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Advancing Synthetic Gene Regulators Development with High-Throughput Sequencing Technologies

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学位論文の要約

題目　Advancing Synthetic Gene Regulators Development with High-Throughput Sequencing Technologies
（ハイスループットシークエンシング技術を用いた革新的遺伝子制御法の開発に関する研究）

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序論

General Introduction:

Next-generation-sequencing (NGS)/High-throughput sequencing technologies enable us to obtain extensive information by deciphering millions of individual DNA sequencing reactions simultaneously. Sequencing on this scale facilitates significant advances in diverse disciplines, ranging from discovery, design, and evaluation of many small molecules and relevant biological mechanisms for personalized therapies. We used this sequencing technology to gain in-depth insight into DNA binding small molecules-triggered biological phenomena and its redesign. We have also used NGS application to identify specific targets of small molecule.

In this thesis, we systematically studied the binding mechanism and its corresponding biological effects of various types of synthetic gene regulators in global genome scale, using the interface of chemical biology and high-throughput sequencing.

Chapter 1:

Next-Generation Sequencing Studies Guide the Design of Pyrrole-Imidazole Polyamides with Improved Binding Specificity by the Addition of β-alanine

In this chapter, we studied the high-affinity binding sites of the previously reported small molecules: SAHA-PIP I and its structurally similar counterpart SAHA-PIP K, both activate entirely different set genes. By using the Next Generation Sequencing based Bind-n-seq approach, we identified the binding specificity of SAHA-PIP I and SAHA-PIP K in a broad context of synthetic oligo libraries. Both compounds showed highly significant binding efficiency compared with PIP I and PIP K (PIPs without SAHA). This prompted us to redesign SAHA-PIPs and replace the SAHA moiety with β-alanine (the β-moiety) in the non-core binding region of the 10-ring hairpin polyamide. The results showed that the β-moiety enforces binding of PIP in the closed form. This discovery is being useful for the current PIP
conjugates developments.

Chapter 2:
Deciphering The Genomic Targets Of Alkylating Polyamide Conjugates Using High-Throughput Sequencing

In this study, we have made use of high-throughput sequencing technology to show the significant sequence specific binding and alkylation of alkylating PIP-indole-seco-CBI conjugates corresponding to the proposed DNA binding rule. Results of this study indicate the structural composition of PIP-indole-seco-CBI conjugates favorably alters the sequence specificity of PIPs. In the future, this method may be an efficient tool compared with conventional PAGE analysis for studying sequence-specific alkylation and designing of small molecules for targeted gene silencing. Apart from the chemical arrangement of PIP, the organization of histone-DNA packed nucleosome may affect the binding sites availability. It is unclear, whether nucleosome dependent binding sites may leads to the reduction in binding specificity of PIP conjugates. We have also developed a method to map the alkylating PIP conjugates binding regions throughout the human genome. Our genome level mapping study recognized a new significant genomic target for alkylating PIP in the field of breast cancer.

Chapter 3:
Genome-wide mapping of artificial transcriptional activators

Here, we studied the effective SAHA PIPs specific binding mechanism in nucleus from living cells. Though, the SAHA PIPs have millenary predicted binding sites, only a few of those trigger a specific gene regulation. We previously showed individual SAHA PIPs can regulate a unique set of genes in human somatic cells (like iPSc and germ cell activating gene network). In a complex genomic space, the reason for strong and specific biological effects of SAHA-PIPs is not clear. Our SAHA PIP specific pull-down based high-throughput sequencing study resulted, SAHA-PIPs specific binding sites among the sequenced reads. Mapping of SAHA-PIPs bound enriched areas on the whole human genome directly indicates its specific biological gene regulations are due to its chromatinized DNA specific binding. Here, we used non-crosslinking small molecules, so it may be suitable to recognize other DNA binding small molecule’s genomic effects based on its binding in the actual chromatinized genome.

In summary: combining various cross-points of chemical biological approaches and high-throughput sequencing applications, we have effectively proves the sequence specific properties of artificial gene regulators in actual genome space. Aftermath of these studies will focus on prospective applications of high-throughput sequencing technologies in small molecule based chemical biological therapeutics development.