様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Ligand-free hepatocyte-targeting of nanomedicines by selective stealth coating of liver reticuloendothelial system scavenger cells

研究課題名（英文）Ligand-free hepatocyte-targeting of nanomedicines by selective stealth coating of liver reticuloendothelial system scavenger cells

研究代表者

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研究者番号：７０７９４３５３

交付決定額（研究期間全体）：（直接経費）　　3,500,000円

研究成果の概要（和文）：肝実質細胞（肝細胞）への核酸治療薬の部位特異的送達は、多くの肝疾患の治療戦略として期待されている。
しかしながら、全身投与された核酸治療薬の肝細胞選択的デリバリーにおける最大の問題は、肝スカベンジャー網状内皮系（RES）［類洞内皮細胞 およびクッパー細胞］による非特異的排除であり、輸送効率の大幅な低下を引き起こす。
本研究では、オリゴカチオンを結合させた2本鎖poly(ethylene glycol)を用いたPEGコーティングにより、RESのスカベンジャー受容体を選択的に覆い、遮断することで、肝RESによる核酸治療薬の認識と排除を抑制し、肝細胞への送達を最大化することでこの問題に対処した。

研究成果の学術的意義や社会的意義
Scientific significance: We developed in situ stealth coating technology for transient blockade of liver scavenger cells, and promoted nucleic acid therapeutic transfection.
Social significance: Our technology can increase the therapeutic potential of nanomedicines with lower doses without ligands.

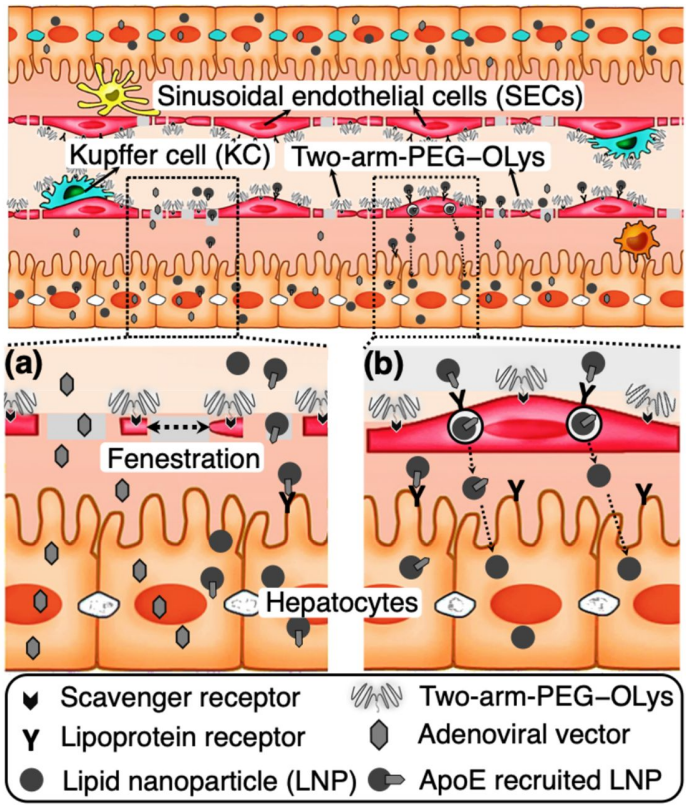
研究成果の概要（英文）：Site-specific delivery of nucleic acid therapeutics to liver parenchymal cells (hepatocytes) is a potential strategy for the treatment of many liver diseases. However, the biggest issue in hepatocyte-selective delivery of systemically administered nucleic acid therapeutics is nonspecific elimination by liver scavenger reticuloendothelial system (RES) [sinusoidal endothelial cells (SECs) and Kupffer cells (KCs)], causing a substantial decrease in delivery efficiency. In this study, we addressed this issue by selective masking of scavenger receptors of RES with PEG coating using a oligocation conjugated two-armed poly(ethylene glycol) to suppress the recognition and elimination of nucleic acid therapeutics by liver RES and thereby maximizing the delivery to hepatocytes.

研究分野：Biomedical engineering

キーワード：Liver blockade　Sinusoidal endothelium　Kupffer cells　Nanomedicine retargeting

1. 研究開始当初の背景

Cell-specific targeting of theranostics is the ultimate goal of nanomedicine, which not only drastically reduces the dose required for therapy but also minimizes toxicity and immunogenicity by avoiding off-target effects. In particular, site-specific targeting of hepatocytes using synthetic and nature-derived gene delivery systems (GDSs) has shown promising therapeutic outcomes for treating a wide variety of liver diseases including hemophilias and cancers. Yet, reported hepatocyte-targeting efficiencies were limited, though the majority of the dose is accumulated in the liver. This limited hepatocyte-targeting is mainly because of two issues. First, most of the dose is nonspecifically captured by the liver scavenger RES wall (SECs and KCs), leaving a very low dose to hepatocytes. Second, rapid nuclease degradation of gene drugs of nonviral- and inactivation of viral-GDS in the lysosomal compartment within scavenger cells causes low gene transfer to hepatocytes. Avoiding these two issues is key to allowing GDS to reach hepatocytes. The PI conceived would it be possible to maximize the hepatocyte-selective targeting of gene therapeutics by minimizing nonspecific entrapment by scavenger wall? Towards finding an answer to this question, PI has recently developed a selective and transient poly(ethylene glycol) (PEG) coating to scavenger wall to avoid the nonspecific recognition and elimination of GDSs, a first-of-its-kind strategy in the world (**Scheme 1**). The Oligo(L-lysine) conjugated two-arm-PEG (two-arm-PEG−OLys) selectively bind to abundantly expressed scavenger receptors (SRs) of SECs and KCs for coating the PEG, leaving other receptors uncoated, and thus, accessible to GDS. Interestingly, PEG coating was ultimately cleared from the wall within 6 hours by excreting to bile, therefore leading to transient PEG coating. Such selective and transient PEG coating effectively prevented nonspecific entrapment of GDS by scavenger wall [A. Dirisala et al.



Scheme 1. In situ PEG coating to scavenger receptors of liver SECs and KCs by two-arm-PEG–OLys to prevent nanomedicine capture via stealth property of PEG. Transendothelial pathways of nanomedicine to reach hepatocytes. (**a**) Passive trafficking through the fenestrae of SECs. (**b**) Specific receptor-mediated uptake in endocytic vesicles after protein corona recruitment and cytoplasmic shuttling via transcytosis.

Sci Adv 2020;6:eabb8133]. This key finding motivated PI to redirect GDS from the scavenger wall to hepatocytes.

2. 研究の目的

Having established that GDSs can maximize protein expression in the liver, it is hypothesized that hepatocytes may majorly be transfected as the nonspecific uptake capacity of the scavenger wall was blocked by PEG coating. Thus, the purpose of this proposal is projected to increase the transfection efficiency of genetic drugs to the liver (**Scheme 1a,b**), and exploit this maximized liver gene expression for therapy. The scientific significance of the proposal is separately described for viral- and nonviral-GDS.

Viral GDS: Despite some clinical success using viral GDSs, severe liver toxicity led to the death of humans in clinical trials due to the high dose required to obtain a therapeutic level of protein expression. This unexpected outcome is strongly associated with adverse inflammatory reactions of the scavenger wall, as it captures a significant portion of the viral dose. It clearly demands the urgency of strategies that reduce the viral dose without compromising the level of therapeutic protein expression.

Nonviral GDS: PI extended the liver coating strategy to messenger RNA (mRNA) adsorbed cationic liposomes (lipoplex). Such lipoplexes for delivering mRNA have enormous potential for genome editing. However, the transfection potential of LNP/mRNA to hepatocytes has not yet been fully maximized due to nonspecific clearance and concomitant nuclease degradation of mRNA within scavenger cells despite varying lipid composition and size. PEG coating to the scavenger wall enabled a 14.1-fold increase in Luc expression in the liver compared to without

3. 研究の方法

BALB/c mice (6 weeks old, female, Charles River Laboratories) were intravenously injected with 1.25 mg of two-arm-PEG-OligoLys, followed by the injection of AdV encoding firefly luciferase (FLuc) driven by the CMV-IVS promoter (Vector Biolabs, Malvern, PA, USA), sequentially at 5-min interval. For the control mice, 10 mM HEPES buffer containing 150 mM NaCl (pH 7.4), instead of two-arm-PEG-OligoLys, was injected before the AdV injection. Three weeks after the AdV injection, the livers were excised. The extracted livers were homogenized using Multibead Shocker in passive lysis buffer, followed by a Luc assay using a Luciferase Assay System and Lumat LB9507. The luminescence intensity values were normalized to the total protein amount in the homogenates determined by the Micro BCA Protein Assay Reagent Kit. The values were presented after subtracting the background values obtained from the tumors harvested from mice without AdV injection.

The mRNA encoding firefly luciferase (FLuc) was coated onto cationic liposomes at a liposome-to-mRNA ratio of 1 to 2. This resulted in anionic lipoplex with an average intensity-weighted mean hydrodynamic diameter of 221 nm, a polydispersity index of 0.11, and an anionic zeta-potential of −30 mV. BALB/c mice (6 weeks old, female,) were intravenously injected with anionic lipoplex loading 2 μg FLuc mRNA with and without two-arm-PEG-OligoLys preinjection (1.25 mg in 10 mM HEPES buffer, pH 7.4). Livers were harvested 6 hours after anionic lipoplex. The Luc assay and data were

analyzed as described in the previous section for the quantification of Luc expression in the tumor tissue.

4. 研究成果

The scientific results are separately described for viral- and nonviral-GDS.

Viral GDS: PEG coating of the scavenger wall would be advantageous because this technology substantially improved gene expression in the liver (12-fold) at an equivalent dose used without PEG coating (**Fig. 1**). Therefore, PEG coating to the scavenger wall can potentially reduce the dose required for viral gene therapy. AdV was chosen because it is most widely used for targeting hepatocytes, though it is intrinsically captured by scavenger wall cells, and, accounts for 32% of all gene therapy clinical trials. The improved Luc expression in the liver after PEG coating (**Fig. 1**), suggests that successful prevention of AdV entrapment to the scavenger wall resulted in an increased transfer to hepatocytes. Most likely, the smaller size of AdV (93 nm) compared to the size of pores (fenestrae) (141 nm) within SECs facilitated them to passively transport across fenestrae to reach hepatocytes for endocytic uptake (**Scheme 1a**).

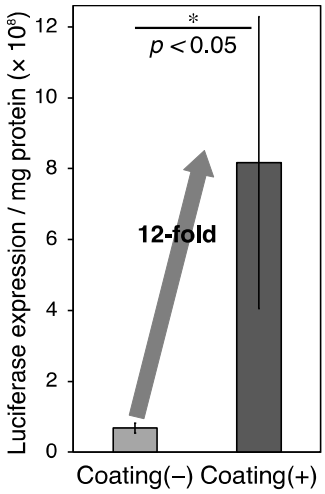


Fig. 1. AdV trasnduction in the liver with and without PEG coating (n = 4). Mean ± STD.

Nonviral GDS: PEG coating (**Fig. 2**), suggesting the delivery potential of LNP to hepatocytes maximized because nonspecific SRs of SECs and KCs were blocked by PEG coating. This remarkable expression in the liver after PEG coating could fulfill the promise of 'hepatocyte-selective distribution' of GDSs and could be explained by two mechanisms. First, direct passive trafficking of LNP or endogenous apolipoprotein E (ApoE) recruited LNP (ApoE/LNP) through fenestrated SECs makes them accessible to hepatocyte endocytic capture (**Scheme 1a**). Second, ApoE/LNP SECs, which are not PEG-coated. This receptor binding triggers rapid transcytosis of ApoE/LNP to hepatocytes, which bypasses lysosomal degradation (Scheme 1b). The ligand-free hepatocyte-selective targeting indispensably maximizes the therapeutic efficacy and minimizes toxicity and immunogenicity of GDS by dose reduction without compromising the therapeutic level of protein expression.

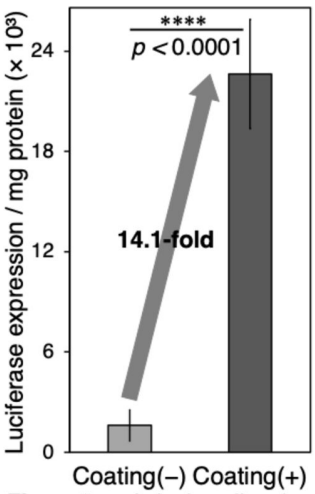


Fig. 2. Anionic lipoplex transfection in the liver with and without PEG coating (n = 4). Mean ± STD.

５．主な発表論文等

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２．論文標題 Stealth and pseudo-stealth nanocarriers	５．発行年 2023年
３．雑誌名 Advanced Drug Delivery Reviews	６．最初と最後の頁 114895～114895
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.addr.2023.114895	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Roy Sayoni Maitra、Garg Vrinda、Sivaraman Sushmitha Pedugu、Barman Sourav、Ghosh Chitrita、Bag Pousali、Mohanasundaram Palanivelmurugan、Maji Partha Sona、Basu Arnab、Dirisala Anjaneyulu、Ghosh Surya K.、Maity Amit Ranjan	４．巻 83
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掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jddst.2023.104408	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

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掲載論文のDOI（デジタルオブジェクト識別子） 10.29011/2577-1515.100218	査読の有無 有
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1．著者名 Van Guyse Joachim F. R.、Abbasi Saed、Toh Kazuko、Nagorna Zlata、Li Junjie、Dirisala Anjaneyulu、Quader Sabina、Uchida Satoshi、Kataoka Kazunori	4．巻 136
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掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/ange.202404972	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

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1．発表者名 Dirisala Anjaneyulu、Uchida Satoshi、Kataoka Kazunori
2．発表標題 Selective and transient stealth coating of liver scavenger wall enables retargeting of nanomedicines
3．学会等名 14th ISAJ Annual Symposium on Integrated Science For a Sustainable Society(招待講演)（招待講演）（国際学会）
4．発表年 2023年

１．発表者名 Dirisala Anjaneyulu、Uchida Satoshi、Kataoka Kazunori
２．発表標題 Transient stealth coating of liver scavenger sinusoidal wall enables retargeting of nanomedicines
３．学会等名 29th International conference on processing and fabrication of advanced materials(国際学会)（国際学会）
４．発表年 2023年

１．発表者名 Dirisala Anjaneyulu、Uchida Satoshi、Kataoka Kazunori
２．発表標題 Transient stealth coating of liver scavenger sinusoidal wall enables retargeting of nanomedicines
３．学会等名 29th International conference on processing and fabrication of advanced materials（国際学会）
４．発表年 2023年

１．発表者名 Dirisala Anjaneyulu、Uchida Satoshi、Kataoka Kazunori
２．発表標題 Selective and transient stealth coating of liver scavenger wall enables retargeting of nanomedicines
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１．著者名 P. K. Hashim, Anjaneyulu Dirisala	４．発行年 2022年
２．出版社 Jenny Stanford Publishing	５．総ページ数 66
３．書名 Nanomaterials in Chemotherapy	

〔出願〕　計4件

産業財産権の名称 脂質ナノ小胞を脾臓に標的化する方法およびそのための組成物	発明者 内田智士,Anjaneyulu Dirisala,片岡 一則	権利者 同左
産業財産権の種類、番号 特許、1	出願年 2023年	国内・外国の別 国内

産業財産権の名称	発明者	権利者
Complex, medicine, therapeutic agent for cancer, kit and conjugate	N Nishiyama et al.	同左
産業財産権の種類、番号	出願年	国内・外国の別
特許、1	2022年	外国

産業財産権の名称	発明者	権利者
脂質ナノ小胞を脾臓に標的化する方法およびそのための組成物	S Uchida et al.	同左
産業財産権の種類、番号	出願年	国内・外国の別
特許、1	2022年	国内

産業財産権の名称	発明者	権利者
複合体、医薬、癌治療剤、キット及び結合体	NISHIYAMA Nobuhiro et al.,	同左
産業財産権の種類、番号	出願年	国内・外国の別
特許、1	2021年	外国

〔取得〕　計1件

産業財産権の名称	発明者	権利者
Composition controlling pharmacokinetics in the body	Kataoka et al.	2024
産業財産権の種類、番号	取得年	国内・外国の別
特許、1	2024年	外国

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関