IONIZING RADIATION PROMOTES CHANGES IN MICROVESICLES PROFILE DERIVED FROM GLIOBLASTOMA CELLS

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ABSTRACT

Gliomas are the most frequent central nervous system (CNS) tumor, and glioblastoma (GB) is the most aggressive and lethal form. Its treatment consists of the set of surgical resection, radiotherapy and chemotherapy. However, the patient survival is a maximum of 12 to 15 months. Studies suggest that changes in the epidermal growth receptor (EGFR) such as amplification and / or mutations are involved with therapeutic failure and a worse prognosis. Recently, our group has demonstrated that oncogenic molecules can be carried by microvesicles (MV) derived from resistant cells, evidencing a possible role of MV in tumorigenesis and tumor resistance. Therefore, our study aimed to evaluate the microvesicles profile derived from glioblastoma by treatment with ionizing radiation.

To that, the U251MG glioblastoma-derived cell line was cultured in DMEM F12 with 10% fetal bovine serum (FBS) and 2mM L-glutamine. The treatment was performed by exposing the cells to 8Gy of ionizing radiation (IR). To obtain MV, cells after IR treatment were maintained for 48h in the presence or absence of 10% FBS, or in the presence of 10% dFBS (filtered FBS in 0.22µm pore filter membrane), and then the supernatant was centrifuged at 16000g for 2h and 30 minutes for MV isolation. The identification of the MV was performed by annexin V labeling and flow cytometry analysis. Protein content of MV and donor cells was evaluated by the Lowry protein assay protocol. Cell viability was measured by the trypan blue exclusion method and cell death was defined by flow cytometry through annexin V and PI labeling.

Our results show that U251 cells spontaneously release MVs in vitro. Furthermore, the results suggest that irradiation with 8Gy of IR leads to a decrease in the production and release of these microvesicles. In addition, the trypan blue exclusion method evidenced a decrease in the cell viability of the IR treated cells compared to the untreated (0Gy) control. In contrast, cells treated with 8Gy of radiation had higher protein content than the untreated control, and the same was observed in the total proteins of the MV. Associated with this, it was showed that the irradiation induces a discrete increase in EGFR levels and its variant III in glioblastoma cells. Thus, our work suggests that glioblastoma cells have a continuous production of MV and that this activity is altered by treatment with IR.

METHODOLOGY

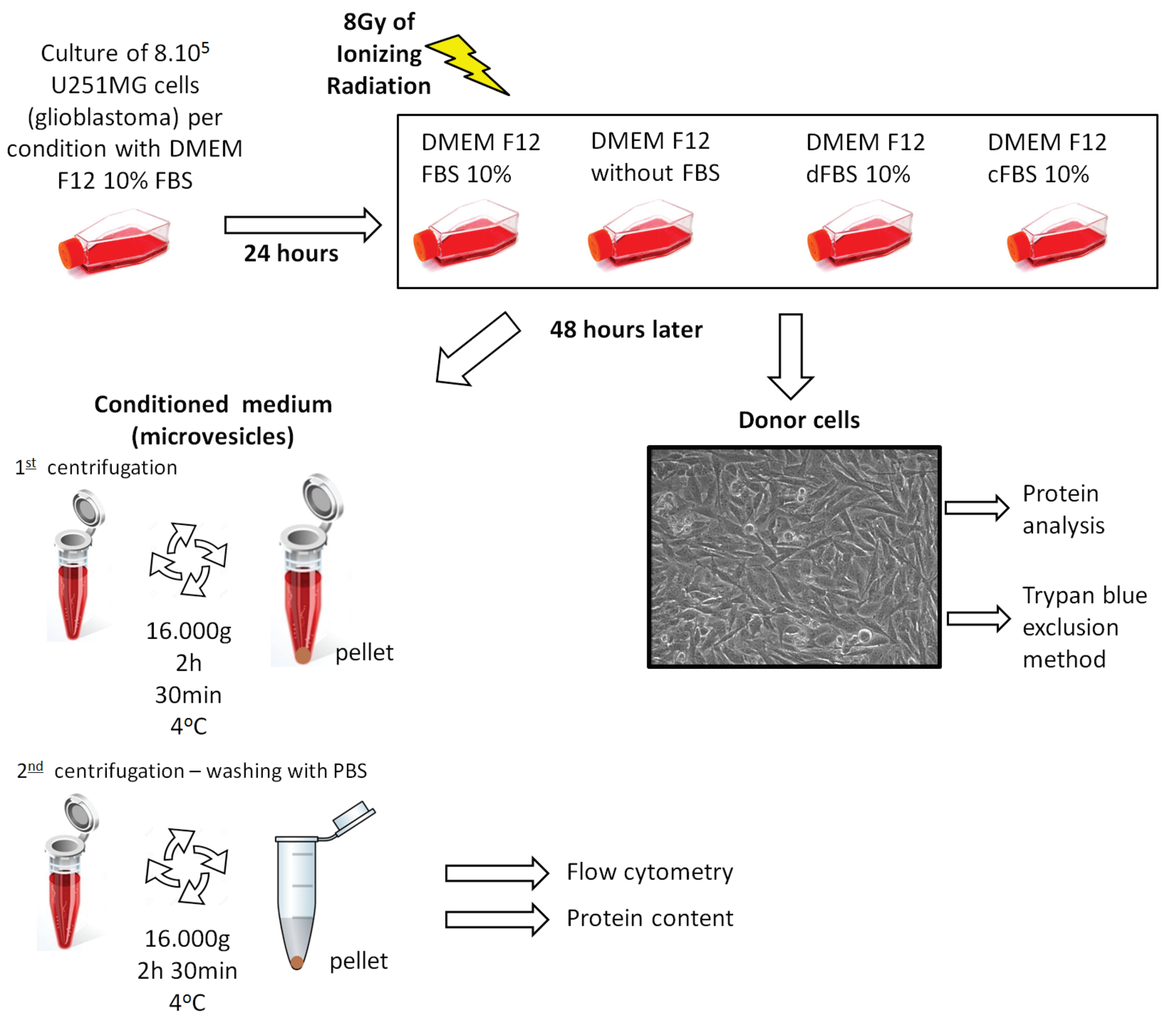

Figure 1: Representative scheme cell culture treatment and microvesicles (MV) isolation. U251 cell line was seeded in 25cm³ bottle and culture for 48 hours with fetal bovine serum (FBS) or centrifuged FSB (cFSB) or filtered FSB (dFSB) or without FSB, after 0 or 8Gy of ionizing irradioation (IR). The conditioned medium was preserved and centrifuged at 16000g for 2:30h at 4°C. After centrifugation, supernatant was discarded and MP at pellet was washed with PBS at 16000g for 2:30h at 4ºC. The MV derived from U251 cells were analyzed by flow cytometry and Lowry protein assay protocol. Treated cells were analyzed by Trypan blue exclusion assay.

Keywords: Microvesicles, Ionizing radiation, Glioblastoma

Financial Support: Programa de Oncobiologia (UFRJ/Fundação do Câncer), FAPERJ, INCT, CNPq, Ministério da Saúde INCA.

RESULTS

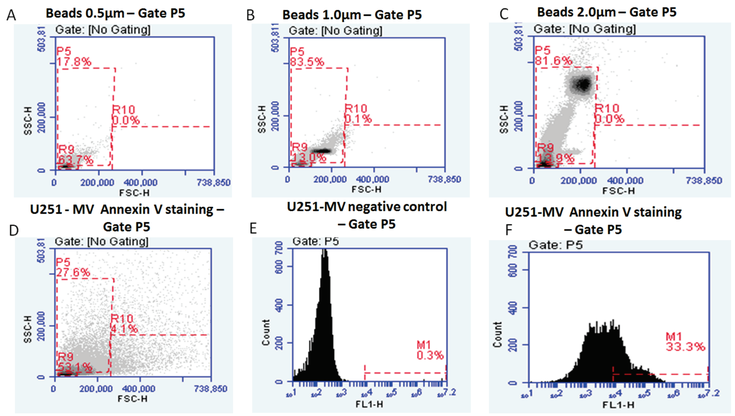

Figure 2: Analysis of MV release by U251 cells by flow cytometry. The gate of interest was delimited using fluorescent beads 0.5µm (A) 1.0µm (B) and 2.0µm (C). U251 shed MV spontaneously *in vitro* (D-F). Population of U251-MV ranges between 0.5 and 1.0µm (D). Positive annexin V staining characterize MV population (F). Negative annexin V control (E).

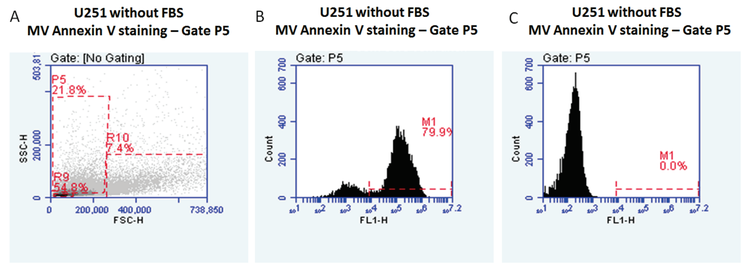

Figure 3: Analysis of microvesicles (MV) derived from U251 cells serum-free culture. Graphics (A,B and C) show the analysis of MV derived from U251 cells after culture with serum-free media. Positive annexin V staining characterize MV population (B). Negative annexin V control (C).

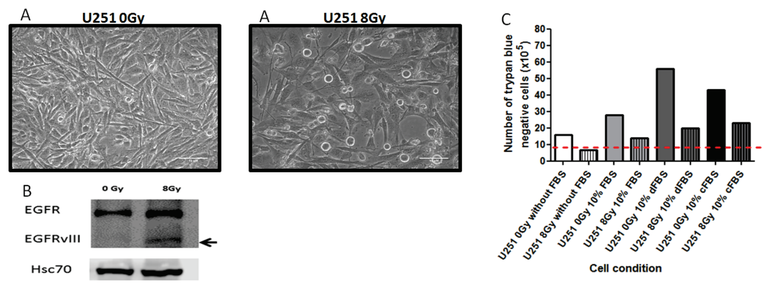

Figure 4: Effects of the ionizing radiation (IR) on U251 cell line. U251 cells were treated with 0 or 8Gy of IR and analyzed after 48 hours. Light microscopy of U251 cells under 0Gy (A – left panel) or 8Gy (A- right panel) of IR. EGFR and EGFRvIII expression levels in U251 cells under 0 or 8 Gy of IR (B). U251 cells were culture with different conditioned medium and analyzed by trypan blue exclusion assay. dFBS (filtered FBS), cFBS (centrifuged FBS).

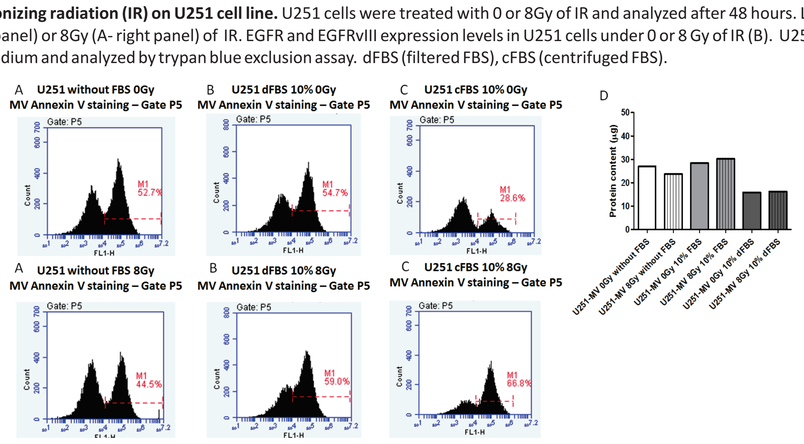

Figure 5: Analysis of different conditions of FBS in microvesicles (MV) release by U251 cells and protein content after ionizing radiation (IR). U251 cells were treated with 0 or 8Gy of IR and analyzed after 48 hours for MV release (A-C) and protein content (D). U251 cells were cultured without FBS (A), with filtered FBS (dFBS) (B) or centrifuged FBS (cFBS) (C). Protein content of MV derived from U251 cells cultured with different conditioned medium (D).

CONCLUSION

Our work suggests that glioblastoma cells have a continuous production of MV and that this activity is altered by treatment with IR.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA