学位論文の要約

題目　Chemical Biology Approaches for Regulating Eukaryotic Gene Expression
(ケミカルバイオロジー的アプローチによる真核細胞の遺伝子発現制御法の検討)

氏名　Junetha Syed Jabarulla
ジュネタ　セイド　ジャバルーラ

序論

General Introduction:

DNA encodes the blueprint of life in the form of nucleotide sequences referred as genes. These genetic information in eukaryotes are tightly synchronized and regulated into a specific transcriptional programme for a particular cell type by various factors such as transcription factors, chromatin remodeling and furthermore recent studies have strongly suggested the functional importance of long non-coding RNAs (lncRNAs). Aberrations at any level of the above mentioned gene regulation could result in dreadful disorders. Treatment strategy employing DNA sequence-specific binding small molecules have new hopes due to its possible reduced side effects. And systems like single –molecule visualization simplifies our understanding of the complex biochemical systems of eukaryotes for designing such small molecules.

Here in this thesis, various chemical biology approaches have been explored in modulating the eukaryotic gene expression as well as to understand its regulation.

Chapter 1: Targeted Suppression of EVI1 Oncogene Expression by Sequence-Specific Pyrrole–Imidazole Polyamide

Pyrrole-imidazole (Py-Im) polyamides (PIPs) are a class of small DNA binding molecules that can be designed to target any destined DNA sequence. They can interfere with the binding of transcriptional regulators and modulate gene expression. Human Ectopic viral integration site 1 (EVI1) is an oncogenic transcription factor whose expression is often deregulated in many aggressive forms of cancer. In this chapter, a novel sequence-specific Py-Im polyamide (PIP1) is developed to target base pairs overlapping the REL and ELK1 binding site in the minimal promoter region of EVI1. PIP1 significantly inhibited the expression of EVI1 mRNA in MDA-MB-231 cells and the whole transcriptome analysis showed that the PIP1 treatment affected the EVI1 mediated gene regulation. In vitro assays suggested this polyamide could effectively inhibit the breast cancer cell migration. Taken together, these results indicate that the

EVI1 targeted Py-Im polyamide could be an effective gene silencer for cancer therapy.

Chapter 2: A Synthetic Transcriptional Activator of Genes Associated with the Retina in Human Dermal Fibroblasts

HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) are potential agents for epigenetic therapy that can induce histone acetylation and facilitate the reexpression of therapeutically important genes. But their mode of action on the target is very non-specific. The sequence specificity to the HDAC inhibitor SAHA can be provided through its conjugation with PIPs. We have developed a library of 32 SAHA–PIP conjugates A to φ such that each conjugate can recognize its unique six-base-pair DNA sequence. In this chapter, we have demonstrated the remarkable ability of the conjugate SAHA-PIP X, in specific activation of therapeutically important retinal genes in HDF by histone modifications. SAHA-PIP X enhanced active H3K27 acetylation marks along specific retinal genes and ChIP-seq peaks exhibited enrichment of motif corresponding the SAHA-PIP X binding site (5'-WCGGWW-3'). The obtained results also provide evidence that each SAHA-PIP conjugate from our chemical library can activate its unique gene circuits in HDFs.

Chapter 3: Single-Molecule Visualization and Characterization of Triple helix formation between the consecutive purines of lncRNA and dsDNA using DNA nanoframe

Triple helix forms in the major groove of the dsDNA by the sequence specific base pairing between the homopolypurine/ homopolypyrimidine sequences in the duplex DNA and the third single stranded triplex forming oligonucleotide (TFO) which can be either DNA or RNA. Biological evidences suggest the presence of such non-canonical structures *in vivo*. Recent studies have shed light on the role of triple helix formation by long non-coding RNA (lncRNA) with genomic DNA that can actively participate in regulating epigenetics. *DBE-T* is a chromatin bound lncRNA, which is selectively expressed in Facioscapulohumeral muscular dystrophy (FSHD) condition and a valuable therapeutic target for treating FSHD. In this chapter, by using our single –molecule visualization method, we have demonstrated the binding of consecutive purines of *DBE-T* to the polypurine tract within the FSHD locus in an anti-parallel orientation generating triple-helical structures. Investigating this mechanism of interaction will provide valuable information at molecular level for developing therapeutics to treat FSHD patients.

In summary, we have employed various chemical biology approaches such as DNA nanotechnology and small molecules to investigate the eukaryotic gene regulation and expression. The outcome of these studies will be focusing on the potential application and development of small molecules as therapeutics in aiding severe human disorders.