学位論文の要約

題目　Synthesis and Evaluation of the Pyrrole-Imidazole Polyamides for Cancer Treatment
（がん治療を目指したピロール-イミダゾールポリアミドの合成と評価）

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DNA is a vital molecule for our life because DNA base sequences composed of four types of bases: adenine (A), thymine (T), guanine(G), and cytosine (C), determine all genetic information. DNA damages or mutations are a primary cause of cancer. Therefore, DNA has been the significant target for many types of anticancer drugs. DNA alkylating agents, a class of anticancer agents targeting DNA, have been widely applied in clinical use for cancer treatment. However, most of these compounds interact nonspecifically with DNA sequences, inducing serious side effects. To address this issue, molecular targeted drugs, such as imatinib for chronic myeloid leukemia (CML) treatment, have been studied. These drugs inhibit cancer growth by interfering with a specific target that is involved in the condition. Targeted drugs are expected to alleviate the side effects of previous DNA alkylating agents that lack selectivity to cancer cells.

To investigate a potential targeted drug, many types of DNA-alkylating pyrrole-imidazole (PI) polyamides have been developed by coupling of PI polyamides and DNA alkylating agents, such as *seco*-CBI and chlorambucil. PI polyamide is a DNA minor-groove binding molecule that recognizes the target sequence according to the pairing rule. DNA-alkylating PI polyamides have attracted attention because of their sequence-specific alkylating activities, which contribute to reducing the severe side effects. Many of these types of conjugates that target specific sequences involved in cancer proliferation have been studied as new candidates for anticancer drugs. In Chapter 1, the author reviews the recent progress into research on DNA-alkylating PI polyamides and their sequence-specific action on target sequences associated with cancer development

This dissertation focused on the synthesis and evaluation of new types of DNA-alkylating PI polyamides acting on the runt-related transcription factor (RUNX) family which has become an emerging target for cancer treatment. The RUNX family has been widely recognized as an essential master transcription factor relating to cancer development. Each of the RUNX family members binds specifically to the consensus DNA sequence, 5’-TGTGGT-3’. The binding of RUNX to specific DNA sequences is hypothesized to promote the expression of downstream genes and to cause cancer proliferation. Based on this proposed mechanism of cancer growth, the chlorambucil-PI polyamide conjugate (conjugate **1**) targeting the RUNX-binding sequence has been developed. It was reported that conjugate **1** had a marked anti-cancer effect in mouse models of acute myeloid leukemia. This result

demonstrated the potential of conjugate **1** as an anticancer drug. However, sufficient characterization and optimization of conjugate **1**, which are required for the clinical application, have not been performed.

In Chapter 2, to address this issue, the author chemically examined the molecular characteristics of conjugate **1** to confirm its potential as a RUNX-inhibiting drug. Conjugate **1** showed favorable results for sequence-specificity and binding affinity to the target sequence. The author also prepared an alternative conjugate **2**, which targets the same DNA sequence with conjugate **1**, by replacing one pyrrole with β-alanine. It was previously reported that the introduction of β-alanine into PI polyamides significantly improved their sequence-specificity because β-alanine allows the crescent-shaped ligand to fit the curvature of the DNA helix. Consistent with this previous report, the chemical characteristics of conjugate **2** were superior to those of conjugate **1**, suggesting that reaction selectivity and binding affinity to the RUNX-binding sequence were effectively improved by the substitution of β-alanine for pyrrole. This study implied the possibility of conjugates **1** and **2** as candidates for cancer therapeutics.

In Chapter 3, based on the attractive results in Chapter 2, a new series of CBI-PI polyamide conjugates targeting RUNX-binding sequence were synthesized to explore the possibility of an alternative structure. These conjugates were modified by switching the DNA alkylating moiety from chlorambucil to *seco*-CBI. It was previously determined based on Bind-n-Seq analysis that these CBI-PI polyamides had specificity to their respective target sites. However, the levels of enrichment at the target sequence were relatively low in that study. Therefore, to confirm this finding, the author elucidated the chemistry of DNA alkylation by these CBI conjugates using PAGE and HPLC analysis, which were used in Chapter 2. These analyses revealed that these conjugates specifically alkylated their targeted adenines. Furthermore, these CBI-PI polyamide conjugates showed about 100 times stronger cytotoxicity than the chlorambucil-PI polyamides which were reported in Chapter 2. It was indicated that the application of *seco*-CBI improved the anticancer effect of alkylating PI polyamides.

In conclusion, this dissertation chemically revealed the potential of DNA-alkylating PI polyamides as candidates for a RUNX-inhibition drug. Since the approach using DNA-alkylating PI polyamides might contribute to overcoming the problem of severe side effects and drug resistance caused by current chemotherapeutic drugs, further investigations of these conjugates are expected to promote the development of new types of anticancer agents. In addition to the chemical biology research described above, the author also worked on the internship and the project to support the social application of fundamental researches in the healthcare field. These achievements are briefly summarized in Appendix.