showed a beneficial binding affinity (Kd = 330 nM). In the further SAR study, for example, we found that the substitution of Lys8 to Leu was strongly enhanced the binding affinity (Kd = 8.1 nM). In the presentation, we will show the detailed SAR studies including secondary structure analysis.

[1] Y. Ito, WO2016186206 A1, Nov., 24, 2016.

PA-101

Synthesis and structure-activity relationship study of an antibody-binding peptide focused on the C-terminal histidine residue

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Antibody-drug conjugate (ADC) is an attractive agent which can selectively deliver the drug to the target cell. We previously developed Fc-selective crosslinking reaction using antibody-binding peptide **1** (GPDC*AYHKGELVWC*TFH: *disulfide, Kd = 220 nM) for the preparation of ADC [1]. However, depending on the feature of the linking drugs, the preparation of desired ADC is sometimes difficult because of the decreased binding affinity of the peptide **1**-drug conjugate. Therefore, to enhance the antibody-binding affinity of the peptide, we performed a structure-activity relationship (SAR) study of the shortened peptide **2** (DC*AYHKGELVWC*TFH: *disulfide, Kd = 330 nM) focused on its C-terminal histidine residue (His17).

In the Fmoc-based solid-phase peptide synthesis, a significant amount of byproduct resulting from C-terminal p-hydroxybenzylation was observed during the cleavage step from the resin. The side-reaction can be suppressed by the addition of 1,3-dimethoxybenzene as a scavenger, as indicated by a previous report [2]. Subsequently, the antibody-binding affinity of the synthesized peptides was evaluated by the Surface Plasmon Resonance assay. The peptide with a substitution of His17 to 2-pyridylalanine showed a 4-fold higher binding affinity (Kd = 76 nM) compared to peptide **2**. However, a similar substitution to 3- or 4-pyridylalanine decreased the affinity, suggesting that the position of nitrogen atom in the side chain is important for the strong antibody binding. In the presentation, we will show the details about the further SAR study.

[1] Y. Ito, WO2016186206 A1, Nov., 24, 2016.
[2] P. Stathopoulos et al., J. Pept. Sci. 2006, 12, 227.

PA-102

Canceled

PA-103

CM-10K, A Novel Peptide Analogue Designed from Cecropin A-Melittin Hybrid Peptide, Showed A Promising Antibacterial Activity, Antibiofilm and Stability

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Antimicrobial peptides (AMPs) have recently attracted a great attention as promising antibiotic candidate. However, therapeutic applications of these AMPs have been hindered by their toxicity, low stability and high cost of production. Designing a novel peptide to overcome these problems is challenging and practicable. Previous studies have shown that cecropin A-melittin hybrid peptide or CM exhibits broad-spectrum antimicrobial activity with high toxicity. In this study, the modification of CM by amino acid substitution with lysine residue at position 10 (CM-10K) showed a remarkable antibacterial activity against *Staphylococcus epidermidis* ATCC 35984 (MIC) and *Pseudomonas aeruginosa* ATCC 27853 (MIC) with a decrease in toxicity as shown by the lower hemolytic activity against human red blood cells compared with a parent peptide, CM. Furthermore, CM-10K retains its activity under challenging physiological conditions (150 mM NaCl, 4.5 mM KCl, 1 mM $MgCl_2$) and high temperature environment up to 100°C. Flow cytometry results demonstrated that CM-10K killed bacterial cells by depolarizing and permeabilizing the cell membrane. Remarkably, CM-10K also inhibits the biofilm formation of *Staphylococcus epidermidis* ATCC 35984. Taken together, the peptide CM-10K may provide a promising antimicrobial agent for therapeutic applications.

Keywords: antimicrobial peptides, cecropin A-melittin, antibacterial activity, antibiofilm

PA-104

Antimicrobial and Antibiofilm Activities of CM-10K14K, A Novel Modified Peptide Analogue, against *Staphylococcus epidermidis*

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The Gram-positive bacteria *Staphylococcus epidermidis* is one of major cause of healthcare-associated infections due to its ability to colonize and form biofilms on medical devices such as catheters and other surgical implants. Moreover, the present of bacteria resistant to commonly used antimicrobial agents in biofilm also play an important role in biofilm treatment failure. CM, a hybrid of two antimicrobial peptides cecropin A (1-7) and melittin (2-9), displays antimicrobial activity with hemolytic effect. In order to improve the activity and overcome the toxicity, a novel peptide analogue was designed by amino acid substitution with lysine at two positions. The modified peptide, CM-10K14K, showed a potent antimicrobial activity against *S. epidermidis* strain (MIC) and also presented lower hemolytic effect than the parent peptide. More importantly, CM-10K14K markedly inhibits biofilm formation up to 70% at 2XMIC concentration. From this study, CM-10K14K could be an effective therapeutic agent against biofilm-related infection.

PA-105

Generation of Helix-Loop-Helix Peptide Inhibitor of the Interaction between Human CTLA-4 and B7

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Cytotoxic T lymphocyte antigen-4 (CTLA-4) which is expressed on T-cells plays a role in dampening the immune response, and inhibitors of this immune check point are capable of treating cancer cells by stimulating the immune system. Here, we tried to generate an inhibitor of the interaction between CTLA-4 and B7-1 from the libraries of Helix-Loop-Helix (HLH) peptide, which we have designed as a molecular scaffold for generating molecular-targeting peptides.

Yeast surface-displayed HLH peptide libraries were screened against human CTLA-4-Ig by using florescence-activated cell sorting (FACS), and yeast clones which selectively bound to CTLA-4 were isolated. Peptide Y-2 which was displayed on yeast cells showed the K_D value of 1.53±0.15 μM and the competitive inhibitory activity for the CTLA-4 and B7-1 interaction by using a flow cytometer. For further affinity maturation of Y-2, the secondary library in which the amino acids were randomly mutated by error prone PCR was constructed, and three favorable clones were isolated from the library by screening with FACS. We synthesized the peptides by standard SPPS, and examined their structures by using CD spectrometry. All of the peptides showed α-helical structures, and surface plasmon resonance analysis demonstrated that peptide ERY2-4 showed high and specific binding activity to the human CTLA-4 with the K_D value of 196.8±2.3 nM, and also inhibited the CTLA-4 and B-7-1 interaction with the IC_{50} value of 1.1±0.03 μM.

HLH peptide, ERY2-4 is expected to be a potent immune checkpoint inhibitor for CTLA-4.

PA-106

Development and Biological Evaluation of Echinomycin Analogues for Antitumor Drug

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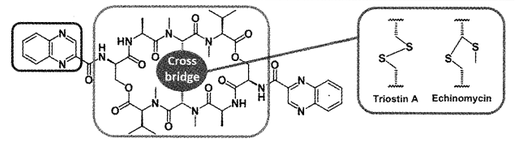
Triostin A(TA) and Echinomycin(Ec), which are naturally occurring quinoxaline antibiotics isolated from *Streptomyces aureus*, are C_2-symmetric cyclic octadepsipeptide with a disulfide and a thioacetal cross-bridge respectively. Ec has highly potent antitumor activity (IC_{50}: 5.5 nM on MCF-7) and inhibitory effect of hypoxia-inducible factor (HIF)-1 (0.35 nM). We recently established a useful liquid-phase procedure of total synthesis of TA. TA was found to have a strong cytotoxicity (IC_{50}: 2.0 μM on MCF-7) and inhibitory effect of HIF-1α transcriptional activity (26.7 nM).

In order to develop a mid-size anticancer drug based on a cyclic depsipeptide scaffold, we designed various derivatives of TA and Ec with the modified amino acid sequences of the depsipeptide ring, different aromatic chromophores, and various cross-bridge structures. These compounds were synthesized by the liquid phase procedure, and the obtained compounds were evaluated for their cytotoxicity on various tumor cells, transcriptional activity of HIF-1α, and anti-angiogenic activity. We found that several Ec derivatives having a modified cross-bridge structure exhibited strong antitumor activity and HIF-1α inhibitory effect comparable to Ec.

Structural modification studies of quinoxaline antibiotics

Modification	Cytotoxicity	HIF-1 suppression
Amino acid sequence of depsipeptide ring	↓	↓
Aromatic chromophore	↓	non
Cross bridge structure	↑↑	↑↑



PA-107

Epinecidin-1 protects mice from LPS-induced endotoxemia and cecal ligation and puncture-induced polymicrobial sepsis

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The antimicrobial peptide, epinecidin-1 (Epi), was identified from Epinephelus coioides and may have clinical application for treating sepsis. Epi has been shown to ameliorate antibiotic-resistant bacteria-induced sepsis in mice, but further evaluation in mixed-flora models and a description of the protective mechanisms are essential to establish this peptide as a potential therapeutic. Therefore, we first tested the protective effects of Epi against polymicrobial sepsis-induced bactericidal infection, inflammation and lung injury that result from cecal ligation and puncture in mice. Furthermore, since lipopolysaccharide (LPS) is a key inducer of inflammation during bacterial infection and sepsis, we also tested the LPS-antagonizing activity and related mechanisms of Epi-mediated protection in mice with LPS-induced endotoxemia and LPS-treated Raw264.7 mouse macrophage cells. Epi rescued mice from both polymicrobial sepsis and endotoxemia after delayed administration and suppressed both lung and systemic inflammatory responses, while attenuating lung injury and diminishing bacterial load. In vitro studies revealed that Epi suppressed LPS-induced inflammatory cytokine production.