Award Number: W81XWH-12-1-0527

TITLE: Function of Brg1 Chromatin Remodeling Factor in Sonic Hedgehog-Dependent Medulloblastoma Initiation and Maintenance

PRINCIPAL INVESTIGATOR: Xuanming Shi

CONTRACTING ORGANIZATION: University of Texas, Southwestern Medical Center at Dallas Dallas, Texas 75390-9133

REPORT DATE: October 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
## 4. TITLE AND SUBTITLE

Function of Brg1 Chromatin Remodeling Factor in Sonic Hedgehog-Dependent Medulloblastoma Initiation and Maintenance

## 6. AUTHOR(S)

Xuanming Shi, Jiang Wu

E-Mail: Xuanming.Shi@UTsouthwestern.edu

## 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Texas, Southwestern Medical Center at Dallas, Dallas, Texas 75390-9133

## 14. ABSTRACT

Medulloblastoma is the most common malignant pediatric brain tumor. Overactive Shh signaling in cerebellum granule neuron precursors (CGNPs) is the leading cause of the childhood medulloblastoma (Shh-subtype). Previously we showed that chromatin remodeler Brg1 deletion resulted in reduced proliferation of CGNPs in developing cerebellum due to impaired Shh-activated target gene expression. Current study focuses on the requirement of Brg1 in mouse model of Shh-subtype medulloblastoma. Evidences showed that Brg1 is required for SmoM2-dependent CGNP mitogenic target gene expression and proliferation in cultures. Through conditional knockout Brg1 in primary cultured medulloblastoma cells, tumor growth was inhibited. Induction of Brg1 deletion in subcutaneous transplantation led to tumor aggression significant blocked. qRT-PCR and Western Blot showed that Shh-dependent mitotic target genes are decreased by knockout of Brg1. Systematic analyses of how BAF complexes regulate tumor growth will be performed to uncover the mechanism of medulloblastoma development at chromatin level. These studies will provide insights for drug development and therapy of pediatric brain tumor and other Shh-dependent tumors.

## 15. SUBJECT TERMS

Medulloblastoma, Sonic Hedgehog, Chromatin remodeling, BAF complex, Brg1, mouse model of shh-subtype medulloblastoma
# Table of Contents

**Introduction** .............................................................................................................................. 4

**Body** ............................................................................................................................................... 6

  Aim1. Determine function of Brg1 in SmoM2-induced medulloblastoma formation .................. 6

  Aim2. Determine function of Brg1 in SmoM2-dependent tumor progression and maintenance......... 7

  Aim3. Identify Brg1/BAF interacting co-activators of Shh signaling in medulloblastoma........... 9

**Key Research Accomplishments** ..................................................................................................... 11

**Reportable Outcomes** ....................................................................................................................... 12

**Conclusions** ................................................................................................................................... 13

**References** ..................................................................................................................................... 14

**Appendices** .................................................................................................................................. 15
Introduction

Brain tumors are the leading cause of cancer-related death in children, and medulloblastoma is the most common malignant pediatric brain tumor (1). Although overall survival rates have improved in recent years, the mortality rate remains significant. Hence, new insights into the molecular mechanisms controlling medulloblastoma development are essential for improving clinical trial design, and developing molecularly targeted therapies (2). Shh signaling pathway plays important roles in many development processes and adult homeostasis (3-6). Elevation of Shh target gene expression has been associated with the initiation and/or maintenance of a large spectrum of cancer types, among which medulloblastoma is one of the most well-known Shh-dependent cancer type (3, 7-9). During early postnatal cerebellum development, Shh is required for CGNP proliferation (10-12). However, overactive Shh pathway causes CGNP over-proliferation and medulloblastoma (7, 13, 14). Among all the genetic defects, mutations resulting in an overactive Shh signaling in cerebellum granule neuron precursors (CGNPs) are the leading cause of the childhood medulloblastoma and are responsible for ~25% of occurrences (Shh-subtype) (2). Shh signaling pathway mediated by Patched (Ptc h1) and Smoothened (Smo) controls target gene expression by differentially regulating activity of Gli family of transcription factors (3, 4, 12). The regulation of mitogenic target genes by Shh/Gli in cerebellum is critical for CGNP proliferation and medulloblastoma formation.

Mammalian SWI/SNF like BAF (Brg1/Brm associated factors) chromatin remodeling complexes regulate transcription by modulating chromatin structures (15, 16). It has been shown that depending on the tissue contexts, BAF complexes can either promote or suppress tumor development by regulating different sets of target gene transcription in a context-dependent manner (17-20). Recently, we have shown that Brg1, the core subunit of BAF complexes, interacts with Gli transcription factors and is required for activating Shh-induced target gene transcription. Brg1-deletion resulted in reduced proliferation of CGNPs in developing cerebellum due to impaired Shh-activated target gene expression, indicating that Brg1 is required for Shh-dependent CGNP proliferation (21). Thus I hypothesize that Brg1 is required for Shh-subtype medulloblastoma growth and progression. In the study I use SmoM2-mouse model and
breed with Brg1 conditional knockout allele to test the hypothesis. Those studies for molecular mechanism of medulloblastoma growth at chromatin level will provide insights for drug development and therapy of pediatric brain tumor and other Shh-dependent tumor.
**Body**

**Aim1. Determine function of Brg1 in SmoM2-induced medulloblastoma formation**

Two subaims in this part are to determine 1) the function of Brg1 in SmoM2-dependent Shh target gene expression and CGNP proliferation, and 2) Brg1 function in SmoM2-dependent medulloblastoma formation.

Using an inducible mouse model of medulloblastoma with a SmoM2-YFP mutant gene (a point mutation in Smoothened) knocked-in at the Rosa26 locus downstream of a LoxP-flanked stop signal (22), and an inducible Actin-CreER transgene, as well as a conditional Brg1 null allele, we bred different genotypes: wt, Brg1 \(^{ikO}\), Smo, and Smo Brg \(^{ikO}\), to determine Brg1 function in CGNPs. We have previous shown that cultured SmoM2 CGNPs display increased expression of Gli1 (the most faithful and sensitive Shh target gene) compared to wild-type cultures. Conditional knockout of Brg1 decreased Gli1 protein level, and CGNP proliferation indicated by a mitotic marker phosphorylated histone 3 (H3P) (data not shown). Currently we found Brg1 deletion reduced the SmoM2-dependent mitogenic target gene expression. In contrast, CGNP marker Math1 was not changed (Figure 1). These experiments suggested that Brg1 is required for SmoM2-induced Shh target gene expression and CGNP proliferation.

![Figure 1](image)

**Figure 1** Brg1-deletion led to reduced SmoM2-dependent Shh target gene expression in CGNP cells using qRT-PCR analysis. Student’s t-test: *, \(P<0.05\); **, \(P<0.01\).

To determine the function of Brg1 in SmoM2-dependent medulloblastoma formation, we bred above-mentioned mice but using Nestin-creER. One injection of tamoxifen is expected to induce the expression of SmoM2 and deletion of Brg1. However, since activity of CreER system depends on the Cre expression level and tamoxifen delivery efficiency, deletion of Brg1 and expression of SmoM2 may occur in a mosaic pattern. The resulting tumor formation rate and survive curve from Brg1 \(^{+/+}\) and Brg1 \(^{F/F}\) mice tended to be different but
not significant (data not shown). Interestingly our preliminary data showed Brg1 +/- heterozygote may have a different phenotype comparing to Brg1 +/- in SmoM2-dependent target gene expression and tumor growth. As an alternated plan, we are breeding Brg1 +/- Nestin-CreER SmoM2 mice to analyze the function of Brg1 in SmoM2-dependent medulloblastoma initiation and formation.

**Aim2. Determine function of Brg1 in SmoM2-dependent tumor progression and maintenance.**

In this part we have determined the roles of Brg1 in primary cultured medulloblastoma and in tumor progression by allograft transplantation.

Development of Shh-dependent medulloblastoma requires an active Shh pathway for maintenance and progression. It has been reported that 40% of SmoM2, Actin-CreER mice develop medulloblastoma due to leakage of the CreER activity (22). Indeed we have observed the occurrence of similar tumors in the Brg1 iKO SmoM2 Actin-CreER mice without tamoxifen induction. However, the weak Cre activity without tamoxifen is not sufficient for Brg1 deletion (data not shown).

**Figure 2** Brg1-deletion in cultured SmoM2-MB decreased mitogenic target gene expression and tumor growth A) Gli1 protein level decreased by knockout of Brg1 in cultured MB cells showed by Western blot. B) qRT-PCR analysis showed the decrease of mitogenic target genes at the mRNA in cultured MB cells. C) ATP viability assay of MB cultures treated with tamoxifen for 3 days. Student’s t-test: *, P<0.05; **, P<0.01.

To determine the role of Brg1 in medulloblastoma growth, we first in vitro cultured medulloblastoma cells formed due to Cre leakage in Brg1 iKO SmoM2 Actin-CreER mice at P60. 4-hydroxy tamoxifen (4OHT) was added to the culture to induce Brg1 deletion (Figure 2A). After 3 days in culture, Brg1 was effectively deleted in 4OHT-treated cultures. Deletion of Brg1 led to significant reduction of Gli1, Ptch1 expression (Figure 2A, B), and the
mitogenic target gene *CcnD1* in medulloblastoma cultures (Figure 2B). Deletion of *Brg1* also inhibited medulloblastoma growth as shown by an ATP viability assay in the cultured cells (Figure 2C).

![Figure 3](image)

**Figure 3** *Brg1* is required for tumor progression by subcutaneously transplantation in SCID-NOD mice. Tumor A) and the genotyping B) dissected from transplanted mice injected with tamoxifen 3 days after transplantation.

To determine the requirement of *Brg1* for *SmoM2* medulloblastoma allograft tumor formation ability, freshly prepared small tumor pieces of *Brg*<sup>F/+</sup> *SmoM2 Actin-CreER* was injected/transplanted subcutaneously into the flank regions of immunodeficient SCID-NOD mice. Three days after transplantation, the recipient SCID-NOD mice were injected with tamoxifen every other day for 10 times to induce *Brg1* deletion in allograft tumors. The *Brg*<sup>F/+</sup> tumor size after injection of tamoxifen (Tam, (Figure 3A)) was significant smaller than the control in which oil was injected. In contrast, no such changes were found in *Brg*<sup>+/+</sup> tumor transplantation (Figure 3A). The genotyping of these tumors further confirmed the transplanted tumor type and deletion of one allele *Brg1* after injection of tamoxifen (Figure 3B). These data together indicated *Brg1* reduction inhibited medulloblastoma progression and maintenance.

To gain enough tissue for further analysis such as target gene expression or immunostaining, tamoxifen was injected after tumor is obvious, which allows to study *Brg1* function and the relevant mechanism in allograft tumor growth and progression. As shown in Figure 4A, *Brg1* deletion significantly decreased Gli1 protein level, as well as mitogenic target genes *CcnD1* and *N-Myc* (Figure 4B).
Taken together, Brg1 is required for medulloblastoma progression and maintenance by regulating Shh-target genes. Since loss of Brg1 in one allele results in significant growth reduction in tumor transplantation, we will determine the function of Brg1 in primary tumor from Brg1 F/+, SmoM2, ActinCreER mice.

**Aim3. Identify Brg1/BAF interacting co-activators of Shh signaling in medulloblastoma.**

Our previous studies have suggested that BAF complex activates Shh-induced transcription by recruiting other unidentified co-activators to Shh target genes. To understand the mechanisms underlying Brg1 function in Shh target gene activation and Shh-dependent medulloblastoma, we proposed to use a proteomic approach (23) to identify BAF-interacting proteins in Shh-activated medulloblastoma. The BAF-interacting co-activators will be good candidates for mediating Shh-induced gene activation and for Shh-dependent tumor formation. We confirmed interaction between Brg1 and Gli1 in the medulloblastoma tissue (Figure 5), indicating the tissue is a feasible resource to probe the interaction proteins important in medulloblastoma development.

In addition, recently we found that an H3K27me3 demethylase Jmjd3 is required for Shh signaling pathway in neural development and medulloblastoma growth through modulating histone modification. It was reported that Brg1 interacts with Jmjd3 to regulate target gene expression (24). Hence, studies on how Brg1 cooperates with histone modifiers, such as Jmjd3, to regulate Shh target genes will help us to understand molecular mechanism of medulloblastoma development.
In summary, Brg1 is required for SmoM2-dependent CGNP mitogenic target gene expression and proliferation in cultures. Through conditional knockout Brg1 in primary cultured medulloblastoma cells, tumor growth was inhibited. Induction of Brg1 deletion in subcutaneous transplantation led to tumor aggression significant blocked. qRT-PCR and Western Blot showed that Shh-dependent mitogenic target genes are decreased by knockout of Brg1. Systematic analyses of how BAF complexes regulate tumor growth will be performed to uncover the mechanism of medulloblastoma development at chromatin level.
Key Research Accomplishments

- Brg1 is required for SmoM2 (gain-of-function mutation) dependent CGNP mitogenic target gene expression and proliferation.
- Brg1 is required for SmoM2 dependent medulloblastoma growth in primary culture.
- Deletion of Brg1 inhibits medulloblastoma progression in subcutaneous transplantation.
- Deletion of Brg1 in subcutaneous transplantation decreases mitogenic target gene expression.
- Brg1 physically interacts with Gli1 in medulloblastoma cells, which is important for tumor growth.
Reportable Outcomes


Conclusions

We have confirmed hypotheses that the chromatin remodeler Brg1 is required for SmoM2-dependent mitogenic target gene expression, and cerebellum granular neural precursor proliferation. We showed the evidence that Brg1 is required for medulloblastoma growth in primary culture. Importantly, deletion of Brg1 in subcutaneous transplantation inhibits medulloblastoma progression, possibly through decreasing the mitogenic target genes. To further understand how BAF complexes regulate medulloblastoma development, RNA-seq, and ChIP-seq for Brg1 and histone modifications may systematically uncover the underlying mechanism.
References

Appendices

1. Curriculum Vitae.

Biographical Sketch

Provide the following information for each individual included in the Research & Related Senior/Key Person Profile (Expanded) Form.

<table>
<thead>
<tr>
<th>NAME</th>
<th>POSITION TITLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>XUANMING SHI</td>
<td>POSTDOCTORAL RESEARCHER II</td>
</tr>
</tbody>
</table>

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training).

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (IF APPLICABLE)</th>
<th>YEAR(S)</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anhui University, Hefei, China</td>
<td>B.S.</td>
<td>1999</td>
<td>Biochemistry</td>
</tr>
<tr>
<td>Sichuan Agricultural University &amp; Institute of Microbiology, Chinese Academy of Science, China</td>
<td>M.S.</td>
<td>2005</td>
<td>Biochemistry &amp; Molecular Biology</td>
</tr>
<tr>
<td>University of Rostock &amp; Research Institute for the Biology of Farm Animals, Germany</td>
<td>Ph.D.</td>
<td>2009</td>
<td>Molecular Biology</td>
</tr>
<tr>
<td>University of Texas Southwestern Medical Center at Dallas</td>
<td>Postdoctoral</td>
<td>2010</td>
<td>Developmental Biology</td>
</tr>
</tbody>
</table>

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List in chronological order the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 4 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INDIVIDUAL.

Positions and Employment

1999-2001 Assistant Engineer, BBCA Biochemistry Group Company, Bengbu, China.
2001-2002 Technician, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.
2002-2005 Graduate student, Sichuan Agricultural University and Institute of Microbiology, Chinese Academy of Sciences. Mentor: Prof. Dr. Keqian Yang & Prof. Dr. Yubi Huang
2005-2009 PhD student, University of Rostock & Research Institute for the Biology of Farm Animals, Germany. Mentor: Prof. Dr. Hans-Martin Seyfert
2010- Postdoctoral researcher, UT Southwestern Medical Center at Dallas, Texas. Mentor: Dr. Jiang Wu
HONORS AND AWARDS


Professional Associations/Affiliations

American Association of Cancer Research 2013

Peer-reviewed Publications (in chronological order)


A signaling induced epigenetic complex regulates Sonic hedgehog dependent development and tumor proliferation by modulating the bivalent chromatin domain

Xuanming Shi, Zilai Zhang, Xiaoming Zhan, Yu Chen, Takashi Satoh, Kai Ge, Jiang Wu

1Department of Physiology and Developmental Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390, 2Osaka University, Japan, 3NIDDK, Bethesda, MD

The Sonic hedgehog (Shh) signaling pathway plays important roles during development and adult homeostasis. Mutations in the Shh signaling are associated with developmental diseases and cancers. Shh signaling elicits diverse biological responses by differentially regulating the transcription outcomes mediated by Gli family of transcription factors. The mechanisms underlying Gli-mediated gene expression remain elusive; it is not clear how Gli proteins activate transcription in response to Shh. Previous work from my lab shows that a complex chromatin environment is critical for Shh signaling output. We discovered that prior to their induction, all known Shh target genes are marked by a bivalent chromatin domain containing a repressive H3K27me3 mark and an active H3K4me3 histone modification, which reflects the poised states of Shh target genes. The removal of the repressive H3K27me3 mark and the increase of the active H3K4me3 mark correlate with Shh target gene activation. Importantly, we showed that the H3K27me3 demethylase Jmjd3 controls the chromatin status of the bivalent domain and is crucial for Shh-induced gene activation. Interestingly, activation of gene expression by Jmjd3 also requires its non-enzymatic activities. Shh treatment significantly enhanced the interaction between Jmjd3 and MLL complex, the methyltransferase responsible for adding the H3K4me3 active mark. Thus the resolution of the bivalent domain by Jmjd3 and MLL in response to Shh are required for Shh-induced gene activation. Using lose of function analyses, we have demonstrated that Jmjd3 is required for Shh-dependent normal cerebellum development and proliferation of Shh-type medulloblastoma cancer cells. Our study may provide new therapeutic targets for treating Shh-related cancers.