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INDUCED BREAST CANCER CELL APOPTOSIS BY SYNCHROTRON-BASED IRRADIATION WITH MONOCHROMATIC MICROBEAMS

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INTRODUCTION

Breast cancer is the most common female malignancy and has the second highest cancer related mortality in western women. Radiotherapy (RT) plays an important role in the modern management of breast cancers of all stages. However, the efficiency of conventional RT continues to be limited by treatment induced radio-toxicity in collateral healthy tissues [1].

In this work, we present the feasibility studies of the monochromatic micro-beam therapy (m-MRT) technique, a novel synchrotron based radiotherapy concept that uses high brilliance, monochromatic X-ray micro-beams smaller than 200 microns, applied to treat breast cancer tissue and cell samples. Two different energies were used for those tests: 50 keV and 100 keV.

The tumor fragments and cells samples were irradiated ex-situ and then analyzed to assess the damages induced by m-MRT irradiation. Eight patient derived xenografted (PDX) tumor fragments were irradiated and implanted in live NOD Severe Combined Immuno-deficient common gamma (NSG) mice to assess the effect of irradiation on tumor growth comparing to the control.

The pilot studies showed that the m-MRT treatment of cancerous tissue slowed down the tumor growth in (NSG) mice as compared to untreated controls. The biomolecule analysis demonstrated that the irradiation induced cancer cell apoptosis by triggering a stress response of the cells at radiation dose of 60 Gy or higher. Future studies will investigate how the cancer cells respond to the irradiation treatment in vivo in the live animals.

METHODS AND MATERIALS

BIOMEDICAL IMAGING AND THERAPY (BMIT) FACILITY

The irradiation therapy experiments were performed at the 05ID-2 beamline of the BioMedical Imaging and Therapy (BMIT) facility at the CLS, which provides synchrotron-based imaging and radiation therapy capabilities [2]. The source for the 05ID-2 beamline used for this research is a multi-pole superconducting 4.3 T wiggler. The high field gives a critical energy over 20 keV. The optics in the POE-1 and POE-3 hutches generate a monochromatic beam that is 22 cm wide in the experimental

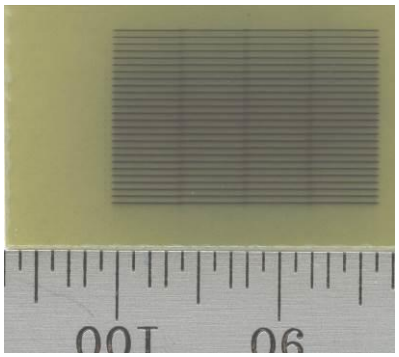

Figure 1. Radio-chromic film (EBT-3) record of the four adjacent micro-beams irradiations (@100 keV) with the total dose of 250 Gy, scale in mm.

hutch SOE-1. The double crystal bent-Laue monochromator provides an energy range appropriate for therapy studies in animals (50-150 keV). In the experimental hutch, the broad beam is divided into 4 mm wide and 50 μm high slices, with 400 microns c-to-c spacing, using tungsten collimator – see Figure 1.

CANCER MODEL AND TUMOR INNOCULATION

Six female NSG mice were used for implantation of tumor tissue fragments The NSG mice (6~8 weeks of age) were implanted with tumor fragments originating from a patient-derived breast cancer tumor (PDX-1) donated by patient treated in Saskatoon. After growing for ~3-5 weeks in the mice, the tumors were excised, cut into small fragments with sizes about ~10 mm^3 and kept in sterile Eppendorf tubes that were irradiated at the BMIT facility.

To prepare the cells samples, the tumor cells were digested from the tumor tissue and equally divided into 10^7 cells in each of 30 Eppendorf tubes. The suspended cells were pelleted immediately before the irradiation treatment. Both the tumor fragments and cell samples were kept on ice for the transportation between the CLS and the Lab Animal Service Unit (LASU) at the campus of the University of Saskatchewan and for the waiting time at the BMIT facility.

MONOCHROMATIC IRRADIATION TREATMENT DESIGN

The tumor fragments were treated with either broadbeam or microbeams. For each experimental group, one fragment was reserved as an untreated control. Right after the irradiation treatment, the tumor fragments, including the control, were delivered back to

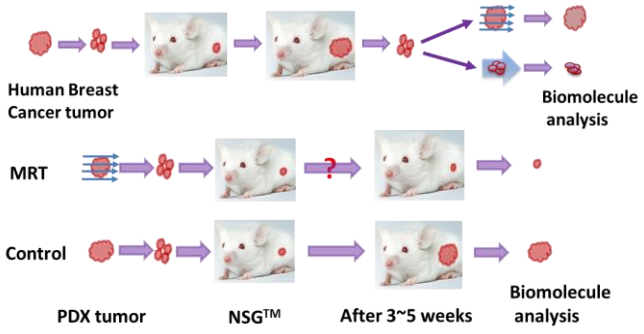

Figure 2: *Irradiation treatment designs to investigate the mechanism of the radiology behind microbeam irradiation treatment.*

the LASU, where the fragments were cut into small pieces about ~2 mm^3 and implanted immediately into NSG mice. Each mouse carried two pieces at two fatty pad sites, while one mouse with two untreated tumor fragments served as a control.

The irradiation treatment designs are depicted in Figure 2. The mice bearing breast cancer tumors were monitored for tumor growth until the animals reached their humane end-points. The tumors were excised for further biomolecule analysis to assess the DNA and protein damage induced by the irradiation, using Western Blot (WB) and sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE) analytical techniques.

The cells samples were irradiated with broad-beam of a wide range of dose from tens to hundreds of Gray. Immediately after the treatment, some cell samples were analyzed to assess the DNA and protein damages. The others were frozen to -80°C for further biomolecule analysis.

Two tumor fragments were treated with monochromatic broadbeam: 10 (H) x 20 (W) mm, with 80 Grays total dose. Thirty tumor cells samples were irradiated with various doses and four other tumor fragments were irradiated with monochromatic microbeams: 50 microns (H) x 4 mm (W) with 250 Gy total dose. All the experiments and animal use protocols have been approved by the animal research ethics board of the University of Saskatchewan.

RESULTS AND DISCUSSION

RESPONSES OF TUMOR CELLS AND TISSUES TO THE M-MRT

The human breast cancer tumor donated for this research was partially resistant to chemotherapy treatment. Previous studies found that expression of 6 genes from the tumors resected from PDX tumor-bearing NSG mice was correlated to their treatment resistance, which was an inherent trait of the human breast cancer tumors chosen for these studies [3]. Cancer cells which are resistant to chemotherapy are characterized by elevated expression of the breast cancer resistance protein (BCRP). To investigate whether monochromatic microbeam irradiation could reverse the drug resistance on these cancer cells by inducing changes on these proteins, BCRP was selected as a protein marker.

Expression of BCRP was analyzed using the WB analysis for the cell samples treated with broadbeam. On the left in Figure 3 shows the result of the WB analysis of the cell samples treated with broadbeam of various doses. It clearly shows the reduction of the BCRP expression induced by the irradiation treatment. The critical dose to induce the changes was 60 Gy and no advantage was observed for increasing the dose to 200 Gy. It is interesting to find that the cancerous cells treated at 60 Gy, also showed elevated expression of the 5' adenosine monophosphate-activated protein kinase (AMPKα1/2), an enzyme playing an essential role in cellular energy homeostasis, which is also a protein marker of the cell stress response. No stress response was observed from the cells treated with higher dose.

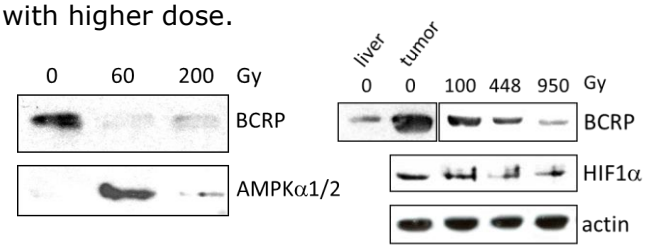

Figure 3. *WB analysis of the cell samples (left) shows the reduction of the BCRP and elevated expression of the AMPKα1/2 and the results for the tissue samples (right), the reduction of both BCRP and HIF1α induced by irradiation treatment and no changes was observed for actin.*

The right side in Figure 3, shows the WB analysis results for the tissue samples irradiated by microbeams. A healthy liver sample was used as a control to depict constitutive levels of BCRP. Significant over-expression of BCRP was obtained in the unirradiated tumor cells. In contrast to the cell samples, reductions in BCRP were also observed for the samples treated with dose of 100 Gy compared to the control. However, a significant reduction of BCRP was observed for the samples treated with very high doses. Similar to BCRP, the protein hypoxia-inducible factor 1, alpha subunit (HIF1α) was also over-expressed in cancerous cells. Hypoxia plays an important role in the formation of a vascular system in tumors. However, after irradiation, HIF1α expression was slightly reduced compared to the control. Meanwhile the housekeeping protein actin, a globular multi-functional protein that forms microfilament, which is not over-expressed in cancer cells, was used as a control in the WB analysis. As expected, it was not affected by the irradiation treatment. These findings indicated that the microbeam irradiation had preferential activity against the proteins which regulate the cancer cells progression, which are consistent with what found from the studies of MRT applied to brain disorder diseases [4]. On the other hand, these findings demonstrate the potential of the m-MRT in helping understand the underlying radiological mechanisms of the radiotherapy. More meaningful conclusion will need further statistic studies.

ASSESSMENT OF MICROBEAM IRRADIATION ON TUMOR GROWTH

The effect of irradiation on tumor growth implanted in NSG mice has been evaluated by monitoring post-irradiation tumor growth over time. For the first pilot experiment, the tumor fragments were treated with broadbeam and implanted in NSG mice. None of the tumors grew within the monitoring period (data not shown). For the second pilot experiment, tumor fragments were irradiated with m-MRT with total dose of 250 Gy. Then each fragment was cut into small pieces post-treatment and immediately engrafted back into NSG mice.

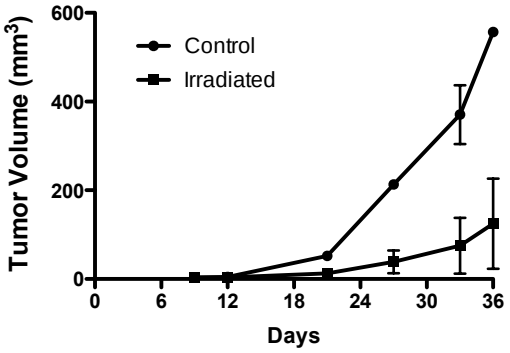

Figure 4. *Mean tumor volume recorded at different days after the irradiation treatment.*

Their post-irradiation growth chart is shown in Figure 4. It is not surprising to see the control tumor fragments grew to a mean volume of 560 mm^3 within 36 days. However,

the growth of the treated tumor fragments was significantly slowed reaching a mean volume of 120 mm^3 within the same timeframes. In fact 50% of the irradiated tumors did not show any growth in the first 36 days (data not shown). More work needs to be done using a larger numbers of mice to enable meaningful statistical analysis relating to the impact of microbeam irradiation treatment on tumor growth. Ideally, we would be able to maintain the tumor-bearing mice for a longer periods to determine whether the irradiated fragments that didn't grow during the 36 days would eventually grow out, or the tumor growth would slow further, or the tumors would involute entirely. It would be interesting to determine the therapeutic potential of in vivo irradiation of tumors in live animals.

ASSESSMENT OF MOTION EFFECT ASSOCIATED WITH LIVE ANIMALS

The motion effects introduced by the animal's heart beat and respiration is a concern in the application in microbeam irradiation therapy [5]. Such motion would cause broadening of the Microbeam, potentially overcoming the advantages of using microbeam configuration, resulting in a smaller peak/valley dose ratio and complicating the radiology interpretation. The motion effect has been evaluated in this work using euthanized mice

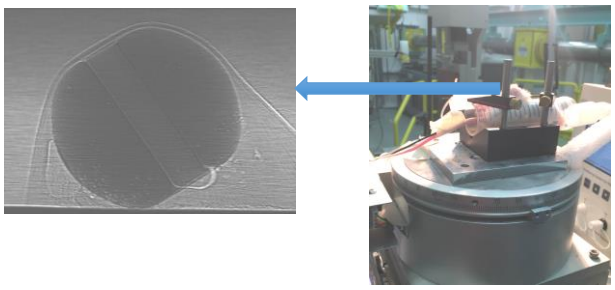

Figure 5. Stacked *CT images (left) recorded within 45 minutes shows the motion effect and the setup of live animal experiment used for the motion assessment (right).*

bearing subcutaneous glass beads and live mice bearing fat pad tumors by recording the computed tomography (CT) images with time. With applying the strategy shown in the right picture shown in figure 5, the motion was limited to ~30 μm over ~30-45 min, which is tolerable for the Microbeam configuration used in this study.

CONCLUSION AND FUTURE PERSPECTIVE

The pilot studies show that the m-MRT treatment of cancerous tissues slowed down the tumor growth implanted in NSG mice as compared to untreated controls. The biomolecule analysis demonstrated that the irradiation induced cancer cell apoptosis by triggering a stress response of the cells at radiation dose of 60 Gy or higher. It shows reductions in expression levels of a protein associated with drug-resistance in cancer cells, BCRP, while the control protein was not affected by irradiation. These findings will help understand the underlying radiology of the irradiation radiology of the clinical importance. Future studies will investigate how the cancer cells respond to the irradiation treatment in vivo in live NSG animals.

ACKNOWLEDGEMENTS

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