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）Overcoming multi drug resistance in 3D breast cancer cell model by pH-sensitive biomimetic nanoparticles

研究課題名（英文）Overcoming multi drug resistance in 3D breast cancer cell model by pH-sensitive biomimetic nanoparticles

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：この研究は、抗がん剤に対する耐性を発達させた乳がんの治療効率を高めるためのドラッグデリバリーシステムの開発を目的としています。この実験では、3次元MDR細胞構造を持つ人体と同様の条件下でその有効性を評価しました。
2年間の研究の結果、この研究で開発されたナノ粒子は食作用を回避し、より大量の薬物をMDR癌細胞に送達することが確認されました。この研究は、一種の膜を使用した以前の研究とは異なり、ハイブリッドタイプの赤血球と耐性癌細胞膜を使用した最初の研究です。その効率は、薬剤感受性の癌細胞膜よりも効率的であることを証明する上でも重要です。

研究成果の学術的意義や社会的意義
This research is the first study using a hybrid type of red blood cells and resistant cancer cell membranes. The developed NPs have the potential to reduce treatment cost. It can be an asset to the Japanese economy as it provides a proprietary market in competition with other countries.

研究成果の概要（英文）：This study aimed to develop a drug delivery system to increase the treatment efficiency of breast cancer that has developed resistance to anticancer drugs. So, the nanoparticles were protected using the red blood cells membrane, which allows them to evade immune cells and increase the delivery efficiency using the cell membrane with the properties of resistant cancer cells. This experiment has evaluated its effectiveness under similar conditions to the human body with a three-dimensional MDR cell structure.
As a result of the two-year study confirmed that the nanoparticles developed in this study avoid phagocytosis and deliver a more significant amount of drug into the MDR cancer cell. This research is the first study using a hybrid type of red blood cells and resistant cancer cell membranes, unlike previous studies using a kind of membrane. Its efficiency is also significant in proving that it is more efficient than drug-sensitive cancer cell membranes.

研究分野：生体材料学関連

キーワード：Multi-drug resistance　Breast cancer　Biomimetic nanoparticles　Cell membrane

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Breast cancer is the most common and lethal cancer type in women worldwide. Numerous therapeutic strategies that include smart biological treatments toward specific cellular pathways are being developed. Yet, inherent and acquired multidrug resistance (MDR) to chemotherapeutic drugs remains a major obstacle to effective breast cancer treatments. Currently, cell membrane-camouflaged NP drug delivery is a new concept that focuses on the targeted delivery of therapeutics by living cells instead of passive factors or surface markers. RBC can easily escape immune system recognition among these camouflaged NP, inhibiting macrophage-mediated phagocytosis using CD47. Compared to other cells, cancer cells' inherent homologous adhesion property for tumor targeting is rarely investigated and exploited. Cancer cells possess intercellular homologous binding capability with membrane proteins, which could be utilized for NP surface functionalization to allow complete replication of surface antigenic diversity. So, I proposed an RBC-cMDR fusogenic NP system as a new theragnostic nanoplatforms for breast cancer.

２．研究の目的

This research aims to develop 1) a human-like 3D tumor microenvironment in vitro system for studying MDR and aid in discovering novel anti-MDR therapies, and 2) RBC-cMDR fusogenic NP with target specificity for overcoming MDR via efficient drug delivery in breast cancer MDR cells.

３．研究の方法

FY 2020, research work was mainly focused on 1) cell preparation and 2) developed pH-sensitive polymer and nanoparticles (NPs). First, the parents and multi-drug resistance (MDR) cell lysates were prepared. Cell line integrated molecular authentication (CLIMA) analysis confirmed the differences between parents and MDR cells. Furthermore, cellular cytotoxicity, MDR-related protein, and gene expression were confirmed. Red blood cell (RBC) and MDR cell membrane were extracted using hypotonic solution and gathering by ultracentrifugation method. Second, the pH-sensitive polymer was synthesized by the RAFT polymerization method and evaluated. I developed pH 6.4 sensitive polymers and formed doxorubicin (DOX) loaded NPs.

FY 2021, research focused on 1) optimizing the cell membrane coating method and 2) building the MDR environment on a 3D spheroid. First, the prepared pH-sensitive NPs were coated with RBC and MDR membrane. The ideal ratio and purification were confirmed with drug loading efficiency and the size of NPs. Second, the 3D spheroid was set up to build an MDR tumor environment. Fibroblast, endothelial cell line, and MDR cancer cells were co-cultured in Gravity TRAP 96-well. We set up 150 μm diameter size of spheroid and delivered NPs.

４．研究成果

I set up cell membrane extraction conditions and RBC, and MDR cell membranes were aliquoted at a 4 mg/mL concentration. The stocks were kept at -80 °C deep freezer until ready to use. The polymer was synthesized and set up Pka 6.4 for destructing at acidic tumor conditions. NMR analysis **(Fig. 1A)** and titration assay **(Fig. 1B)** showed that polymer synthesis succeeded. After optimizing the polymer, I synthesize anti-cancer drug (DOX) loaded NPs. Before the cell membrane was coated on the surface, the NPs showed 150 nm-160 nm size and had a positive charge **(Fig. 1C)**. Post cell membrane coated on the NPs, size become slightly increased about 160-170 nm and has a slightly negative charge **(Fig. 1D)**. The TEM results showed clearly that the RBC-cMDR fusogenic NPs were formed fusogenic NPs **(Fig. 1E).**

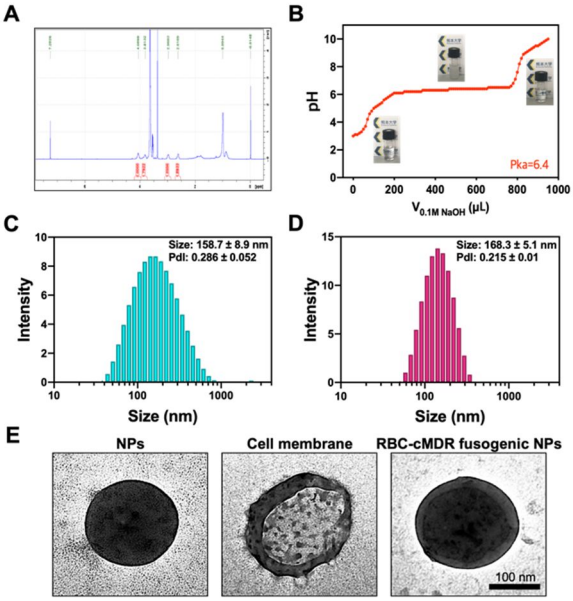

Figure 1. Material preparation. **A.** NMR of polymer, **B.** Titration of polymer, **C.** Size of NPs, **D.** Size of RBD-cMDR fusogenic NPs, E. TEM image of NPs, cell membrane, and RBC-cMDR fusogenic NPs

The cell membrane modification was performed by western blot. The membrane-targeted cocktail antibody proved that the fusogenic NPs coated with the cell membrane **(Fig. 2A)**. 3D spheroid was built using a microtissue mold **(Fig. 2B)** and to reduce the hypoxic/necrotic zone under 2%), the spheroid size was set up to a maximum of 150 µm diameter. In **Fig. 2C**, MDR cancer cell 2D images showed a low uptaken of DOX inside the nucleus. Compared to free DOX, RBC-cMDR fusogenic NPs accumulate inside the nucleus and start to induce apoptosis. 3D spheroid showed high accumulative RBC-cMDR fusogenic NPs imaged in all MDR single-cells **(Fig. 2D)**. For paper publication, SPIM imaging is planning on mid of June.

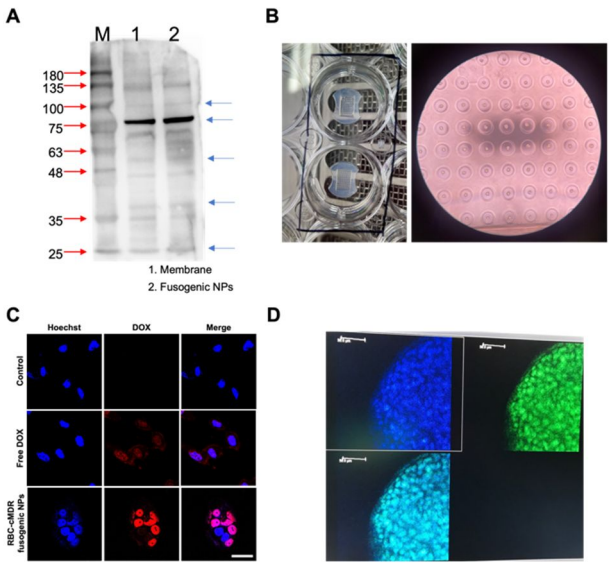

Figure 2. In vitro evaluation. **A.** Western blot of RBC-cMDR fusogenic NPs, **B.** Gravity TRAP microtissue mold and 3 days cultured spheroid, **C.** 2D cell culture, **D.** 3D cell culture.

Herein, I developed low immunogenicity and highly internalized RBC-cMDR fusogenic NPs for MDR treatment. The RBC-cMDR fusogenic NPs have a 160-170 nm spherical uniform character, and the drug release could control depending on the pH. Furthermore, the 2D and 3D micro-sized spheroid showed that RBC-cMDR fusogenic NPs could internalize deeply and efficiently deliver the drug. This research revealed that the RBC-cMDR fusogenic NPs have the potential to overcome the MDR.

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　3件／うちオープンアクセス　3件）

１．著者名 Sho Ueno, Min Woo Kim, Gibok Lee, Yong Il Park , Takuro Niidome, Ruda Lee	４．巻 12
２．論文標題 Development of ErbB2-Targeting Liposomes for Enhancing Drug Delivery to ErbB2-Positive Breast Cancer	５．発行年 2020年
３．雑誌名 pharmaceutics	６．最初と最後の頁 585
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/pharmaceutics12060585	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

１．著者名 Sajid Fazal, Ruda Lee	４．巻 13
２．論文標題 Biomimetic Bacterial Membrane Vesicles for Drug Delivery Applications	５．発行年 2021年
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掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/pharmaceutics13091430	査読の有無 有
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１．著者名 Chinmaya Mahapatra, Ruda Lee, Manash K. Paul	４．巻 27
２．論文標題 Emerging role and promise of nanomaterials in organoid research	５．発行年 2022年
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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.drudis.2021.11.007	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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３．学会等名 The Japanese Society for Biomaterials
４．発表年 2021年

１．発表者名
Sho Ueno, Min Woo Kim, Takuro Niidome, Ruda Lee

２．発表標題
Development of ErbB2-targeting Liposomes for Enhancing Drug Delivery to ErbB2-positive Breast Cancer

３．学会等名
The 36th Annual Meeting of the Japan Society of Drug Delivery System

４．発表年
2020年

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Sho Ueno, Takuro Niidome, Ruda Lee

２．発表標題
Development of ErbB2-targeting Liposomes for Enhancing Drug Delivery to ErbB2-positive Breast Cancer.

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４．発表年
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１．発表者名
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２．発表標題
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１．発表者名
Sho Ueno, Ruda Lee

２．発表標題
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３．学会等名
Biosensors 2021（国際学会）

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２．発表標題 ErbB2/EGFデュアルターゲティングリポソームのDOX/Rapa併用研究における相乗効果
３．学会等名 The 37th Annual Meeting of the Japan Society of Drug Delivery System
４．発表年 2021年

〔図書〕　計0件

〔産業財産権〕

〔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			
韓国	Korea Basic Science Institute (KBSI)	Kangwon National University	Chonnam National University	他2機関
米国	University of Utah	University of California Los Angeles		