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Development of novel structural related analogs of PFI-3 (SRAPs): Selective BRG1/BRM bromodomain inhibitors

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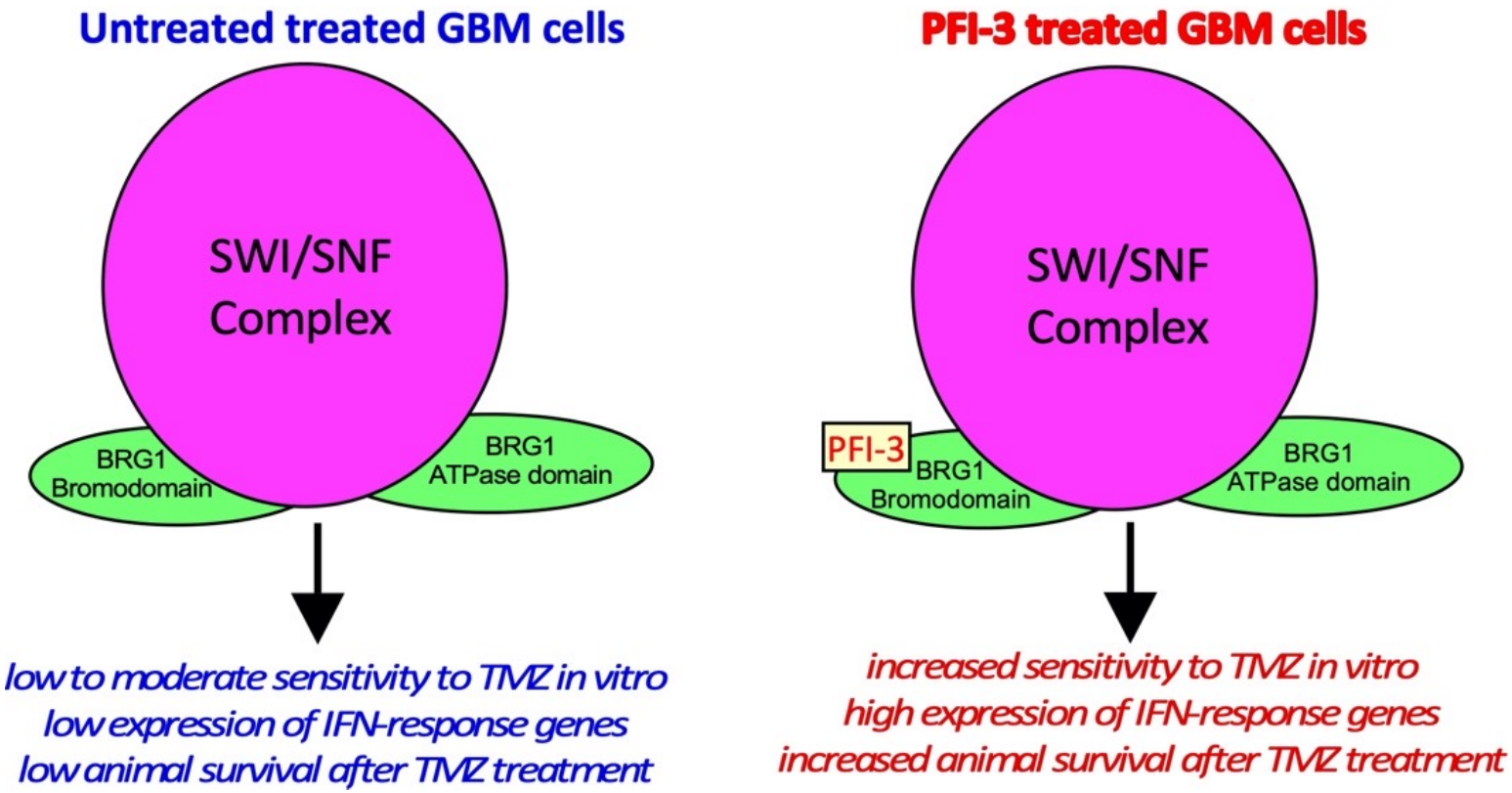
UTHSC CANCER

Development of novel structural related analogs of PFI-3 (SRAPs): Selective BRG1/BRM bromodomain inhibitors

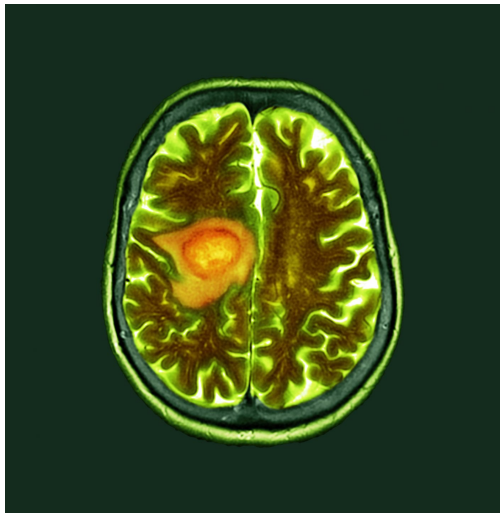


Abstract:
Glioblastoma (GBM) is the most aggressive and treatment-refractory malignant adult brain cancer. After standard of care therapy, the overall median survival for GBM is only ~6 months with a 5-year survival <10%. Although some patients initially respond to the DNA alkylating agent temozolomide (TMZ), unfortunately most patients become resistant to therapy and brain tumors eventually recur. We previously found that knockout of BRG1 or treatment with PFI-3, a small molecule inhibitor of the BRG1 bromodomain, enhances sensitivity of GBM cells to temozolomide *in vitro* and *in vivo* GBM animal models. Those results demonstrated that the BRG1 catalytic subunit of the SWI/SNF chromatin remodeling complex appears to play a critical role in regulating TMZ-sensitivity. In the present study we designed and synthesized **S**tructurally **R**elated **A**nalogs of **P**FI-3 (SRAPs) and tested their bioactivity *in vitro*. Among of the SRAPs, **9f** and **11d** show better efficacy than PFI-3 in sensitizing GBM cells to the antiproliferative and cell death inducing effects of temozolomide *in vitro*, as well as enhancing the inhibitor effect of temozolomide on the growth of subcutaneous GBM tumors.

Keywords: Bromodomain; BRG1; BRG1 bromodomain inhibitors; Glioblastoma (GBM); SWI/SNF; Temozolomide (TMZ)

Introduction

Clinical characteristics of GBM

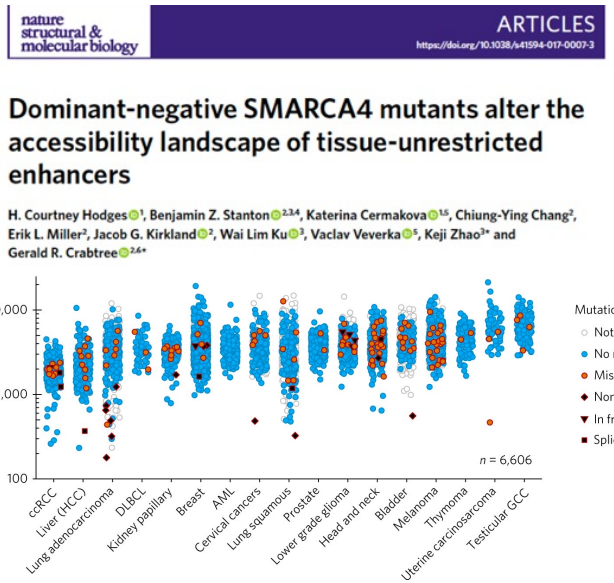


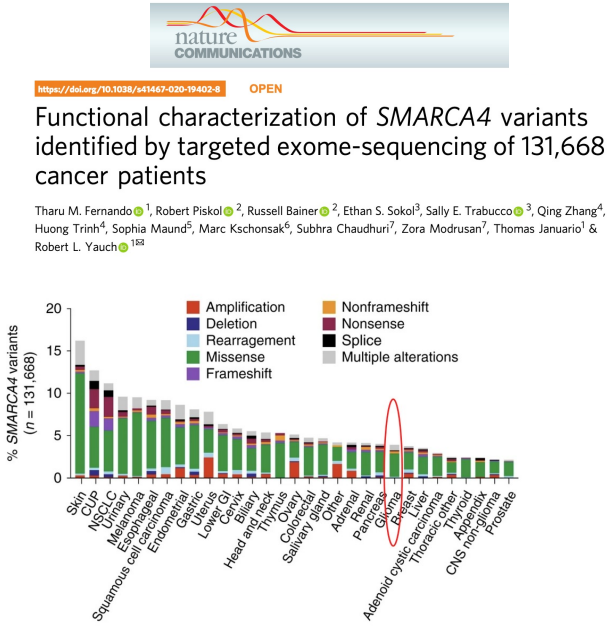
- Glioblastoma (GBM) is the most common and most aggressive form of primary brain cancer in adults
- Median overall survival is 12-14 months from diagnosis.
- Standard of care is surgery with adjuvant radiotherapy and treatment with temozolomide (TMZ), a DNA alkylating agent. This regimen has been the same for ~20 years
- Tumor recurrence is frequent (~80% of patients) and median survival is only 4-5 months
- Despite extensive genomic analysis and introduction of targeted therapies, patient survival in GBM remains relatively unchanged for decades
- Therefore, new therapeutic approaches in GBM are needed

Introduction

The BRG1 subunit of the SWI/SNF complex in tumorigenesis:

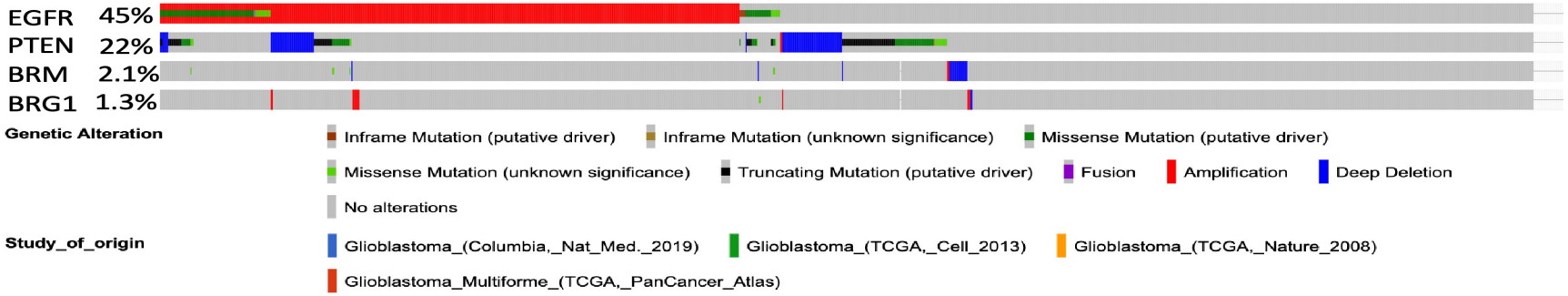
- **The BRG1 (*SMARCA4*) catalytic subunit of the SWI/SNF complex regulates gene expressio differentiation, DNA repair and development**
- **Silencing and loss of function mutations in BRG1 are frequent in certain cancers of the lu ovaries, skin, and blood (Tumor suppressor).**
- **The tumor suppressor action of BRG1 is well described**
- **So BRG1 is always a tumor suppressor!**





Introduction

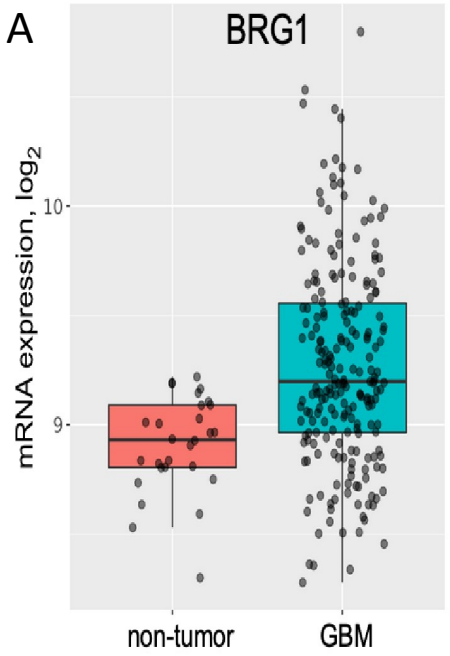
OUR HYPOTHESIS: The BRG1 subunit of the SWI/SNF complex promotes tumorigenesis in GBM.

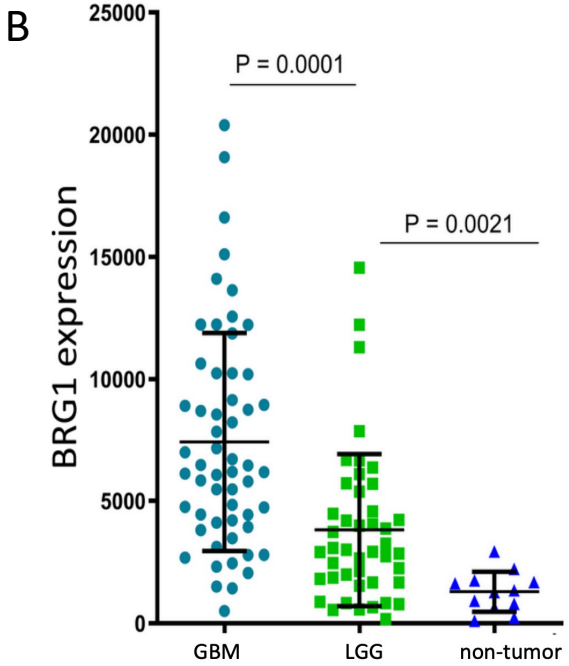


- GBM patient samples were queried for EGFR, PTEN, BRM and BRG1 mutations, deletions and amplifications using the cBioPortal tool.
- Although EGFR and PTEN mutations are common in GBM, BRG1 and BRM mutations are rare

Introduction

Analysis of BRG1 expression in glioma is consistent with BRG1 being pro-tumorigenic



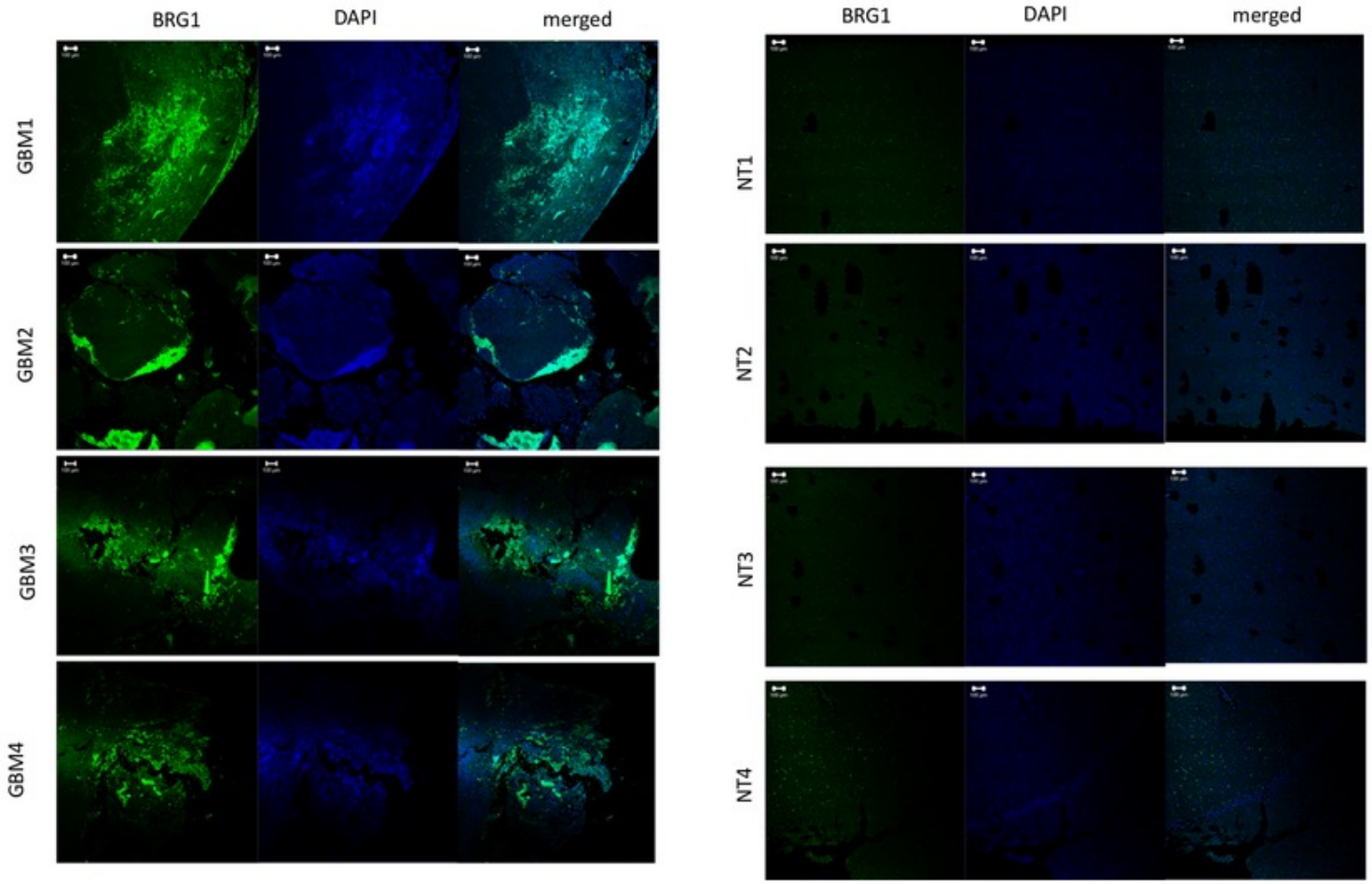


A. BRG1 expression in non-tumor tissue and GBM patient samples in Rembrandt database was compared.

B. RNA was extracted from de-identified patient biopsies from GBM, LGG or normal brain tissue (55, 44 and 12 samples, respectively), and BRG1 expression was determined by qPCR (n = 3) and normalized to actin expression.

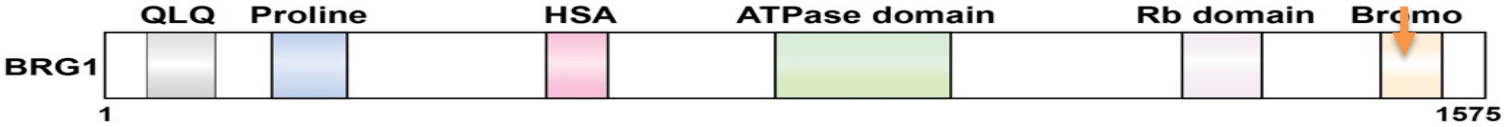
Introduction

Regions of high BRG1 mRNA expression in GBM patient tissue determined by RNA in situ hybridization



Introduction

PFI-3 target



PFI-3 targets BRG1/BRM bromodomains

Journal of Medicinal Chemistry
Article
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Identification of a Chemical Probe for Family VIII Bromodomains through Optimization of a Fragment Hit

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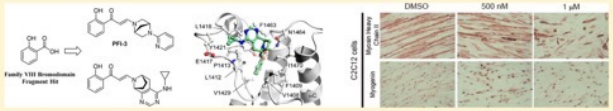
Supporting Information



ABSTRACT: The acetyl post-translational modification of chromatin at selected histone lysine residues is interpreted by an acetyl-lysine specific interaction with bromodomain reader modules. Here we report the discovery of the potent, acetyl-lysine-competitive, and cell active inhibitor PFI-3 that binds to certain family VIII bromodomains while displaying significant, broader bromodomain family selectivity. The high specificity of PFI-3 for family VIII was achieved through a novel bromodomain binding mode of a phenolic headgroup that led to the unusual displacement of water molecules that are generally retained by most other bromodomain inhibitors reported to date. The medicinal chemistry program that led to PFI-3 from an initial fragment screening hit is described in detail, and additional analogues with differing family VIII bromodomain selectivity profiles are also reported. We also describe the full pharmacological characterization of PFI-3 as a chemical probe, along with phenotypic data on adipocyte and myoblast cell differentiation assays.

PFI-3 does not inhibit cancer cell proliferation

Therapeutics, Targets, and Chemical Biology
Cancer Research

The SMARCA2/4 ATPase Domain Surpasses the Bromodomain as a Drug Target in SWI/SNF-Mutant Cancers: Insights from cDNA Rescue and PFI-3 Inhibitor Studies

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Abstract

The SWI/SNF multisubunit complex modulates chromatin structure through the activity of two mutually exclusive catalytic subunits, SMARCA2 and SMARCA4, which both contain a bromodomain and an ATPase domain. Using RNAi, cancer-specific vulnerabilities have been identified in SWI/SNF-mutant tumors, including SMARCA4-deficient lung cancer; however, the contribution of conserved, druggable protein domains to this anticancer phenotype is unknown. Here, we functionally deconstruct the SMARCA2/4 paralog dependence of cancer cells using bioinformatics, genetic, and pharmacologic tools. We evaluate a selective SMARCA2/4 bromodomain inhibitor (PFI-3) and characterize its activity in chromatin-binding and cell-functional assays focusing on cells with altered SWI/SNF complex (e.g., lung, synovial sarcoma, leukemia, and rhabdoid tumors). We demonstrate that PFI-3 is a potent, cell-permeable probe capable of displacing ectopically expressed, GFP-tagged SMARCA2-bromodomain from chromatin, yet contrary to target knockdown, the inhibitor fails to display an antiproliferative phenotype. Mechanistically, the lack of pharmacologic efficacy is reconciled by the failure of bromodomain inhibition to displace endogenous, full-length SMARCA2 from chromatin as determined by *in situ* cell extraction, chromatin immunoprecipitation, and target gene expression studies. Furthermore, using inducible RNAi and cDNA complementation (bromodomain- and ATPase-dead constructs), we unequivocally identify the ATPase domain, and not the bromodomain of SMARCA2, as the relevant therapeutic target with the catalytic activity suppressing defined transcriptional programs. Taken together, our complementary genetic and pharmacologic studies exemplify a general strategy for multidomain protein drug-target validation and in case of SMARCA2/4 highlight the potential for drugging the more challenging helicase/ATPase domain to deliver on the promise of synthetic-lethality therapy. *Cancer Res; 75(18); 3865–78. ©2015 AACR.*

Introduction

Epigenetic dysregulation plays a fundamental role in the development of cancer (1). Large-scale genome sequencing has uncovered recurrent somatic mutations and copy-number (CN) changes in histone-modifying enzymes and chromatin remodeling complexes supporting a causal role for altered epigenetic states in tumorigenesis (2–4). Although the mechanistic consequences of these alterations remain poorly understood, it is appreciated that such events promote acquisition of cell oncogenic capabilities through deregulation of nucleosome-dynamics, gene transcription, DNA replication, and repair (5). Indeed, chromatin regulators are emerging as therapeutic targets and inhibitors of histone-modifying enzymes, as well as bromodomains, which "read" the histone marks, have recently shown efficacy in preclinical and clinical settings through their ability to reverse oncogenic transcriptional programs (6–8).

The Switch/Sucrose Non Fermentable (SWI/SNF) is a multisubunit chromatin remodeling complex that consists of one of two mutually exclusive helicase/ATPase catalytic subunits, SMARCA2 and SMARCA4. Together with core and regulatory subunits, SMARCA2/4 couple ATP hydrolysis to the perturbation of histone-DNA contacts. This sculpting of the nucleosomal landscape at promoters provides access to transcription

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

T.A. Paul and P.K. Shah contributed equally to this article.

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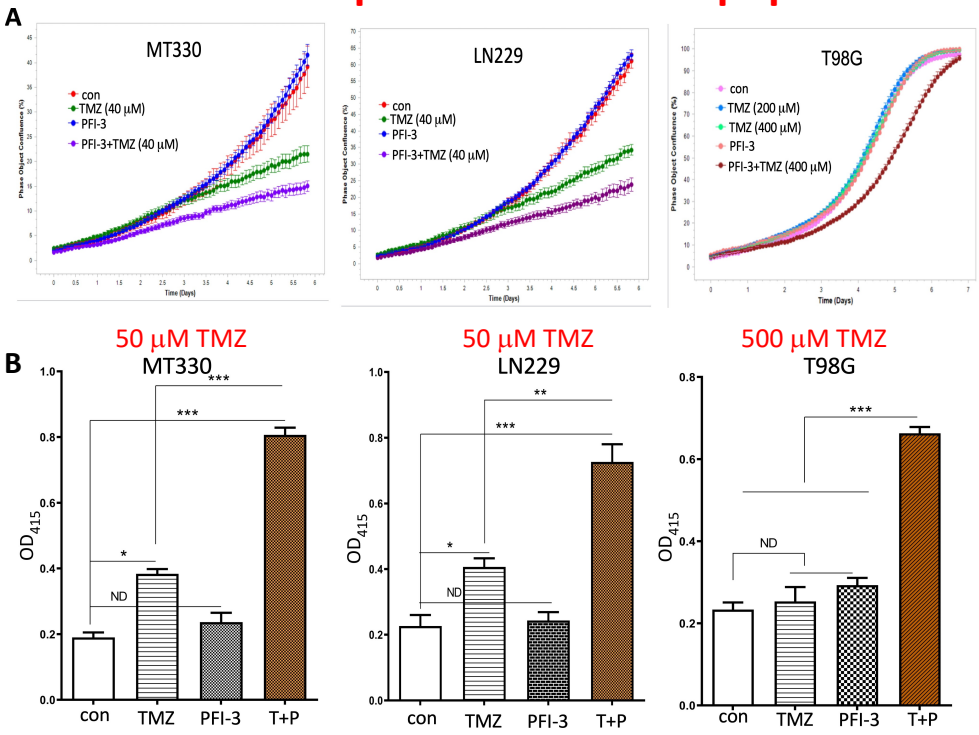
Introduction

PFI-3 sensitizes GBM cells to the antiproliferative and apoptotic inducing action of TMZ

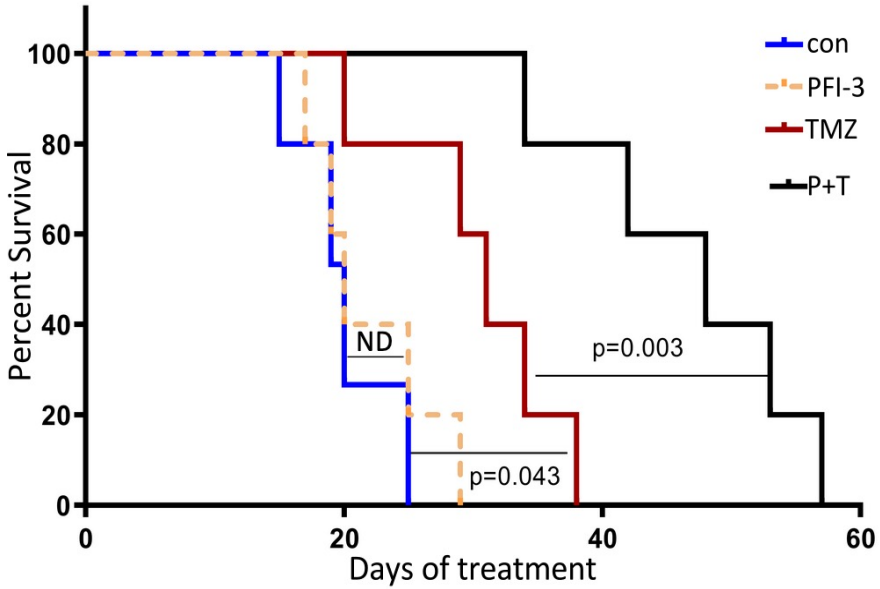


(A) MT330, LN229 and T98G GBM cells were treated with TMZ as indicated and PFI-3 (2 μM), and cell proliferation was determined by Incucyte live cell analysis.

(B) MT330, LN229 and T98G GBM cells were treated with TMZ (50, 50 and 500 μM, respectively) and PFI-3 (2 μM), and apoptosis was determined at 48 hr by a cell death ELISA.

Introduction

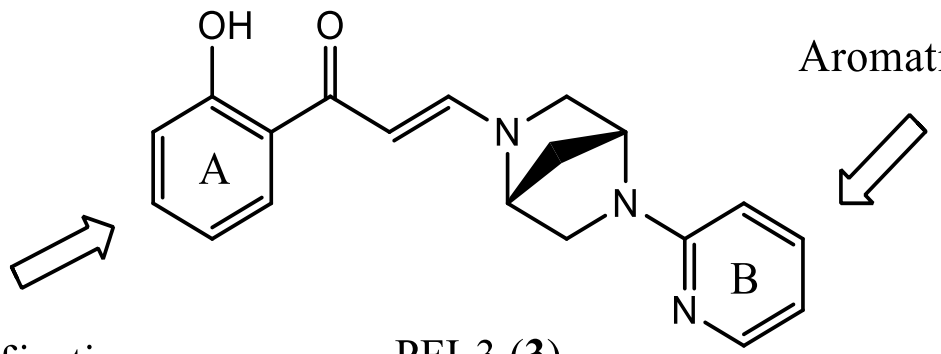
PFI-3 enhances the anticancer activity of TMZ on GBM brain tumors



Luciferase-expressing MT330 cells were injected into the brains of 5-week-old female immunocompromised NSG mice. After tumors were identified by bioluminescence (~10 days after tumor cell injection), mice were intraperitoneally injected thrice weekly with TMZ (60 mg/Kg body weight) or PFI-3 (10 mg/Kg body weight) alone, or in combination. Kaplan-Meier analysis of the survival data (8 mice/group) was performed.

Results and discussion

The general strategy to alter the structure of PFI-3 to produce SRAPS (**S**tructurally **R**elated **A**nalogs of **P**FI-3)



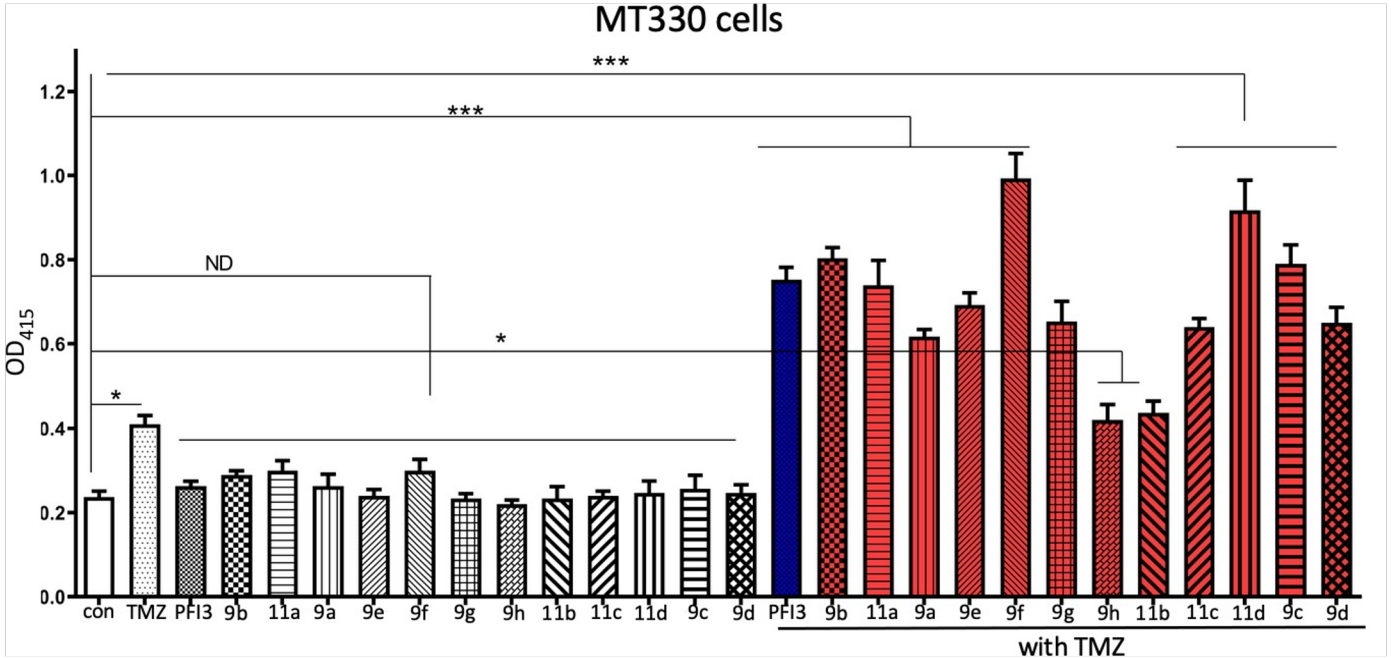
Aromatic B-ring Modification

Aromatic A-ring Modification

PFI-3 (**3**)

Results and discussion

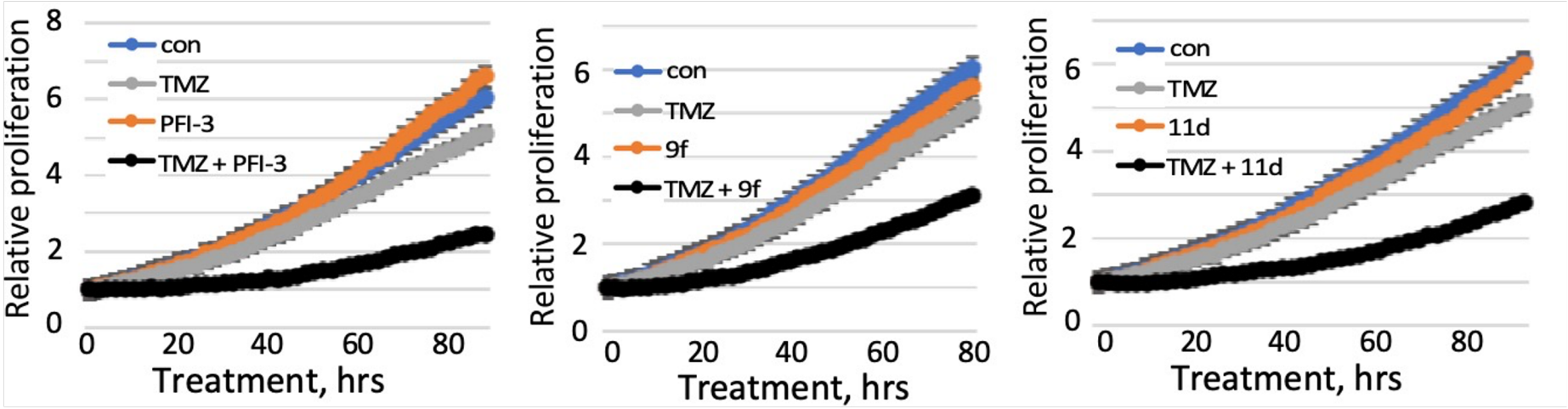
The effect of SRAPs on TMZ-induced apoptosis of GBM cells.



MT330 GBM cells were treated with PFI-3 or the indicated SRAPs alone (2 μM), and with TMZ (50 μM). Apoptosis was determined by a cell death ELISA at 72 hr.

Results and discussion

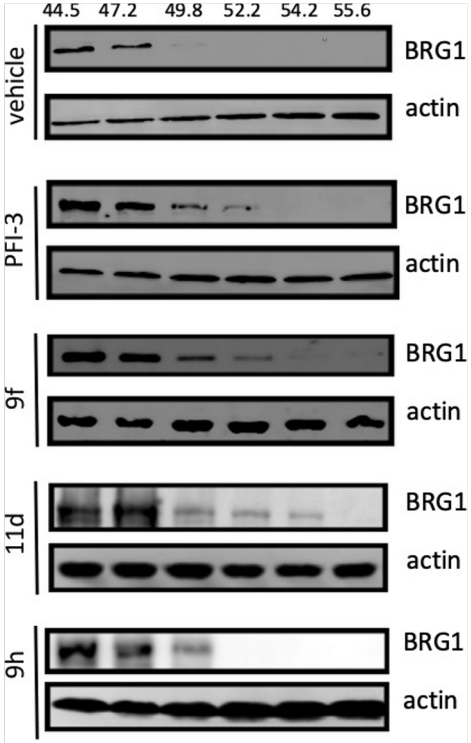
SRAPs sensitize GBM cells to the antiproliferative effect of TMZ



LN229 were treated with TMZ (50 μM), PFI-3 or the SRAPs (2 μM), and cell proliferation was determined by Incucyte live cell assays.

Results and discussion

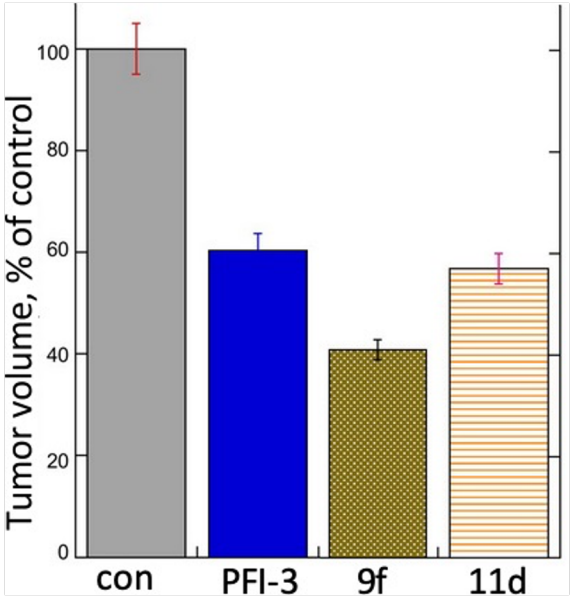
Cellular thermal shift assays (CESTAs) show SRAPs bind to the BRG1 bromodomain



MT330 cells expressing an epitope tagged bromodomain construct of BRG1 were treated with either PFI-3 (30 μM), SRAPs (30 μM), or DMSO for 3 hr. After heating over a temperature range from 44.5 to 55.6 °C for 5 min, the cells were lysed, placed on ice at 4°C and then immunoblotted for BRG1 or actin.

Results and discussion

SRAPs enhance the anticancer activity of TMZ on GBM tumor growth



The effect of TMZ and SRAPs on the tumorigenicity of MT330 cells was assessed by injection of 10^6 tumor cells into the flanks of NSG mice. After confirming tumor initiation by live animal imaging, mice were treated with SRAPs (10 mg/Kg body weight), PFI-3 (10 mg/Kg body weight), or vehicle (con) in combination with TMZ (60 mg/Kg body weight) by intraperitoneal injection on alternate days, and the reduction in tumor volume was calculated at 2 weeks post-treatment.

Results and discussion

We used Cyclica's Ligand Express artificial intelligence and computational biophysics platform to make these predictions about the synthesized SRAPs:

- Likely to penetrate the blood-brain barrier (probability BBB penetrant 0.87 - 0.99)
- Likely to have high intestinal absorption (probability of human intestinal absorption 0.92 - 0.99)
- Unlikely to be mutagenic in the Ames assay (probability Ames positive 0.44 - 0.60)
- Unlikely to interact with the cannabinoid (CNR2) and androgen (ANDR) receptors (probability of interaction 0.33 - 0.65 and 0.12 - 0.60, respectively).
- Likely to have similar solubility to PFI-3
- Likely to interact with SMARCA4/BRG1 in preference to BRM

Conclusions

- We designed and synthesized **S**tructurally **R**elated **A**nalogs of **P**FI-3 (SRAPs)
- We found SRAPs that are better than PFI-3 in sensitizing GBM cells to the antiproliferative and cell death inducing effects of TMZ *in vitro.*
- These SRAPs bound to the bromodomain of BRG1
- SRAPs enhanced the anticancer activity of TMZ on GBM tumor growth.
- SRAPs were predicted to have good drug-like properties

Acknowledgments

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