Biological characterization of a glioblastoma radioresistant subpopulation cells

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Abstract

Most of the cancers seem to be initiated by stem-like cells. They are characterized by a strong resistance to radiation and chemotherapy and they have a specific molecular signature. Besides this *in vitro* characteristic when injected in immune-deficient mice those cells are able to grow and to form a tumor. These stem-like cells are now known as cancer stem cells (CSCs or also tumor initiating cells TIC). Hypoxia, the lack of oxygen that often occurs in tumor seems to be the perfect niche for those cancer stem cells. In our work we present preliminary results concerning irradiation of TIC in oxic and hypoxic (1% oxygen) conditions with X-ray and carbon ions up to 20 Gy.

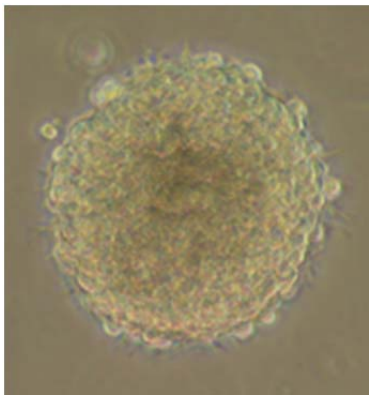

Figure 1: Tumor initiating cells growing as spheroid.

Material and Methods

Cell line: #10-IR cells were established by Dr. E. Kim [1]. **Carbon ion exposure:** energy range 114-160 MeV/u. Mean LET 70 keV/µm**. X-ray exposure:** carried out with an Isovolt DS1 X-ray machine (Seifert) operated at 250 kV and 16 mA with 7 mm beryllium, 1 mm aluminum and 1 mm copper filtering and a dose rate of 3 Gy/min. **Neurosphere formation assay:** Cells were irradiated as single cells and then seeded in 25 cm^2-tissue culture flask. After two weeks the formed spheres bigger than 50 µm were counted as survivors. **3D Spheroid Culture Cell Invasion Assay (Amsbio):** Two weeks after exposure, 50 µl of cell sphere solution were seeded into a microtiter plate with 50 µl of invasion matrix. Pictures were taken seven days after seeding. **Cancer Stem Cells RT² Profiler™ PCR Array (Qiagen):** Performed following the manufacturer's protocol; expression of 27 genes (triplicates) involved in pathways related to cancer stem cells were investigated. **ELISA analysis (Life technologies):** Performed following the manufacturer's protocol.

Results

Survival curve of irradiated cells revealed a subpopulation of radioresistant cells. This subpopulation (around 20% in oxia and 50% in hypoxia) is resistant up to 20 Gy of X-ray (Fig 2) and carbon ion irradiation (data not shown).

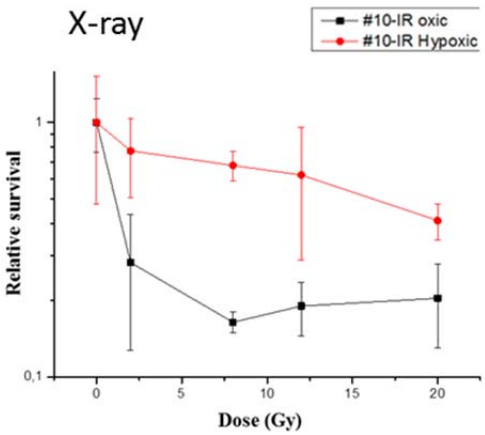

Figure 2: #10-IR cells irradiated with X-ray up to 20 Gy in oxic and hypoxic conditions.

Vascular endothelial growing factor is a cytokine that stimulates vasculogenesis and angiogenesis. Few studies claim that glioblastoma radioresistance may be enhanced by VEGF release [2] and recent studies have demonstrated that VEGF is responsible for the cells' increase in stemness [3].

Results show that #10-IR cells after irradiation and/or hypoxia increased their VEGF release. The increase was 8 times after carbon ion irradiation (8 Gy) and more than 6 times after 8 Gy of photon irradiation (Fig 3).

Hypoxia seems to be a strong enhancer of the VEGF production. At 8 Gy of carbon ion irradiation, the amount of cytokine release is over 40 times higher compared to the oxic control (data not shown).

DOI:10.15120/GR-2015-1-APPA-HEALTH-24

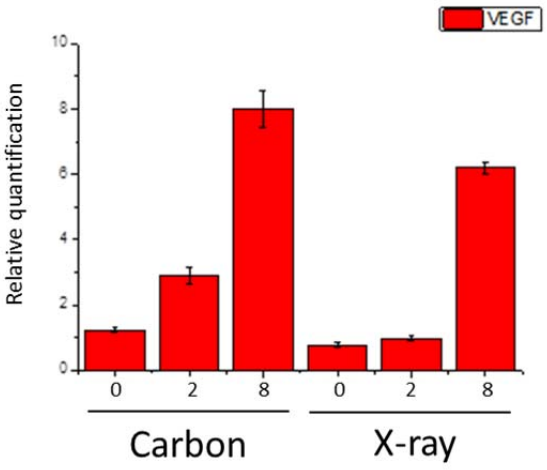

Figure 3: #10-IR Relative quantification of VEGF after exposure to carbon ions and X-ray.

Further investigations were performed to identify the molecular and phenotypical signature of the cells' subpopulation.
First, the invasion capacity was studied two weeks after irradiation (Fig 4). Pictures of the spheres were taken for the sham-irradiated control (panel A) and spheres derived from the progeny of cells exposed to 8 Gy of carbon (B) or X-ray (C). Analysis was performed measuring the outer area of the spheres as well as the inner sphere part (i.e. not migrated) of at least 20 spheres per dose. The two values were then set in relation to estimate the sphere's invasion capacity (data not shown).

The control was found to be highly invasive (panel A), whereas radiation exposure lead to a reduction in the invasiveness. Carbon ions were found to be more effective with respect to the invasiveness reduction at iso-doses of 8 Gy (panel B and panel C).

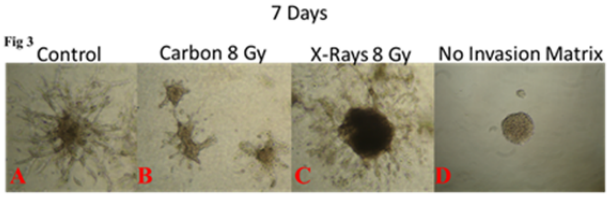

Figure 4: Invasion assay. Panel show sham-irradiated controls (A) and spheres derived from cells exposed to 8 Gy of carbon ions (B) or X-ray (C). Panel D displays a negative control where no matrix gel was added.

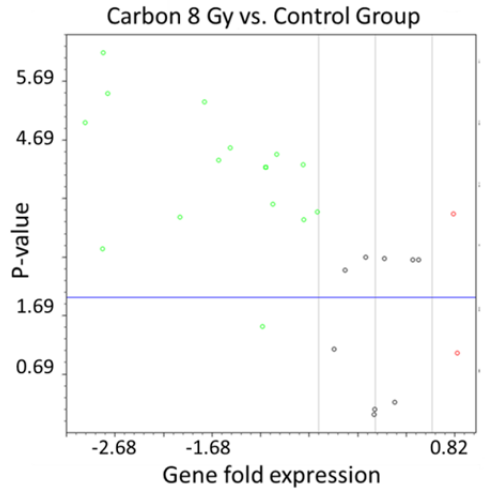

Figure 5: Fold-change results using ΔΔC(t) calculations. The x-fold expression of genes is plotted versus the p-value (green dots: down-regulated genes, red dots: up-regulated, black dots: fold-change is not significantly changed as compared to the control.

Most of the genes involved in the cancer stem cell pathways were down-regulated after exposure to 8 Gy carbon ion.

Conclusion

A radioresistant subpopulation of cells was found in the glioblastoma cell line used in this study. VEGF is well known to stimulate angiogenesis and is discussed to be responsible for an increased stemness and radioresistance of cells. In this work a correlation between radiation under hypoxic conditions and higher VEGF release was found. The increase of dose or the lack of oxygen is directly correlated with the increase in VEGF release. Tridimensional gel assay was used to investigate the invasiveness of this resistant subculture. Particle radiation was found to be more effective than photon radiation to reduce the *in vitro* invasiveness. Moreover, our results suggest that particle radiation affects the expression of genes related to cancer stem cell pathways. Additional experiments are currently performed to confirm these results and to further characterize the subpopulation.

References

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DOI:10.15120/GR-2015-1-APPA-HEALTH-24