**Aberrant Transcriptional Regulation of Super-enhancers by RET Finger Protein-histone Deacetylase 1 Complex in Glioblastoma: Chemoresistance to Temozolomide**

Atsushi NATSUME,1 Masaki HIRANO,1 Melissa RANJIT,1 Kosuke AOKI,1 and Toshihiko WAKABAYASHI1

1Department of Neurosurgery, Nagoya University School of Medicine, Nagoya, Aichi, Japan

**Abstract**

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 such as DNA mismatch repair and base excision repair systems, by which glioma cells acquire chemoresistance to some extent (5% and 95%, respectively). Histone3 Lysin27 residue-acetylation (H3K27ac) status regulates cis-regulatory elements, which increases the likelihood of gene transcription. Histone deacetylase (HDAC) complex 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. The cis-regulating and three-dimensional transcriptional mechanism is called “super-enhancer”. 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. We conclude that the downregulation of RFP or the disruption of the RFP/HDAC1 complex leads to an increase in TMZ efficacy in glioblastoma by changing histone modifications which lead to changes in cell division, cell cycle and apoptosis.

Key words: glioma, chemoresistance, super-enhancers, RET finger protein, HDAC1

**Introduction**

Glioblastoma (GBM) is the most aggressive of all brain tumors. Its prognosis is bleak, with a median survival time of 14.6 months. Temozolomide (TMZ) is an oral chemotherapeutic alkylating agent that offers some promise. Nevertheless, it only confers a 5-year survival rate in <10% of cases.1,2 TMZ is a prodrug, administered orally, but activated in the more alkaline environment of the brain tumor tissue.3–5 TMZ induces tumor cell cytotoxicity by methylating genomic DNA. The major site of methylation is at the N3 position of adenine (~20%), the N7 of guanine (~80%) and the O6 of guanine (5%). Acquired resistance to TMZ is a common phenomenon in the patient population.6,7 DNA repair mechanisms such as DNA mismatch repair8–10 and base excision repair (BER)11,12 contribute to TMZ resistance. Temozolomide methylates guanine residues in glioma cells, which results in cell death due to the failure of the DNA mismatch repair system to find a complementary base for methylated guanine. However, MGMT creates a DNA repair system by demethylating the guanine residues methylated by TMZ. Thus, MGMT plays a vital role in TMZ resistance.13 Poly(ADP-ribose) polymerase 1 (PARP1) plays a role in TMZ resistance via BER. PARP1 binding protein (PARPB) or C12orf48 binds directly to PARP1, leading to an increase in PARP1 activity. The expression of PARPB is evidently increased in many types of cancer (Fig. 1). It has been observed that PARP1 inhibitors augment the therapeutic effect of TMZ in glioma.15

In this review article, we describe that how the disruption of histone deacetylase (HDAC) complex affects the status of Histone3 Lysin27 residue-acetylation (H3K27ac)-mediated cis-regulatory elements (super-enhancer), leading to chemoresistance to TMZ.

**Gene expression regulated by super-enhancer**

The variety of DNA regulatory elements in the genome includes silencers, insulators, and enhancer
regions. Those control and maintain gene expression that occurs during mammalian development. Enhancers are particularly important in their regulatory roles that help determine cell fates. They are key cis-regulatory elements that can influence transcription of genes that differ in orientation or are thousands to millions pairs away from promoters and transcriptional start sites.\textsuperscript{16,17} Recent studies have sought to identify enhancers globally by focusing on the histone marker H3-lysine4-monomethylated (H3K4me1), with the aim of identifying many cell type-specific enhancer sites.\textsuperscript{18–21} However, many of the enhancers that are enriched by H3K4me1 have proximal gene transcriptional activity\textsuperscript{22} and many H3K4me1 associated enhancer regions are inactive.\textsuperscript{18,22} Conversely, H3K27ac is able to distinguish between inactive (poised) and active enhancer elements.\textsuperscript{23}

Even though a single enhancer can activate the expression of a nearby gene, high levels of cell type-specific and/or signal-dependent gene expression require enhancers located heterogeneously, with some genes residing in enhancer-rich regions of the genome. Such enhancer-rich regions have recently been termed as “super-enhancers”.\textsuperscript{24–26} Super-enhancers were initially defined as large genomic loci tens of kilobases in length with an unusually high density of enhancer-associated markers, such as binding of the mediator complex, relative to most other genomic loci.\textsuperscript{24,25} These regions can also feature high density and/or extended (>3 KB) depositions of the histone marker H3K27ac. Super-enhancers can be differentiated from regular enhancers on the basis of differences in the density of mediator complex-binding sites or of H3K27ac markers. These differences have revealed the presence of 300–500 super-enhancers in most types of cells.\textsuperscript{24} Many super-enhancers and nearby genes are cell type-specific, and the gene sets that are associated with super-enhancers in a given cell type are highly enriched for the biological processes that define the identities of the cell types (Fig. 2).

In gliomas, tumor malignancy is associated with DNA hypomethylation, including altered cis-regulatory elements and promoter hypomethylation that leads to transcriptional upregulation of genes.\textsuperscript{27} CpG sites are found in gene promoters as well as in gene bodies or cis-regulatory elements.

---

**Fig. 1** MGMT demethylation is a known factor in the resistance of temozolomide (TMZ), which is the mainstream drug. The PARP1–PARP binding protein (PARPB) complex also contributes to TMZ resistance by the BER pathway. Depletion of RET finger protein (RFP) decreases the transcription of PARPB, and destroys the formation of complex, leading to cytotoxicity.

**Fig. 2** Histone3 Lysin27 residue-acetylation (H3K27ac) status regulates cis-regulatory elements, which increases the likelihood of gene transcription. Histone deacetylase (HDAC) complex deacetylate lysine residues on core histones, leading to a decrease in gene transcription. In cis-regulatory element regions (super-enhancer), complexes with HDAC repress histones by H3K27ac deacetylation.
such as enhancers, silencers, and insulators.\textsuperscript{28,29} These regulatory elements contain binding sites for transcription factors and act to increase or decrease transcription. The protein–DNA complex folds a three-dimensional chromatin loop called a topology associated domain (TAD). TADs are regulated by insulators, namely CCCTC-binding factors (CTCFs), which can block interactions between enhancers and promoters in the intergenic regions (Fig. 3). In IDH mutant gliomas, hypermethylation at CTCF binding sites inhibits the binding of CTCF, leading to aberrant enhancer-gene interplays and the upregulation of oncogenes such as platelet-derived growth factor receptor-\textalpha.\textsuperscript{30} The glioma-CpG island methylator phenotype-low subgroup has been speculated to exhibit a loss of genome-wide DNA methylation, including at CTCF binding sites, thereby affecting the chromatin architecture.\textsuperscript{31}

**RET finger protein**

RET finger protein (RFP, also termed as tripartite-motif-containing 27, TRIM27) is a transcription factor that can become oncogenic when fused with RET tyrosine kinase.\textsuperscript{32–34} RFP is involved in cell growth\textsuperscript{35} and apoptosis\textsuperscript{36} and is expressed in diverse types of cancer cells.\textsuperscript{33,37–41} In these cells, RFP forms a tripartite complex with HDAC1 and nuclear transcription factor Y (NF-Y). The knockdown of RFP (RFP-KD) disrupts the complex formed between RFP, HDAC1, and NF-Y, which increases the expression of thioredoxin-binding protein-2 (TBP-2).\textsuperscript{42} These events increase the chemosensitivity of cancer cells to cisplatin (Fig. 4A).

---

**Fig. 3** (A) The protein–DNA complex folds a three-dimensional chromatin loop called a topology associated domain (TAD). (B) TADs are regulated by insulators, namely CCCTC-binding factors (CTCFs), which can block interactions between enhancers and promoters in the intergenic regions. Hypermethylation at CTCF binding sites inhibits the binding of CTCF, thereby affecting the chromatin architecture.

**Fig. 4** RET finger protein (RFP, also termed as tripartite-motif-containing 27, TRIM27) is a transcription factor that can become oncogenic when fused with RET tyrosine kinase. RFP forms a tripartite complex with histone deacetylase 1 (HDAC1) and nuclear transcription factor Y (NF-Y). The knockdown of RFP (RFP-KD) disrupts the complex formed between RFP, HDAC1, and NF-Y, which increases the expression of thioredoxin-binding protein-2 (TBP-2) that is related to reactive oxygen species (ROS) and chemoresistance.
RET finger protein/HDAC1 complex and super-enhancer in GBM in association with cell cycles

RET finger protein is able to form a complex with HDAC1 in GBM cells, similar to Hela cells.41 Destruction of the complex using RFP siRNA can increase the efficacy of TMZ against RFP-expressing glioma cells. To investigate the mode of action, Ranjit et al.43 studied aberrant H3K27ac-controlled active cis-regulatory elements using chromatin immunoprecipitation next-generation sequencing and RNA-seq. The analyses revealed that disruption of the RFP–HDAC1 complex in turn disrupted the functioning of the H3K27ac-controlled active cis-regulatory elements. At first, the authors expected that the depletion of RFP–HDAC1 would lead to the upregulation of H3K27ac in most cis-regulatory-element regions. However, increases in the amount of H3K27ac were detected in some regions while decreases were evident in other regions. The findings implied that the RFP–HDAC1 complex directly controls the “H3K27ac gain” and upregulated genes and indirectly controls the “H3K27ac loss” and downregulated genes.

Gene Ontology analyses indicated that the genes in the “H3K27ac loss” group, whose expression levels significantly decreased, included genes with functions in the cell cycle, cell division, and DNA replication.

RET finger protein/HDAC1 complex and super-enhancer in GBM in association with the BER pathway

RET finger protein binds to the promyelocytic leukemia (PML) gene,44 and PML interacts with the mSin3a-HDAC1 corepressor,45 which in turn inhibits the expression of MGMT.46 Furthermore, RFP is linked with p53 sumoylation,47,48 and p53 downregulates MGMT in astrocytes.48 The PARPBP–PARP1 complex is a key BER agent that causes TMZ resistance. Ranjit et al.43 observed that RFP-KD led to a significant decrease in PARPBP expression. In addition, PARP1 inhibitors sensitize other cancers to antitumor agents by inhibiting the production of thioredoxin and reactive oxygen species.49,50

 Knockdown of RFP disrupts the RFP–HDAC1 complex, which results in the upregulation of Forkhead box1 (FOXO1) and TBP-2 proteins and the subsequent generation of ROS and induction of apoptosis. HDAC1 inhibits FOXO1,51 which induces the expression of TBP-2. Overexpression of TBP-2 results in the inactivation of thioredoxin (TRX) and increased oxidative stress.42 The inhibition of HDAC1 by RFP-KD results in the increased expression of FOXO1 and TBP-2 (Thioredoxin-interacting protein). RFP depletion and TMZ treatment induces oxidative stress in GBM cells (Fig. 4B).

Conclusion

In conclusion, RFP-KD or the disruption of the RFP–HDAC1 complex increases TMZ chemosensitivity of GBM cells by altering the pattern of histone modification, which alters oxidative stress and cell division.

Conflicts of Interest

All authors have no conflicts of interest to declare.

References

1) Stupp R, Mason WP, van den Bent MJ, et al.: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352: 987–996, 2005
2) 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 10: 459–466, 2009
3) Rottenberg DA, Ginos JZ, Kearfott KJ, Junck L, Bigner DD: In vivo measurement of regional brain tissue pH using positron emission tomography. Ann Neurol 15 Suppl: S98–S102, 1984
4) Denny BJ, Wheelhouse RT, Stevens MF, Tsang LL, Slack JA: NMR and molecular modeling investigation of the mechanism of activation of the antitumor drug temozolomide and its interaction with DNA. Biochemistry 33: 9045–9051, 1994
5) Tisdale MJ: Antitumor imidazotetrazines—XV. Role of guanine O6 alkylation in the mechanism of cytotoxicity of imidazotetrazinones. Biochem Pharmacol 36: 457–462, 1987
6) Happold C, Roth P, Wick W, et al.: Distinct molecular mechanisms of acquired resistance to temozolomide in glioblastoma cells. J Neurochem 122: 444–455, 2012
7) Kato T, Natsume A, Toda H, et al.: Efficient delivery of liposome-mediated MGMT-siRNA reinforces the cytotoxicity of temozolomide in GBM-initiating cells. Gene Ther 17: 1363–1371, 2010
8) Liu L, Markowitz S, Gerson SL: Mismatch repair mutations override alkyltransferase in conferring resistance to temozolomide but not to 1,3-bis(2-chloroethyl)nitrosourea. Cancer Res 56: 5375–5379, 1996
9) Hirose Y, Katayama M, Stokoe D, Haas-Kogan DA, Berger MS, Pieper RO: The p38 mitogen-activated protein kinase pathway links the DNA mismatch repair system to the G2 checkpoint and to resistance to chemotherapeutic DNA-methylating agents. Mol Cell Biol 23: 8306–8315, 2003
10) Cahill DP, Levine KK, Betensky RA, et al.: Loss of the mismatch repair protein MSH6 in human glioblastomas is associated with tumor progression during temozolomide treatment. Clin Cancer Res 13: 2038–2045, 2007

Neurol Med Chir (Tokyo) 59, August, 2019
Super-enhancers-mediated Chemoresistance to Temozolomide

11) Tang JB, Svilar D, Trivedi RN, et al.: N-methylpurine DNA glycosylase and DNA polymerase beta mediate BER inhibitor potentiation of glioma cells to temozolomide. *Neuro Oncol* 13: 471–486, 2011

12) Goellner EM, Grimme B, Brown AR, et al.: Overcoming temozolomide resistance in glioblastoma via dual inhibition of NAD+ biosynthesis and base excision repair. *Cancer Res* 71: 2308–2317, 2011

13) Hermisson M, Klumpp A, Wick W, et al.: O6-methylguanine DNA methyltransferase and p53 status predict temozolomide sensitivity in human malignant glioma cells. *J Neurochem* 96: 766–776, 2006

14) Piao L, Nakagawa H, Ueda K, et al.: C12orf48, termed PARP-1 binding protein, enhances poly(ADP-ribose) polymerase-1 (PARP-1) activity and protects pancreatic cancer cells from DNA damage. *Genes Chromosomes Cancer* 50: 13–24, 2011

15) Tentori L, Leonetti C, Scarsella M, et al.: Systemic administration of GPI 15427, a novel poly(ADP-ribose) polymerase-1 inhibitor, increases the anti-tumor activity of temozolomide against intracranial melanoma, glioma, lymphoma. *Clin Cancer Res* 9: 5370–5379, 2003

16) Visel A, Rubin EM, Pennacchio LA: Genomic views of distant-acting enhancers. *Nature* 461: 199–205, 2009

17) Heintzman ND, Ren B: Finding distal regulatory elements in the human genome. *Curr Opin Genet Dev* 19: 541–549, 2009

18) Heintzman ND, Hon GC, Hawkins RD, et al.: Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature* 459: 108–112, 2009

19) Ghisletti S, Barozzi I, Mietton F, et al.: Identification and characterization of enhancers controlling the inflammatory gene expression program in macrophages. *Immunity* 32: 317–328, 2010

20) Heinz S, Benner C, Spann N, et al.: Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 38: 576–589, 2010

21) Kim TK, Hemberg M, Gray JM, et al.: Widespread transcription at neuronal activity-regulated enhancers. *Nature* 465: 182–187, 2010

22) Visel A, Blow MJ, Li Z, et al.: ChIP-seq accurately predicts tissue-specific activity of enhancers. *Nature* 457: 854–858, 2009

23) Creighton MP, Cheng AW, Welstead GG, et al.: Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc Natl Acad Sci USA* 107: 21931–21936, 2010

24) Hnisz D, Abraham BJ, Lee TI, et al.: Super-enhancers in the control of cell identity and disease. *Cell* 155: 934–947, 2013

25) Whyte WA, Orlando DA, Hnisz D, et al.: Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell* 153: 307–319, 2013

26) Dowen JM, Fan ZP, Hnisz D, et al.: Control of cell identity genes occurs in insulated neighborhoods in mammalian chromosomes. *Cell* 159: 374–387, 2014

27) Mazor T, Pankov A, Johnson BE, et al.: DNA methylation and somatic mutations converge on the cell cycle and define similar evolutionary histories in brain tumors, *Cancer Cell* 28: 307–317, 2015

28) Yang X, Han H, De Carvalho DD, Lay FD, Jones PA, Liang G: Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell* 26: 577–590, 2014

29) Singer M, Kosti I, Pachter L, Mandel-Gutfreund Y: A diverse epigenetic landscape at human exons with implication for expression. *Nucleic Acids Res* 43: 3498–3508, 2015

30) Flavahan WA, Drier Y, Liau BB, et al.: Insulator dysfunction and oncogene activation in IDH mutant gliomas. *Nature* 529: 110–114, 2016

31) Malta TM, de Souza CF, Sабedot TS, et al.: Glioma CpG island methylator phenotype (G-CIMP): biological and clinical implications. *Neuro Oncol* 20: 608–620, 2018

32) Reymond A, Meroni G, Fantozzi A, et al.: The tripartite motif family identifies cell compartments. *EMBO J* 20: 2140–2151, 2001

33) Takahashi M, Inaguma Y, Hiai H, Hirose F: Developmentally regulated expression of a human “finger”-containing gene encoded by the 5’ half of the ret transforming gene. *Mol Cell Biol* 8: 1853–1856, 1988

34) Hasegawa N, Ishiwata T, Asai N, et al.: A RING finger motif regulates transforming activity of the rfp/ret fusion gene. *Biochem Biophys Res Commun* 225: 627–631, 1996

35) Shimon Y, Murakami H, Hasegawa Y, Takahashi M: RET finger protein is a transcriptional repressor and interacts with enhancer of polycomb that has dual transcriptional functions. *J Biol Chem* 275: 39411–39419, 2000

36) McNab FW, Rajasbaum R, Stoye JP, O’Garra A: Tripartite-motif proteins and innate immune regulation. *Curr Opin Immunol* 23: 46–56, 2011

37) Tezel G, Nagaoka T, Iwahashi N, et al.: Different nuclear/cytoplasmic distributions of RET finger protein in different cell types. *Pathol Int* 49: 881–886, 1999

38) Isomura T, Tamiya-Koizumi K, Suzuki M, et al.: RFC is a DNA binding protein associated with the nuclear matrix. *Nucleic Acids Res* 20: 5305–5310, 1992

39) Takahashi M, Ritz J, Cooper GM: Activation of a novel human transforming gene, ret, by DNA rearrangement. *Cell* 42: 581–588, 1985

40) Tsukamoto H, Kato T, Enomoto A, et al.: Expression of Ret finger protein correlates with outcomes in endometrial cancer. *Cancer Sci* 100: 1895–1901, 2009

41) Iwakoshi A, Murakumo Y, Kato T, et al.: RET finger protein expression is associated with prognosis in lung cancer with epidermal growth factor receptor mutations. *Pathol Int* 62: 324–330, 2012

42) Kato T, Shimoso Y, Hasegawa M, et al.: Characterization of the HDAC1 complex that regulates...
the sensitivity of cancer cells to oxidative stress. Cancer Res 69: 3597–3604, 2009
43) Ranjit M, Hirano M, Aoki K, et al.: Aberrant active cis-regulatory elements associated with downregulation of RET finger protein overcome chemoresistance in glioblastoma. Cell Rep 26: 2274–2281.e5, 2019
44) Cao T, Duprez E, Borden KL, Freemont PS, Etkin LD: Ret finger protein is a normal component of PML nuclear bodies and interacts directly with PML. J Cell Sci 111 (Pt 10): 1319–1329, 1998
45) Khan MM, Nomura T, Kim H, et al.: Role of PML and PML-RARalpha in Mad-mediated transcriptional repression. Mol Cell 7: 1233–1243, 2001
46) Bobustuc GC, Baker CH, Limaye A, et al.: Levetiracetam enhances p53-mediated MGMT inhibition and sensitizes glioblastoma cells to temozolomide. Neuro Oncol 12: 917–927, 2010
47) Matsuura T, Shimono Y, Kawai K, et al.: PIAS proteins are involved in the SUMO-1 modification, intracellular translocation and transcriptional repressive activity of RET finger protein. Exp Cell Res 308: 65–77, 2005
48) Blough MD, Zlatescu MC, Cairncross JG: O6-methylguanine-DNA methyltransferase regulation by p53 in astrocytic cells. Cancer Res 67: 580–584, 2007
49) Komuro A, Raja E, Iwata C, et al.: Identification of a novel fusion gene HMGA2-EGFR in glioblastoma. Int J Cancer 142: 1627–1639, 2018
50) Yin ZX, Hang W, Liu G, et al.: PARP-1 inhibitors sensitize HNSCC cells to APR-246 by inactivation of thioredoxin reductase 1 (TrxR1) and promotion of ROS accumulation. Oncotarget 9: 1885–1897, 2018
51) Yang Y, Zhao Y, Liao W, et al.: Acetylation of FoxO1 activates Bim expression to induce apoptosis in response to histone deacetylase inhibitor depsipeptide treatment. Neoplasia 11: 313–324, 2009

Address reprint requests to: Atsushi Natsume, MD, PhD, Department of Neurosurgery, Nagoya University School of Medicine, 65 Tsurumai, Showa-ku, Nagoya, Aichi 466-8550, Japan.
e-mail: anatsume@med.nagoya-u.ac.jp