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Article

Interaction between FAK/αB-crystalline is important for viability of the glioblastoma cells

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Glioblastoma multiforme is a tumor of the central nervous system. Focal Adhesion Kinase (FAK) and αB-crystalline are two proteins involved in glioblastoma development. In this study, we investigated whether the FAK/αB-crystalline interaction is important for glioblastoma cells, we aimed to investigate the interaction of these two proteins in the glioblastoma multiforme cell line U87-MG. Two peptides named FP01 peptide (derived from αB-crystalline) and FP02 peptide (derived from FAK) were synthesized for this study. Treatment of U87-MG with the peptides FP01 and FP02 in the concentration at 50 μM reduced the viability cellular to around 41% and 51%, respectively. Morphological alterations in the cells treated with the peptides when compared to the control were observed. This study suggests that the interaction between FAK and αB-crystalline is important for the viability of glioblastoma cells.

Keywords: Glioblastoma. FAK. αB-crystalline. Peptides. Protein interaction.

INTRODUCTION

Glioblastoma multiforme (GBM) is the most common type of brain tumor and is known as one of the most aggressive, destructive, and invasive human cancers (Wechsler-Reya, Scott, 2001; Stupp, Hegi, van den Bent, 2006). According to the morphological diagnostic criteria defined by the World Health Organization, GBM is classified as grade IV malignancy, which refers to the majority of malignant tumors with a mean survival of 6 to 12 months (Kleihues *et al*., 1995). Concomitantly, GBM responds poorly to conventional therapy (Robins, Chang, Butowski, 2007). Due to its high proliferative capacity, this tumor infiltrates surrounding tissues, so its complete removal by surgery is usually impossible and radiotherapy is not always efficient (Karcher *et al*., 2006). The bloodbrain barrier (BBB) is also an additional problem for treatment and tumor cells found in areas of hypoxia are resistant to radiotherapy (Schröder *et al*., 1991). Chemotherapy, associated with surgery and radiotherapy can lead to tumor regression and increase patient survival (Chang *et al*., 2007). However, it is not always possible to associate neurosurgery, radiotherapy, and chemotherapy, which reduces the survival of patients diagnosed with glioblastoma (Robins, Chang, Butowski, 2007).

Several factors seem to be involved in the development of GBM, such as changes in the activity and expression of specific proteins (Natarajan, Hecker, Gladson, 2003). Different proteins have high

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expression in this tumor, but in the present study, we focused on FAK (Focal Adhesion Kinase) and αB-crystalline (CryAB) proteins. Previous studies have shown that the level of αB-crystalline expression is elevated in glioblastomas (Schultz *et al.*, 2005) and this elevated expression seems to have a functional role in apoptosis resistance (Goplen *et al.*, 2010). Regarding FAK, several studies have shown that increased activity of tyrosine kinases such as FAK is associated with the development of glioblastomas. This protein also promotes the proliferation, survival, and migration of glioblastoma cells both in vitro and in vivo (Natarajan, Hecker, Gladson, 2003; Haskell *et al.*, 2003; Singh *et al.*, 2003; Dunn *et al.*, 2012). In previous studies, an interaction between αB-crystalline and FAK in cardiac cells was demonstrated (Pereira *et al.*, 2014). In that study, the authors demonstrated that the interaction between αB-crystalline and FAK is essential for the maintenance of the survival of cardiac myocytes submitted to mechanical stress. Indeed, αB-crystalline depletion resulted in a significant reduction in FAK protein levels in cardiac myocytes subjected to stretching and in mouse hearts subjected to coarctation of the aorta. Once the interaction is interrupted, the cells die by apoptosis, due to the reduction in FAK levels and consequent activation of the signaling cascade that culminated in apoptosis (Pereira *et al.*, 2014). Importantly, the structural bases of the FAK/αB-crystalline interaction in cardiac myocytes were investigated through a combination of molecular and structural approaches. Thus, the peptides involved in this interaction were mapped and identified (Pereira *et al.*, 2014). The interaction between FAK/αB-crystalline proteins is important for cell survival. Thus, the development of specific peptides that reduce this interaction could be a new therapeutic target for pathologies with disorders in cell survival, such as glioblastoma, leading these cells to cell death. The authors demonstrated through biochemical studies that FAK and αB-crystalline interact in glioblastoma cells and this interaction is important for cell viability. Decoy peptides corresponding to a short amino acid sequence in the αB-crystalline ACD domain (FP01) and the FAK-FAT domain (FP02) were designed to study. Studies have demonstrated that such peptides can reduce the interaction between FAK/αB-crystalline promoting cell death (Pereira *et al.*, 2014). Thus, these results encouraged us to investigate whether the interaction between FAK and αB-crystalline plays an important role in maintaining cell viability in glioblastoma and whether reducing this interaction can lead to the death of these cells.

MATERIAL AND METHODS

Cell Culture

The glioblastoma multiforme cell line, U87-MG (The European Collection of Cell Culture/Sigma Aldrich, St Louis, USA) was gently provided by Prof. Aline Mara dos Santos (Institute of Biology, State University of Campinas, Brazil). U87-MG cells were grown in a culture medium composed of RPMI 1640 (Roswell Park Memorial Institute) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin, at 37°C, 95% O_2 atmosphere supplemented and 5% CO_2. Upon reaching 95% confluence, the U87-MG cells were removed from the flask using trypsin solution, 0.25% (Sigma), and centrifuged. The precipitate was homogenized in a culture medium, and the cells were counted, with the trypan blue, in a Neubauer chamber and plated in different concentrations according to the experiment.

Western blotting Assay

Total proteins were extracted, for this the culture cells were lysed by RIPA buffer (ThermoFisher, 89900) and then the lysates were centrifuged at 14,000 rpm for 10 minutes (min) to remove cellular debris. Western blot was performed using protein extracts that were fractionated on a 12% SDS-PAGE gel and then transferred to nitrocellulose membranes. Membranes were probed with mouse anti-αB-crystalline (BIO-RAD, MCA2697), anti-FAK (ThermoFisher Scientific, AHO0502), anti-actin (BIORAD, VMA00078); anti-cleaved caspase-3 (c-Casp3) antibodies (Cell Signaling Technology, 9664), followed by anti-rabbit (GE

Amersham, NA931V) or anti-mouse (GE Amersham, NA934V) IgG antibodies as secondary antibody. Band quantification was performed using Image J (Image Processing and Analysis in JAVA/National Institutes of Health NIH program).

Immunoprecipitation Assay

U87-MG cells in lysis buffer were normalized (500 µg of total protein) and incubated with an anti-αB-crystalline monoclonal antibody in excess (5 µl; BIORAD, MCA2697). The proteins were collected by the addition of 30 µl of protein A-G plus (Merck, US1lP05). Immunoblots of the immunoprecipitates were performed with anti-FAK (1:1000; ThermoFisher Scientific, AHO0502), anti-αBcrystalline (1:5000; BIO-RAD, MCA2697), anti-actin(1:1000, BIORAD, VMA00078). Quantification of bands was performed using Image J (Image Processing and Analysis in JAVA-program/National Institutes of Health NIH).

Peptide Synthesis and Molecular Docking

Peptides previously identified in FAK/αB-crystalline interaction assays (Pereira *et al.*, 2014) were synthesized (FP-01-αB-crystalline$_{85\text{-}94}$; FP-02 - FAK$_{921\text{-}930}$; Company: AminoTech Research and Development). These peptides were linked by an N-terminal disulfide bond to the biological membrane translocator peptide TAT$_{47\text{-}57}$ (YGRKKRRQRRR), which is known to internalize peptides and proteins into cells. To access the complete sequences referring to the proteins, we used the identifications: GenBankTM: NP_ Q52L78 (αB-crystalline) and GenBankTM: NP_032008.1 (FAK).

Molecular docking was performed using the GRAMM-X web server using the αB-crystalline (PDB: 2KLR)* and the FAK FAT region (PDB: 1 K40)* models. Docking was performed freely (without contact conditions as input to the program) three times and 300 solutions were obtained in each run. The 10 best solutions from each run were tested using the CRYSOL28 program. The deviation between the models selected in each run was evaluated using the RMSD (Root mean square deviation) using the MultiProt Server (http://bioinfo3d.cs.tau.ac.il/MultiProt/). *Protein Data Bank code.

MTT Assay

U87-MG cells were seeded in a 96-well plate at a density of $2{,}0 \times 10^4$ cells per well one day before treatment. The cells were treated with 10, 25, or 50 µM of each peptide (FP01 or FP02) or 50 µM control TAT per well or saline solution (PBS buffer). As a positive control, we performed the treatment with the antitumoral drug temozolomide (10 µM). After 24 or 48 hours of treatment, the 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay was used to determine cell viability. Absorbance was read at 570 nm with a ThermoFisher Multiskan FC microplate reader (51119000). Cell morphology was visualized with a light optical inverted microscope (TL-LED, Leica Microsystems, Germany) after 24 hours or 48 hours of treatment cells with peptides FP01, FP02, or TAT in the concentration 50 µM.

Statistical analysis

Variance analysis ANOVA and Bonferroni's post hoc comparison were used to compare control groups (treated with TAT) and treated groups (with peptides FP01 or FP02). $p < 0.001$ was considered statistically significant. Data are presented as mean ± standard deviation (sd). for the indicated number of experiments.

RESULTS AND DISCUSSION

FAK may be associated with αB-crystalline in glioblastoma cells

The FAK/αB-crystalline interaction in glioblastoma cells was evaluated through the expression of both proteins in these cells. Immunoblot analysis revealed the expression of αB- crystalline and FAK proteins in glioblastoma cells (Figure 1A). Previous studies demonstrated high expression of αB-crystalline and FAK proteins in glioblastoma cells (Natarajan, Hecker, Gladson, 2003; Schultz *et al.*, 2005; Goplen *et*

al., 2010; Haskell *et al.*, 2003; Singh *et al.*, 2003; Dunn *et al.*, 2012). Concerning αB-crystalline expression a study showed that the level of this protein is elevated in glioblastomas (Schultz *et al.*, 2005) and this elevated expression seems to have a functional role in apoptosis resistance (Goplen *et al.*, 2010). FAK, on the other hand, is often overexpressed and activated in tumor cells, responsible for promoting tumor progression and metastasis (Natarajan, Hecker, Gladson, 2003; Schultz *et al.*, 2005; Goplen *et al.*, 2010; Haskell *et al.*, 2003; Singh *et al.*, 2003; Dunn *et al.*, 2012).

The FAK/αB-crystalline interaction was evaluated by immunoprecipitation of protein extracts with anti-αB-crystalline antibody. The immunoprecipitation result demonstrated the presence of the FAK/αB-crystalline interaction in glioblastoma cell extract (Figure 1 B). Pereira *et al.*, 2014 have demonstrated the interaction between the αB-crystalline chaperone and FAK, revealing that this interaction is essential for maintaining the survival of cardiac myocytes subjected to mechanical stress and important for the protection of FAK against proteolysis by calpain. Therefore, in the present study, we confirmed the interaction between FAK and αB-crystalline in glioblastoma cells, which is probably an important mechanism of resistance to apoptosis in glioblastoma cells.

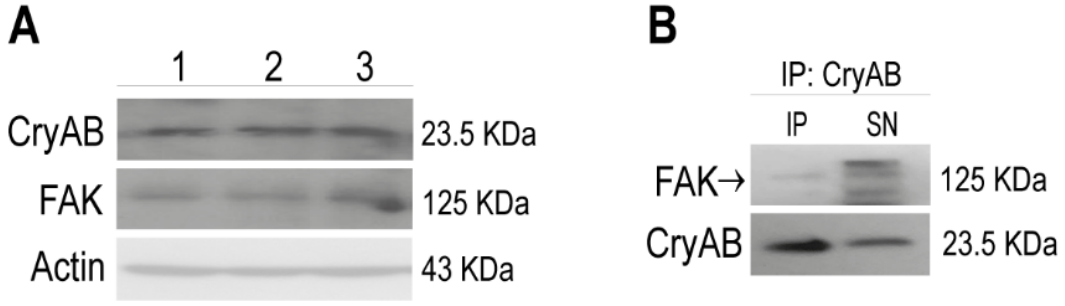

FIGURE 1 - FAK interacts with αB-crystalline in glioblastoma cells. (a) Representative immunoblots of three independent experiments showing extracts from gliblastoma cells probed with anti-FAK and anti-αB-crystalline (CryAB) antibodies. Actin served as loading control. (b) Representative (n=3) anti-FAK immunoblot of αB-crystalline immunoprecipitates (IP). Anti-αB-crystalline immunoblot served as loading control. SN (supernatant).

Molecular docking shows the interaction between FP01 and FP02 peptides and the FAK/αB-crystalline proteins

In a previous study, it was demonstrated that the interaction FAK/αB-crystalline is essential for the maintenance of the survival of cardiac myocytes submitted to mechanical stress (Pereira *et al.*, 2014). From these previous data, it can be confirmed in this current study that the interaction between FAK and αB-crystalline also occurs in glioblastoma cells. The peptides involved in the FAK/αB-crystalline interaction in cardiac myocyte cells were synthesized by the company AminoTech Research and Development. Thus, such peptides were used for study in glioblastoma. Figure 2A shows the chromatographic profile by HPLC coupled to mass spectrometry of both peptides, where the high purity index and the identity of the amino acid residues are observed. FP01 peptide corresponding to αB-crystalline residues spanning 85-94 of the α-crystalline domain (ACD) ($_{85}$SPEELKVKVL$_{94}$) and FP02 peptide corresponding to FAK residues spanning 921-930 of the Focal adhesion targeting domain (FAT) ($_{921}$NDKVYENVTG$_{930}$). Molecular docking analyses were performed to evaluate the regions of interaction between the peptides proposed in the study and FAK and αB-crystalline proteins. The crystallographic structure of the FAT domain of FAK (PDB: 1K40) and the structure obtained by Nuclear Magnetic Resonance (NMR) of the ACD domain of αB-crystalline (PDB:

2KLR) were used as inputs for the program. Initially, molecular coupling was carried out between the FAT domain of FAK and the ACD domain of αB-crystalline (Figure 2B). The FP01 and FP02 peptides were highlighted in each domain to demonstrate the interaction region between the proposed proteins and peptides (Figure 2B).

Furthermore, the residues that constitute the FP01 peptide (SPEELKVKVL) were selected from the PyMol program and molecular docking was performed between the FP01 peptide and the FAT domain of FAK (Figure 2C). The results showed that the FP01 peptide is closer to the α4 helix region of the FAK FAT domain. The region of interaction extended to the α3 helix. Figure 2D highlights the FP02 peptide region in the structure of the FAK FAT domain. This peptide is located in the α1 helix region and the docking results showed that there was no contact between this region and the FP01 peptide. The results found showed that the FP01 peptide interacts with the FAT domain of FAK near the regions of the α3 and α4 helices.

The residues that constitute the FP02 peptide (NDKVYENVTG) were selected using the PyMol program and a molecular docking study was carried out between the peptide and the ACD domain of αB-crystalline (Figure 2 E). PDB 2KLR code refers to the dimeric conformation of the ACD domain of αB-crystalline and the docking result showed that the FP02 peptide was associated close to the dimer interface, which is formed by β6+7 and β3 sheets. The region in which the peptide interacted was opposite to that demonstrated by molecular docking between the entire domains (Figure 2 B). Figure 2 F highlights the FP01 peptide region in the structure of the ACD domain of αB-crystalline. This peptide is found in the β4 leaf region and the docking results showed that there was no contact between this region and the FP02 peptide.

The results obtained through molecular docking analyses showed that the regions of interaction between the peptides synthesized proposed in this study with the full proteins do not were similar to those presented by Pereira *et al.* in 2014.

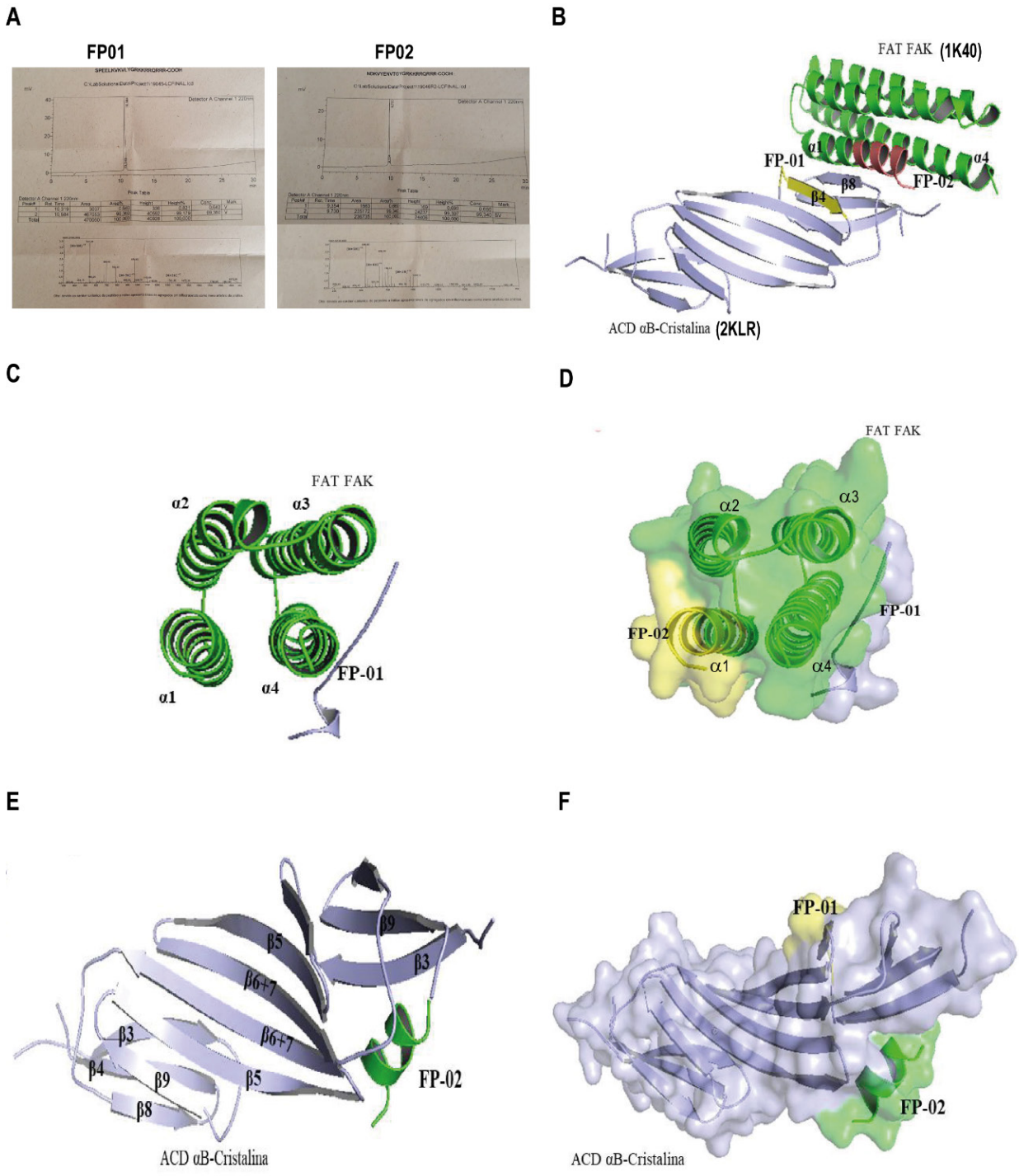

FIGURE 2 - Interaction region of FP01 and FP02 peptides and proteins FAK and αB-crystalline by molecular docking. (a) HPLC chromatographic profile and ESI MS/MS spectrum of the peptide named FP01 of the αB-crystalline protein and of the peptide named FP02 of the FAK protein (b) Molecular docking between the FAT domain of FAK and the ACD domain of αB-crystalline. Ribbon representation of the ACD domain of αB-crystalline with the peptide FP01 (SPEELKVKVL) in yellow and ribbon representation of the FAK FAT domain with the peptide FP02 (NDKVYENVTG) in purple. (c) Frontal image of the model highlighting the contact surface between the FP01 peptide and the α 3 and α 4 helices of the FAK FAT domain. (d) In yellow, the region of the FP02 peptide present in the α1 helix of the FAT domain of FAK. (e) Oblique image of the model with indications of the interaction interface involved, highlighting the contact surface between the FP02 peptide and the β6 + 7 and β3 sheets of the αB-crystalline ACD domain. (f) In yellow, the region of the FP01 peptide present in the β4 sheet of the ACD domain of αB-crystalline.

Treatment with FP01 and FP02 reduces cell viability, promotes apoptosis, and alters the morphology of U87-MG cells

The effect of peptides on cell viability was investigated by MTT assay with the peptides FP01, FP02, TAT, and PBS buffer as control negative and temozolomide as positive control. The cell viability results showed that the treatment of cells for 24 hours with the FP01 peptide showed a reduction in cell viability of 31%, 35%, and 41% for concentrations of 10 µM, 25 µM, and 50 µM respectively in comparison to PBS buffer. Treatment of cells with TAT (50 µM) peptide or temozolomide (10 µM) showed a reduction in cell viability of 21% and 10% respectively. Treatment of cells for 48 hours with the FP01 peptide showed no significant alteration in cell viability (Figure 3 A-B). Treatment with the FP02 peptide for 24 hours resulted in a reduction of cell viability around 26%, 47%, and 51% for concentrations of 10 µM, 25 µM, and 50 µM respectively, compared to PBS buffer. Treatment of cells for 48 hours with the FP02 peptide showed no significant alteration in cell viability (Figure 3 C-D). These results suggest that FP01 and FP02 interfere with the FAK/αB crystalline interaction leads to reduced cell viability. The results observed after 48 hours of treatment were probably because the cells in which the peptides were not internalized proliferated and, probably, there was a reduction of the effect of peptides in relation to the number of cells.

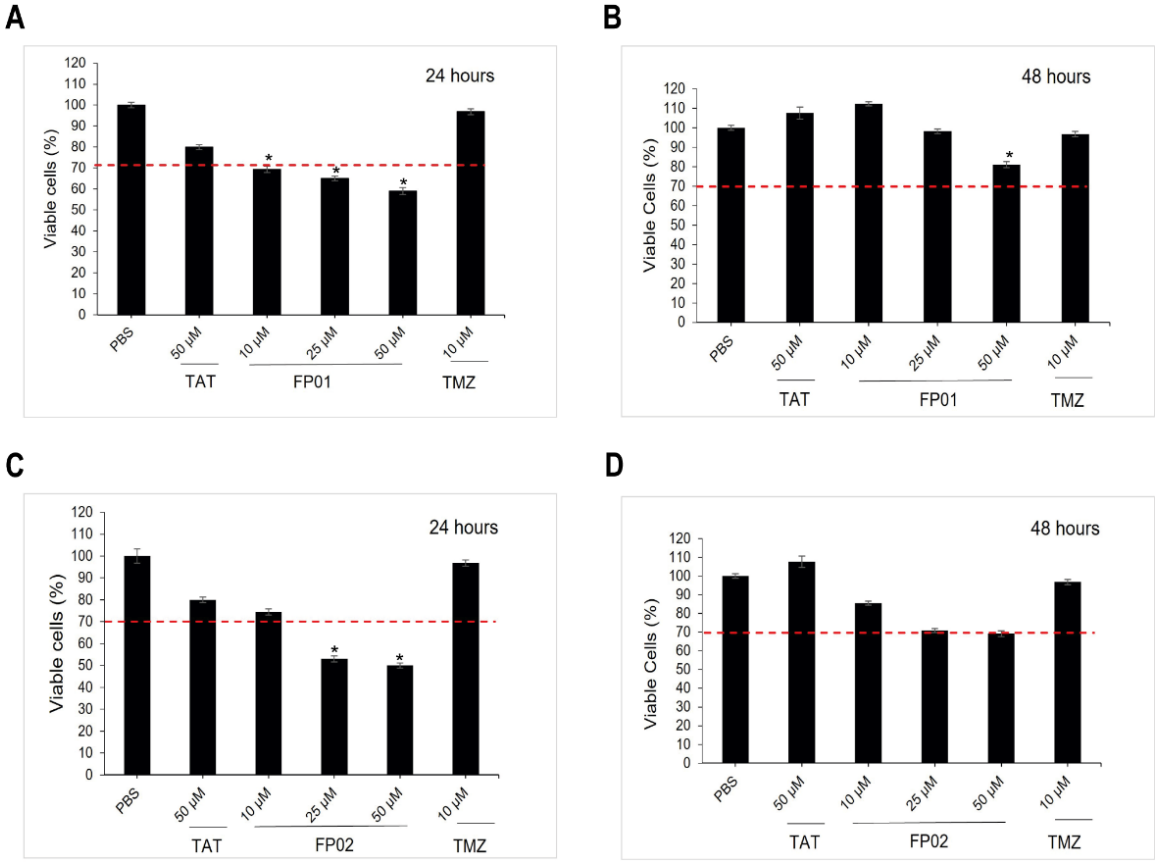

FIGURE 3 - Treatment with peptides FP01 or FP02 reduces viability celullar in glioblastoma cells (a) Cytotoxic effect of FP01 peptide at different concentrations (10µM, 25µM, 50µM) at 24 hours, represented as a percentage in relation to the control group (PBS buffer). (b) Cytotoxic effect of FP01 peptide at different concentrations (10µM, 25µM, 50µM) at 48 hours, represented as a percentage in relation to the control group (PBS buffer). (c) Cytotoxic effect of FP02 peptide at different concentrations (10µM, 25µM, 50µM) at 24 hours, represented as a percentage in relation to the control group (PBS buffer). (d) Cytotoxic effect of FP02 peptide at different concentrations (10µM, 25µM, 50µM) at 48 hours, represented as a percentage in relation to the control group (PBS buffer). *$p < 0.0001$ versus PBS. Red dated line indicates the minimum percentage of significant cell viability reduction.

In order to evaluate the cell death mechanism that FP01 and FP02 peptides cause in U87-MG cells, a western blotting assay was performed to verify the expression of the cCasp-3 protein (apoptosis marker). U87-MG cells treated with FP01 or FP02 at 50 µM for 24 hours showed a clear increase in cCasp-3. We did not observe the expression of cleaved caspase-3 in cells treated with PBS buffer (Figure 4). Caspase-3 is the main executioner caspase that is cleaved and activated by caspases initiated from caspase-8 and caspase-9. cCasp-3 degrades multiple cellular proteins and is responsible for morphological changes and DNA fragmentation in cells during apoptosis (Mcilwain, Berger, Mak, 2013; Liu *et al.*, 2021). The results obtained indicated that FP01 and FP02 promote cell death by apoptosis.

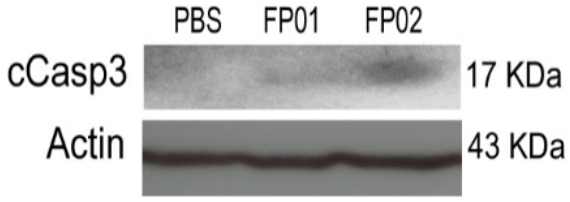

FIGURE 4 - Treatment with peptides FP01 or FP02 promoves apoptosis in glioblastoma cells. (a) Representative immunoblots of three independent experiments showing the expression of c-Casp3 after treatment with the peptides FP01 or FP02. Actin served as loading control.

We evaluated the morphology of cells after treatment with FP01 and FP02 peptides at that concentration of 50µM for 24 hours. Changes were observed in the morphology and number of cells treated with FP01 and FP02 peptides in relation to the control (TAT 50 µM or PBS buffer) (Figure 5 – Panels A-D). It is known the extremely complex challenges involved in the treatment of GBM and the need for new strategies for cancer therapies that can counteract the problems that arise particularly in chemotherapy due to resistance to current drugs and their low specificity (Riedl, Zweytick , Lohner, 2011). Currently, new alternatives have been proposed in cancer therapy, mainly represented by hybrid molecules coupled to conventional chemotherapy drugs, computer-assisted drugs, and the use of bioactive peptides (Cui *et al.*, 2020; Dallavalle *et al.*, 2020; Eghtedari, Porzani, Nowruzi, 2021; Trinidad-Calderón, Varela-Chinchilla, García-Lara, 2021). Peptides have attracted special attention as an alternative method to traditional cancer therapy. This is mainly due to some very positive characteristics such as high affinity, specificity, and low toxicity, offering useful and optimistic substitutes compared to chemical drugs (Segura-Campos *et al.*, 2011; Wang *et al.*, 2017). In this study an unprecedented association was observed between FAK and αB-crystalline in glioblastoma cells. The role of this interaction was validated through the design of decoy peptides corresponding to residues 85-94 of the ACD domain of αB-crystalline ($_{85}$SPEELKVKVL94). This peptide was named FP01 and the peptide named FP02 corresponding to residues 921-930 of the FAT domain of FAK ($_{921}$NDKVYENVTG$_{930}$) was also used in this study. Peptide treatment reduces cell viability and alters the morphology of U87-MG cells.

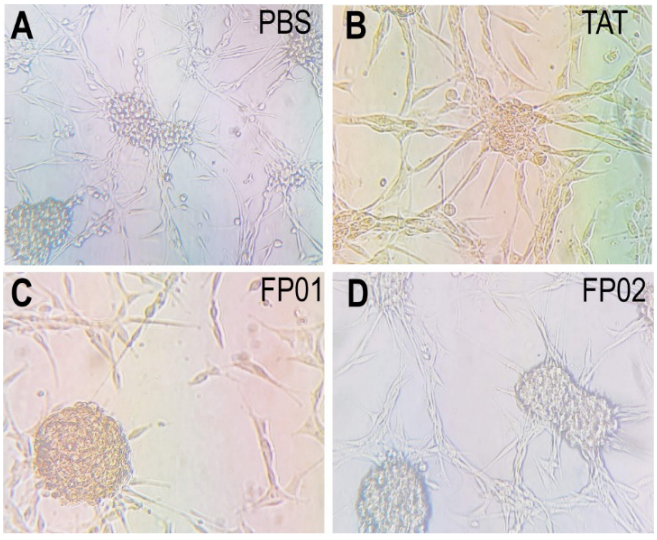

FIGURE 5 - Treatment with peptides FP01 or FP02 alters morphology of glioblastoma cells. Representative image of U87-MG glioblastoma cells treated for 24 hours with PBS buffer (negative control) (panel A), cells treated with 50 µM TAT peptide (panel B), cells treated with 50 µM FP01 peptide (panel C) and cells treated with 50 µM FP02 peptide (panel D). 40x magnification.

In conclusion, considering the role of αB-crystalline and FAK em glioblastoma cells and the findings of the present study we suggest that reduced the interaction between FAK/ αB-crystalline can lead to the death of the glioblastoma cells. Further

investigations regarding FAK and αB-crystalline complex in the glioblastoma cells may contribute to the knowledge of glioblastoma pathophysiology and the discovery of new molecular targets.

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AUTHOR CONTRIBUTIONS

JEA and MBMP conceived and supervised the study; JEA and MBMP designed experiments; MLCF and LL performed experiments; JEA, RRT, and MBMP provided new tools and reagents; MLCF, JEA, and MBMP analyzed data; JEA, MBMP wrote the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Chang JE, Khuntia D, Robins HI, Mehta MP. Radiotherapy and radiosensitizers in the treatment of glioblastoma multiforme. Clin Adv Hematol Oncol. 2007;5(11):894-902.

Cui W, Aouidate A, Wang S, Yu O, Li Y, Yuan S . Discovering Anti-Cancer Drugs via Computational Methods. Front Pharmacol. 2020;11:1-20.

Dallavalle S, Dobricic V, Lazzarato L, Gazzano E, Machuqueiro M, Pajeva I. Improvement of conventional anti-cancer drugs as new tools against multidrug-resistant tumors. Drug Resistance Updates. 2020;50:100682.

Dunn GP, Rinne ML, Wykosky J, Genovese G, Quayle SN, Dunn IF. Emerging insights into the molecular and cellular basis of glioblastoma. Genes Dev. 2012;26(8):756-784.

Eghtedari M, Porzani SJ, Nowruzi B. Anticancer potential of natural peptides from terrestrial and marine environments: a review. Phytochem Lett. 2021;42: 87-103.

Goplen D, Bougnaud S, Rajcevic U, Boe SO, Skaftnesmo KO, Voges J et al. αB-crystallin is elevated in highly infiltrative apoptosis-resistant glioblastoma cells. Am J Pathol. 2010;177(4):1618-1628.

Haskell H, Natarajan M, Hecker TP, Ding Q, Stewart J Jr, Grammer JR, et al. Focal adhesion kinase is expressed in the angiogenic blood vessels of malignant astrocytic tumors in vivo and promotes capillary tube formation of brain microvascular endothelial cells. Clin Cancer Res. 2003;9(6):2157-2165.

Karcher S, Steiner HH, Ahmadi R, Zoubaa S, Vasvari G, Bauer H, et al. Different angiogenic phenotypes in primary and secondary glioblastomas. Int J Cancer. 2006;118(9):2182-2189.

Kleihues P, Soylemezoglu F, Schäuble B, Scheithauer BW, Burger PC. Histopathology, classification, and grading of gliomas. Glia. 1995;15(3):211-221.

Liu T, Peng F, Cao X, Liu F, Wang Q, Liu L et al. Design, synthesis, antibacterial activity, antiviral activity, and mechanism of myricetin derivatives containing a quinazolinone moiety. ACS Omega. 2021;6(45):30826-30833.

Mcilwain DR, Berger T, Mak, TW. Caspase Functions in Cell Death and Disease. Cold Spring Harbor Perspect Biol. 2013;5(4):8656-8656 11.

Natarajan M, Hecker TP, Gladson CL. FAK signaling in anaplastic astrocytoma and glioblastoma tumors. Cancer J. 2003;9(2):126-133.

Pereira MBM, Santos AM, Gonçalves DC, Cardoso AC, Consonni SR, Gozzo FC, et al. αB-crystallin interacts with and prevents stress-activated proteolysis of focal adhesion kinase by calpain in cardiomyocytes. Nat Commun. 2014;5:5159-5175.

Riedl S, Zweytick D, Lohner, K. Membrane-active host defense peptides – Challenges and perspectives for the development of novel anticancer drugs. Chemistry And Physics of Lipids. 2011;164(8):766-781.

Robins HI, Chang S, Butowski N. Therapeutic advances for glioblastoma multiforme: current status and future prospects. Curr Oncol Rep. 2007;9:66-70.

Schröder R, Bien K, Kott R, Meyers I, Vössing R. The relationship between Ki-67 labeling and mitotic index in gliomas and meningiomas: demonstration of the

variability of the intermitotic cycle time. Acta Neuropathol. 1991;82(5):389-394.

Schultz S, Pinsky GS, Wu NC, Chamberlain MC, Rodrigo AS, Martin SE. Fine needle aspiration diagnosis of extracranial glioblastoma multiforme: Case report and review of the literature. Cytojournal. 2005;2:1-6.

Segura-Campos M, Guerrero LC, Betancur D, Hernandez-Escalante VM. Bioavailability of Bioactive Peptides. Food Rev Int. 2011;27(3):213-226.

Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J et al. Identification of a cancer stem cell in human brain tumors. Cancer Res. 2003;63(18):5821-5828.

Stupp R, Hegi ME, van den Bent MJ. European Organization for Research and Treatment of Cancer Brain Tumor and Radiotherapy, G and National Cancer Institute of Canada Clinical Trials, G. Oncologist. 2006;11:165-180.

Trinidad-Calderón PA, Varela-Chinchilla CD, García-Lara S. Natural Peptides Inducing Cancer Cell Death: mechanisms and properties of specific candidates for cancer therapeutics. Molecules. 2021;26(24):7453-7474.

Wang C, Dong S, Zhang L, Zhao Y, Huang L, Gong X, et al. Cell surface binding, uptaking and anticancer activity of L-K6, a lysine/leucine-rich peptide, on human breast cancer MCF-7 cells. Sci Rep. 2017;7:8293-8306.

Wechsler-Reya R, Scott MP. The developmental biology of brain tumors. Ann Rev Neurosci. 2001;24:385-428.

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