学位論文の要約

題目　Chemical Biology Approaches for the Molecular Recognition of DNA Double Helix
（DNA 二重らせんの分子認識に関するケミカルバイオロジー研究）

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序論

General Introduction: DNA double helix has a unique structure that allows it to regulate most important biological processes including gene regulation, charge transport and its damage/repair. DNA minor groove are considered to be of great interest for developing new drugs since it is a site of non-covalent high sequence specific interactions for a large variety of organic small molecules including natural products. Taking a clue from nature synthetic transcriptional activator targeting specific DNA minor groove sequences was designed and developed. Again the DNA itself can be used as a tool for the detection of binding site of DNA binding small molecule, and also for investigating crucial biological phenomena that regulate transcription by the substitution of 5-bromouracil (^{Br}U).

Here in this thesis, using chemical biology approaches we have thoroughly studied the molecular recognition of DNA.

Chapter 1: Synthesis and Biological Evaluation of Targeted Transcriptional Activator with HDAC8 Inhibitory Activity

Recently, we developed a differential gene activating multifunctional small molecule SAHA-PIP (Sδ) by conjugating a histone deacetylase (HDAC) inhibitor, SAHA, to a selective DNA-binding pyrrole-imidazole polyamide (PIP). In this chapter we synthesized a derivative of Sδ, called Jδ to evaluate the role of surface recognition domain (–phenyl) of SAHA in Sδ-mediated transcriptional activation. In Vitro studies revealed that Jδ displayed potent inhibitory activity against HDAC8. Jδ retained the pluripotency gene-inducing ability of Sδ when used alone and in combination with Sδ; a notable increase in the pluripotency gene expression was observed. Interestingly, Jδ significantly induced the expression of HDAC8-controlled Otx2 and Lhx1.

Chapter 2:

Chemical Modification of a Synthetic Small Molecule Boosts its Biological Efficacy against Pluripotency Genes in Mouse Fibroblast

Here, we carried out chemical modification on SAHA-PIP (Sδ) to improve its biological efficacy and show that the biological activity of Sδ got significantly ($P = <0.05$) improved against the core pluripotency

genes after the incorporation of an isophthalic acid (IPA) in its C-terminus. The resultant IPA conjugate 2 dramatically induced Oct-3/4 to demonstrate a new chemical strategy for developing PIP conjugates as next-generation genetic switches.

Chapter 3: Electron injection at specific sites using polyamide in BrU substituted DNA: A versatile photo affinity cleavage method

In this chapter we addressed a powerful photo-affinity cleavage method to detect the binding sites of small molecules in a long 5-bromouracil (^{Br}U) substituted DNA (thymine replaced by ^{Br}U) via electron injection under UV irradiation. By attaching an electron donor pyrene to pyrrole imidazole polyamides, which can bind to the DNA minor groove in a sequence specific manner, we were successfully able to inject an electron at their binding sites in just 5 sec UV irradiation (365 nm) in two long DNA fragments.

Chapter 4: Efficient generation of Uracil-5-yl radical ion in ^{Br}U substituted DNA using DNA minor groove binder Hoechst 33258 under photo-irradiation condition.

In this chapter we used similar photoreaction scheme to detect the binding site of another DNA minor groove binding small molecule Hoechst 33258. This dye is well known as a staining agent and also recently in micro irradiation technology has been used to visualize repair protein recruitment process in the DNA damage site. Our results suggest that this dye under 365 nm irradiation can efficiently donate electron to ^{Br}U residues at its binding site and generate the reactive uracil-5-yl radical.

Chapter 5: Evidence of cooperative binding partnership between Sox2 and Pax6 proteins via electron transfer from protein to DNA

In this chapter we have demonstrated electron transfer process from protein to the DNA under photo irradiation condition. Using two transcription factors such as Sox2 and Pax6 which contain tryptophan, we demonstrated electron transfer to the ^{Br}U residue at the binding site of protein under UV 280 nm light. These two proteins are co-expressed in neuronal and retinal tissues and work together in neuronal and retinal development through cooperative binding on the minimal enhancer (DC5) of the δ1-crystallin gene. We modified the DC5 enhancer by replacing thymine with ^{Br}U and after photo irradiation with protein we successfully detected their cooperative binding affinity via electron transfer.

In summary combining various chemical biology approaches from small molecules to proteins we have successfully demonstrates molecular recognition into the double helix. Outcome of these studies will focus on potential application of the compound for generating iPSC and the idea of manipulation in DNA itself will provide a highly sensitive ^{Br}U based detection assay.