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– Biofunctional Design-Chemistry –

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Scope of Research

The ultimate goal of our research is the regulation of cellular functions by designed peptides and proteins. Current research subjects include (1) development of novel intracellular delivery systems aiming at elucidation and control of cellular functions using designed membrane permeable peptide vectors, (2) elucidation of the DNA or RNA binding modes of nucleic acid binding proteins, and design of artificial regulators of gene expression, (3) elucidation and control of membrane curvature, and (4) design of stimulation-responsible artificial peptides and proteins.

KEYWORDS

Membrane-Permeable Peptides
Intracellular Delivery
Peptide Design
DNA/RNA Binding Protein
Membrane Curvature

Recent Selected Publications

Kuriyama M.; Hirose H.; Masuda T.; Shudou M.; Arafiles J.V.V.; Imanishi M.; Maekawa M.; Hara Y.; Futaki S., Piezo1 Activation Using Yoda1 Inhibits Macropinocytosis in A431 Human Epidermoid Carcinoma Cells, *Sci. Rep.*,**12,** 6322 (2022).

Hirose H.; Hirai Y.; Sasaki M.; Sawa H.; Futaki S., Quantitative Analysis of Extracellular Vesicle Uptake and Fusion with Recipient Cells, *Bioconjug. Chem.*, **33,** 1852-1859 (2022).

Nakagawa Y.; Arafiles J.V.V.; Kawaguchi Y.; Nakase I.; Hirose H.; Futaki S., Stearylated Macropinocytosis-Inducing Peptides Facilitating the Cellular Uptake of Small Extracellular Vesicles, *Bioconjug. Chem.*, **33,** 869-880 (2022).

Yoshida A.; Oyoshi T.; Suda A.; Futaki S.; Imanishi M., Recognition of G-quadruplex RNA by a Crucial RNA Methyltransferase Component, METTL14, *Nucleic Acids Res.*, **50,** 449-457 (2022).

Nagano Y.; Arafiles J.V.V.; Kuwata K.; Kawaguchi Y.; Imanishi M.; Hirose H.; Futaki S., Grafting Hydrophobic Amino Acids Critical for Inhibition of Protein-Protein Interactions on a Cell-Penetrating Peptide Scaffold, *Mol. Pharm.*, **19,** 558-567 (2022).

Piezo1 Activation Using Yoda1 Inhibits Macropinocytosis

Macropinocytosis is a type of endocytosis accompanied by actin reorganization-driven membrane ruffling, followed by the formation of large vesicles called macropinosomes. Because macropinosomes (0.2–10 μm in diameter) are considerably larger than vesicles produced by other endocytic pathways (~100 nm in diameter), micropinocytosis can supply cells with large amounts of amino acids and proteins that are important for cell growth. Especially, it is well known that cancer cells with oncogenic RAS mutations promote micropinocytosis and efficiently acquire nutrients for their growth through macropinocytosis. Thus, inhibition of macropinocytosis is a promising way for cancer therapy. However, few specific agents that inhibit macropinocytosis have been developed because macropinocytosis-specific functional proteins and lipids have not been identified. Macropinocytosis is accompanied by large membrane deformations, but it is not known whether mechanosensitive channels that sense membrane tension are involved in macropinocytosis. In this study, we focused on Piezo1, one of the the mechanosensitive ion channels, and investigated if Piezo1 activation affects micropinocytosis (Fig. 1) [1]. We found that Yoda1, a specific Piezo1 agonist, potently inhibits macropinocytosis induced by epidermal growth factor (EGF) in A431 cancer cells. Treatment with Yoda1 efficiently inhibited membrane ruffle induction and subsequent macropinosome formation. The inhibition of ruffle formation by Yoda1 was dependent on the extracellular Ca^{2+} influx through Piezo1. Piezo1 activation aberrantly activated the calcium-activated potassium channel KCa3.1 and inhibited Rac1 activation which is crucial for membrane ruffle formation. These results suggest that Ca^{2+} ions can regulate EGF-stimulated macropinocytosis. This study paves the way for the development of methods to manipulate macropinocytosis by regulating Ca^{2+} channel activity such as mechanosensitive channels using chemical tools.

References

[1] Kuriyama, M. *et al*., *Sci. Rep.*, **12,** 6322. (2022).

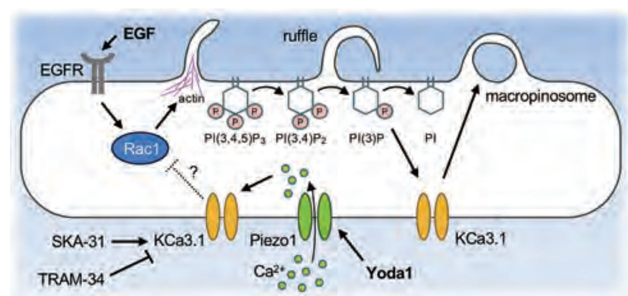

Figure 1. Proposed mechanism of macopinocytosis inhibition by Piezo1 activation. Reprinted from [1].

G-Quadruplex Specific Binding of the METTL14 RGG Domain

RNA G-quadruplexes (rG4s) are non-canonical four-stranded structures formed by G-rich sequences. Previous reports have shown the importance of rG4s in various biological processes. Recent bioinformatics analyses pointed out that potential RNA G4-forming sequences and *N*6-methyladenosines (m6A) are colocalized in viral RNA (ZIKV and HIV)[2]. m6A is the most abundant RNA modification which involves in various important aspects of RNA metabolism. The m6A modification is catalyzed by the m^6A writer complex composed of the METTL3/METTL14 heterodimer and additional adaptor proteins. So far, the mechanism of the relationship between rG4 and RNA adenosine methylation has not been elucidated at all. Here, we focused on the RNA binding property of METTL3/METTL14 methyltransferase heterodimer, especially the RNA binding specificity of the RGG repeats of METTL14 to RNA G-quadruplexes.

Methyltransferase domain (MTD) heterodimer of METTL3 and METTL14 with the RGG repeats of METTL14, MTD3/MTD14-RGG, were prepared. Gel mobility shift assays demonstrated that the RNA binding affinities of MTD3/MTD14-RGG to G4-forming RNAs were higher than those to non-G4 forming RNAs in K^+ buffer but comparable in Li^+ buffer. These results indicate that MTD3/MTD14-RGG specifically recognized G4-structured RNAs. In addition, *in vitro* methylation assays in the mixtures of G4 forming RNA and non-G4 forming RNA in the presence of HeLa total RNA showed that MTD3/MTD14-RGG selectively methylated adenosines close to rG4. These results provide a possible process for recruiting METTL3/METTL14 to G4 forming regions and a new insight of rG4 in epitranscriptomic regulation (Fig. 2) [3].

Reference

[2] Fleming, A.M. *et al.*, *ACS Cent. Sci.*, **5**, 218-228 (2019).
[3] Yoshida, A.*et al.*, *Nucleic Acids Res.*, **50**, 449-457 (2022).

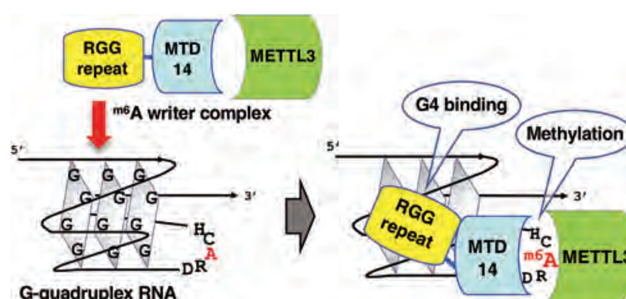

Figure 2. m6A writer complex binds to G-quadruplex RNA.