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論文題目	Macropinocytosis-Inducing Peptides: Identification, Utility, and Mechanism-of-Action 新規マクロピノサイトーシス誘導ペプチドの同定、細胞内送達への有用性と作用様式		

（論文内容の要旨）

Understanding the mechanisms for intracellular delivery is paramount to develop smarter and more efficient strategies. Among well-known cell entry routes, endocytosis pathways, particularly macropinocytosis, are attractive for the delivery of biomacromolecules. Macropinocytosis is an inducible, actin-dependent form of endocytosis. This process is performed by cells to ingest large amounts of extracellular fluid and materials into vesicles known as macropinosomes. External stimuli, such as growth factors, could activate this process to facilitate large scale and cell-wide uptake. Utilizing macropinocytosis as a cellular entry route could improve the efficiency of intracellular delivery.

This dissertation illustrates the identification of novel macropinocytosis-inducing peptides and their potential as a tool for intracellular delivery (Chapter 1), and the active chemical species and their mechanism for macropinocytosis induction (Chapter 2).

Chapter 1: Nucleic acid and Protein Delivery Using a Macropinocytosis-Inducing Peptide Combined with a Membrane-Lytic Peptide
(第1章　マクロピノサイトーシス誘導ペプチドと膜溶解ペプチドとを併用した核酸およびタンパク質送達)

Intracellular delivery using endocytic pathways require (1) the accumulation of cargo molecules into endosomes, and (2) an effective method for endosome disruption to release the cargoes into the cytosol. Macropinocytosis non-preferentially engulfs extracellular materials in large quantities and accumulates them in macropinosomes. This characteristic of macropinocytosis should have merits for the intracellular delivery of large functional cargoes. This chapter will discuss the use of an identified macropinocytosis-inducing peptide, fused with a physicochemical disruptor of endosomes, to deliver functional nucleic acids and proteins into cells.

It has been reported that the interaction of CXC-chemokine receptor 4 (CXCR4) with its ligand, stromal cell-derived factor (SDF)-1α, leads to macropinocytosis. To identify potent macropinocytosis-inducing peptides, N-terminal peptide segments of SDF-1α were synthesized and their macropinocytosis inducing activity was evaluated in terms of 70 kDa dextran uptake. A peptide corresponding to the 21-residue peptide (SN21: KPVSLSYRCPCRFFESHVARA-amide) was identified as a potent macropinocytosis inducer. Its activity was also confirmed by inhibitor experiments and by visualizing actin reorganization. SN21 was then combined with the membrane-lytic peptide LK15 (SN21-LK15) to facilitate cargo release into the cytosol. Effective intracellular delivery of nucleic acids (plasmid DNA and siRNA), immunoglobulins (IgGs), and genome-editing proteins (Cre recombinase and TALE-VP64) were achieved using SN21-LK15, suggesting that macropinocytosis-based delivery successfully accommodates biomacromolecules with different physicochemical properties.

Chapter 2: Important Chemical Species and Their Mechanism to Activate Macropinocytosis (第2章　マクロピノサイトーシス活性化に重要な化学種と作用機序)

Although SN21 is derived from the N-terminal segment of SDF-1α, SN21 lacks interaction with CXCR4 to activate macropinocytosis. Structure-activity relationship, analyzed by alanine scanning, revealed the importance of residues Y7 to F14 for the activity. More importantly, mutations on the positions 9 or 11 abolished the activity of SN21. A 8-residue derivative of SN21 (P4A) was established, showed a similar increase in dextran uptake, and was used to study the modes of action of the peptides. HPLC analysis suggested the formation of active P4A species immediately after addition to culture media. Amino acids in the media are critical in forming active P4A species. More importantly, dextran uptake was not stimulated in the absence of formed products, and chemically synthesized active P4A species induced high levels of dextran uptake, suggesting them as the active components in the activity of P4A.

These peptides activate phosphoinositide 3-kinase (PI3K). This finding suggests that SN21/P4A-mediated macropinocytic uptake may share a similar mode of macropinocytosis activation with growth factors such as through the epidermal growth factor receptor (EGFR) or the platelet-derived growth factor receptors (PDGFR).

This dissertation highlights unique aspects of macropinocytosis induced by SDF-1α-derived peptides, its applicability to intracellular delivery, and the mechanism-of-action. These should contribute to the development of intracellular delivery systems of macromolecules and should have implications on the fundamental understanding of macropinocytosis.

（続紙 2 ）

（論文審査の結果の要旨）

マクロピノサイトーシスは外的刺激により誘導されるアクチン駆動型の液相エンドサイトーシスで、細胞外液および溶質の細胞内への取込を促進する働きを持つ。この取込経路の利用により、バイオ高分子の細胞内送達の促進も期待される。

本論文の第1章では、ストロマ細胞由来因子(SDF)-1αのN末端21アミノ酸配列に対応するペプチドSN21がマクロピノサイトーシス誘導能を有し、SN21存在下、モデル高分子70 kDaデキストランの細胞内取込が顕著に促進されることが示された。さらに、SN21と膜傷害性ペプチドLK15を連結したペプチド(SN21-LK15)存在下、プラスミドDNA、siRNA、免疫グロブリンG(IgG)、Creリコンビナーゼや人工転写因子 TALE-VP64などが高効率で細胞内に送達され、所望の活性が得られることが確認され、SN21-LK15のバイオ高分子の細胞内送達への有用性が示された。

第2章では、SN21の構造活性相関を通して、マクロピノサイトーシス誘導における9位、および11位のシステインの重要性が指摘された。さらなるSN21の配列の検討により、マクロピノサイトーシス誘導に重要な8アミノ酸が同定された。その類縁体P4Aは、SN21を上回る70 kDaデキストランの細胞内取込促進効果を有していた。また、P4Aによるマクロピノサイトーシス誘導には培地中のシステインが重要な役割を果たすことが明らかになった。

以上、本論文は、新規マクロピノサイトーシス誘導ペプチドSN21の同定と細胞内送達への有用性の確認を行うとともに、作用様式に検討を加えたものであり、バイオ高分子の細胞内送達に有用な方法論を提示するものと考えられる。

よって、本論文は博士（薬科学）の学位論文として価値あるものと認める。また、令和2年8月21日、論文内容とそれに関連した事項について試問を行った結果、合格と認めた。

要旨公表可能日：　　年　　月　　日以降