Pyrrole Imidazole Polyamide–Based Genetic Switches for Intractable Diseases/Cancer

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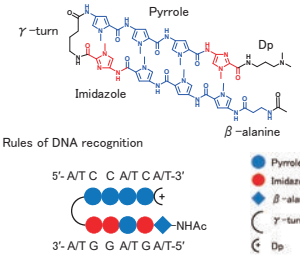
Pyrrole imidazole (PI) polyamides

Bioorg. Med. Chem. 2018; 26 (8): 1393. H. Sugiyama et al.
Sequence-specific DNA binding Pyrrole-imidazole polyamides and their applications.
(Review)

Chemical structure of a hairpin PI polyamide



Rules of DNA recognition

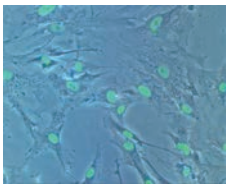
Pyrrole unit can be replaced by β-alanine unit.

PI polyamides bind to DNA minor groove sequence-selectively.

J. Am. Chem. Soc. 2010; 132 (41): 14521. P. B. Derven et al.
Structural basis for cyclic Py-Im polyamide allosteric inhibition of nuclear receptor binding.

Cell permeability of PI polyamides

J. Pharmacol. Exp. Ther. 2005; 315 (2) :571. H. Sugiyama et al.
Synthetic pyrrole-imidazole polyamide inhibits expression of the human transforming growth factor-beta1 gene.

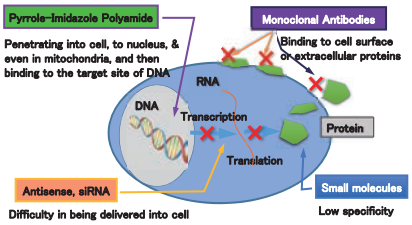


Human vascular smooth muscle cells + 

(1 nM, 2 hours)

The use of confocal microscopy to visualize subcellular localization of fluorophore-labeled molecules is a convenient method to study uptake and trafficking of polyamides in living cells. Nuclear localization of more than 100 hairpin PI polyamide-fluorophore conjugates in several human cell lines has been examined.

Target of PI polyamides



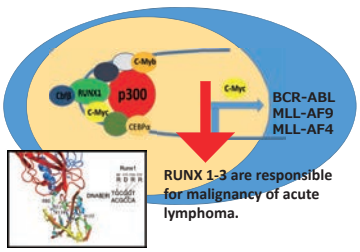
Because the DNA-binding affinities of PI polyamides are comparable to those of transcription factors, silencing of gene expression is able to be achieved by competitive binding of PI hairpin polyamides to consensus DNA binding sequences of transcription factors.

By conjugation of a PI polyamide with a DNA alkylating reagent such as chlorambucil, the PI polyamide is covalently connected with DNA in a sequence selective manner. Such DNA alkylating PI polyamides can cause gene silencing even if the recognition site of the PI polyamide is in a mRNA coding region of a gene.

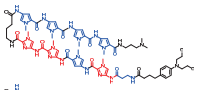
RUNX inhibitor (Please see the other poster.)

Cancer Sci. 2018 ;109 (8): 2358. Y. Kamikubo
Genetic compensation of RUNX family transcription factors in leukemia. (Review)

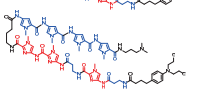
J. Am. Chem. Soc. 2019; 141 (10): 4257. H. Sugiyama et al.
Molecular Characteristics of DNA-Alkylating PI Polyamides Targeting RUNX Transcription Factors.



Chb M prime



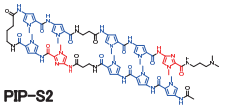
Chb M



The chlorambucil-PI polyamide conjugates, were designed to target the RUNX-binding sequence. These conjugates are proposed to suppress the expression of RUNX-target genes, such as BCL11A and TRIM24, by blocking RUNX-DNA binding.

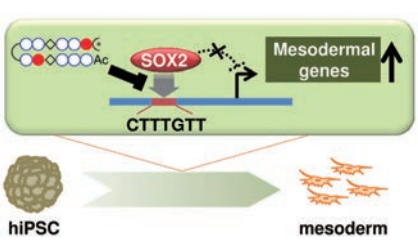
PI polyamides for suppression of SOX2 (Please see the other poster.)

Nucleic Acids Res. 2017; 45 (16): 9219. H. Sugiyama et al.
A synthetic DNA-binding inhibitor of SOX2 guides human induced pluripotent stem cells to differentiate into mesoderm.



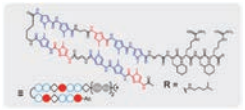
PIP-S2

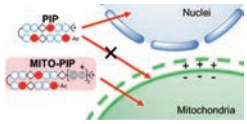
Harnessing knowledge about the key transcriptional changes during induction of cardiomyocyte, we developed a DNA-binding inhibitor termed PIP-S2, targeting the 5'-CTTTGTT-3' and demonstrated that inhibition of SOX2-DNA interaction by PIP-S2 triggers the mesoderm induction in hiPSCs.

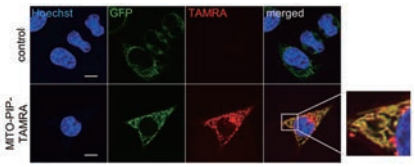


PI polyamides for mitochondria DNA (MITO PIP)

J. Am. Chem. Soc. 2017; 139 (25): 8444. H. Sugiyama et al.
Creation of a Synthetic Ligand for Mitochondrial DNA Sequence Recognition and Promoter-Specific Transcription Suppression.







Mitochondria and nuclei were visualized by CellLight Mitochondria-GFP, BacMam 2.0 (Thermo Fisher Scientific), and Hoechst 33342, respectively. Consistent with the gene expression studies, MITO-PIP-TAMRA showed strong signals in the cytosol, and the merged image clearly showed it overlapped the signal of mitochondria-GFP, thereby confirming that the MITO-PIPTAMRA are efficiently localized in the mitochondria.

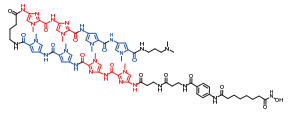
Epigenetic ON switch of gene expression

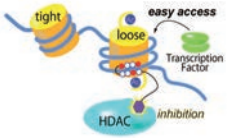
The mechanism of epigenetic upregulation by a conjugate of a pyrrole-imidazole polyamide (PI-polyamide)

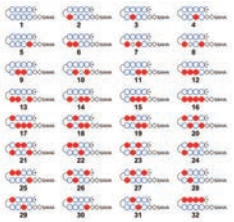
- A PI polyamide conjugate and a histone deacetylase (HDAC) inhibitor activate histone acetyltransferase (HAT).
- This acetylation loosens nucleosomes, which initiates binding of transcription factors and results in selective gene expression at PI-polyamide binding sites (ON switch).

SAHA PI polyamides

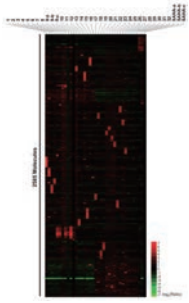
Sci Rep. 2014;4:3843. H. Sugiyama et al.
Distinct DNA-based epigenetic switches trigger transcriptional activation of silent genes in human dermal fibroblasts.







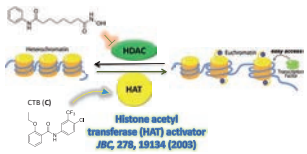
Through microarray studies and functional analysis, we demonstrate for the first time the remarkable ability of thirty-two different SAHA-PIPs to trigger the transcriptional activation of exclusive clusters of genes and noncoding RNAs. QRT-PCR validated the microarray data, and some SAHA-PIPs activated therapeutically significant genes like KSR2 . We propose the potential use of SAHA-PIPs as reagents capable of targeted transcriptional activation.



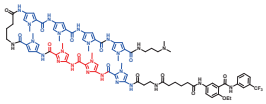
An unsupervised hierarchical clustering analysis of top 100 up-regulated genes in SAHA-PIP 1-32 treated fibroblasts.

CTB PI polyamides

Angew. Chem. Int. Ed. 2015; 54(30): 8700. H. Sugiyama et al.
A Synthetic DNA-Binding Domain Guides Distinct Chromatin-Modifying Small Molecules to Activate an Identical Gene Network.



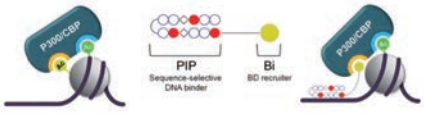
CTB-I



CTB-I and SAHA-I induced similar gene expression profiles. Although the actual mechanism underlying the identical bioactivity is not straightforward, site-specific acetylation triggered by SAHA-mediated HDAC inhibition or CTB-mediated HAT activation at a particular key sequence could cause the activation of OCT-3/4 and its regulated genes.

Bi PI polyamides

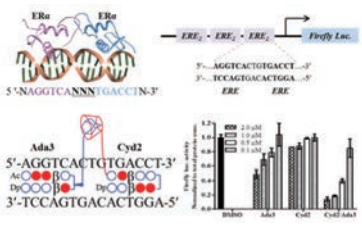
J. Am. Chem. Soc. 2018; 140 (23): 7108. H. Sugiyama et al.
Biomimetic Artificial Epigenetic Code for Targeted Acetylation of Histones.



Biochemical assays verified that Bi-PIPs recruit P300 to the nucleosomes having their target DNA sequences and extensively accelerate acetylation. Bi-PIPs also activated transcription of genes that have corresponding cognate DNA sequences inside living cells.

Cooperative binding PI polyamides

J. Am. Chem. Soc. 2018; 140 (7): 2426. H. Sugiyama et al.
Pip-HoGu: An Artificial Assembly with Cooperative DNA Recognition Capable of Mimicking Transcription Factor Pairs.



Cooperation between pairs of transcription factors (TFs) has been widely demonstrated to play a pivotal role in the spatiotemporal regulation of gene expression, but blocking cooperative TF pair—DNA interactions synergistically has been challenging. To achieve this, we designed programmable DNA binder pyrrole-imidazole polyamides conjugated to host—guest assemblies (Pip-HoGu) to mimic the cooperation between natural TF pairs. The system also has a longer recognition sequence (two-PIP binding length plus gap distance), favorable sequence selectivity, higher binding affinity, and in particular, a flexible gap distance (0–5 bp).

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Graduate School of Science, Kyoto University
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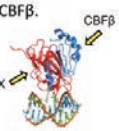
RUNX transcription factor

- RUNX (Runt-related transcription factor) is an evolutionally conserved transcription factor which binds to 5'-TGPyGGT-3' (Py = T or C) sequence of the genome.

Ito Y et al. Nat Rev Cancer. 2015 Feb;15(2):81-95.

- RUNX forms a hetero-dimeric complex with CBFβ.



- RUNX family is consisted of three members.
  - RUNX1: Definitive hematopoiesis...
  - RUNX2: Bone development...
  - RUNX3: Neurogenesis, thymopoiesis...

- Historically, RUNX1 is considered as a tumor suppressor in acute myeloid leukemia (AML).

Kamikubo Y et al Blood. 2017 Apr 13;129(15):2070-2082.
Kamikubo Y et al. Blood. 2013 Jan 24;121(4):638-42.
Silva FP et al. Oncogene. 2001 Jan 30;22(4):538-47.

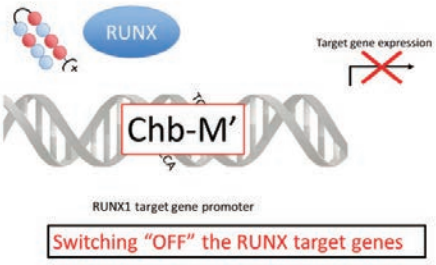
- Recently, RUNX1 is found to have an oncogenic property in the development of AML with inv(16).

Kamikubo Y et al. Cancer Cell. 2010 May 18;17(5):455-68.
RK Hyde et al. Leukemia. 2015 Aug; 29(8): 1771–1778.

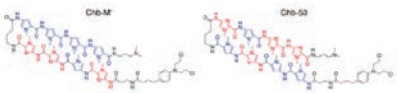
- Overexpression of *RUNX* family genes are implicated in the progression of various cancers.
  - *RUNX1* in leukemia, prostate cancer, neuroblastoma...
  - *RUNX2* in breast cancer, prostate cancer, osteosarcoma, non-small cell lung cancer...
  - *RUNX3* in head and neck cancer, Ewing sarcoma...

Ito Y et al. Nat Rev Cancer. 2015 Feb;15(2):81-95.

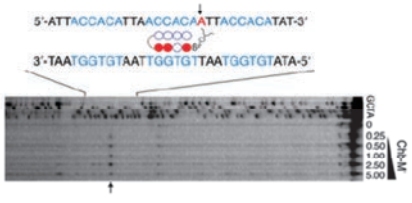
PI polyamide targeting consensus binding sequence of RUNX



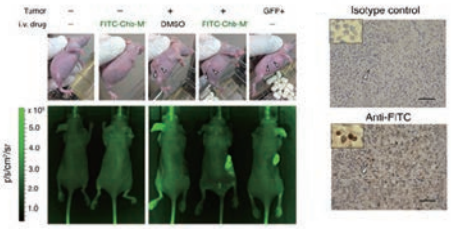
Chemical structures of the synthetic Chb/PI polyamide conjugates targeting 5'-TGTGGT-3' (Chb-M') and 5'-TGCGGT-3' (Chb-50)



Chb-M' – dependent alkylation and cleavage

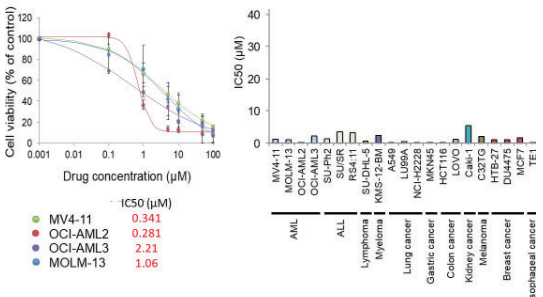


Specific accumulation of Chb-M' in tumor

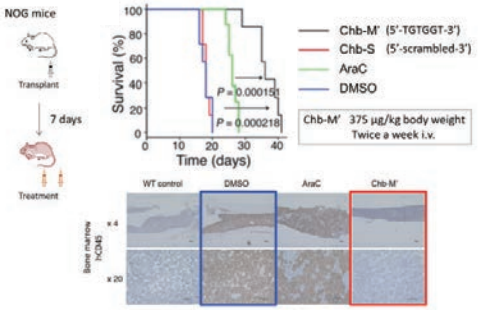


Genetic regulation of the RUNX transcription factor family has antitumor effects.
J Clin Invest. 2017 Jun 30;127(7):2815-2828. doi: 10.1172/JCI91788. Epub 2017 May 22.

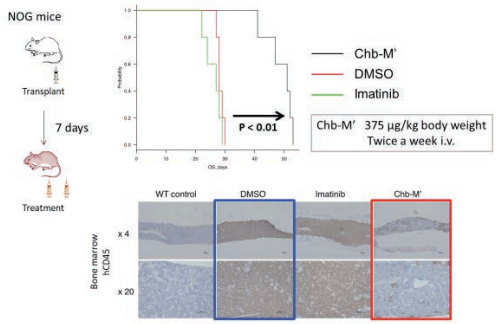
Chb-M' exerts strong antitumor effect against various types of cancers



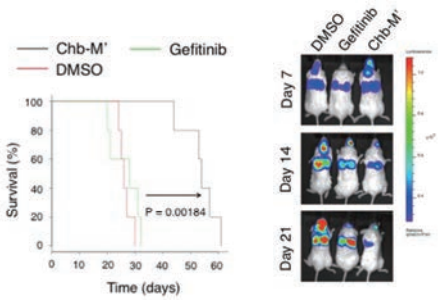
Acute myeloid leukemia model with MV4-11 (FLT3 mut)



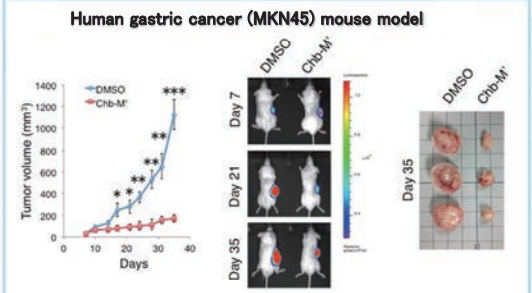
Acute lymphoblastic leukemia model with SU/SR (T351I mut)



Gefitinib resistant non-small cell lung cancer (A549) mouse model

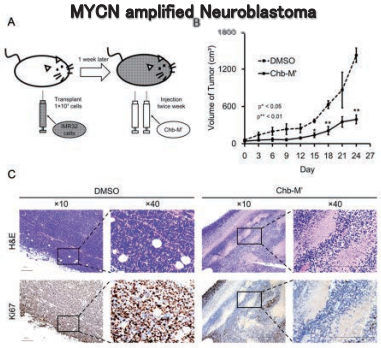


Human gastric cancer (MKN45) mouse model

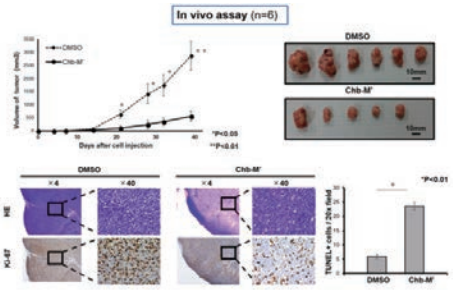


RUNX1 positively regulates ErbB2/HER2 signaling pathway through modulating SOS1 expression in gastric cancer cells.
Sci Rep. 2018 Apr 23;8(1):6423. doi: 10.1038/s41598-018-24969-w.

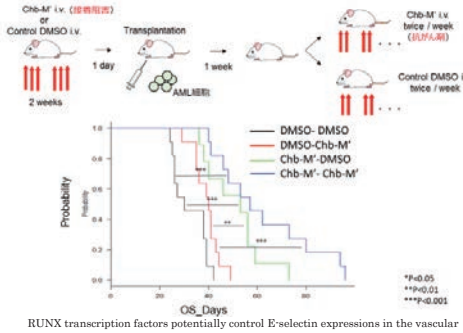
MYCN amplified Neuroblastoma



Chb-M' has proliferative inhibitory effect against MRT *in vivo*



RUNX1 enhances leukemia cell engraftment in the vascular niche positively through modulating E-Selectin expressions



RUNX transcription factors potentially control E-selectin expressions in the vascular niche of mice bone marrow.
Blood Adv. 2018 2:509-515: doi: https://doi.org/10.1182/bloodadvances.2017009324

Chb-M' completely inhibited metastasis through Organ Niche regulation

Double Negative Prostatic Cancer (DNPC)

PC-3