

News Release

Title

Aberrant active *cis*-regulatory elements associated with downregulation of RET finger protein overcome chemoresistance in glioblastoma

Key Points

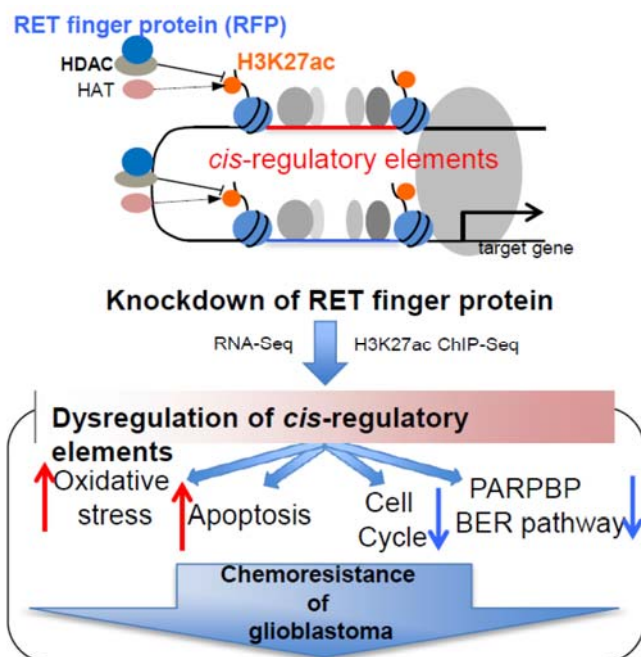
◦RET Finger Protein and histone deacetylase1 make a complex which modulates H3K27 acetylation and *cis*-regulatory elements in glioma cells

◦RET Finger Protein knockdown disrupts the RFP-HDAC1 complex, and alters the determinants of oxidative stress and base excision repair

◦RET Finger Protein knockdown enhances temozolomide chemosensitivity both *in vitro* and *in vivo* in glioblastoma

Summary

Glioblastoma (GBM), the most common primary brain tumor, is the most aggressive human cancers, with a median survival rate of only 14.6 months. Temozolomide (TMZ) is the frontline chemotherapeutic drug in GBM. Drug resistance is the predominant obstacle in TMZ therapy. Drug resistance occurs via multiple pathways. Histone3 Lysin27 residue (H3K27)-acetylation status regulates *cis*-regulatory elements, which increases the likelihood of gene transcription. Histone deacetylase complex (HDAC)s deacetylate lysine residues on core histones, leading to a decrease in gene transcription. In *cis*-regulatory element regions, complexes with HDAC repress histones by H3K27ac deacetylation. RET Finger Protein (RFP) is a protein that is expressed in many kinds of cancer. RFP forms a protein complex with HDAC1. The disruption of the RFP-HDAC1 complex has resulted in increased drug sensitivity in other cancers (Figure 1).



(Figure 1)

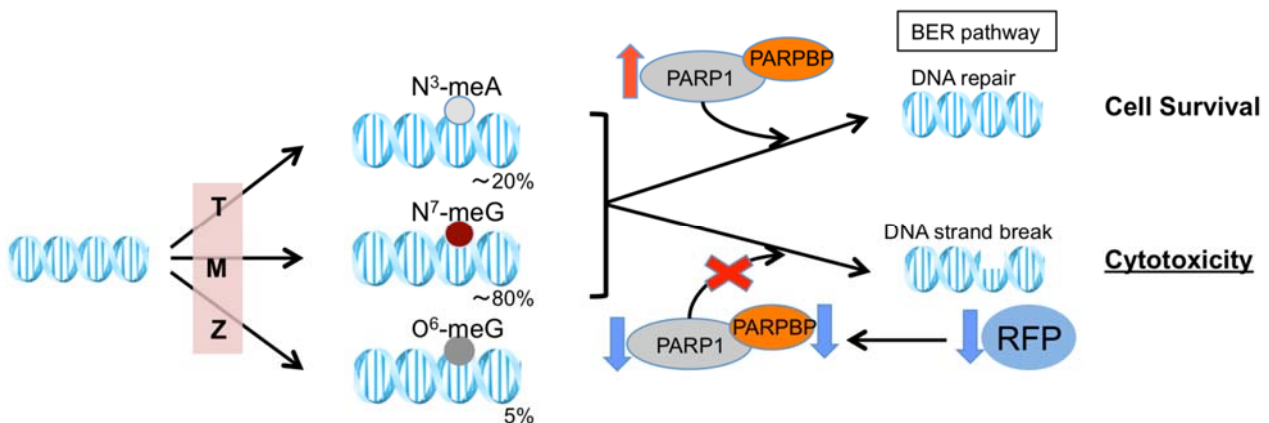
A research teams led by Dr. Atsushi Natsume, Associate professor in the Department of

Neurosurgery (Dean: Kenji Kadomatsu, M.D., Ph.D.) in collaboration with Dr. Takuya Kato, Assistant Professor in the Department of Pathology in Kitasato University School of Medicine (Dean: Toshiyuki Miyashita, M.D., Ph.D.) and Drs. Atsushi Enomoto, Associate professor, and Masahide Takahashi, Professor in the Department of Pathology in Nagoya University School of Medicine, Drs. Melissa Ranjit and Masaki Hirano studied the effects of downregulation of RFP in glioma cells, and its combination with TMZ treatment. RFP depletion with TMZ treatment showed a considerable decrease in glioma cell growth and increased the survival time of intracranial tumor bearing mice. RFP depletion abated numerous cis-regulatory elements and decreased the expression of genes with functions of apoptosis, mitosis, cell cycle and DNA replication. The RNA for PARPBP, a protein that increases TMZ resistance, was also diminished, while those of FOXO1 and TBP-2, both of which enhance oxidative stress, were increased. RFP plays an augmentative role in TMZ resistance by H3K27 deacetylation and disorganization of cis-regulatory elements related to RFP.

RFP shows promise as a suitable target for an effective combination therapy with TMZ.

Research Background

New treatment strategies to improve glioblastoma patient survival have been a pressing need in recent years. MGMT demethylation is a known factor in the resistance of Temozolomide(TMZ), which is the mainstream drug. PARP1 also contributes to TMZ resistance by the BER pathway. PARP-binding protein (PARPBP) is increased in various types of cancer. (Figure 2)



(Figure 2)

The RET Finger Protein and its role in various types of cancer has been explored in recent years. Also known as TRIM27, RFP is a transcription factor that becomes oncogenic upon fusion with RET tyrosine kinase. RFP is involved in cellular processes like cell growth and apoptosis, and is expressed in many types of cancer. RFP forms a tripartite complex along with Histone deacetylase 1(HDAC1) and NF-Y.

It was previously made apparent that the breakdown of the RFP-HDAC1 complex increases the

chemosensitivity of cisplatin in other cancers. The RFP-HDAC1 complex binds to the thioredoxin binding protein-2 promoter, resulting in inhibited TBP-2 expression. Thioredoxin is a protein that lessens oxidative stress by scavenging Reactive oxygen species. Thioredoxin binding protein-2 (TBP-2) binds to thioredoxin, leading to an increase in reactive oxygen species and oxidative stress, and resulting in cell death. But the mechanism and effects of the disruption of the RFP-HDAC1 complex in TMZ sensitivity in glioma remained to be characterized. In this study, we determined whether RFP-KD causes TMZ sensitivity in TMZ-resistant glioma and whether the RFP-HDAC1 complex breakdown causes aberration of H3K27ac- controlled cis-regulatory elements.

Research Results

RFP-KD increases the efficacy of TMZ in RFP-expressing glioma cells.

We performed cell viability assessments of high RFP-expressing TMZ-resistant glioma cell lines T98, U87-MGMT and TGS-01 transfected with siRFP treated with TMZ. We found that the combination of siRFP and TMZ treatment yielded the lowest cell viability. The combined treatment with siRFP and TMZ also had a consistent effect on the survival of mice with the U87-MGMT orthotopic xenograft (Figure 3).

RFP-KD impairs cis-regulatory-elements-mediated regulation of genes related to cell division, the cell cycle, DNA replication and apoptosis

We located regions of enrichment (RoEs) with the marker H3K27ac of active *cis*-regulatory elements via ChIP in U87-MGMT and T98 cells that had undergone RFP-KD. We determined the corresponding gene expression levels by RNA-seq. Upon GO analysis, we found that RFP-KD upregulates apoptosis and downregulates the pathways for cell division, the cell cycle and DNA replication in glioma cells. We also found that the gene expression levels and H3K27ac peaks of thioredoxin binding protein-2 (TBP-2, TXNIP) and FOXO1 were both activated upon RFP-KD, while that of PARPBP was significantly decreased (Figure 4).

RFP and PARPBP expression correlates with poor prognosis in patients with glioma

We analyzed the overall survival of 633 patients with glioma which was stratified by expression of RFP [$\log_2(\text{RPKM} + 0.001)$, cutoff = 10.39 (median)] by means of a Kaplan-Meier plot. Also, we found that PARPBP expression correlates with poor prognosis in patients with glioma [$\log_2(\text{RPKM} + 0.001)$, cutoff = 5.115 (median)] through analysis via a Kaplan-Meier plot. This shows that RFP and PARPBP expression correlates with poor prognosis in patients with glioma (Figure 5).

RFP-KD induces oxidative stress leading to apoptosis and inhibition of cell division.

U87-MGMT cells treated with siRFP and/or TMZ showed fluorescence in a CellROX Orange assay that indicated an increase in oxidative stress. Cell cycle analysis suggested that siRFP

alone decreased the percentage of cells in the S and G2/M phase, and increased the percentage of cells in the G0/G1 phase in U87-MGMT cells. TMZ alone increased the percentage of cells in the G2/M phase. TUNEL assay showed that the combination of siRFP and TMZ induced apoptosis in U87-MGMT cells (Figure 6).

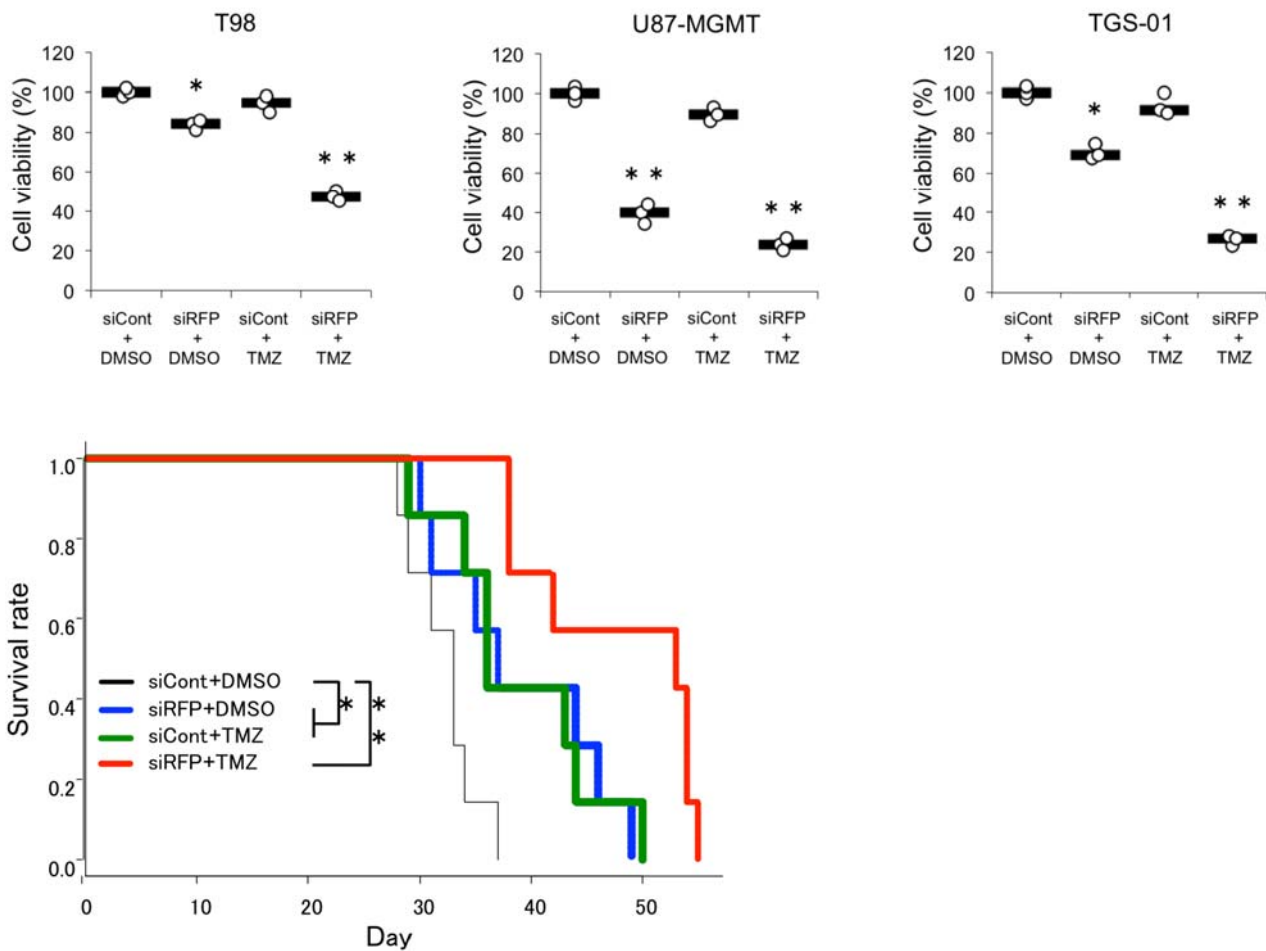


Figure 3. RFP-KD increases the efficacy of TMZ in RFP-expressing glioma cells.

H3K27ac-gained and upregulated adjacent gene : 62 genes	
Upregulated pathways	p-values
GO:0043065: positive regulation of apoptotic process	$10^{-4.49}$
GO:0008637: apoptotic mitochondrial changes	$10^{-3.57}$
GO:0030155: regulation of cell adhesion	$10^{-2.79}$

H3K27ac-lost and downregulated adjacent gene : 293 genes	
Downregulated pathways	p-values
GO:0007059: chromosome segregation	$10^{-10.29}$
R-HSA-1640170: Cell Cycle	$10^{-8.85}$
GO:0006260: DNA replication	$10^{-6.67}$
GO:0071103: DNA conformation change	$10^{-5.66}$

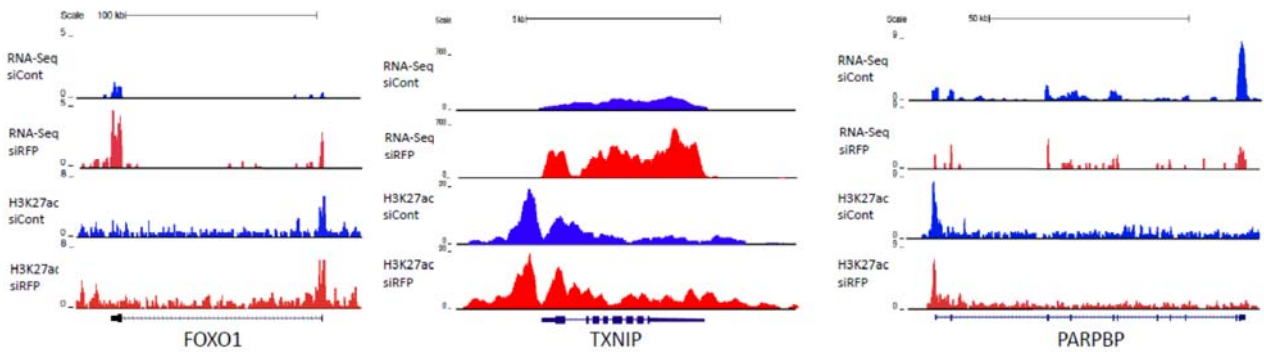


Figure 4. RFP-KD impairs cis-regulatory-elements-mediated regulation of genes related to cell division, the cell cycle, DNA replication and apoptosis

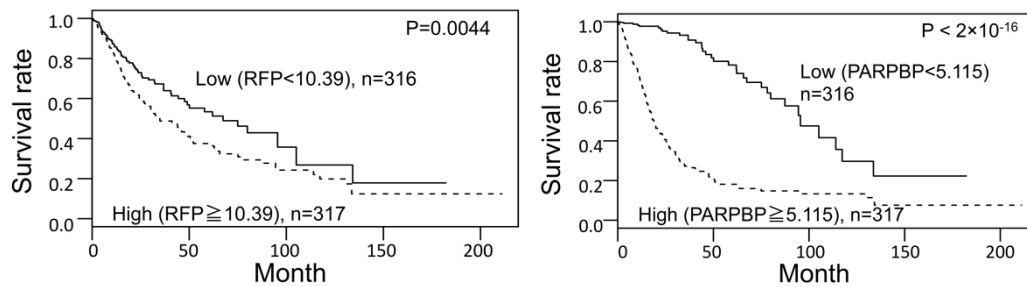


Figure 5. RFP and PARPBP expression correlates with poor prognosis in patients with glioma

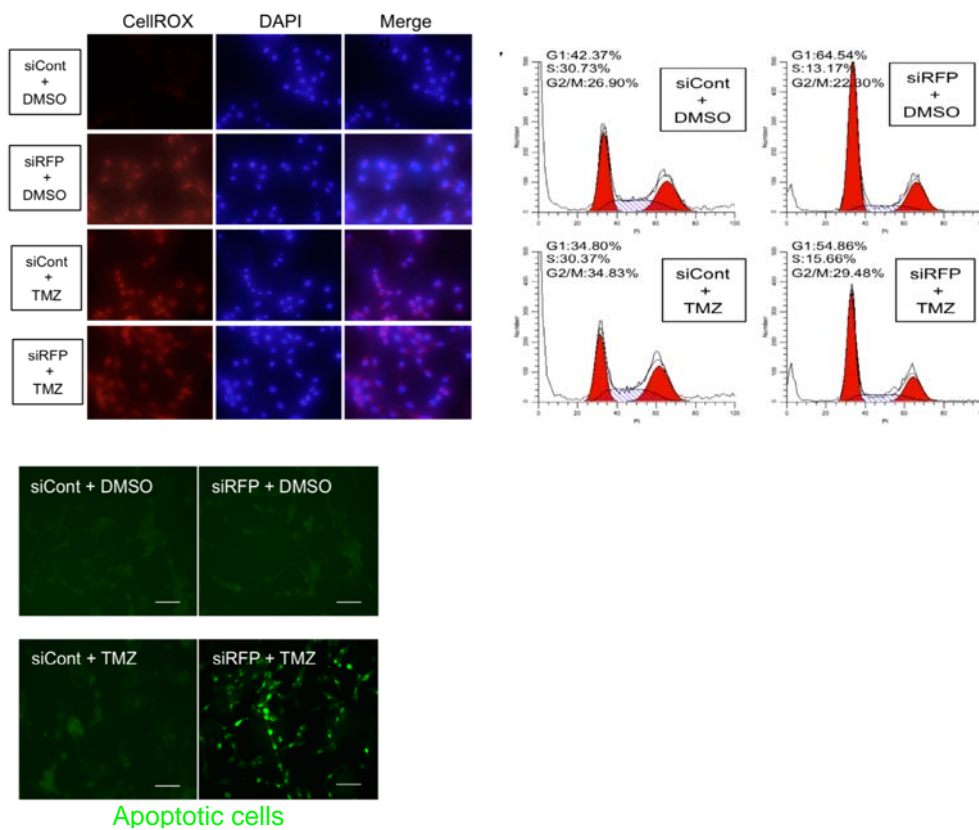


Figure 6. RFP-KD induces oxidative stress leading to apoptosis and inhibition of cell division.

Research Summary and Future Perspective

Downregulation of RFP in glioma cells causes changes in histone H3K27 modification patterns, which further leads to increase in oxidative stress levels, inhibition in cell division and cell cycle, apoptosis, and depletion of genes that abet TMZ resistance. The precise mechanism by which the RFP-HDAC1 complex directly controls the “H3K27ac gain” and upregulated genes, but controls the “H3K27ac loss” and downregulated genes indirectly remains to be explained. Although further studies on some of these changes are necessary, RFP has proven to be a pertinent target in combined therapy with temozolomide in glioblastoma.

Publication

Melissa Ranjit, Masaki Hirano, Kosuke Aoki, Yusuke Okuno, Fumiharu Ohka, Akane Yamamichi, Sachi Maeda, Kazuya Motomura, Keitaro Matsuo, Atsushi Enomoto, Yasushi Ino, Tomoki Todo, Masahide Takahashi, Toshihiko Wakabayashi, Takuya Kato and Atsushi Natsume. Aberrant active cis-regulatory elements associated with downregulation of RET finger protein complex overcome chemoresistance in glioblastoma. *Cell Reports*, published online on February 26, 2019.

DOI : [10.1016/j.celrep.2019.01.109](https://doi.org/10.1016/j.celrep.2019.01.109)

Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Cell_R_20190227.pdf