THE NEW LQB-223 COMPOUND MODULATES ERK1/ERK2 MAP KINASE PATHWAY, AND MICRORNA-7, IN GLIOBLASTOMA CELL LINES EXHIBITING DIFFERENT RADIORESISTANCE PROFILES

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INTRODUCTION AND OBJECTIVE

Glioblastoma (GB) is a highly aggressive grade IV astrocytoma. Patients with GB present 12 months average overall survival (OS). The standard treatment is based on a combination of surgery, radiotherapy and temozolamide combined chemotherapy. The epidermal growth factor receptor (EGFR) plays a major role in the carcinogenesis, evolution and response to treatment for patients with GB. Therefore, EGFR and its pathways, like MAP kinases (MAPK) ERK1/ERK2, are highly overexpressed in GB. The tumor suppressor microRNA-7 (miR-7) is able to inhibit the genic expression of EGFR and Raf1 regulating multiple levels of its signaling cascade resulting in increased sensibility to ionizing radiation in GB cell lines. However, patients with GB acquire resistance to the treatment, which explains the low OS rate. This justifies the need to research new drugs capable of modulating EGFR expression. This project aims to characterize the radioresistance, and to evaluate the new LQB-223 compound effect and mode of action on the GB cell lines: A172 (PTEN mutated), T98G (TP53 and PTEN mutated) and U251 (TP53, EGFR and PTEN mutated).

MATERIAL AND METHODS

- Human GB cell lines: U251 (TP53, EGFR and PTEN mutated), T98G (TP53 and PTEN mutated) and A172 (PTEN mutated);
- Cell viability was evaluated by MTT assay;
- Cell cycle profile was evaluated by flow cytometry;
- Cell death and apoptosis were evaluated by annexin V/PI labeling, pro-caspase-3 and cleaved caspase-3 expression;
- Pro-caspase-3, cleaved caspase-3, p-H2AX, ERK, p-ERK and Ras protein expression were assessed by Western blotting;
- MicroRNAs, miR-125b and miR-7 expression was evaluated by Quantitative Real time QT-PCR.

RESULTS

T98G and A172 cell lines showed an expressive G2/M cell accumulation after exposure to de 8, 16 e 24Gy doses of radiation. These doses induced an increased DNA fragmentation only in U251 and T98G cell lines. All cell lines were more resistant to low ionizing radiation and A172 was also resistant to high doses of radiation. LQB-223 reduced the cell viability and cell proliferation, leading to an accumulation in the G2/M phase of the cell cycle, increased DNA fragmentation and cell apoptosis in the GB cell lines. Increased miR-7 expression, reduction of ERK protein phosphorylation and total Ras expression could partially explain the LQB-223 anti-tumor effect. Recent preliminary studies of T98G cell line showed an increase in H2AX phosphorylation suggesting that LQB-233 induces a double break in the DNA strand.

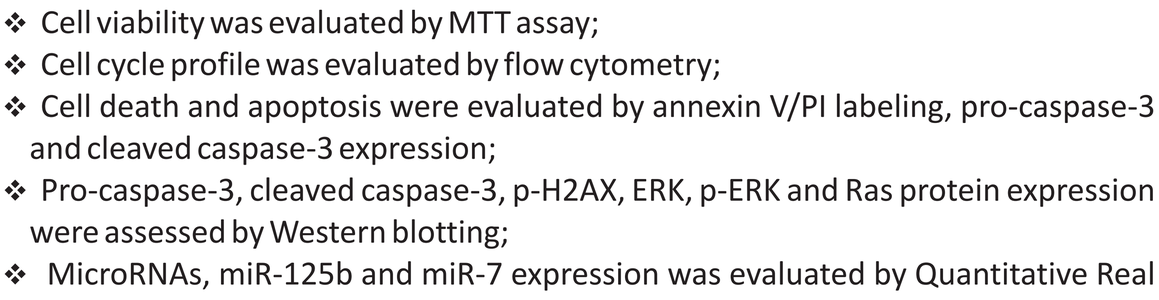

Fig 1. DNA fragmentation evaluated by flow cytometry after exposure ionizing radiation. Graphic showing percentage of U251 (A), T98G (B) and A172 (C) cells in Sub-G0/G1 phase of cell cycle after exposure to different doses of ionizing radiation for 6, 24, 48 and 72h. Mean of two independent experiments ± standard error. *p<0.05, p<0.01, ***p<0.001.

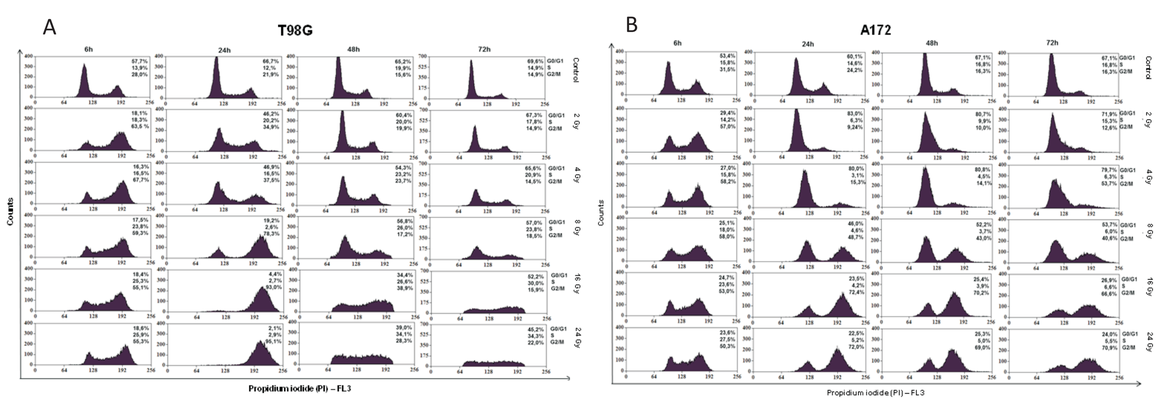

Fig 2. Ionizing radiation effect on cell cycle. The T98G and A172 cell lines were exposed to different doses of ionizing radiation for 6, 24, 48 and 72h and DNA content was evaluated by flow cytometry. Representative histograms showing percentage of T98G (A) and A172 (B) cells in each phase of cell cycle . Representative histograms of two independent experiments.

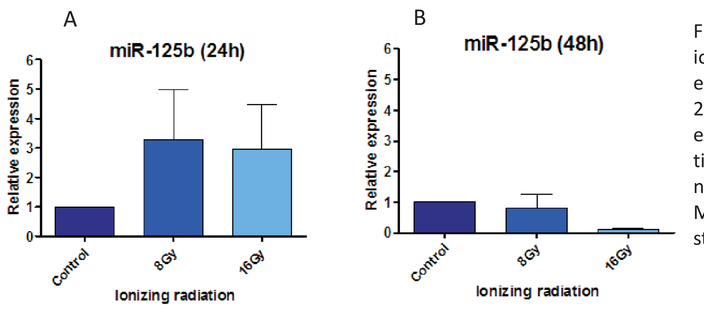

Fig 3. microRNA-125b expression after exposure ionizing radiation. The U251 cell line was exposed to 8 and 16Gy of ionizing radiation for 24 (A) and 48h (B) and microRNA-125b expression was evaluated by Quantitative Real time QT-PCR. Expression of microRNA-125b was normalized to the expression level of RNU6B. Mean of three independent experiments ± standard deviation.

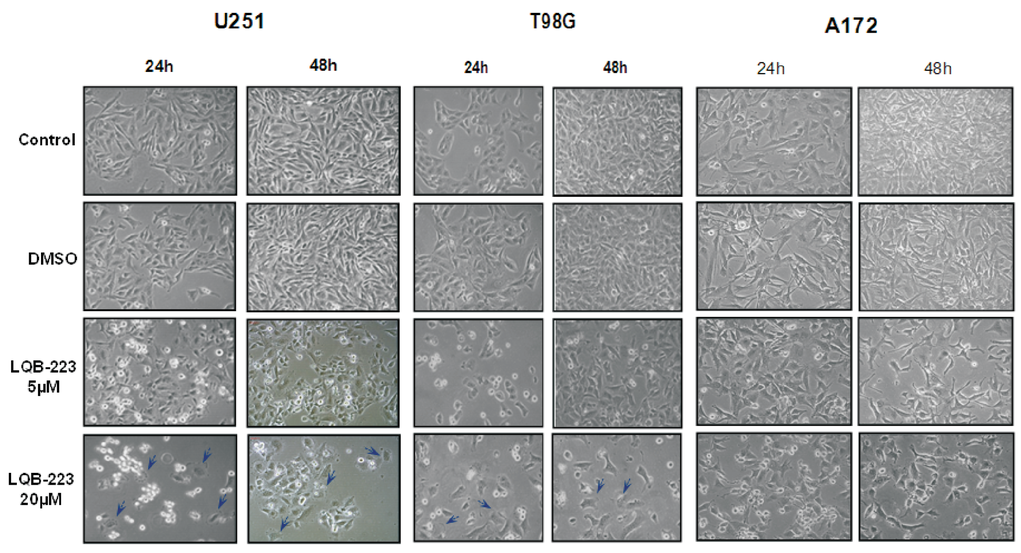

Fig 4. Morphological features after LQB-223 treatment in GB cells line. The U251, T98G and A172 cell lines were treated with 5 and 20 μM of LQB-223 for 24 and 48h. Contrast phase photomicrography showing morphological features observed after treatment with LQB-223 obtained in 10 times magnification. Representative photomicrography of three independent experiments.

Fig 5. LQB-223 effect on cell viability. Percentage of U251 (A), T98G (B) and A172 (C) viable cells after treatment with increasing concentrations of LQB-223 for 24, 48 and 72h. The graphs represent the mean of three independent experiments ± standard error. *p<0.05, p<0.01, ***p<0.001 compared to DMSO.

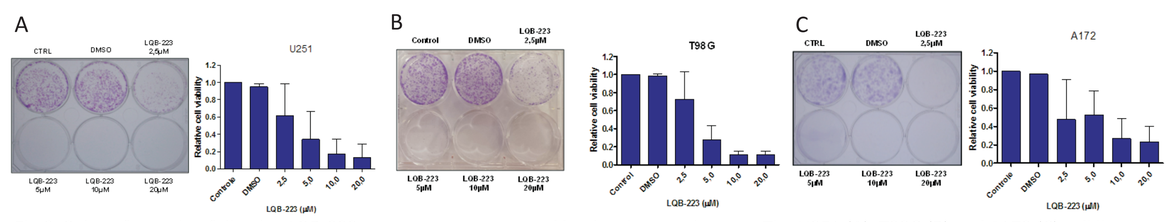

Fig 6. Colony formation following LQB-223 treatment was assessed by the clonogenic assay. The U251 (A), T98G (B) and A172 (C) cell lines were treated with increasing concentrations of LQB-223 for 48h, after which the drug was removed. After colony formation, cells were stained with crystal violet. Colonies were dissolved and optical density was measured. The graphs represent the mean of two independent experiments ± standard error.

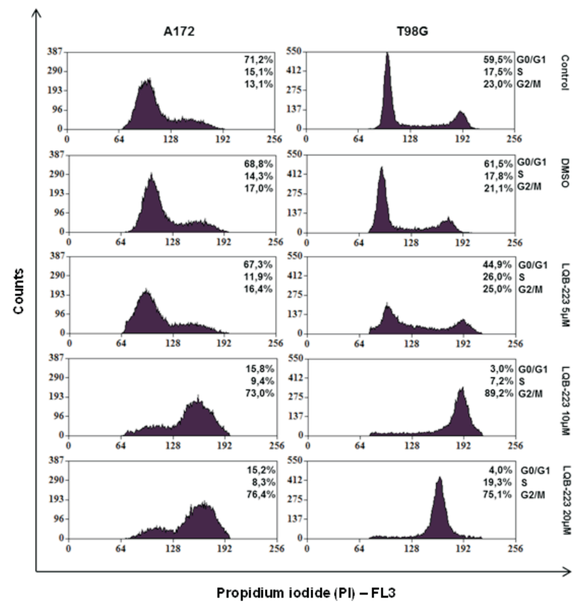

Fig 7. LQB-223 induces G2/M arrest in GB cancer cells. The A172 and T98G cell lines were exposed to 5, 10 and 20μM of LQB-223 for 24h and the DNA content was evaluated by flow cytometry. The histograms are representative of three independent experiments. The values represent the percentage of cells in each phase.

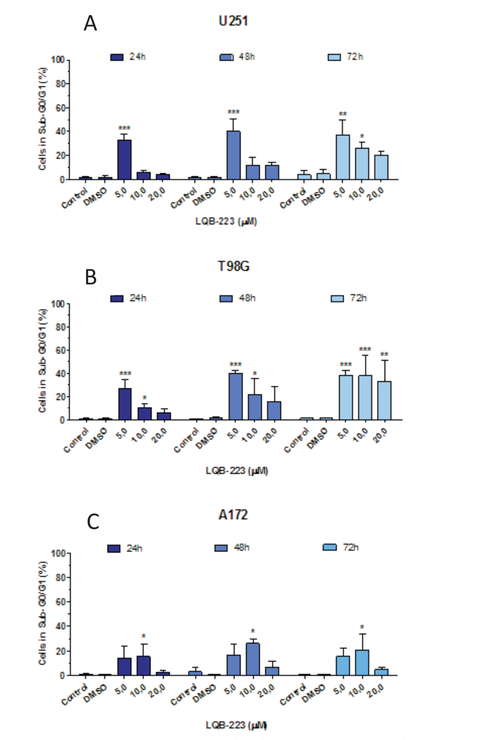

Fig 8. DNA fragmentation evaluated by flow cytometry after LQB-223 treatment. Graphic showing percentage of U251 (A), T98G (B) and A172 (C) cells in Sub-G0/G1 phase of cell cycle after exposure to 5, 10 and 20μM of LQB-223 for 24, 48 and 72h. Mean of three independent experiments ± standard error. *p<0.05, p<0.01, ***p<0.001 compared to DMSO.

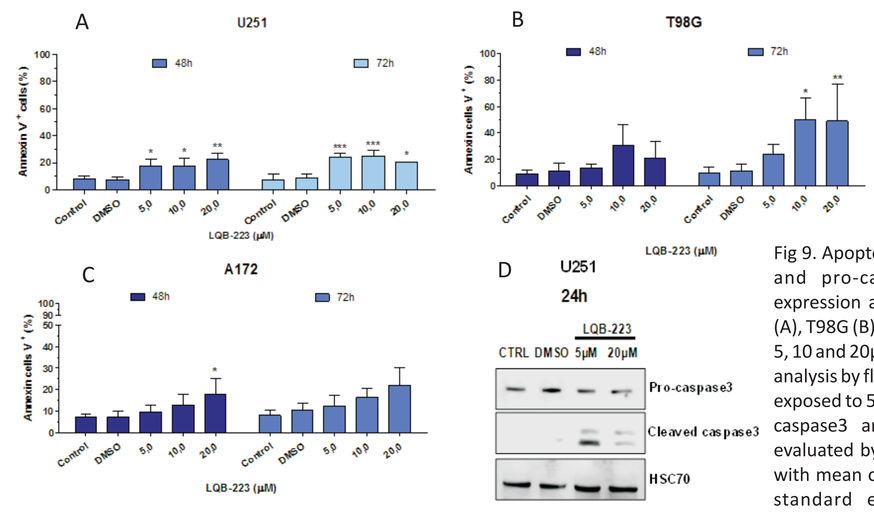

Fig 9. Apoptosis evaluated by annexin V/PI assay and pro-caspase3 and cleaved caspase3 expression after LQB-223 treatment. The U251 (A), T98G (B) and A172 (C) cells were treated with 5, 10 and 20μM LQB-223 for annexin V/PI labeling analysis by flow cytometry. The U251 cell line was exposed to 5 and 20μM LQB-223 for 24h and pro-caspase3 and cleaved caspase3 levels were evaluated by Western blotting (D). Bar graphics with mean of three independent experiments ± standard error (annexin positive cells = annexinV$^+$/ PI$^-$ + annexinV$^+$/ PI$^+$) were plotted. HSC-70 (70kDa) expression was used as endogenous control. *p<0.05, **p<0.01, ***p<0.001 compared to DMSO.

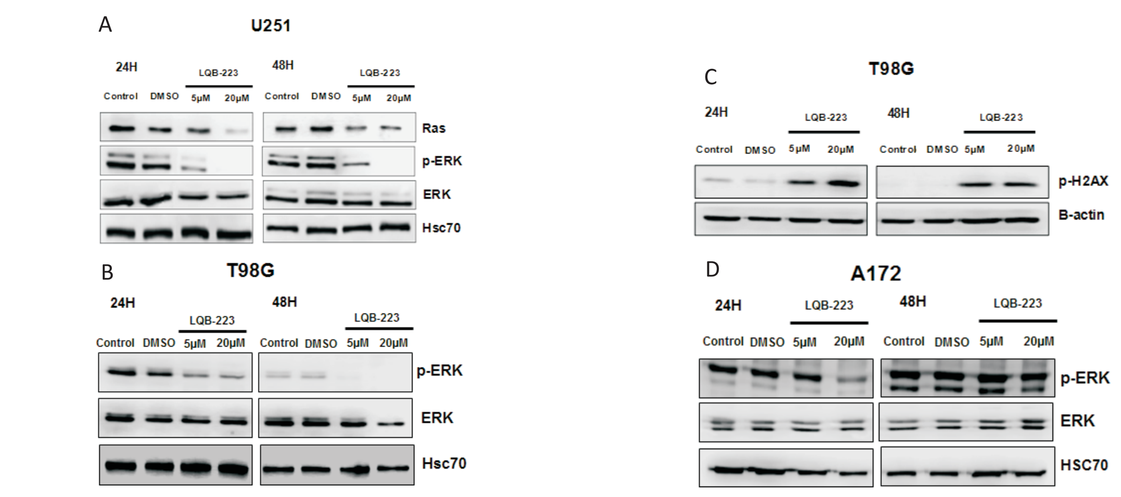

Fig 10. Cell signaling proteins expression evaluated after LQB-223 treatment. The U251 (A), T98G (B and C) and A172 (D) cell lines were treated with 5 and 20μM of LQB-223 for Ras, ERK1/2, p-ERK1/2 and p-H2AX expression analysis after 24 and 48h. HSC-70 (70kDa) and β-actin (42kDa) expression was used as endogenous control. Figure representative of three ind

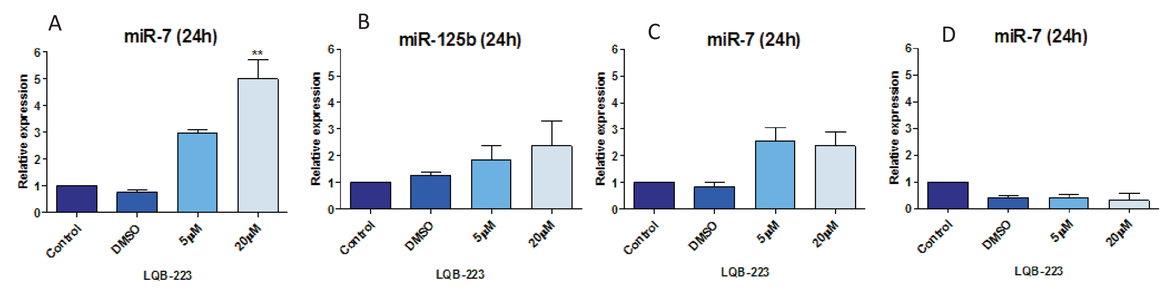

Fig 11. microRNAs expression evaluated after LQB-223 treatment. The U251 (A and B), T98G (C) and A172 (D) cell lines were treated to 5 and 20μM for 24h and microRNA-125b and microRNA-7 expression was evaluated by Quantitative Real time QT-PCR. Expression of microRNAs was normalized to the expression level of RNU6B. Mean of three independent experiments ± standard deviation.

CONCLUSION

Taken together, our data show that LQB-223 compound has a great anti-tumor potential, not only on the ionizing radiation sensitive but also on the resistant GB cell lines. Also, the observed changes may suggest different levels of EGFR inhibition perhaps through miR-7 increase.

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