News Release

Title

Identification of a potent therapeutic agent for the treatment of lethal glioma
~ Elucidation of epigenetic mechanisms of tumor formation in a subset of glioma~

Key Points

- Precision medicine is an emerging approach for cancer treatment. Based on the characteristics of each cancer type, the most appropriate treatment was given for each cancer patient.
- Glioma is one of the most frequent brain tumors with dismal prognosis. Even lower-grade gliomas (LGGs) show poor clinical outcome, although they are relatively less aggressive compared to the high-grade gliomas. LGGs are divided into two distinct subgroups based on *IDH* mutation status. Although *IDH*-wild-type LGGs show more aggressive phenotypes than *IDH*-mutated LGGs, lack of knowledge regarding relevant molecular drivers for this type of tumors has hindered the development of therapeutic agents.
- Using mouse model, which spontaneously develops *IDH*-wild-type LGGs, we found that EZH2, a histone methyltransferase, plays pivotal roles in tumor formation. An EZH2 inhibitor (EPZ6438) suppressed growth of human *IDH*-wild-type LGG cell lines and mouse tumors as well.
- Our combined analyses of human LGGs and the mouse model clarify an important molecular pathway and provide a strong rationale for targeting EZH2 as an effective treatment of this type of gliomas.

Summary

Gliomas are classified by combining histopathological and molecular features including isocitrate dehydrogenase (*IDH*) status. Although *IDH*-wild-type lower-grade gliomas (LGGs) show more aggressive phenotype than those with *IDH* mutation, a potent therapeutic agent for treatment of this type of glioma was not identified yet. Here, we examined human *IDH*-wild-type LGGs and a glioma mouse model with a mosaic analysis with double markers (MADM) system, which concurrently lacks *p53* and *NF1* and spontaneously develops tumors highly comparable with a subset of human *IDH*-wild-type LGG. During tumor formation, enhancer of zeste homolog (EZH2), a histone methyltransferase, was upregulated even at an early stage of tumorigenesis, together with an increased number of genes with H3K27me3 modifications which are EZH2-catalyzed histone modifications. Pharmacologic inhibition of EZH2 in MADM mice showed significant reduction of tumor size. An EZH2 Inhibitor also inhibits cell proliferation of human *IDH*-wild-type LGG cell line.

Our study clarifies a pathogenic molecular pathway of a subset of *IDH*-wild-type LGG that depends on EZH2 activity. Clinical trial of an EZH2 inhibitor for patients with hematopoietic malignancy is now ongoing. In near future, an EZH2 inhibitor might be available for various cancer patients. Our study

provides a strong rationale for targeting EZH2 as a promising precision medicine for this type of glioma.

Research Background

Gliomas represent approximately 80% of malignant central nervous system tumors. The World Health Organization (WHO) classification system subdivides diffuse gliomas into grade II to IV based on histopathological findings. Recent comprehensive studies revealed that WHO grade II and III gliomas, called lower-grade gliomas (LGGs), are classified by combining histopathological and molecular features including isocitrate dehydrogenase (*IDH*) status. Mutations in the *IDH* gene have been found in approximately 80% of LGGs, which show a characteristic feature of relatively favorable prognosis, while *IDH*-wild-type LGGs display an aggressive phenotype. The molecular basis underlying the tumorigenesis of the often-devastating *IDH*-wild-type LGG is still largely unclear. Here, we used a glioma mouse model with a mosaic analysis with double markers (MADM) system (MADM mouse model) in which the genetic and gene expression profile and histopathological findings resemble *IDH*-wild-type LGG. In this study, we aim to identify a potent treatment target in *IDH*-wild-type LGG using combined analyses of MADM mouse model and human glioma samples.

Research Results

Among the 116 epigenome-associated genes, that were substantially expressed and altered at a genetic level in human gliomas (n=707) in public database of the Cancer Genome Atlas (TCGA), *Ezh2* was the most upregulated gene in MADM tumors. Expression level of *Ezh2* was consistently highly upregulated from the early precancerous stage to glioma resulting in gradually increased level of H3K27me3 in precancerous cells (Figure 1). Next, we generated *Ezh2*$^{\Delta/wt}$ MADM mice, in which the *Ezh2* catalytic SET domain was heterozygously deleted by the Cre/loxP system. Tumor growth in *Ezh2*$^{\Delta/wt}$ MADM mice was significantly suppressed compared with that observed in *Ezh2* wild-type MADM mice, resulting in significantly prolonged survival (*P<0.05).* Selective EZH2 catalytic inhibitors are reported to be effective against hematopoietic malignancy. Therefore, we examined the effects of pharmacological EZH2 inhibition on MADM tumors by the post-oral (PO) administration of EPZ6438, a

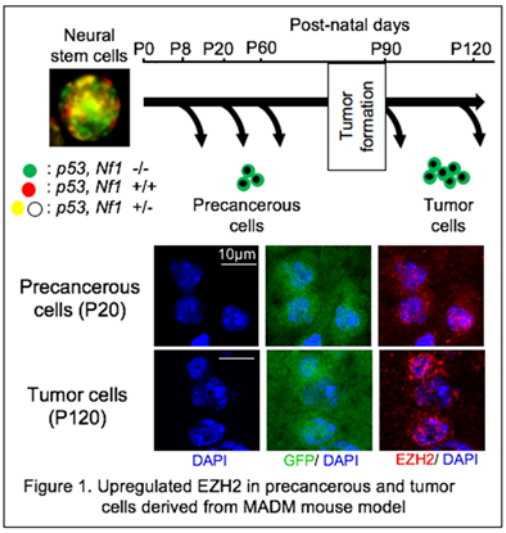

Figure 1. Upregulated EZH2 in precancerous and tumor cells derived from MADM mouse model

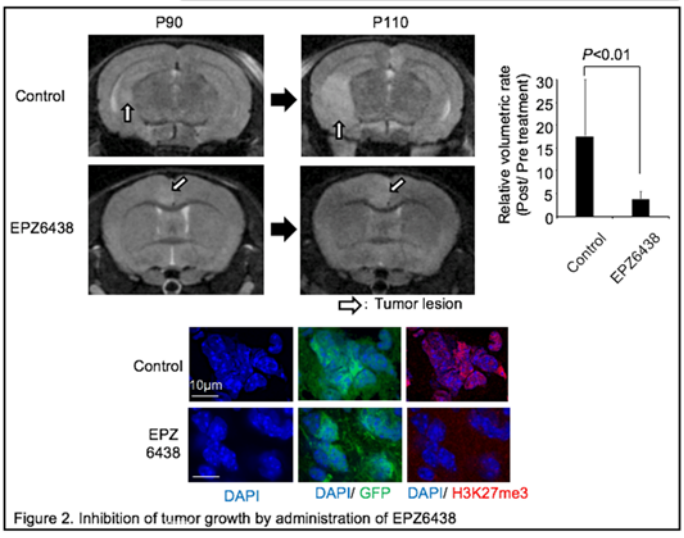

Figure 2. Inhibition of tumor growth by administration of EPZ6438

selective small molecule inhibitor of EZH2. Consistent with the genetic inhibition of *Ezh2*, EPZ6438 treatment significantly suppressed tumor growth compared with controls, along with the downregulation of H3K27me3 in tumor tissues (Figure 2). Of note, EPZ6438 significantly inhibited the cell growth of TM31, a representative human *IDH*-wild-type LGG cell line exhibiting alterations of *p53* and *NF1* genes.

Research Summary and Future Perspective

In the current study, we identified the dysregulation of EZH2 in both human *IDH*-wild-type LGG and a MADM glioma mouse model in which the genetic and gene expression profile and histopathological findings resemble this type of glioma. We aim to perform clinical trial of an EZH2 inhibitor for patients with *IDH*-wild-type LGG exhibiting genetic alterations of *p53* and *NF1* in order to demonstrate that inhibition of EZH2 is a hopeful precision medicine for this type of glioma patients (Figure 3).

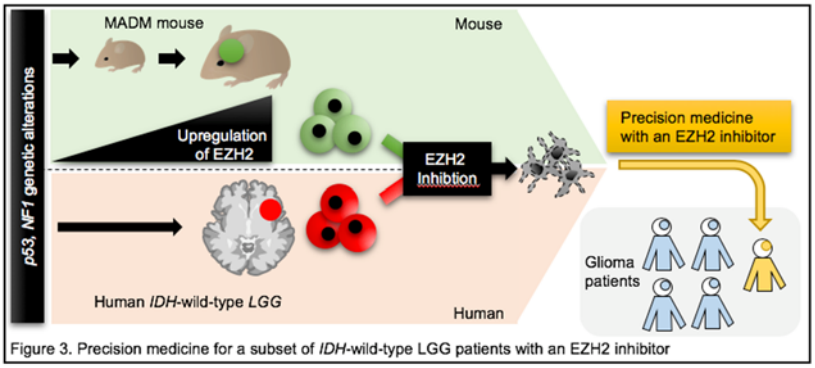

Figure 3. Precision medicine for a subset of *IDH*-wild-type LGG patients with an EZH2 inhibitor

Publication

Fumiharu Ohka[1,2,13], Keiko Shinjo[1,13], Shoichi Deguchi[1,2], Yusuke Matsui[3], Yusuke Okuno[4], Keisuke Katsushima[1], Miho Suzuki[1], Akira Kato[2], Noboru Ogiso[5], Akane Yamamichi[1,2], Kosuke Aoki[2], Hiromichi Suzuki[2], Shinya Sato[6], Nirmala Arul Rayan[7], Shyam Prabhakar[7], Jonathan Göke[7], Teppei Shimamura[8], Reo Maruyama[9], Satoru Takahashi[6], Akio Suzumura[10], Hiroshi Kimura[11], Toshihiko Wakabayashi[2], Hui Zong[12], Atsushi Natsume[2]*, and Yutaka Kondo[1]*.

1.Division of Cancer Biology, Nagoya University Graduate School of Medicine, Nagoya, Japan

2.Department of Neurosurgery, Nagoya University Graduate School of Medicine, Nagoya, Japan

3.Laboratory of Intelligence Healthcare, Nagoya University Graduate School of Medicine, Nagoya, Japan

4.Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, Nagoya, Japan

5.Laboratory of Experimental Animal, National Center for Geriatrics and Gerontology (NCGG), Obu, Japan

6.Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan.

7.Computational and Systems Biology, Genome Institute of Singapore, Singapore, Singapore

8.Division of Systems Biology, Nagoya University Graduate School of Medicine, Nagoya, Japan

9.Project for Cancer Epigenomics, Cancer Institute of Japanese Foundation for Cancer Research, Tokyo, Japan

10.Department of Neuroimmunology, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

11.Cell Biology Center, Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Japan
12.Department of Microbiology, Immunology and Cancer biology, University of Virginia School of Medicine
13.These authors equally contributed to this work
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