Study of morphological changes and survival fraction in EMT6 cell line post- gamma ray irradiation

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Abstract. Radiotherapy plays an important role in cancer treatment; however, the incidence of radio-resistance remains become major challenges in cancer treatment. The presence of epithelial-mesenchymal transition (EMT) has become a clinical hallmark for cancer to relapse after radiation treatment. The aim of this study was to investigate the morphological changes and cell survival of EMT6 cell line post–gamma ray irradiation. The irradiation of EMT6 cells was conducted with two approaches, first, EMT6 cells were treated with single dose of gamma-ray ranging low to high dose of gamma-ray (0-16 Gy) and second, EMT6 cells were exposed to fractionated gamma-ray irradiation (2 Gy per cycle) where cells that survive the initial irradiation were further irradiated with 2 Gy dose. Results showed that there were morphological changes in treated cell compared to sham-control cells in both irradiation approaches which include loss of glandular pattern, vacuolated cell plasma, pleomorphic nuclei and enlarged size. For survival fraction, the survival fractions were reduced with increasing dose compared to the sham-control in single dose reaction while for the fractionated irradiation, survival fractions were reduced until 3rd cycles and increased in 4th cycles and maintained until 6 cycles which showed resistance of EMT6. In conclusion, radiation therapy causes cell morphological changes and fractionation of gamma-ray irradiation lead to resistance EMT6 after 4 cycles.

1. Introduction

Acquired radio-resistance remains a crucial problem in cancer treatment that may lead to relapse of cancer incident for example more than 90% mortality in breast cancer patient is due to the cancer metastasis rather than the primary cancer [1]. Recent studies have shown that there are multiple factors that causes the development of radio-resistance including resistance of survival cells population towards radiation therapy [2]. Epithelial mesenchymal transition (EMT) cell is defined as a biological process of transition of epithelial cell to become mesenchymal cells. The presence of EMT
has become a medical criterion of recurrent cancer in cancer patients. EMT is a process in which enabling cell escape from treatment that lead to metastasis [3]. Understanding the incidence of EMT development post fractionated radiotherapy may explain the underlying mechanism of radio-resistance. Hence, the purpose of this study was to develop an understanding on morphological changes and survival fraction of EMT cell lines post gamma-ray irradiation.

2. Materials and Methods

2.1 Cell culture and irradiation
Mouse breast cancer cell lines EMT6 was obtained from the American Type Culture Collection (ATCC, USA). Cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic (penicillin + streptomycin) and incubated at 37°C in a humidified atmosphere with 5% CO₂. Cell were grown to 70%-80% confluency in vented T25cm² culture flasks prior to irradiation. Cells were irradiated using a Gamma cell 220 Excel (MDS NORDION / GC 220 E, Ottawa, Canada) at the Department of Nuclear Science, Faculty Science and Technology, Universiti Kebangsaan Malaysia, Selangor, Malaysia at operating dose rate of approximately 18.67 Gy/ min. The irradiation of EMT6 cells was conducted with two approaches, first, EMT6 cells were treated with single dose of gamma-ray ranging low to high dose gamma-ray (0-16 Gy) and second, EMT6 cells were exposed to fractionated gamma-ray irradiation (2 Gy per cycle) where cells that survived the initial irradiation were further irradiated with 2 Gy dose. Unirradiated EMT6 cells at the same passage number served as control.

2.2 Clonogenic survival assay and morphology
EMT6 cell line survival was determined by a clonogenic survival assay based on previous study [4] with slight modification. Control and treated cells were seeded in 100 mm petri dish. Cells were let to growth to form colonies for 7 days. Then colonies were fixed and stained by removing the culture medium and toluidine blue in 70% ethanol. Dishes scanned using Epson V600 scanner (Malaysia) and the colonies were counted using ImageJ software (NIH, USA). The survival fraction of the cell to gamma-ray irradiation was calculated and average number of colonies were plotted (Mean ± standard deviation (SD), n = 3). Morphological changes of control and treated cell were observed using a phase contrast microscope (Olympus Optical Co., Ltd, Tokyo).

2.3 Statistical analysis
Data are expressed as means ± SDs of at least three independent experiments.

3. Results and Discussions

3.1 Various Morphology changes in EMT6 treated cell compared to control in fractionated irradiation
Results showed changes in morphological of EMT6 treated cells as compared to sham-control cells. Figure 1A and 1B showed the normal morphology of untreated cells after 3 days and 7 days respectively. After 3 days post-irradiation of the first cycle (2 Gy), EMT6 cells showed a loss of glandular pattern, vacuolated cell cytoplasm, pleomorphic nuclei and enlarged cell size as compared to untreated EMT6 cells (normal morphology) (Figure 1C). After 7 days post-irradiation in the first cycle, the treated EMT6 cells showed oedema and swelling with irregular size and shape including round, ellipse and fusiform shape (Figure 1D). The morphology characteristics for treated cells compared to control for 2nd cycle until 4th cycle were similar. However, after 5th cycle (10 Gy), the treated cells showed oedema and swelling with irregular size compared to sham control (Figure 1F) after 3 days post-irradiation. While after 7 days post irradiation showed that, the cells were recovered from radiation treatment. The EMT6 cells became polygonal in shape with regular dimensions (Figure 1G).

The most significant morphological changes recorded were the presence of pleomorphic nuclei and cell enlargement. This is due to irradiation-induced caused arrest to the G2 phase of the cell cycle which associated with an increase the size of the cell by increasing the size of the nucleus [5]. Consequently, pleomorphic nuclei were expressed in this study due to disruption to DNA in the
nucleus in line with previous study that reported that after irradiation the morphological of radiotherapy-treated CaP cells changes including vacuolated plasma [6]. Though the cells were different, the mechanism action of radiation was similar thus similar results were reported.

Figure 1. Morphology of untreated cells (sham control) after 3 days (A) and 7 days (B) respectively. Morphology of cells irradiated with 2 Gy doses (First cycle) after 3 days (C) and 7 days (D) post-irradiation. Red arrow indicated there were pleomorphic nuclei. Black arrow indicated cells showed oedema. Yellow arrow showed cells in fusiform shape. F) Morphology of cell 3 days post-irradiation in the 5th cycle of radiation (total cumulative dose is 10 Gy). Cells started to swell as early as 3 days after irradiation. G) Seven days post-irradiation in the 5th cycle, EMT6 cell lines showed recovery from radiation treatment.

3.2 Different pattern of survival fraction were observed between single dose irradiation compared to fractionated irradiation.

Figure 2A showed the survival fraction of EMT6 cell treated from low to high dose in single fraction where the result showed that the mean survival fraction of EMT6 decreased in with increasing dose from 0 to 16 Gy. While Figure 2B showed the survival of EMT6 treated with fractionated dose of 2 Gy up to 7 cycles (14 Gy) where the mean survival fraction of EMT6 decreased until 3rd cycle (6 Gy). However, the mean survival fraction was increased back after 4th cycle and the survival fraction relatively maintained in 5-7 cycles. This indicated that the EMT6 cells line developed resistance towards gamma-ray irradiation in fractioned exposure of radiation.

Gamma-ray irradiation has been proved to have the greatest penetrating effect and highest energy of ionizing radiation due to its high frequency and shortest wavelength. Exposure to gamma-ray irradiation induces apoptosis and DNA damage by alterations in the expression of genes that leads in alteration in the cell cycle process. In this study, two different results were obtained when EMT6 cell lines were directly radiated with single doses ranging of 0 Gy – 16 Gy or with fractionated dose 2 Gy per cycle. In the first approached, the survival fraction of treated cells was decreased the dose of radiation increased. A possible explanation for these results may be high dose of radiation causes extensive damage thus inducing more apoptosis and greater DNA damage [7]. In the second approached, contrary to the first approached, the results obtained showed that treated cell developed radio-resistance after 4th cycles. This result was supported with the fact that cells were recovery similar like untreated cell 7 days post-irradiation in 5th cycles. Previous study has demonstrated the
development of radio-resistance cancer cell following single exposure of 6 Gy after 7 days post-irradiation and the author suggested that inhibition of TGF-B pathway might help to overcome radio-resistance [8].

![Graph A](image1.png) ![Graph B](image2.png)

**Figure 2.** A) Showed the survival fraction of EMT6 cell treated from low to high dose in single fraction. B) Survival fraction graph for fractionated radiation of EMT6 cell lines with 2 Gy dose for each cycle.

### 4. Conclusion

In conclusions, this study has shown that the effect of fractionated gamma-ray irradiation on EMT6 morphological changes and the development of radio-resistance cells compared to single dose irradiation. However, the current study has only using gamma-ray source and it would be interesting to compare the effect of other sources of radiation such as x-ray.

### 5. References

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