氏　　名	小松　将之
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論文審査委員	委員長　教　授　西垣　功一 委　員　教　授　松岡　浩司 委　員　准教授　石丸　雄大 委　員　准教授　根本　直人

論文の内容の要旨

Although molecular target drugs have shown their potential for various disease therapies, most of them are made of small molecules and antibodies and thus have drawbacks of low specificity and / or high production cost. Therefore, peptide aptamers can be other candidates for clinical purpose because of their usage of being of high affinity and manageability. However, from the view point of pharmacodynamics and pharmacokinetics, there left many to be improved to the goal of good drugs. As a step toward this goal, we designed various types of conjugates: especially paired peptides (P&P) and peptide-antibody conjugates usable for cancer research and therapy.

P&P is a novel concept which can elaborately enhance the functions of peptide aptamers, where the primary step has a meaning of finding high affinity / function peptides (peptide aptamers) as a set and the secondary step is to find the positive cooperativity of those peptides combinatorially. In the preceding study, our laboratory has already established how to select a set of high affinity / function peptides: *i.e.* the initial selection utilizing the cDNA display which enables us to identify various peptides of considerable affinity / function, and secondary library method termed ASAC (all-steps-all-combination). In this study, not only inhibitory but also activatory activities could be obtained. The latter, usually very hard to develop, could be acquired by employing another novel technology *selection-by-function*. Following these technologies and an additional one which enabled to screen a different activity of cathepsin E at neutral pH (primary physiological function is believed to be optimized at pH4.5 and the function at pH7.2 is derivatized and shifted from such function). We could successfully develop the P&P of a high affinity (K_d = 2 nM) and activating ability (180%) to protease cathepsin E at neutral pH, improving the properties much higher than the previous peptide aptamers. Besides such excellent bio-pharmacochemical properties, which can be exploited for developing diagnostic reagent, one of these peptides was confirmed have an ability to enhance the cancer cell apoptosis through the activation of cathepsin E, indicating the competence as a cancer drug seed.

Another research was carried out to develop a peptide-antibody conjugate for cancer immunotherapy and chemotherapy, pursuing a novel function which cannot be realized by individual molecules. We have developed two

types of such designing: (1) Cell-cell linking antibody-peptide aptamer conjugate, *i.e.*, Immunoglobulin-conjugated anti-CD16a peptide aptamer for induction of ADCC (antibody-dependent cell-mediated cytotoxicity)-dependent cancer cell killing; and (2) Pep-toxin antibody-peptide aptamer conjugate, *i.e.*, Immunoglobulin-guided cytotoxicity-expressing peptide. Both were theoretically discussed with preliminary experiments.

Most importantly, diversities of high-quality peptide aptamers (providable by the systemic in vitro evolution method) can be designed to work elaborately, flexibility, and effectively in combination with the other functional peptide and/or protein (such as antibody).

In conclusion, we succeeded in developing methodological concepts and resultant products such as a paired peptide activator with anti-cancer activity by introducing novel useful conjugates.

論文の審査結果の要旨

The applicant (hereafter called as author) had challenged to develop a new field in the peptide aptamer application related to cancerous drugs and succeeded in pioneering to pave a road toward this goal.

Although molecular target drugs have shown their potential for various disease therapies, most of them are made of small molecules and antibodies and thus have drawbacks of low specificity and / or high production cost. Therefore, peptide aptamers can be other candidates for clinical purpose because of their usage of being of high affinity and manageability.

However, from the view point of pharmacodynamics and pharmacokinetics, there left many to be improved to the goal of good drugs. As a step toward this goal, the author designed various types of conjugates: especially paired peptides (P&P) and peptide-antibody conjugates usable for cancer research and therapy.

P&P technology was developed in our laboratory and formerly applied to panning cathepsin E (CE) activity-modulating peptides under acidic conditions (pH 4.5). In this study, it was examined whether it can work to evolve the CE-activating peptides under a neutral pH. Through this study, the author tried to expand the applicability of the P&P method and generalize the effectiveness of PLM (progressive library method), confirming the central concept that can lead to generate and sophisticate functional peptide aptamers: The successive steps of the primary one that has a meaning of finding high affinity/function peptides (peptide aptamers) as a set, the secondary one (ASAC: all-steps-all-combination) that is to find the positive coopera-tivity of those peptides combinatorially, and the third one (paired peptide library) that is to establish the most functional peptides in this systemic approach make up the strategy for advancing the function of peptides with certainty. In this study, the author also contributed to establish the way of identifying the activator peptide aptamers for enzymes. This is usually very hard to develop but could be acquired by employing another technology *selection-by-function*.

Following these and others, authors could successfully develop paired peptide aptamers of a high affinity (K_d = 2 nM) and activating ability (180%) to protease cathepsin E at a neutral pH. Besides such excellent bio-pharmacochemical properties, which can be exploited for developing diagnostic reagents, one of these peptides was confirmed to have an affinity to enhance the cancer cell apoptosis through the activation of cathepsin E, indicating the competence as a cancer drug seed.

Therefore, the author has enabled a great advance in the peptide aptamer study by introducing exact property analyses such as SPR (Surface Plasmon Resonance) and FIS (Fluoresence Intensity Shift; a kind of gel shift assay) together with bioassays of cell apoptosis, caspase activation, TRAIL induction, and others.

The other research was carried out to develop a peptide-antibody conjugate for cancer immunotherapy and chemotherapy, pursing a novel function which cannot be realized by individual molecules. The author have developed two types of conjugates for such a purpose: (1) Cell-cell linking antibody-peptide aptamer conjugate (***lariat type***), *i.e.*, Immunoglobulin-conjugated anti-CD16a peptide aptamer for induction of ADCC (antibody-dependent cell-mediated cytotoxicity)-dependent cancer cell killing; and (2) Pep-toxin antibody-peptide aptamer conjugate, *i.e.*, Immunoglobulin-guided cytotoxicity-expressing peptide (***payload type***). Both were theoretically discussed and practically examined to some extent.

Most importantly, multiple high-quality peptide aptamers provided by the PLM approach, of which methodological effectiveness has been confirmed by the current study of the author, allowed the author to design and preliminarily fabricate useful conjugate molecules in combination with the other functional peptide and/or protein (especially, antibody), which is a pioneering endeavor in this field.

In conclusion, the author succeeded in developing a method and resultant products such as paired peptide activators with anti-cancer activity and schemes, exploiting peptides to fabricate functional conjugates.