Development of macrocyclic peptide for the control of VP24-KPNA interaction and conformation dynamic of channelrhodopsin

論文の内容の要旨

Development of macrocyclic peptide for the control of VP24-KPNA interaction and conformation dynamic of channelrhodopsin

（VP24−KPNA 相互作用とチャネルロドプシン構造を制御する大環状ペプチドの開発）

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Peptides are a class of molecules that bind to protein with exquisite binding affinity and specificity, which allows them to be used as molecules to disrupt protein-protein interaction (PPI) or serve as co-crystallization ligands. The RaPID (Random nonstandard Peptide Integrated Discovery) system is peptide discovery platform of which enables a rapid selection of protein-binding peptides from a genetically encoded peptide libraries. In this study, I will describe my work towards the discovery of peptides using the RaPID system for modulation of a PPI (**Chapter 2**) as well as being a co-crystallization ligand to trap an ion channel into a specific conformation (**Chapter 3**).

Chapter 1: General introduction

I introduce the superior features of peptides as well as the advantages of the RaPID system as a peptide discovery platform. At the same time, I also introduce the binding mode of macrocyclic to their targets and give a brief introduction of each chapters.

Chapter 2: Macrocyclic peptides inhibitors for the protein-protein interaction between Ebola virus protein VP24 and KPNA5

The protein-protein interaction of VP24-KPNA5 is critical for the pathogenesis of Ebola virus disease. Therefore identification of a molecule that modulates the VP24-KPNA5 interaction not only will facilitate our understanding towards the pathogenesis of Ebola virus, but also allows for the development of new therapy against Ebola virus disease. In this chapter, I

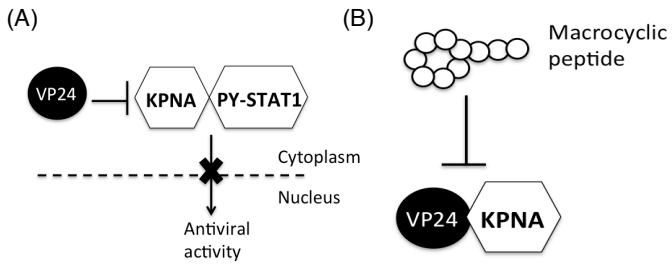

Figure 1. (A) Biological function of VP24. VP24 plays a critical role in suppressing host innate immune system. (B) VP24-binding peptides were developed to target VP24-KPNA protein-protein interaction.

described an affinity selection against Zaire Ebola virus protein VP24 by means of the RaPID system. Three macrocyclic peptides exhibit a remarkable high affinity to VP24 (K_D was in the single-digit nM range) as well as inhibitory activity against VP24-KPNA5 interaction (IC_{50} was in the μM range). This work demonstrates for the first time that a chemical probe is capable of modulating the PPI interaction, which provides a good starting point for the development of starting point for the development of unique anti-viral drugs against Ebola virus.

Chapter 3: Development of peptides that trap the open-state structure of Channelrhodopsin.

Channelrhodospsin is a light-gated ion channel, which is considered as a widely applicable tool in neuroscience. However, so far little is known about to its gating mechanism. In order to facilitate our understanding toward it, to obtain an "open state" structure is crucial. As the open state is transit, it is impossible to capture the open state structure by x-ray crystallography. In this chapter I described a light-based selection strategy to identify potential open state binders. Based on ligated-gated features of channelrhodopsin, I developed a selection strategy based on the RaPID system to identify potential open state binders. Using the modified system, I performed a selection against channelrhodopsin using two independent peptide libraries, a macrocyclic peptide library and a linear peptide library. For both of the selections, I was able to identify potential channelrhodopsin binding clones. Further, I used a single- clone assay to qualitatively determine their binding ability and selectivity toward the open state of channelrhodopsin. The result revealed that one clone of which was selected from the macrocyclic peptide, could be the most promising clones to have high-affinity to channelrhodopsin. On the other hand, a linear peptide of which show less potency in high-affinity binding, however showed selectivity toward the open state, suggesting that structural flexibility is critical for selectively binding to open state. In addition, I the co-crystallization of one of the macrocyclic peptide with channelrhodopsin was conducted and results was also described in details.

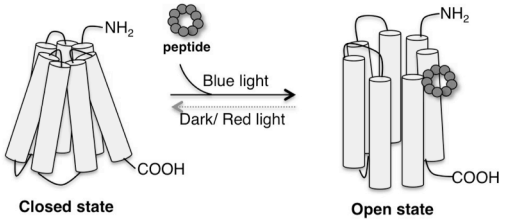

Figure 2. Schematic presentation of the aim of the study. The aim of study is to use peptide to trap the open state of channelrhodopsin (ChR).