ROLE OF GLIOBLASTOMA-DERIVED MICROVESICLES ON CELLULAR MALIGNANCY

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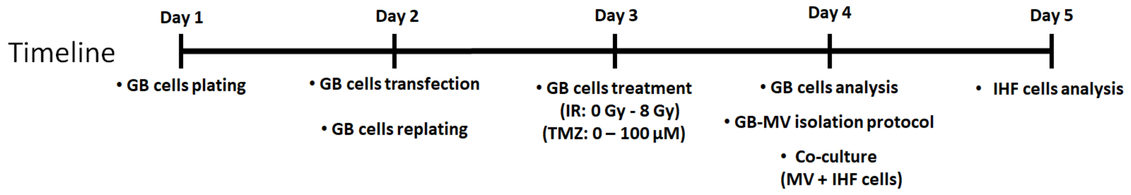
ABSTRACT

Introduction: Glioblastomas (GB) are highly malignant brain tumors, being one of the most aggressive and lethal human cancers. Despite intensive treatment including surgical resection, radiotherapy and temozolomide (TMZ) chemotherapy, the overall survival of glioblastoma patients ranges between 12 to 15 months. Microvesicles (MV) released from GB cells (GB-MV) are important to cellular communication in brain microenvironment and they are also associated with tumor progression. Previously our group demonstrated that oncogenic molecules are transported and transferred by MV to tumor cells. Moreover, studies have shown that EGFR and its mutant EGFRvIII isoform can be carried by GB-MV. In this study, we hypothesized that GB-MV have the potential to promote malignant changes in healthy microenvironment cells. Thus, the aim of this study was to investigate the role of MV secreted by GB cells in promoting healthy cells malignancy. **Methods:** GB cell lines (U251, A172 and T98G) were submitted to EGFR or EGFRvIII gene overexpression, using plasmid vectors (EGFR-GFP and MSCV-XZ066-EGFRvIII, respectively), and further treated with 100μM of TMZ or 8Gy of ionizing radiation (IR). After determining protein and mRNA levels of EGFR or EGFRvIII of the GB-donor cells, and their downstream pathways, by Western Blotting and RT-qPCR, GB-MV were isolated using centrifugal sedimentation and characterized. Next, non-tumoral cells (IHF) were co-cultured with GB-MV, and the phenotypic profile of these cells was analyzed with Western Blotting and RT-qPCR. Besides, their growth (proliferation) was analyzed with the crystal violet assay. **Results and Conclusion:** Our data showed that IR induced downregulation of EGFR and YB-1 mRNA levels, and a reduction of pEGFR and pAKT protein levels in EGFR overexpressing cells. Additionally, EGFR overexpressing cells showed low levels of pAKT protein after TMZ incubation. In opposition, GB cells overexpressing EGFRvIII showed low effect on AKT and pAKT protein levels after IR or TMZ treatment. Analysis of GB-MV revealed high levels of EGFR protein and mRNA, independently of TMZ or IR treatment. The data obtained by co-culturing IHF cells and GB-MV showed induction of high EGFR and YB-1 mRNA levels in non-tumor cells. In addition, GB-MV promoted increased proliferation rate in IHF cells and changes on protein and mRNA molecules associated with oncogenic pathways. Altogether, our data suggest that IR and TMZ treatment promote downregulation of EGFR pathway in GB cells. However, GB-MV carrying EGFR may contribute to malignancy of non-tumor cells, independently of cellular treatment. Thus, studying the role of MV in the communication among GB and healthy cells will enable a better understanding of the mechanisms involved in GB progression.

METHODS



RESULTS

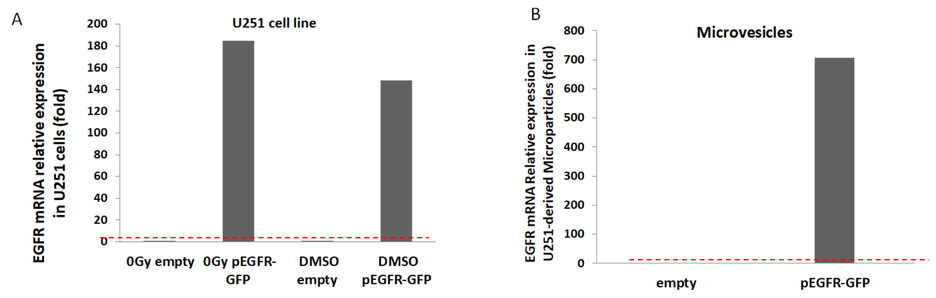


Figure 1: Analysis of EGFR mRNA levels in U251 cell lines and U251-derived microvesicles (U251-MV) after pEGFR-GFR transfection. Analysis of EGFR mRNA levels in U251 cell line (A) and in U251-MV (B) by quantitative real time PCR. GAPDH mRNA was used as endogenous control.

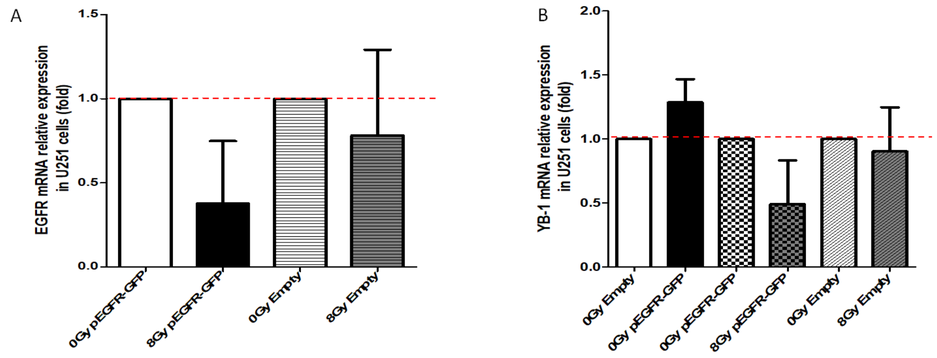


Figure 2: Analysis of EGFR and YB-1 mRNA levels in U251 cell lines untreated and treated with 8Gy after pEGFR-GFR transfection. Analysis of EGFR mRNA levels in U251 cell line (A) and YB-1 (B) by quantitative real time PCR. GAPDH mRNA was used as endogenous control.

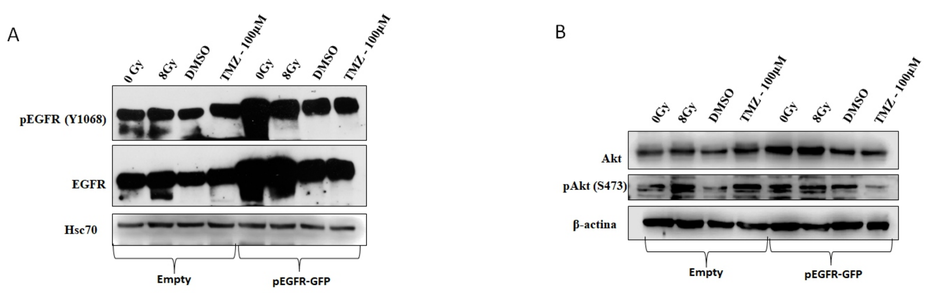


Figure 3: Analysis of U251 protein levels after pEGFR-GFR transfection. U251 cells were trasfected with pEGFR-GFP and then treated with ionizing radiation (IR) or temozolomide (TMZ). (A) pEGFR and EGFR protein levels; (B) Akt and pAkt protein levels. Hsc70 and β-actin were used as endogenous control.

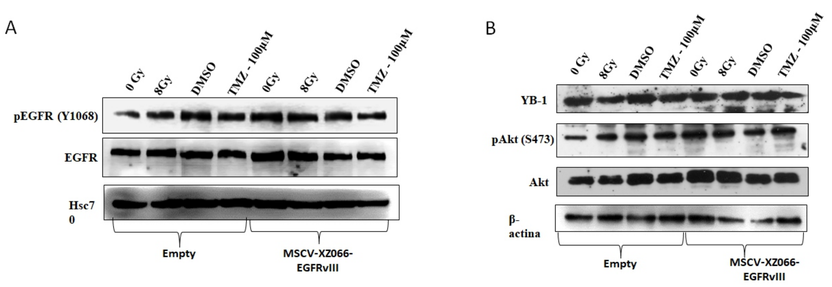


Figure 4: Analysis of U251 protein levels after pMSCV-XZ066-EGFRvIII transfection. U251 cells were trasfected with pMSCV-XZ066-EGFRvIII and then treated with ionizing radiation (IR) or temozolomide (TMZ). (A) pEGFR and EGFR protein levels; (B) YB-1, Akt and pAkt protein levels. Hsc70 and β-actin were used as endogenous control.

Figure 5: Analysis of protein content in (A) U251-derived microvesicles (U251-MV) after pEGFR-GFR transfection and (B) T98G-derived microvesicles (T98G-MV) after pMSCV-XZ066-EGFRvIII transfection. U251-MV and T98G-MV were obtained after donor cells trasfection and treatment with ionizing radiation (IR) or temozolomide (TMZ).

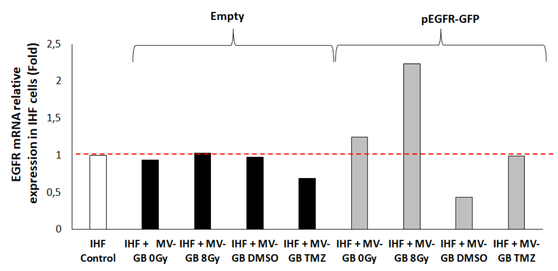


Figure 6: Analysis of EGFR mRNA levels in the non-tumor cell line (IHF). Microvesicles (MV) were obtained after U251 cells transfection with pEGFR-GFR and treatment with ionizing radiation (IR) or temozolamide (TMZ). EGFR mRNA expression levels in IHF cells after co-culture with U251-MV, by quantitative real time PCR. GAPDH mRNA was used as endogenous control.

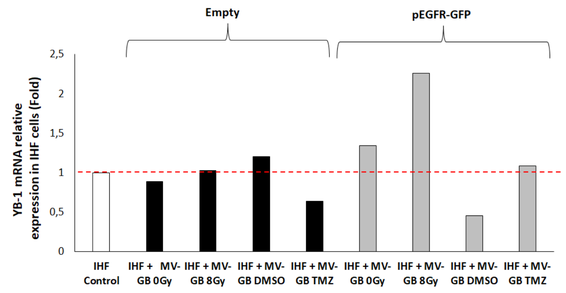


Figure 7: Analysis of YB-1 mRNA levels in the non-tumor cell line (IHF). Microvesicles (MV) were obtained after U251 cells transfection with pEGFR-GFR and treatment with ionizing radiation (IR) or temozolamide (TMZ). EGFR mRNA expression levels in IHF cells after co-culture with U251-MV, by quantitative real time PCR. GAPDH mRNA was used as endogenous control.

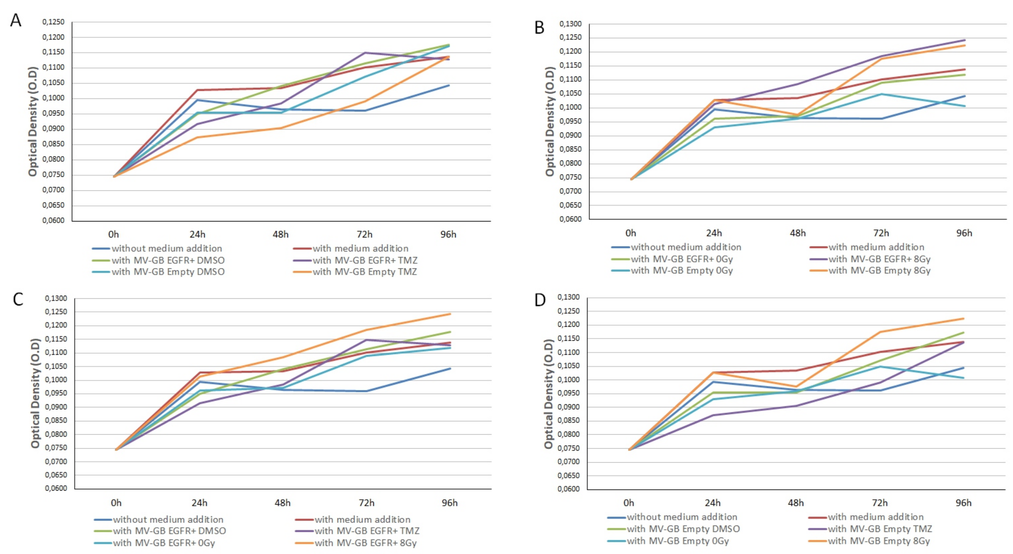


Figure 8: Proliferation kinetic analyses by crystal violet staining. IHF cells were analyzed for proliferation daily by incorporation of crystal violet after co-culture with microvesicles obtained from U251 cells transfected with pEGFR-GFP and treated with ionizing radiation (IR) or temozolamide (TMZ). (A) Shows the data from TMZ treatment, (B) ionizing radiation treatment, (C) pEGFR-GFP and (D) empty.

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Keywords: Glioblastoma, Microvesicles, EGFR and EGFRvIII

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