INTRODUCTION

Microtubule actin crosslinking factor 1 (MACF1) is a member of the plakin protein family discovered a little over two decades ago as a cytoskeletal cross-linking protein between actin microfilaments and microtubules that has been shown to contribute to cell proliferation and migration across diverse cellular systems [1]. More specifically it was demonstrated that MACF1 was prominently involved in neuronal cell migration [2], while subsequent investigation by Hu et al. [3], provided evidence that MACF1 when knocked down compromised osteoblasts proliferation. Despite established roles of MACF1 in cell behaviors that contribute to the propagation and maintenance of cancers, few studies have evaluated it as a therapeutic target in this disease. To this end we recently provided...
Inhibition of MACF1 Sensitizes Glioblastomas to Radiation

Evidence that MACF1 was expressed at high levels in glioblastomas but not in lower grade brain tumors and normal brain tissue [4]. Additionally, down-regulation of MACF1 which has been described to have genetic mutations in renal cell carcinomas and endometrial cancers [5,6], had an anti-tumorigenic effect on glioblastoma cell viability and migration [4]. The inhibition of protumorigenic glioblastoma cell behaviors as a consequence of silencing MACF1 function were associated with down-regulation of Wnt signaling mediators, Axin1 and beta-catenin [4]. These data were consistent with findings in a seminal study by Chen et al. [7], which also described the interaction of MACF1 with Wnt signaling mediators, and are significant because Wnt signaling is a known mechanistic pathway that contributes to the progression of several cancers including glioblastomas [8]. From a therapeutic standpoint we also demonstrated that temozolomide, the gold standard chemotherapeutic drug used to treat glioblastomas induced MACF1 expression in these tumors and had a synergistic effect when combined with MACF1 inhibition [4].

Expanding upon our findings, we evaluated here the effects of inhibiting MACF1 as a means to enhance and sensitize the response of glioblastomas to radiation therapy in vitro glioblastoma model systems. Radiation is typically used clinically to treat patients diagnosed with glioblastomas following surgical resection. An unfortunate caveat has been the limited efficacy of radiation as a single treatment option that enhances overall survival of patients diagnosed with this disease [9,10]. However, pioneering work by Stupp et al. [11], established a clinical precedence for the utility of combinatorial radiation therapy approaches for the treatment of glioblastomas, when they demonstrated an enhanced therapeutic benefit to patients that received radiation plus the chemotherapeutic agent temozolomide, as compared to patients that received radiation treatment alone. To date however, the primary targets of combinatorial radiotherapy approaches in glioblastomas have been limited to DNA repair proteins and protein kinase signaling cascades [12-16]. Inhibitory targeting of MACF1 as a radiosensitizer, represents a novel experimental strategy that broadens combinatorial radiotherapy approaches in genetically heterogeneous glioblastomas that is essential to improving and managing disease progression. Additionally, we examined and identified ribosomal protein S6, a pro-tumorigenic downstream signaling mediator in the mTOR pathway [17,18] and whose expression has been associated with poor survival of glioblastoma patients [19] as a mechanistic contributor of the combinatorial impact of MACF1 inhibition and radiation treatment.

MATERIALS AND METHODS

Cells culture conditions and reagents

U251 human glioblastoma cells were purchased from Sigma-Aldrich (St. Louis, MO, USA; 09063001) and A172 human glioblastoma cells from the American Type Culture Collection (Manassas, VA, USA; ATCC-CRL 1620). All cell lines were maintained in Dulbecco’s Modified Eagles Medium-DMEM (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 2 mM L-glutamine (Invitrogen), 100 nM MEM non-essential amino acids (Invitrogen), and penicillin-streptomycin (Invitrogen) at 37°C and 5% CO2. GIPZ lentiviral shRNAs were purchased from Dharmacon (Chicago, IL, USA) and a Mark 1 Cesium-137 source was used to treat cells with a single 5 Gy dose of radiation in the Department of Radiation Oncology at Vanderbilt University Medical Center [20].

MACF1 inhibitory silencing

shRNA lentiviral transduction was performed with one of three lentiviral shRNAs targeting MACF1 (1-V2LHS_28596; 2-V3LHS_306210; 3-V3LHS_306213-3) in 1×10⁵ U251 and A172 cells in serum free media with a multiplicity of infection (MOI) of 0.9 overnight at 37°C and 5% CO2; cells were transduced with non-silencing shRNA as a control. Next complete growth media was added to U251 cells containing lentiviral shRNAs and allowed to incubate for 3–4 days. Following initial transduction of A172 cells, serum free media containing lentiviral shRNAs was replaced with normal growth media for 24 hours. Subsequently, growth media was removed and replaced with normal growth media containing 187 ng/mL of puromycin and incubated for 72 hours. Cells were then trypsinized, replated, and incubated in fresh growth media with puromycin for an additional 24 hours prior to treatment with radiation. MACF1 expression was subsequently examined in cells treated with control shRNAs and cells transduced with shRNAs targeting MACF1 using immunofluorescence procedures. Experiments were each performed at least three times. Means were determined between three separate experiments and statistical analysis was performed using Student’s t-test to evaluate significance between experimental conditions using Graphpad Prism 8 (GraphPad Software, Inc., San Diego, CA, USA).

Cell viability assay

U251 and A172 cells were either treated with shRNAs alone, irradiated with 1×5 Gy, or treated with shRNAs (Dharmacon, Chicago, IL, USA) prior to irradiation as described above. Subsequently, 7×10³ cells were plated and allowed to incubate for 1, 2, 3, or 5 days in DMEM (Invitrogen) containing 10% fetal...
bovine serum (Invitrogen). A172 cells were incubated in media containing puromycin. At the end of each time point tissue culture media was removed, the cell monolayer fixed with 100% methanol for 5 minutes and stained with 0.5% crystal violet in 25% methanol for 10 minutes. Cells were then washed three times 5 minutes each with distilled water to remove excess dye and allowed to dry overnight at room temperature. Cells were then manually counted or the incorporated dye was solubilized in 0.1 M sodium citrate (Sigma-Aldrich) in 50% ethanol. Next, 100 μL of experimental samples were transferred to 96 well plates and optical densities read at 595 nm using a X-mark microplate absorbance spectrophotometer (BioRad, Hercules, CA, USA). Experiments were each performed at least three times with duplicate samples and means determined by averaging duplicate samples within each independent experiment. ANOVA followed by Tukey’s post-hoc analysis were used to evaluate significance between means of experimental conditions using Graphpad Prism.

Cell motility
Motility assays were conducted according to manufacturer’s instructions (CellBiolabs Inc., San Diego, CA, USA). Media containing 10% fetal bovine serum was added to the lower chamber of the migration well, while 2×10⁴ irradiated and shRNA treated irradiated cells were prepared in 300 μL of serum free media, added to the Boyden chamber insert, and allowed to incubate for 24 hours at 37°C and 5% CO₂. Subsequently, non-migratory cells were removed from plate inserts (per manufacturer’s instructions) and migratory cells stained with proprietary staining solution. Images were captured using an Olympus IX53 (Olympus, Center Valley, PA, USA) microscope and optical densities read at 560 nm in proprietary solubilization solution using a X-mark microplate absorbance spectrophotometer (BioRad). Experiments were each performed at least three times with duplicate samples and means determined by averaging duplicate samples within each independent experiment. ANOVA followed by Tukey’s post-hoc analysis were used to evaluate significance between means of experimental conditions using Graphpad Prism.

Immunofluorescence labeling
For immunofluorescence labeling 1.5×10⁴ cells were plated that were naïve controls, treated with shRNAs alone, radiation alone, or the combination of shRNAs and radiation. Cells were then incubated overnight at 4°C with antibodies that detect MACF1 (377534; Santa Cruz Biotechnology, Dallas, TX, USA) and p-S6 ribosomal protein (4858; Cell Signaling Technology, Danvers, MA, USA). Samples were then rinsed three times with PBS, incubated with an Alexa 488 goat-anti-mouse conjugated secondary antibody (4412; Cell Signaling Technology) for 1 hour in the dark, rinsed again and examined with an Olympus IX53 microscope. Quantification of fluorescence was determined by measuring the fluorescence intensity from a total of 50 individual cells from three separate independent experiments and averaged. Statistical analysis was performed using ANOVA followed by Tukey’s post-hoc analysis to evaluate significance between means of experimental conditions using Graphpad Prism. Antibody validation was performed via western blotting that assessed molecular weight and specificity of target proteins. Experiments were each performed at least three times.

RESULTS
MACF1 expression in glioblastoma patients
In an effort to expand findings from our previous investigation that showed MACF1 was expressed in glioblastoma tissue but not in normal brain tissue [4], The Cancer Genome Atlas (TCGA: ciobortalp) was used to gain further insight regarding the association of MACF1 expression and glioblastoma patient survival [21]. A comparative survival curve analysis was performed on patient cohorts stratified by MACF1 expression and showed a relative decrease of patient survival in glioblastomas with high MACF1 expression as compared to low MACF1 expressing tumors between 5–15 months (Fig. 1). Additionally, correlative expression analyses between MACF1 and aberrantly expressed genes known to contribute to the etiology and progression of these tumors revealed a statistically significant positive correlation between MACF1 and epidermal growth factor receptor (EGFR) expression, while a negative correlation was observed between MACF1, PTEN, CDKN2A, and TP53 expression (Fig. 1).

Anti-tumorigenic effects of radiation and MACF1 inhibition in glioblastoma cells
At present there are no pharmacological inhibitors to impair MACF1 cross-linking and signaling functions. To this end we have previously shown that genetic inhibition of MACF1 reduced glioblastoma cell proliferation in established and patient derived xenograft glioblastoma cell lines, which provided the technical feasibility and foundation for the present study [4]. Therefore, MACF1 expression and function here were down-regulated in glioblastoma cells using a lentiviral transduction shRNA genetic inhibitory approach that induced
Inhibition of MACF1 Sensitizes Glioblastomas to Radiation

Fig. 1. Correlative expression of MACF1 to patient survival and glioblastoma associated genes. Comparative survival curve analysis of glioblastoma patients with low MACF1 expression (<1.048; n=74) and high MACF1 mRNA expression (>1.048; n=83). MACF1 co-expression with EGFR, PTEN, p53, and CDKN2A.
Fig. 2. Down-regulation of MACF1 in genetically silenced glioblastoma cells. U251 glioblastoma cells treated with non-targeting shRNAs (A-C) and shRNAs targeting MACF1 (D-F); A172 glioblastoma cells treated with non-targeting shRNAs (G-I) and shRNAs targeting MACF1 (J-L); MACF1 expression (green); DAPI (blue); cytoplasmic MACF1 expression (arrows). Displayed are immunofluorescent images representative of three separate independent experiments with comparable results. Image J was used to measure the fluorescence intensity from a total of 50 individual cells from three separate independent experiments (n=3) and averaged. Student's t-test was used to determine statistical significance (**p<0.01; error bars: standard error of the means). Scale bar=100 µm; total magnification ×200; RFU: relative fluorescence units.
a considerable reduction in the cytoplasmic expression of this cytoskeletal protein (Fig. 2). Reduced MACF1 protein expression to shRNAs targeting MACF1 was accompanied by decreased glioblastoma cell viability. Specifically, a 28% and 48% decrease in U251 cell viability was observed in response to shRNAs targeting this cytoskeletal cross-linker as compared to non-targeting shRNA controls, while shRNAs targeting MACF1 in A172 cells had a 50% and 17% reduction in cell viability when compared to non-targeting controls (Fig. 3).

As a single treatment modality radiation has not been clinically beneficial in improving the overall survival of glioblastoma patients [2,3]. This is a consequence of intrinsic and acquired radiation resistance that can be attributed to genomic instability and radiation induced expression of DNA damage response proteins [22-24] that contribute to the invasive nature of these tumors and their inevitable recurrence [25-29]. Consistent with this notion cbioportal data showed that MACF1 positively correlated with mRNA expression of several DNA damage repair proteins in clinical glioblastoma patients (Table 1). Experimentally, the combinatorial efficacy of radiation and down-regulation of MACF1 protein expression had a demonstrative effect on glioblastomas, with as much as an 82% ($p<0.01$) and 32% ($p<0.05$) statistically significant decrease in U251 and A172 cell viability, respectively, when compared to non-targeting shRNA control cells treated with radiation (Fig. 3). Additionally, the combinatorial effects of radiation

### Table 1. Co-expression of MACF1 and DNA damage repair proteins in glioblastomas

| MACF1  | Spearman’s correlation | $p$ value |
|--------|------------------------|-----------|
| p53BP1 | 0.349                  | 7.05e-6   |
| ATM    | 0.252                  | 1.429e-4  |
| BRCA2  | 0.331                  | 2.133e-5  |
| RAD50  | 0.276                  | 4.394e-4  |

[Fig. 3. MACF1 inhibition sensitizes glioblastoma cells to radiation. Impairing MACF1 function reduces cell viability in non-irradiated cells and enhances the efficacy of radiation. Data displayed is of a single experiment performed in duplicate and equivalent to three separate independent experiments performed in duplicate with comparable outcomes. ANOVA analysis followed by Tukey’s post-hoc analysis revealed statistical significant differences between means of experimental conditions ($^*p<0.05$; $^{**}p<0.01$; error bars: standard error of the means).]
and MACF1 inhibition on the migratory capacity of glioblastoma cells were determined using Boyden chamber cell migration assays. These data revealed that U251 and A172 cell migration was abolished in cells treated with shMACF1-2 and radiation, albeit A172 glioblastoma cell migration was more susceptible to combination treatments ($p<0.05$; $p<0.01$) as compared to controls (Fig. 4). It should also be noted that the anti-migratory response of glioblastoma cells to the combination of radiation and MACF1 inhibition is not likely a consequence of cell death, as a decrease in the number of viable cells was not observed at time points relative to the number of viable cells at the day zero time point (data not shown).

Radiosensitization of glioblastoma cells via down-regulation of ribosomal protein S6

Mechanistic evaluation of the radiosensitization effects of

| Ribosomal protein S6 | Spearman’s correlation | $p$ value |
|----------------------|------------------------|-----------|
| p53BP1               | 0.482                  | 2.12e$^{-6}$ |
| ATM                  | 0.608                  | 2.79e$^{-15}$ |
| BRCA2                | 0.381                  | 3.913e$^{-6}$ |
| RAD50                | 0.286                  | 6.73e$^{-4}$ |

Table 2. Co-expression of ribosomal protein S6 and DNA damage repair proteins in glioblastomas

Fig. 4. Radiation and suppression of MACF1 impairs glioblastoma cell migration. U251 (A-D) and A172 (E-H) cells treated with shMACF1 and irradiated with a single 5 Gy dose. Displayed are stained migrating cells in Boyden chambers and data from a single experiment performed in duplicate representative of at least three separate independent experiments performed in duplicate with comparable results 24 hours after radiation exposure. ANOVA analysis followed by Tukey’s post-hoc analysis revealed statistical significant differences between means of experimental conditions ($*p<0.05$; $**p<0.01$; error bars: standard error of the means). Scale bar=200 µm; stained with crystal violet; total magnification $\times100$. 

49
impairing MACF1 in glioblastoma cells was assessed by evaluating changes in ribosomal protein S6 of the mTOR signaling pathway, a contributor in tumor cell proliferation and metastatic invasion. Like MACF1, ribosomal protein S6 had a positive correlative expression with several DNA damage repair genes in clinical glioblastoma patients (Table 2). Effector molecules of ribosomal protein S6, mTOR and its immediate downstream target, RPS6KB1 (p70s6k), whose expression have been...
associated with shorter overall survival of glioblastoma patients [30] were positively correlated with MACF1 in a cohort of patients (Fig. 5). Additionally, the activated form of ribosomal protein S6 that is regulated by p70s6k was also assessed as a mechanistic contributor of the radiosensitization effects of MACF1 inhibition. Although mRNA co-expression analysis did not reveal a positive correlation between MACF1 and ribosomal protein S6, an association of MACF1 and activated ribosomal protein S6 as a mechanistic signaling mediator in glioblastomas was observed here. To this end an 81% and 80% decrease of p-ribosomal protein S6 expression (p<0.0001) was seen in U251 cells treated with radiation and shMACF1 as compared to naïve untreated controls and cells treated with radiation alone (Fig. 5).

**DISCUSSION**

Diagnostic biomarkers as targets and predictors of radiotherapy response for the clinical treatment of glioblastomas have been somewhat ambiguous and limited to the presence of genetic alterations of the EGFR and methylation status of O-6-methylguanine DNA methyltransferase (MGMT) [31-36]. However, genetic and cellular glioblastoma heterogeneity warrant the identification of expanded targets that can improve overall survival outcomes. We previously demonstrated that the spectraplakin protein, MACF1, is a potential novel diagnostic therapeutic target in glioblastomas and when negatively regulated induces an antitumorigenic response to the propagation and progression of these tumors [4]. In furtherance of this, we showed here that MACF1 enhanced and sensitized glioblastoma cells to radiation via a synergistic combinatorial effect when genetically silenced. These findings parallel a recent preclinical investigation of the microtubule poison, eribulin that concomitantly sensitized glioblastomas to radiation alone (Fig. 5).

In conclusion, we demonstrated here that inhibiting MACF1 enhances and sensitizes glioblastomas to radiation that extends findings observed with temozolomide and further supports the inhibitory targeting of MACF1 with clinical treatment modalities for these tumors. Furthermore, the mechanistic involvement of MACF1 in intracellular signaling processes has been broadened to include ribosomal protein S6 as part of the mTOR-p70s6k signaling axis that has recently been described as a prognostic indicator related to poor survival of glioblastoma patients [19]. Taken together this investigation has identified MACF1 as a novel radiosensitization target in glioblastomas.

**Conflicts of Interest**

The authors have no potential conflicts of interest.

**Acknowledgments**

Quincy Quick is supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number SC-3GM121178.

**ORCID iDs**

Quincy A. Quick https://orcid.org/0000-0002-0173-6728

Kala Bonner https://orcid.org/0000-0003-0518-3961

**REFERENCES**

1. Bernier G, Mathieu M, De Repentigny Y, Vidal SM, Kothary R. Cloning and characterization of mouse ACF7, a novel member of the dystonin subfamily of actin binding proteins. Genomics 1996;38:19-29.

2. Ka M, Jung EM, Mueller U, Kim WY. MACF1 regulates the migration and characterization of mouse ACF7, a novel member of the dystonin subfamily of actin binding proteins. Genomics 1996;38:19-29.

3. Hu L, Su P, Li R, et al. Knockdown of microtubule actin crosslinking factor 1 inhibits cell proliferation in MC3T3-E1 osteoblastic cells. BMB Rep 2015;48:583-8.

4. Afghani N, Mehta T, Wang J, Tang N, Skalli O, Quick QA. Microtubule actin cross-linking factor 1, a novel target in glioblastoma. Int J Oncol 2017;50:310-6.
Inhibition of MACF1 Sensitizes Glioblastomas to Radiation

5. Arai E, Sakamoto H, Ichikawa H, et al. Multilayer-omics analysis of renal cell carcinoma, including the whole exome, methylome and transcriptome. Int J Cancer 2014;135:1330-42.

6. Chang YS, Huang HD, Yeh KT, Chang JG. Identification of novel mutations in endometrial cancer patients by whole-exome sequencing. Int J Oncol 2017;50:1778-84.

7. Chen HJ, Lin CM, Lin CS, Perez-Olle R, Leung CL, Liem RK. The role of microtubule actin cross-linking factor 1 (MACF1) in the Wnt signaling pathway. Genes Dev 2006;20:1933-45.

8. McCord M, Mukoyama YS, Gilbert MR, Jackson S. Targeting WNT signaling for multifaceted glioblastoma therapy. Front Cell Neurosci 2017;11:318.

9. Shapiro WR, Green SB, Burger PC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009;10:159-66.

10. Khosla D. Concurrent therapy to enhance radiotherapeutic outcomes in glioblastoma. Ann Transl Med 2016;4:54.

11. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma: a randomised phase III study. Lancet Oncol 2005;6:985-96.

12. Kahn J, Hayman TJ, Jamal M, et al. The mTORC1/mTORC2 inhibitor NVP-BEZ235 as a strategy for radiosensitization of glioblastoma. Clin Cancer Res 2006;12:3935-41.

13. Kil WJ, Cerna D, Burgen WE, et al. In vitro and in vivo radiosensitization induced by the DNA methylating agent temozolomide. Clin Cancer Res 2008;14:931-8.

14. Khosla D. Concurrent therapy to enhance radiotherapeutic outcomes in glioblastoma. Ann Transl Med 2016;4:54.

15. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009;10:159-66.

16. Kahn J, Hayman TJ, Jamal M, et al. The mTORC1/mTORC2 inhibitor NVP-BEZ235 as a strategy for radiosensitization of glioblastoma. Clin Cancer Res 2006;12:3935-41.

17. Kil WJ, Cerna D, Burgen WE, et al. In vitro and in vivo radiosensitization induced by the DNA methylating agent temozolomide. Clin Cancer Res 2008;14:931-8.

18. Sheng Y, Xu M, Li C, et al. Nm23-H1 is involved in the repair of ionizing radiation-induced DNA double-strand breaks in the A549 lung cancer cell line. BMC Cancer 2018;18:710.

19. Martinou M, Giannopoulou E, Malatara G, Argyriou AA, Kalofonos HP, Kardamakis D. Ionizing radiation affects epidermal growth factor receptor signalling and metalloproteinase secretion in glioma cells. Cancer Genomics Proteomics 2011;8:33-8.

20. Contessa JN, Hampton J, Lammering G, et al. Ionizing radiation activates Erb-B receptor dependent Akt and p70 S6 kinase signaling in carcinoma cells. Oncogene 2002;21:4032-41.

21. Kim Y, Kim KH, Lee J, et al. Wnt activation is implicated in glioblastoma radiosensitization. Lab Invest 2012;92:666-73.

22. Wild-Bode C, Weller M, Rimmer A, Dichtans J, Wick W. Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. Cancer Res 2001;61:2744-50.

23. Lammering G, Hewit TH, Valerie K, et al. EGFRVIII-mediated radiation resistance through a strong cytoprotective response. Oncogene 2003;22:55-63.

24. Pelloski CE, Lin E, Zhang L, et al. Prognostic associations of activated mitogen-activated protein kinase and Akt pathways in glioblastomas. Clin Cancer Res 2006;12:13935-41.

25. Li B, Yuan M, Kim IA, Chang CM, Bernhard EL, Shu HK. Mutant epidermal growth factor receptor displays increased signaling through the phosphatidylinositol-3 kinase/AKT pathway and promotes radioresistance in cells of astrocytic origin. Oncogene 2004;23:4594-602.

26. Liu Q, Nguyen DH, Dong Q, et al. Molecular properties of CD133+ glioblastoma stem cells derived from treatment-refractory recurrent brain tumors. J Neurooncol 2009;91:1-19.

27. Shih HA, Betensky RA, Dorfman MV, Louis DN, Loefler JS, Batchelor TT. Genetic analyses for predictors of radiation response in glioblastoma. Int J Radiat Oncol Biol Phys 2005;63:704-10.

28. Barker FG 2nd, Simmons ML, Chang SM, et al. EGFR overexpression and radiation response in glioblastoma multiforme. Int J Radiat Oncol Biol Phys 2005;63:704-10.

29. Contessa JN, Hampton J, Lammering G, et al. Ionizing radiation activates Erb-B receptor dependent Akt and p70 S6 kinase signaling in carcinoma cells. Oncogene 2002;21:4032-41.

30. Kim Y, Kim KH, Lee J, et al. Wnt activation is implicated in glioblastoma radiosensitization. Lab Invest 2012;92:666-73.

31. Wild-Bode C, Weller M, Rimmer A, Dichtans J, Wick W. Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. Cancer Res 2001;61:2744-50.

32. Lammering G, Hewit TH, Valerie K, et al. EGFRVIII-mediated radiation resistance through a strong cytoprotective response. Oncogene 2003;22:55-63.

33. Pelloski CE, Lin E, Zhang L, et al. Prognostic associations of activated mitogen-activated protein kinase and Akt pathways in glioblastomas. Clin Cancer Res 2006;12:13935-41.

34. Li B, Yuan M, Kim IA, Chang CM, Bernhard EL, Shu HK. Mutant epidermal growth factor receptor displays increased signaling through the phosphatidylinositol-3 kinase/AKT pathway and promotes radioresistance in cells of astrocytic origin. Oncogene 2004;23:4594-602.

35. Liu Q, Nguyen DH, Dong Q, et al. Molecular properties of CD133+ glioblastoma stem cells derived from treatment-refractory recurrent brain tumors. J Neurooncol 2009;91:1-19.

36. Shih HA, Betensky RA, Dorfman MV, Louis DN, Loefler JS, Batchelor TT. Genetic analyses for predictors of radiation response in glioblastoma. Int J Radiat Oncol Biol Phys 2005;63:704-10.

37. Barker FG 2nd, Simmons ML, Chang SM, et al. EGFR overexpression and radiation response in glioblastoma multiforme. Int J Radiat Oncol Biol Phys 2005;63:704-10.

38. Contessa JN, Hampton J, Lammering G, et al. Ionizing radiation activates Erb-B receptor dependent Akt and p70 S6 kinase signaling in carcinoma cells. Oncogene 2002;21:4032-41.

39. Kim Y, Kim KH, Lee J, et al. Wnt activation is implicated in glioblastoma radiosensitization. Lab Invest 2012;92:666-73.

40. Wild-Bode C, Weller M, Rimmer A, Dichtans J, Wick W. Sublethal irradiation promotes migration and invasiveness of glioma cells: implications for radiotherapy of human glioblastoma. Cancer Res 2001;61:2744-50.

41. Lammering G, Hewit TH, Valerie K, et al. EGFRVIII-mediated radiation resistance through a strong cytoprotective response. Oncogene 2003;22:55-63.