ウェブサイト公開用

（様式 10）
（海外特別研究員事業）

令和　5　年　11　月　25　日

海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人日本学術振興会 理事長　殿

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受 付 番 号　202160042
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海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：　アラバマ州バーミングハム　（国名：　米国　）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
悪性脳腫瘍に対するエピジェネティクスを標的とした新規治療法の開発

3. 派遣期間：令和　3　年　4　月　1　日　～　令和　5　年　9　月　4　日（　887　日間）

4. 受入機関名及び部局名
受入機関名：　アラバマ大学バーミングハム校
部局名：　脳神経外科

5. 所期の目的の遂行状況及び成果…書式任意　**書式任意（A4 判相当 3 ページ以上、英語で記入も可）**

【記載事項】
- 研究・調査実施状況及びその成果の発表・関係学会への参加状況等
- 新型コロナウイルス感染症の影響にかかる特例措置のうち、国内採用開始・採用期間延長・翌年度渡航のいずれかの適用を受けた場合は、当該措置の適用による影響等

（注）「6.研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

（はじめに）
採択された研究計画書のとおり、KCC-07 およびその compounds による epigenetic 治療の開発のため、これらの薬剤による BAI1 発現の活性効果および抗腫瘍効果を調査した。予想に比して BAI1 発現の再活性化によるタンパク発現レベルが低かった(Figure1)ことから、より抗腫瘍効果を高めるべく、BAI1 の分子細胞生物学的働きの解明と抗腫瘍効果を高める方法の探索を行い、下記成果を得た。

（背景）
Medulloblastoma (MB) is the most common lethal pediatric brain tumor. About 30% of patients with MB will die within 5 years of diagnosis, largely due to metastasis to the lining of the brain and spinal cord. MB patients are currently treated with multimodal combined therapy which includes maximal safe resection, chemotherapy, and craniospinal radiation. Despite such aggressive therapy, many patients remain incurable or suffer neurological, intellectual and physical disabilities as side effects of the treatment, which largely result from the cranial irradiation. Hence, novel therapeutic approaches are urgently needed to reduce the

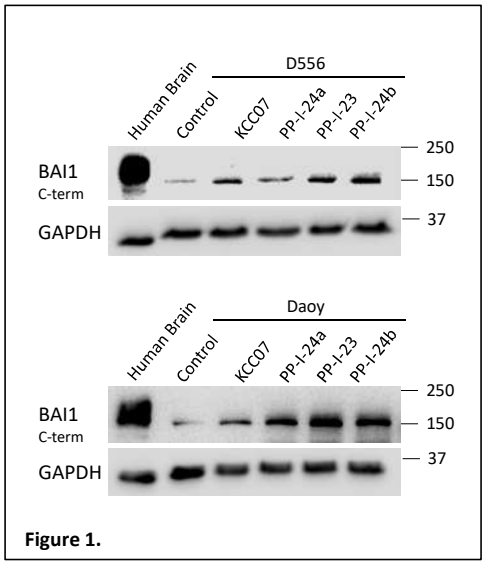

Figure 1.

mortality rates and improve the post-treatment quality of life in MB patients. Importantly, we previously evidenced that ADGRB1, the gene that encodes BAI1 is epigenetically silenced in all 4 MB groups due to the methylation of a CpG island in the gene promoter. This DNA methylation is accompanied by binding of methylated DNA binding protein MBD2 (Methyl-CpG-Binding Domain protein2) and transition to a suppressive chromatin conformation. Reactivation of BAI1 expression in ADGRB1 silent MB cells with KCC-07, a small molecule inhibitor of MBD2 reduced MB cell proliferation and tumor growth. In addition, MBD2 knockout is viable in mice with only minor physiological alterations. Supporting that side-effects of transient KCC-07 treatment are expected to be mild and this approach has promising potential for clinical translation. Our prior data further evidenced that BAI1 can prevent nuclear activity of E3 ubiquitin ligase MDM2 by trapping it at the cell surface, and in the process suppress MB tumor formation by stabilizing p53

Because MDM2 ubiquitinates multiple proteins for degradation, we considered that its membrane trapping by BAI1 might alter the stability of other proteins, which have important roles in MB. In particular, we examined whether the cell membrane association of MDM2 might increase the degradation of oncogenic tyrosine kinase receptors. We discovered that BAI1 destabilizes Insulin Like Growth Factor 1 Receptor (IGF1R) protein levels through MDM2 and defined the related mechanism.

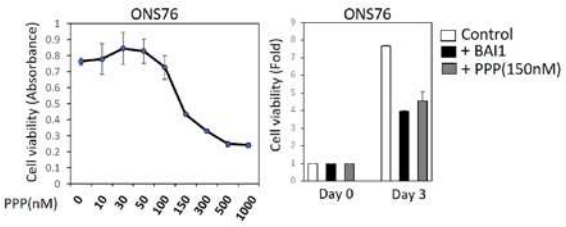

Figure 2.. IGF1R inhibitor (PPP) treatment decreases cell growth of ONS76 MB cells.

（成果）

IGF1R expression is elevated in MB patients and correlates with their survival

To confirm prior observations that IGF1R expression is elevated in MB and may contribute to malignant MB growth, we analyzed 3 distinct transcriptome datasets comprising normal brain and MB of 13 normal brain and 22 MB, 8 normal brain and 9 MB, and 16 normal brain and 19 MB. In all three datasets, IGF1R transcript expression was increased in MB. We next performed survival analysis in published MB datasets and confirmed that low IGF1R mRNA expression is significantly correlated with better prognosis.

Inhibition of IGF1R expression reduces human MB cell growth

To determine whether IGF1R plays an important oncogenic role in human MB cells, we treated ADGRB1 silent human MB cell lines: D556 (Group 3), ONS76 (SHH group-TP53 wildtype) with IGF1R ligand (IGF1) and confirmed that this treatment can increase MB cell growth. Conversely, we treated the cells with IGF1R inhibitor (PPP) and observed a rapid decrease in cell growth, similar to that mediated by transient (D556, ONS76) and stable (D556 tet-on) BAI1 overexpression (Figure 2). These data clearly show that IGF1R is an attractive target in MB.

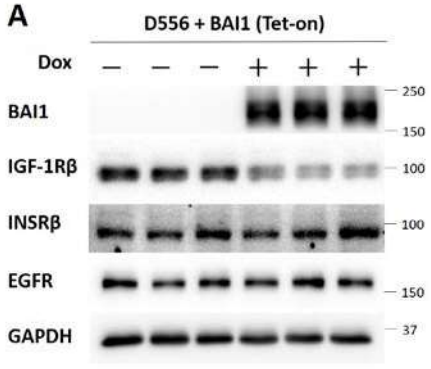

Figure 3. (A) IGF1R protein expression is decreased upon dox-induced BAI1 expression in human D556 medulloblastoma cells.

BAI1 Expression suppresses IGF1R expression in mouse brain and human MB cells.

To determine whether BAI1 expression can change the stability of IGF1R, we first compared IGF1R expression in mouse brain between wildtype and Adgrb1-/- mice. At postnatal day 10 (P10), EGL thickening in Adgrb1-/- mouse cerebellum was observed as we reported before and IGF1R expression was increased in Adgrb1-/- mouse cerebellum and cerebrum, especially in the EGL. We next determined whether IGF1R protein stability is altered by restoration of BAI1 expression in MB cells. Both transient (D556, ONS76) and stable (D556 tet-on) BAI1 transfection led to inhibition of IGF1R expression (Figure 3A). In contrast, the expression levels of other RTKs (INSR, EGFR) used

as controls remained unchanged. This data indicates that BAI1 reactivation selectively targets oncogenic IGF1R in MB

ここまでの成果を Society for Neuro-Oncology 27th Annual Meeting, November 18, 2022 で発表した。これらの成果を得る過程で新型コロナウイルス感染症の影響で本研究計画の要である KCC-07 の流通が滞る、研究室での研究活動が制限される等の事案が発生した。
採用期間の延長措置を受けたことでその後も研究活動の継続を行うことができ、下記に述べる KCC-07 や in vivo 実験の成果を得た。

BAI1 radio-sensitizes MB cells in vitro and reduces tumor growth in mouse cerebellum

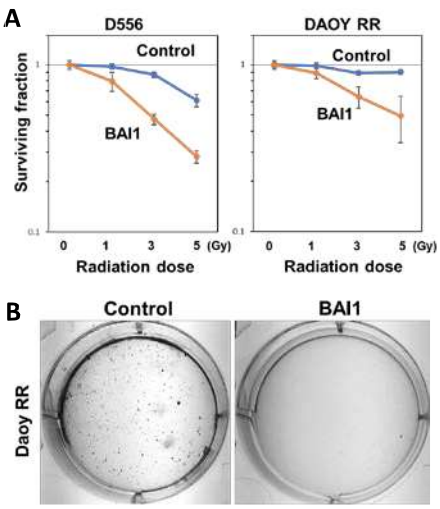

Figure 4(A, B) SRB assay (A) and soft agar colony formation assay after 3GyX4 irradiation (B) demonstrate that expressing BAI1 can radio-sensitize MB cells.

Long-term side effects of the MB therapies which is believed to be due in great part to cranial irradiation is a major clinical problem. Cancer cells can adapt to radiation by IGF1R signaling and its inhibition enhances radiosensitivity by delaying double-strand break repair through non-homologous end-joining and homologous recombination. Cancer drugs that induce autophagy can also induce tumor radiosensitivity. First, we treated MB cells with IGF1R inhibitor (PPP) and autophagy inducer (rapamycin) to confirm that IGF1R suppression and autophagy stimulation can radio-sensitize MB cells. As expected, the surviving fraction after irradiation decreased with PPP and/or rapamycin treatments compared to non-treated cells. Based on these results, we hypothesized that BAI1's anti-IGF1R signaling and pro-autophagy effects might induce radiosensitivity in radio-resistant MB cells. To test this hypothesis, we first generated radioresistant MB cells by progressive in vitro adaptation to low dose irradiation. Interestingly, IGF1R expression was increased in the radiation-adapted cells compared to parental cells.
To test for potential BAI1-mediated radio-sensitizing effects, we transfected the resistant cells with BAI1 and measured cell survival in 2D culture (SRB assays) and radio-resistance by sphere formation assays (SFA) in radiation dose-response experiments. In the presence of BAI1, the surviving fraction after irradiation remarkably decreased in D556, D556 p53KO and ONS76 cells, while it was more moderate in DAOY cell. However, surviving fraction after the irradiation remarkably decreased in DAOYRR with BAI1 expression (Figure 4). Next, SFA revealed that expressing BAI1 in MB cells reduce the number of spheres in repeated irradiation experiment and radiation dose-response experiment (Figure 4). These results demonstrate the radio-sensitizing

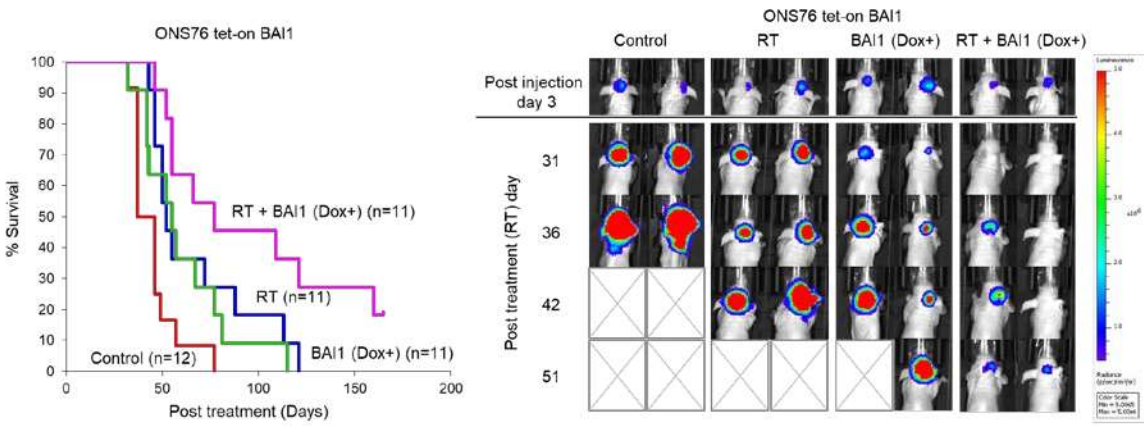

Figure 5

effects of BAI1. In addition, the radio-sensitizing effect of BAI1 was reduced in combination with siIGF1R. Radio-resistance of MB, especially the p53 mutated SHH subgroup is an important clinical problem. Our obtained data suggest that suppressing IGF1R signaling and stimulating autophagy by expressing BAI1 induces radiosensitivity in MB cells regardless of p53 status.

To examine BAI1's effects on in vivo MB growth, we used orthotopic human MB xenograft models with or without BAI1 expression. Immunocompromised nu/nu mice were implanted with tet-on BAI1 inducible MB cell lines (D556 tet-on BAI1 and ONS76 tet-on BAI1) in the cerebellum using stereotactic neurosurgery. Intracranial tumor development was monitored by bioluminescence imaging (BLI) and a subset of the animals were treated with radiation (5x 2Gy, 3 times/week). Kaplan-Meier survival curves and BLI show that combination of BAI1 expression and radiation treatment decreased the tumor growth and increased the survival than radiation treatment only (Figure 5).

Epigenetic reactivation of BAI1 accelerates the effect of irradiation and suppresses MB growth in vivo and in vitro

MBD2 binds methylated DNA and plays a direct role in ADGRB1 transcriptional silencing. To gain insight into clinical translation, we next used MBD2 inhibitor KCC-07, an epigenetic drug candidate that can reactivate BAI1 expression in MB cells. Importantly, MBD2 knockout is viable in mice with no major problem. In addition, KCC-07 is a potent, selective, and brain-penetrant. We treated MB cells with KCC07 and confirmed the decrease of IGF1R. To determine whether KCC-07 treatment can accelerate the effect of irradiation in MB regardless of p53 status, we irradiated MB cells with or without KCC-07 treatment and performed SPA. The number of spheres significantly decreased with combination of KCC-07 treatment and irradiation, indicating that reactivating BAI1 expression with KCC-07 treatment can potentiate the effect of irradiation and decrease the MB cell growth.

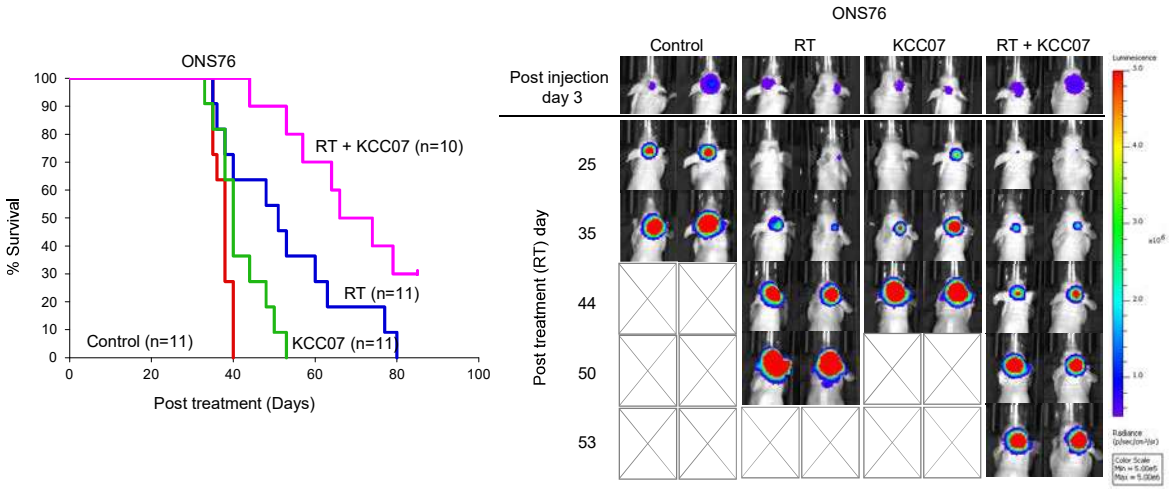

Figure 6

We next tested the therapeutic effect of KCC-07 combined with radiation therapy on orthotopic human MB xenograft models. Kaplan-Meier survival curves and BLI show that reactivating BAI1 with KCC-07 increased the effect of radiation therapy and increased the survival (Figure 6). To note, we previously demonstrated that KCC-07 has no activity in MB xenografts with shRNA depletion of p53 and/or BAI1. However, our data clearly shows that when KCC-07 treatment is combined with irradiation, this epigenetic reactivation of BAI1 accelerates the effect of irradiation regardless of p53 status and contribute to MB treatment.

In summary, our data demonstrate that epigenetic reactivation of BAI1 with KCC07 can radio-sensitize MB cells, indicating that this epigenetic approach is feasible and has potential to address an unmet medical need in MB treatment.

これらの成果は筆頭著者として論文化を進めており、年内の学術誌への投稿を目指している。