学位論文の要約

題目　Elucidation of the Molecular Mechanisms of Gene Expressions-Epigenetics Regulation by Chemical Biology
(ケミカルバイオロジーによる遺伝子発現-エピジェネティクス制御の分子機構の解明)

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序論

Genetics is the field of studying information of DNA sequence：biological phenomena which inherit genes from parents to children and mechanisms of gene expression. In contrast, epigenetics is the field of studying the transmission of genetic information to the next generation and the regulation of gene expression without changing the nucleotide sequence of DNA. It has been found that mainly methylation modification of DNA cytosine, chemical modification of histone, polycomb protein and chromatin remodeling factor are involved in epigenetic phenomena.

In this study, we attempted to elucidate the molecular mechanisms of gene expression-epigenetics regulation using pyrrole-imidazole polyamide and DNA origami. The compound regulating histone modification was linked to pyrrole-imidazole polyamide, which activated the pluripotency-related genes in human skin cells. In addition, the pyrrole-imidazole polyamides targeting the DNA sequence containing methylcytosine were synthesized. The pyrrole-imidazole polyamides inhibited the binding of TET1 protein on the DNA. It was also observed by AFM that TET1 protein favored relaxed both the dsDNA and the dsDNA including fully-methylated cytosine which were fixed on the DNA origami.

1. “Identification of a Small Molecule That Turns ON the Pluripotency Gene Circuitry in Human Fibroblasts”

It has been reported that HDAC inhibitors have anti-tumor activity and positively regulate cellular reprogramming. However, these small molecules cannot act on the locus-specific to genomic DNA. In this study, we synthesized a library of 32 pyrrole-imidazole polyamides conjugated with a HDAC inhibitor, SAHA. Human fibroblasts were treated with each SAHA-PIPs and evaluated. Interestingly, microarray analysis and q-PCR analysis showed that SAHA-PIP (I) mainly increased the expression levels of Oct4, Sox2 and MET-related genes. ChIP-qPCR and ChIP-seq confirmed that SAHA-PIP (I)

enhanced acetylation of the reprogramming genes. It was suggested that SAHA-PIP (I) which has both DNA sequence selectivity and HDAC inhibitory potency induced acetylation of the promoter region of the reprogramming genes, resulting in increasing those gene expressions. This tool would prove useful for activating genes as a genetic switch.

2. “Orientation Preferences of Hairpin Pyrrole–Imidazole Polyamides toward the mCGG site”

The orientation preferences of hairpin pyrrole-imidazole polyamides is still elusive, although it has been reported that replacement of pyrrole with β-alanine modulates the orientation preferences of some hairpin pyrrole-imidazole polyamides. In this study, we synthesized a series of hairpin pyrrole-imidazole polyamides and explored their binding affinities and orientation preferences to methylated DNA with the mCGG target sequence. Thermal denaturation assays revealed that the five hairpin pyrrole-imidazole polyamides, which were anticipated to recognize mCGG in a forward orientation, bind to nontarget DNA, GGmC, in a reverse orientation. Therefore, we designed five pyrrole-imidazole polyamides that could recognize mCGG in a reverse orientation. It was found that the two pyrrole-imidazole polyamides containing Im/β pairs preferentially bound to mCGG in a reverse orientation. The reverse binding hairpin polyamides successfully inhibited TET1 binding on the methylated DNA. Taken together, this study illustrated the importance of designing reverse binding pyrrole-imidazole polyamides for the target sequence, mCGG, which paved the way for pyrrole-imidazole polyamides that can be used with otherwise difficult to access DNA with CG sequences.

3. Direct observation and Analysis of the TET-mediated Oxidation Process in a DNA Origami Nanochip

DNA methylation and demethylation play an important role in the epigenetic regulation of gene expression. Although it has been reported that TET enzymes demethylated 5-methyl group of cytocine with a series of oxidation reactions, demethylation process is not fully understood. To elucidate the relationship between the oxidative processes and structural factors of DNA, we analyzed the behaviour of TET-mediated 5mC-oxidation by incorporating structural stress onto a dsDNAs (double-stranded DNAs) containing 5mC using a DNA origami nanochip. The reactions and behaviors of TET enzymes were systematically monitored by biochemical analysis and single-molecule observation using AFM (atomic force microscopy). We tested whether TET oxidized the dsDNAs in the tense and relaxed states within a DNA nanochip. It was revealed that TET preferred a relaxed substrate regardless of the modification types of 5-oxidated-methyl cytosine. Strikingly, TET enzymes preferred the fully methylated site over the hemi-methylated site. This analysis also permited the direct observations of dynamic movements of TET such as sliding and interstrand transfer by high-speed AFM. In addition,

the thymine DNA glycosylase-mediated base excision repair process was characterized in the DNA nanochip. Thus, we have convincingly established the system's ability to physically regulate enzymatic reactions, which could prove useful for the observation and characterization of coordinated DNA demethylation processes at the nanoscale.