

論文 / 著書情報
Article / Book Information

題目(和文)	免疫チェックポイント阻害剤のための小分子複合体の設計
Title(English)	Design of small molecular compound conjugates for immune checkpoint inhibitor
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出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第11622号, 授与年月日:2020年9月25日, 学位の種別:課程博士, 審査員:小畠 英理,丸山 厚,小倉 俊一郎,堤 浩,三重 正和
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第11622号, Conferred date:2020/9/25, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

(博士課程)
Doctoral Program

論文要旨

THESIS SUMMARY

系・コース：	生命理工学	系	申請学位 (専攻分野)：	博士	(工学)
Department of, Graduate major in	生命理工学	コース	Academic Degree Requested	Doctor of	Engineering
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要旨 (英文 800 語程度)

Thesis Summary (approx.800 English Words)

Immunotherapy has recently emerged as a fourth modality for cancer therapy, together with surgery, chemotherapy, and radiation therapy. The immunotherapy promotes T-cells to kill cancer cells by the blockade of immune checkpoint pathways. One of the major immune checkpoint pathways is inactivated by the binding of programmed cell-death 1 protein (PD-1), which is largely expressed on T cells, and its ligand PD-L. The PD-L1 is mainly expressed on antigen-presenting cells under physiological conditions, but it is upregulated on cancer cells. Interaction between PD-1 and PD-L1 suppresses T-cell function, including cytolytic activity, leading to downregulation of the anti-tumor immune response. Therefore, inhibition of PD-1/PD-L1 interaction is important for cancer immunotherapy. For this inhibition, monoclonal antibodies have been developed and they have been used as therapeutic drugs. However, the high cost and low stability of antibodies are still serious problems. To overcome these problems, small molecular compounds have been screened from chemical library and the derivatives have been synthesized for replacement of antibodies.

Recently, biphenyl compound derivatives have been reported as potent small molecules for inhibition of PD-1/PD-L1 interaction. The derivatives are reported to directly bind to PD-L1 and induce homodimerization of PD-L1 to disturb the interaction between PD-1 and PD-L1. However, the inhibition activity of the biphenyl compound derivative is not so much high. Improvement of the inhibition activity is required to become a therapeutic inhibitor. Therefore, in this thesis, 1-[[3-bromo-4-[(2-methyl[1,1'-biphenyl]-3-yl)methoxy]phenyl]methyl]-2-piperidinecarboxylic acid was chosen as a starting compound which was previously reported as a small molecular inhibitor. The starting compound was modified with amino acids and polymers to improve the inhibition activity.

First, the starting compound was fragmented, and the biphenyl derivative was modified with amino acids. The inhibitory activity was measured by AlphaLISA® immunoassay. The immunoassay is based on photo-induced energy transfer between donor and acceptor beads conjugated with PD-1 and PD-L1. The measured half maximal inhibitory concentration (IC₅₀) of anti-PD-1 monoclonal antibody nivolumab (Opdivo®) was 5.1 nM. Considering the value approximately corresponded with the previously reported value, the immunoassay was judged to be appropriate. When the biphenyl compound derivative was modified with 3-bromotyrosine, it showed about 5 times higher activity (IC₅₀ = 1.5 μM) than that of starting compound (IC₅₀ = 7.2 μM). For the further modification, the biphenyl-conjugated bromotyrosine was modified with various amino acids. However, the further addition of amino acids reduced the inhibitory activity. Thus, in silico docking simulation was conducted to reveal the correlation between the reduced inhibition activity and the docking

modes of the amino acid conjugates in PD-L1 binding pocket. As a result, it demonstrated that the amino acid conjugation altered the docking conformation in binding pocket, and the changed conformation disturbed the formation of PD-L1 homodimer. In other words, improper interactions between conjugated amino acids and binding pocket induced displacement of amino acids and it finally led to reduced inhibition activity. It provides understanding of how small molecule could be rationally derivatized for higher inhibition activity.

Second, polymer and nanoparticle conjugates were prepared to induce multivalent effect. The multivalent binding mechanisms is considered to be the increase of binding chances due to increase of effective concentration and the higher statistical re-binding after dissociation of the original interaction. The biphenyl compounds were conjugated with linear or branched polyethylene glycol (PEG) in this thesis. The immunoassay showed the decrease of inhibitory activity by conjugation with linear PEG. This result suggested that the PEG moiety reduced the interaction of the biphenyl compound with PD-L1. On the other hand, the branched PEG conjugates enhanced the inhibition of PD-1/PD-L1 interaction. In addition, as the number of conjugated biphenyl compound increased, the higher inhibition activity was observed. The highest IC_{50} value was 0.33 μM , which was about 5 times higher than that of non-conjugated monovalent compound. Indeed, comparing with the starting compound, the inhibition activity was enhanced by more than twenty times. In addition, gold nanoparticle (GNP) was also used for the multivalent effect. The biphenyl molecule was immobilized on the surface of GNP with or without PEG spacer. The immobilization on GNP enhanced the inhibitory activity of biphenyl molecule and the insertion of PEG spacer additionally increased the effect.

In conclusion, one of the amino acid-modified biphenyl compounds increased the inhibitory activity of originally reported biphenyl compound against PD-1/PD-L1 interaction. In silico docking simulation demonstrated the molecular mechanism how amino acid modification affected the inhibition of PD-1/PD-L1 interaction in PD-L1 binding pocket. The inhibitory activity of biphenyl compound was significantly increased by polymer and nanoparticle modification. The activity increased with the increase of the number of conjugated biphenyl compound in polymer conjugation.

This thesis demonstrated that the inhibition activity of biphenyl compound against PD-1/PD-L1 interaction can be regulated by chemical modification of amino acids and can be enhanced by multivalent effect through polymer and nanoparticle conjugation.