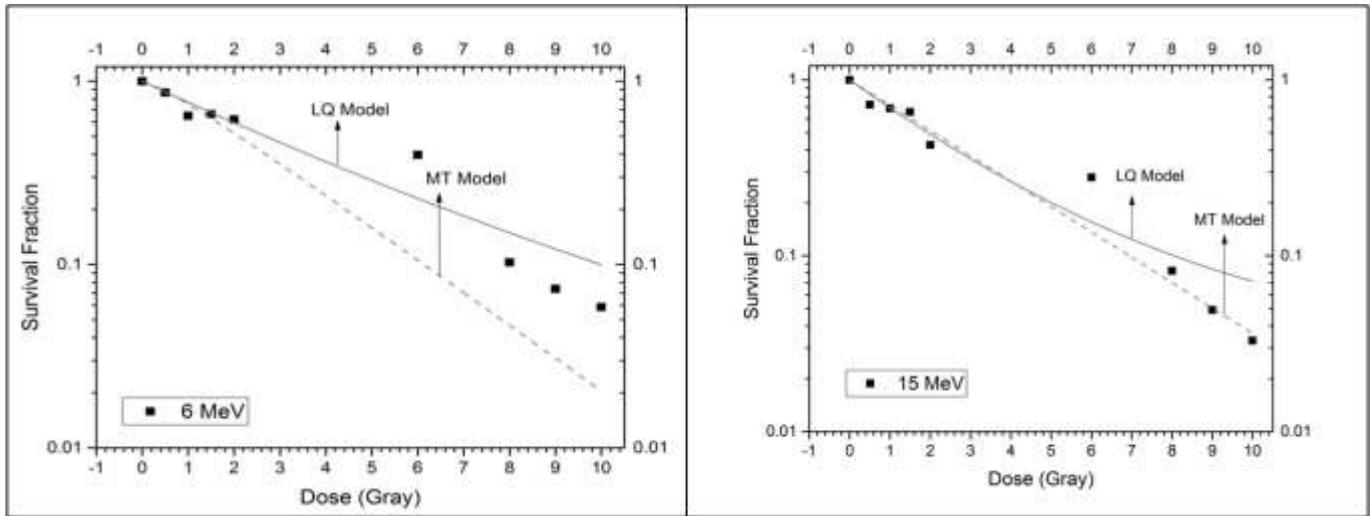
Cell survival curves indicate the relationship between survival fractions with the radiation dose (Gy). The cell survival curves for HeLa cells irradiated with photon beam of energy 6 and 10 MV was depicted in Figure 1. The cell survival curves are generated based on LQ and MT model. The curves generated from MT model are found to fit the experimental data and better than LQ model for 6 MV photon beam. Meanwhile the cell survival curves for 10 MV photon beam have been generated with LQ and MT model are almost identical.

Figure 2 shows the cell survival curves for 6 and 15 MeV electron beams fitted with LQ and MT model. The fitting curve of LQ and MT models were found to fit the experimental data better at low dose compare to high dose. The fitting curves for LQ seem to fit the experimental data better than MT model for 6 MeV electron beams. Meanwhile for 15 MeV electron beams, the results are contradictory in which the MT model fitted the experimental data better at low and high dose compare to LQ model.

In general, survival fractions generated by MT model are slightly higher compare to LQ model for dose ranges from 0.5 to 1.5 Gy. At higher dose up to 10 Gy, LQ model indicates higher survival fraction compare to MT model. LQ model fitted the cell survival data well for photon beams and 6 MeV electron beams. However, MT model is more close to experimental data and fitted better at higher doses.



**Figure 1.** The cell survival curves of HeLa cells irradiated with 6 and 10 MV photon beams fitted with LQ and MT models.



**Figure 2.** The cell survival curves of HeLa cells irradiated with 6 and 15 MV electron beams fitted with LQ and MT models.

**Table 1.** The parameters values from Linear Quadratic model.

Energy	$\alpha$ (Gy <sup>-1</sup> )(SE)	$\beta$ (Gy <sup>-2</sup> )
6 MV	0.30721 (±0.04618)	0.00799 (±0.01225)
10 MV	0.03054 (±0.04231)	0.01607 (±0.00665)
6 MeV	0.26771(±0.05593)	-0.00373 (±0.00863)
15 MeV	0.37901(±0.05895)	-0.01154 (±0.00987)
6 MV	0.30721 (±0.04618)	0.00799 (±0.01225)
10 MV	0.03054 (±0.04231)	0.01607 (±0.00665)

**Table 2.** The parameters values from Multi-target Model

Energy	D <sub>1</sub>	D <sub>0</sub>	N
6 MV	3.75489E19 (±0)	2.42385 (±0.42417)	1.27096 (±0.24639)
10 MV	18.12067 (±16.7689)	3.39997 (±2.12358)	5.60019 (±9.52601)
6 MeV	3.75489E19 (±0)	2.42385 (±0.42417)	1.27096 (±0.24639)
15 MeV	3.01362 (±0.40776)	4022.75834 (±0)	981.21178 (±0)

Radiobiological analysis of the cell survival based on the LQ model parameters is shown Table 1. Parameter for  $\alpha$  (Gy<sup>-1</sup>) represents single radiation hit to the cells where the cells are presumed to be unable to repair themselves after the radiation exposure. Value  $\beta$  (Gy<sup>-2</sup>) represent double radiation hits to the cells where it can repair after the damage caused by the radiation.<sup>3</sup> Based on results in Table 1, both 6 MV and 10 MV photon beams shows the value of  $\alpha$  (Gy<sup>-1</sup>) is higher than  $\beta$  (Gy<sup>-2</sup>). Therefore, it indicates that more cells died due to single radiation hit to the cells.<sup>4</sup> The  $\alpha$  (Gy<sup>-1</sup>) value of 6 MV photon beam is higher than 10 MV photon beam while  $\beta$  (Gy<sup>-2</sup>) value for 10 MV photon beam is higher than 6 MV photon beam. So the cells that were irradiated with 10 MV photon beam has higher tendency to be repaired, compare to 6 MV photon beam. The comparison of parameters value of  $\alpha$  (Gy<sup>-1</sup>) and  $\beta$  (Gy<sup>-2</sup>) between 6 MV

and 10 MV photon beams has demonstrated that low energy photon beams kill much more cells than the high energy. However for electron beams, the value  $\alpha$  ( $\text{Gy}^{-1}$ ) of 15 MeV is higher than 6 MeV, and much more impressively higher compare to photon beams. The LQ model parameters analysis has signified the experimental cell survival obtained to the single radiation hits as the main cause of cell death.<sup>4</sup>

Table 2 shows the parameters value derived from MT model. The value of  $D_1$  presents the initial slope of the curve due to single event killing while  $D_0$  presents the value of final slope of the curve due to multiple event killing. The  $n$  value is the extrapolation number. MT model parameter analysis for 15 MeV electron beam shows the highest values of  $D_0$  compare to other energies used. This shows that multiple event killing have greatest effect to cells death. Similar to LQ model analysis, electron beam causing more biological damage compare to photon beam as indicate by lower value of  $D_0$  for photon beams.

#### 4. Conclusion

The MT model was found to be close to experimental cell survival and generate better fitting curve than LQ model, especially at high dose. The analysis from both models parameter also presents a correlation within cell survival data and could be applied to predict the cells' radiosensitivity and biological damage.

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