論文の内容の要旨

Development of cyclic peptide scaffolds against large undruggable transmembrane proteins
（アンドラッガブルな膜タンパク質を標的にした特殊環状ペプチド創薬）

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Introduction

Transmembrane proteins are known to be an attractive choice for drug development due to the accessibility of their extracellular domains which can be targeted by non-membrane permeable drugs. As a result, transmembrane proteins make up approximately 60% of all drug targets to date. However, a large number of transmembrane proteins with high therapeutic potential as drug targets still remain elusive and “undruggable” by conventional high-througput screening methods. Therefore it has become necessary to explore other possible drug classes.

Recently, peptides have gained increasing attention as drugs for having potentially higher target specificity compared to small molecule drugs while having the potential for bioavailability and membrane permeability not seen in larger biologic drugs. In this study, I aimed at developing bioactive thioether macrocyclic peptides against three large transmembrane proteins, Plexin-B1, Nicastrin and α6β1-integrin. In addition, there is no available crystal structure for monomeric Plexin-B1 and absolutely no crystal structures exist for Nicastrin (human) or α6β1-integrin. Macrocyclic peptides have show some success as crystalization chaperones and thus any bioactive peptides that I discover will be used for structural studies as well.

In the sections below (2, 3, 4, 5 章) I will briefly discuss the results from my thesis.

2 章　Selection against Plexin using the RaPID system.

Plexins are a family of single-pass transmembrane proteins which interact with semaphorins, another class of transmembrane or secreted proteins. Plexin-semaphorin interactions have been known to be involved in the proper development and directional regulation of neurons. In recent years, their roles in bone remodeling, tumor progression, and cell motility have been revealed. Accordingly, plexins have received great attention as potential drug targets.

To date, no small molecule drugs or biologics have been successful at targeting Plexin. Therefore, the RaPID (Random non-standard Peptide Integrated Discovery) system could be used to generate peptides that bind Plexin. In this chapter I discuss my work in generating peptides that bind Plexin

3 章　Selection against integrin using the Rapid system

Integrins are involved in the attachment of cells to their specific tissue systems or cellular niches. Being involved in attachment, abnormal expression of integrins can allow cells to attach to incorrect locations which allows tumor cells to become metastatic. Therefore, inhibitors against integrins can be used as cancer drugs. In this chapter I describe my work in selecting for peptides that bind integrins

4 章　Ribosomally synthesized fused thioether tricyclic peptides

Although macrocyclic peptides in the range of 11 to 15 amino acids have shown to be able to mediate protein-protein interactions, larger scaffolds with more complex structures may yield more biologically active peptides. Various peptides found in nature, such as cyclotides, are composed of upwards of 40 amino acids and bear complex multicyclic structures. These peptides have been shown to have a large variety of biological activites from antiviral to PPI inhibitory abilities. Therefore, the ability of being able to screen such peptides using mRNA display has the potential to discover peptides with similar features such as the cyclotides found in nature.

The RaPID system, takes advantage of genetic code reprogramming to add an N-terminal chloroacetyl group to macrocylize peptides with a downstream cysteine to from a thioether bond. Previous characterization has shown that this N-terminal chloroacetyl group selectively forms thioether bonds with the closest cysteine in an amino acid sequence but is not able to form a thioether bond with an adjacent cysteine. Taking advantage of this unique selectivity, it was hypothesized that a peptide scaffold with four cysteines, with one adjacent to the N-terminus, could be reacted with tris bromomethyl benzene (TBMB) to create a larger fused tricyclic peptide.

First, to confirm if this cyclization selectivity is retained in longer peptides with multiple cysteines, peptides bearing an N-terminal chloroacetyl group followed by an adjacent cysteine and three more downstream cysteines positioned seven amino acids apart were ribosomally synthesized. In addition, genetic code reprogramming was used to introduce ester bonds between each cysteine on different templates which can be hydrolyzed under basic conditions. This way, the fragmentation pattern can be used to reflect the mode of cyclization. Indeed, only fragments representing cyclization occurring between the chloroacetyl group and the second cysteine were observed. Subsequently, various conditions were tested to optimize the addition of TBMB to form fused tricyclic thioether peptides. It was found that after translation, the addition of tris 2-carboxyethyl phosphine (TCEP) in 0.6 M HEPES-KOH pH 7.5 to a final concentration of 20 mM (TCEP) followed by the addition of TBMB in DMF to a final concentration of 8 mM TBMB resulted in successful tricyclization confirmed by MALDI-TOF MS by a mass shift corresponding to the desired tricyclic product.

5 章　Selection against secretase using the RaPID system

Secretases are intramembrane proteases that are involved in the cleavage of various transmembrane proteins which result in signal transduction. Although, inhibitors for secretase complexes are available they are not specific. Therefore, I chose to target specific protein subunits of secretase complexes with an aim of discovering substrate specific inhibitors. In this chapter, I discuss my work in selecting for peptides that bind proteins of secretase complexes.