September 13, 2017
Ohara Pharmaceutical Co., Ltd.

A Cooperative Research Study with the National Cancer Center Japan

Ohara Pharmaceutical Co., Ltd. (Head Office: Shiga, CEO: Dr. Seiji Ohara; hereinafter referred to as "Ohara Pharma") and the Division of Epigenomics, National Cancer Center Research Institute (Chief: Dr. Toshikazu Ushijima) are currently conducting a cooperative research study on drug development aiming to improve epigenetic abnormalities.

Epigenetics is widely known as a mechanism for regulating the expression of genetic information in a base sequence-independent manner, with DNA methylation being the first epigenetic factor to be discovered. Methylation is usually controlled by DNA methyltransferase (DNMT). However, in cancer patients, aberrant DNA methylation inactivates key tumor suppressors, indicating that epigenetic abnormalities also play a role in oncogenic transformation. In particular, many hematologic malignancies are considered to be associated with such abnormalities, and DNMT inhibitors are clinically applied to patients with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). In Japan, azacytidine (Trade name: Vidaza®) has been available as an injectable drug for MDS since 2011. In other countries throughout the world including the U.S., decitabine (Trade name: Dacogen® for injection) is used, as well as azacytidine. In addition, several new DNMT-related drugs and technologies are currently under development, as follows.

Recently, Dr. Ushijima and his colleagues announced the successful development of a new system to assess DNMT inhibitors (Epigenetics, 2016 Dec 9:0. doi: 10. 1080/15592294. 2016. 1267887. [Epub ahead of print]). This assessment system was used to compare a candidate compound for DNMT inhibition developed by Ohara Pharma with existing DNMT inhibitors used in a clinical setting, and comparative data have been published (E0RTC-NCI-AACR 2016, Munich: See the attached materials).

Dr. Ushijima and his group commented that, "Regarding aberrant DNA methylation, we place great expectations on a new drug which is orally available and more highly stable in blood level than decitabine. Epigenomic drugs actually have great potential, although their indications are limited to blood cancers, at present. Seeing the latest trend in the development of new DNMT inhibitors, we can find some clinical developments for solid cancers, including brain tumors, nasal and laryngeal cancer, and head and neck cancer. They also retain the potential to broaden their indications to other diseases in which epigenetic abnormalities are confirmed, such as diabetes mellitus, immune diseases, Alzheimer's disease, and schizophrenia. We are waiting for the arrival of a new oral product whose dose and regimen can be easily adjusted in order to optimize treatment."

Ohara Pharma is making full use of its know-how regarding the synthesis and formulation of nucleic acid medicines to develop a novel DNMT inhibitor. On the other hand, regarding an oral preparation, an optimal preparation to be introduced into a clinical setting is under development through a collaborative research study undertaken not only with Dr. Ushijima's

group, but also with the Department of Drug Discovery and Biomedical Sciences at Saga University, where we are promoting discussions to select an effective dose and regimen.

We will attempt to develop both a cancer diagnostic method and an optimum drug/treatment for aberrant DNA methylation, and carry out continuous research so that they can be applied in a clinical setting, as soon as possible.

For more information, please visit the website of the National Cancer Center Japan using the following link:
http://www.ncc.go.jp/jp/ri/division/epigenomics/project/020/20170906143626.html

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257 : Development of Novel DNA Demethylating Agents with Greater Stability and Less Toxicity

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Conclusion

Two novel DNA demethylating agents with strong demethylation activity and less toxicity were developed.

Background

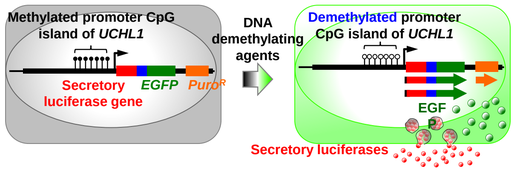
- Epigenetic therapy is expanding from hematological tumor to solid tumor.
- Two approved DNA demethylating agents, 5-azacytidine (azacitidine, AZA) and 5-aza-2'-deoxycytidine (decitabine, DAC), suffer from rapid deamination by cytidine deaminase and hydrolytic ring-cleavage of the base moiety.
- This rapid elimination in plasma makes it challenging to determine the maximum biological dose for individual patients.

Materials & Methods

Screening system

- Quantification of DNA demethylation by luciferase activity
- Isolation of optimal subclones with high S/N ratio
- Suitable system for high throughput screening

[Okochi-Takada, Hattori et al., Epigenetics, *Revision Submitted*]



DNA methylation and apoptosis analyses

- qMSP and Illumina Infinium HumanMethylation 450K
- TF-1 : a cell lines of AML developed from MDS
- Apoptosis assay : Caspase-Glo® 3/7 assay

Identification of novel agents

- Cell viability assay : Cell Count Reagent SF (WST-8)
- SGI-110 : second generation demethylating agent that is resistant to cytidine deaminase

In vivo drug tolerability

- Female athymic nu/nu mouse
- Evaluation of body weight, morbidity, leukocyte count, and hematocrit

Objective

To develop novel and stable DNA demethylating agents

Result

Identification of two compounds as novel and stable demethylating agents

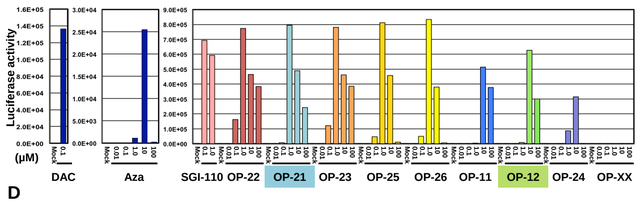
A

Original backbone		No. of compounds	No. of hits*
Azacytidine		33	7
Decitabine		14	12
Others		12	0
	Total	59	19

*Compounds showing the luciferase activity equal to or more than that of Aza or one-tenth of that of DAC

B

C



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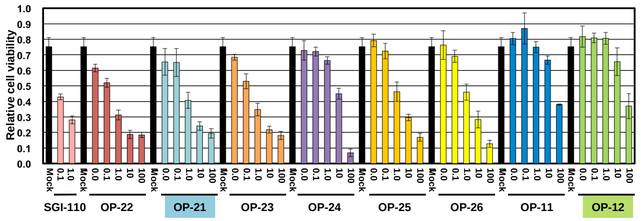
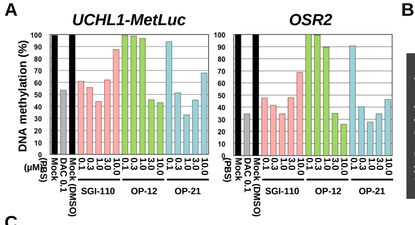


(A) Fifty-nine derivatives of AZA and DAC were synthesized as potentially more resistant to the deamination by cytidine deaminase and/or hydrolytic ring-cleavage. Nine-teen compounds among the 59 were considered to have DNA demethylating activity.
(B) Schedule of drug treatment.
(C) Seven compounds showed luciferase activity equal to that of SGI-110.
(D) OP-12 and OP-21 showed a moderate and a strong cell-growth suppressive effect, respectively.

Result

Induction of genome-wide DNA demethylation and moderate apoptosis

A



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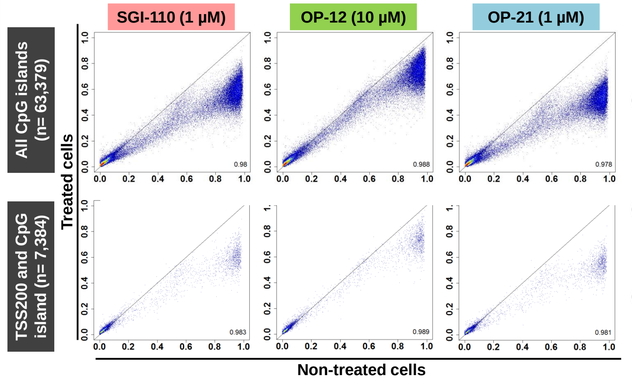

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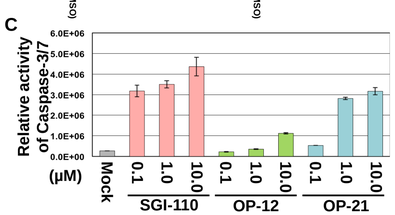
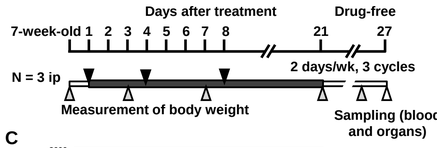


(A) Demethylation of marker genes was observed in the cells treated with OP-12 or OP-21.
(B) Genome-wide DNA demethylation was induced by treatment of OP-12 or OP-21.
(C) Apoptosis was induced by SGI-110 or OP-21 but not by OP-12 in TF-1 cells.

Low *in vivo* toxicity of two novel agents

A

SGI-110	OP-12	OP-21	DAC
	2.85	1.85	
6.35	5.70	3.70	2.50
	11.4	7.40	*MTD of DAC (reference value)
	22.8	14.8	
(mg/kg)	45.6	29.6	



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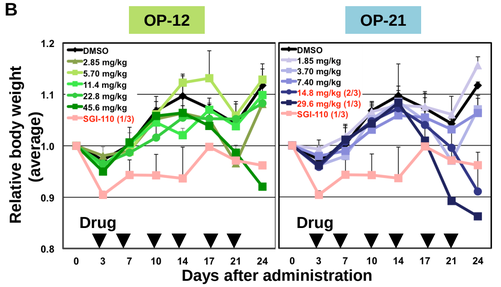

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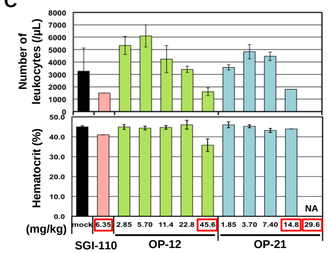

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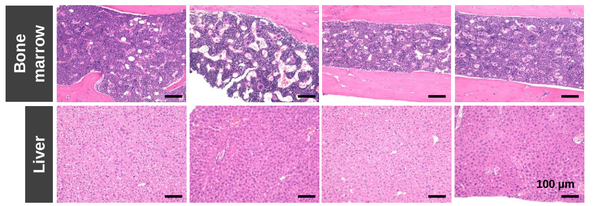

(A) Administration doses (upper panel) and schedule (lower panel) of SGI-110, OP-12, and OP-21.
(B) Severe body weight loss and increase of mortality were observed in 6.35 mg/kg of SGI-110 and high-doses of OP-12 and OP-21 groups.
(C) Leukopenia was absent in mice with OP-12 and OP-21 without body weight loss. Doses with severe body weight loss are marked with red rectangles.
(D) Myelosuppression and hepatotoxicity were absent in mice with OP-12 and OP-21 without body weight loss.

Summary & Discussion

- OP-12 and OP-21 showed strong demethylating activities with less toxicity than SGI-110 and DAC.
- OP-12 might have a distinct mode of demethylating action.
- Anti-tumor activities of OP-12 and OP-21 are evaluating using xenografts of a colorectal cancer cell line (HCT116), and preliminary assessment showed that OP-12 and OP-21 were equally effective to DAC.