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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）Therapeutic polymer nanoreactor with site-specific activation of targeting Warburg metabolism for metastatic breast cancer treatment

研究課題名（英文）Therapeutic polymer nanoreactor with site-specific activation of targeting Warburg metabolism for metastatic breast cancer treatment

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交付決定額（研究期間全体）：（直接経費）　　3,000,000円

研究成果の概要（和文）：1. A generic method has been developed to construct smart enzyme-loaded nanoreactors for targeting tumor metabolism.
2. As a accident discovery, nanoreactors could induce an immunostimulatory form of cell death by pyroptosis, which has the great potential to prime anti-tumor immune responses.

研究成果の学術的意義や社会的意義
1. Scientific significance: a generic method has been developed to construct smart oxidase-loaded nanoreactor for specifically targeting tumor metabolism.
2. Social significance: Our nanoreactors can increase the potential of immunotherapy to bring more benefits to cancer patients.

研究成果の概要（英文）：We developed a oxidase-loaded nanoreactor that could specifically render tumor cells immunogenic by pyroptosis, a highly pro-inflammatory programmed cell death. Of particular interest, the vesicular system based on PICsomes is highly modular, generic, and simple and could be adapted to customize various kinds of activatable nanoreactors based on triggerable cross-linking membrane networks. As a consequence, the nanoreactor with self-boosting catalytic glucose oxidation could protect oxidase to initiate pyroptosis in the long term. We confirmed that not only glucose oxidase-loaded but also lactate oxidase-loaded nanoreactors could induce pyroptosis. In combination with immunotherapy, the antitumor efficacy has been confirmed.

研究分野：Biomaterials

キーワード：Immunotherapy　Nanoreactors　Pyroptosis　Self-assembly　Vesicles　Tumor metabolism

１．研究開始当初の背景

Mounting evidence indicates that cancer is a metabolic disease, in which tumor cell could rewire nutrients utilization and cellular metabolism to satisfy the demands of growth and metastasis. A well-known dysregulation in cancer metabolism is Warburg effect that cancer cells exhibit a marked increase in glucose uptake and produce more lactate (LA) than normal tissues. The corresponding therapeutic opportunity for targeting glucose metabolism is to use glycolytic inhibitor, 2-deoxyglucose (2-DG), as an anticancer drug, which could lead to intracellular ATP depletion and induction of autophagy. However, its use in clinical trials has shown very little efficacy in solid tumors and the dosing necessary to achieve antitumor effects in patients resulted in adverse toxicity. As same as that of 2-DG for antitumor application, most of other therapeutic agents for inducing the stress of nutrients metabolism in clinical and preclinical stage are native enzymes or small molecular inhibitors, both of which suffer from immunogenicity, short half-life, low bioavailability, and substantial toxicity issues. On the other hand, lactate, the end product of glycolysis from Warburg metabolism, initially considered a waste product. Recently, lactate has been recognized as a metabolic driver to rewire the tumor microenvironment and power tumor malignancy, including acidifying the extracellular space, modulating immune cell function and promoting invasion and metastasis. Evidence further indicates tumor use tumor-derived and blood-borne lactate from Warburg metabolism as a fuel to feed the tricarboxylic acid cycle (TCA cycle) and generate energy. The scarcity of nutrients promotes this metabolic symbiosis, that is to say, tumor could switch to prefer lactate as respiratory fuel over glucose when glucose bioavailability is limited. However, lactate has rarely been employed as a direct target to manipulate tumor metabolism and simultaneous targeting glucose and lactate metabolism is also rarely investigated.

Polymer nanomedicine is promising for cancer treatment especially in site-specific therapeutic agents delivery compared to administration of free agents, as it has obvious advantages in terms of biosafety, stability, drug loading efficiency, targeting, and multifunctionality. Based on the tumor microenvironment or the available exogenous stimuli, the design of responsive polymeric nanocarrier is rising for tissue-specific activation.

Cancer is the first leading cause of death in Japan since 1981 and mortality rate is further increasing year by year, which makes Japan face ballooning healthcare costs. Among females, breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death. The growing incidence and mortality of breast cancer during the past two decades in Japan highlights the importance of developing new therapeutics (Lancet. Oncol. 2016, 17, E305- E312). Herein, the applicant would try to use the marriage of nanomedicine and targeting tumor metabolism to yield an offspring that is expected to selectively fight against breast cancer growth and metastasis.

２．研究の目的

The purpose of this proposal is to develop site-specific nanomedicine-directed targeting breast cancer metabolism.

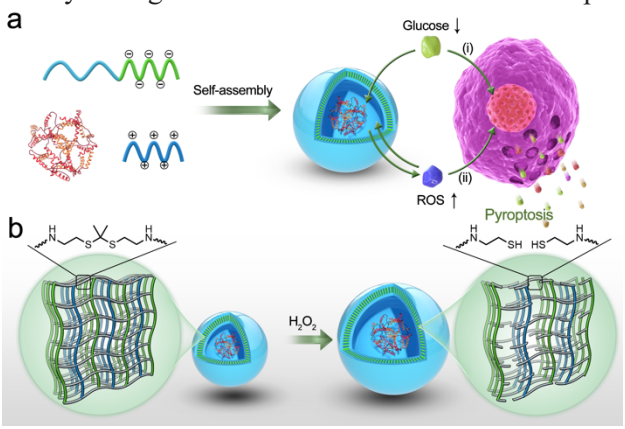
３．研究の方法

A series of glucose oxidase (GOD), or lactate oxidase (LOD)-loaded vesicles will be constructed based on electrostatic self-assembly of a pair of oppositely charged PEG-*b*-polyanion and homopolycation. Glucose/lactate starvation and ROS burst in tumor site are induced by GOD/LOD-catalyzed the oxidation of glucose/lactate. The primary objective is to find a rational design for oxidase-loaded redox-responsive vesicular nanoreactor to target breast tumor metabolism. Tumor redox microenvironment provide distinct opportunity that can be exploited by redox-responsive nanocarrier. However, in the tumor, redox microenvironments are numerous, complex, and heterogeneous. Mounting evidence indicates that a ‘‘one size fits all’’ redox microenvironment does not exist in tumor. This is a great challenge that utilization of redox-responsive nanocarriers as a delivery system. Here, three kind of redox microenvironment-responsive nanocarriers have been screened for tumor-specific activable nanoreactor. Three different redox-responsive linkers are engineered into polycations to construct oxidation-responsive (thioketal bond), reduction-responsive (disulfide bond), and oxidation/reduction dual-responsive (diselenide bond) segments, respectively. The secondary objective is to exploit the benefit of optimal glucose oxidase and lactate oxidase-loaded nanoreactor on improvement in the tumor-specific activation to simultaneously suppress breast tumor growth and metastasis. Most

women with breast cancer do not succumb to primary tumor but instead to metastatic progression that become apparent after surgical resection. Disseminated cells are much dependent on altered metabolism to withstand oxidative and fuel starvation stress. 4T1, MDA-231-D3H1, and MDA 231-LM2-4175 orthotopic breast cancer models will be utilized to investigate the influence of nanoreactor-directed targeting tumor metabolism on tumor growth and metastasis.

４．研究成果

This work provided a general platform to engineer vesicular nanodevices with tunable permeability without involving trade-offs between structural integrity, flexibility and functionality by integrating stimuli-responsive linkers into crosslinking membrane network. Given that varying polycations have already been used to form PICsomes, it is feasible to extend application of this flexible strategy to other responsive vesicles. We can tailor the responsive properties in on-demand manner, for example, smart tumor microenvironment-responsive nanoreactors design. Successful fabrication of several redox-responsive PICsomes and its triggered expansion without fracture affirmed this strategy. Based on high oxidative stress environment and high glucose/lactate in tumor site, it is reasonable to design ROS-responsive GOD-loaded nanoreactor for site-specific activation and amplification of oxidative stress, avoiding the production of excessive ROS in blood and normal tissue. However, mounting evidence indicates that ‘‘one size fits all’’ microenvironment does not exist in tumor. There is clearly an urgent need to construct a series of responsive vesicles with similar physicochemical properties for screening optimal nanoreactor. Furthermore, we found that LOD/GOD-loaded PICsomes as therapeutic nanoreactor with self-boosting catalytic ROS production capability and long-term cytocidal function could induce an immunostimulatory form of cell death termed as pyroptosis, which has the great potential for priming anti-tumor immunotherapy and opens new avenues for using LOD/GOD-loaded nanocarriers as a cancer treatment modality.



Scheme 1. (a) Schematic illustration of the ROS-responsive GOD-loaded therapeutic nanoreactor with self-boosting catalytic glucose oxidation due to membrane permeability enhancement during ROS production for achieving cytocidal function via pyroptosis-mediated immunogenic cell death followed by glucose starvation (i) and oxidative stress induction (ii). (b) Schematic illustration of vesicle swelling from membranal crosslinking density decreasing and membranal hydrophobic-to-hydrophilic transition followed by H2O2-triggered cleavage of hydrophobic thioketal linker into hydrophilic thiols.

５．主な発表論文等

〔雑誌論文〕　計10件（うち査読付論文　10件／うち国際共著　4件／うちオープンアクセス　1件）

１．著者名 Han Yu、Wen Panyue、Li Junjie、Kataoka Kazunori	４．巻 345
２．論文標題 Targeted nanomedicine in cisplatin-based cancer therapeutics	５．発行年 2022年
３．雑誌名 Journal of Controlled Release	６．最初と最後の頁 709～720
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jconrel.2022.03.049	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 Dirisala Anjaneyulu、Uchida Satoshi、Li Junjie、Van Guyse Joachim F. R.、Hayashi Kotaro、Vummaleti Sai V. C.、Kaur Sarandeep、Mochida Yuki、Fukushima Shigeto、Kataoka Kazunori	４．巻 2100754
２．論文標題 Effective mRNA Protection by Poly(l-ornithine) Synergizes with Endosomal Escape Functionality of a Charge-Conversion Polymer toward Maximizing mRNA Introduction Efficiency	５．発行年 2022年
３．雑誌名 Macromolecular Rapid Communications	６．最初と最後の頁 2100754～2100754
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/marc.202100754	査読の有無 有
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２．論文標題 Enzymatically Transformable Polymersome‐Based Nanotherapeutics to Eliminate Minimal Relapsable Cancer	５．発行年 2021年
３．雑誌名 Advanced Materials	６．最初と最後の頁 2105254～2105254
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/adma.202105254	査読の有無 有
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１．著者名 Zhou Qinghao、Mohammed Fathelrahman、Wang Yuheng、Wang Jingbo、Lu Nannan、Li Junjie、Ge Zhishen	４．巻 339
２．論文標題 Hypoxia-responsive block copolymer polyprodrugs for complementary photodynamic-chemotherapy	５．発行年 2021年
３．雑誌名 Journal of Controlled Release	６．最初と最後の頁 130～142
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jconrel.2021.09.023	査読の有無 有
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2．論文標題 Tumor-dilated polymersome nanofactories for enhanced enzyme prodrug chemo-immunotherapy	5．発行年 2021年
3．雑誌名 Journal of Controlled Release	6．最初と最後の頁 418～429
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jconrel.2021.10.015	査読の有無 有
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1．著者名	4．巻
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2．論文標題 Self‐Boosting Catalytic Nanoreactors Integrated with Triggerable Crosslinking Membrane Networks for Initiation of Immunogenic Cell Death by Pyroptosis	5．発行年 2020年
3．雑誌名 Angewandte Chemie International Edition	6．最初と最後の頁 13526～13530
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/anie.202004180	査読の有無 有
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2．論文標題 Chemo-physical Strategies to Advance the in Vivo Functionality of Targeted Nanomedicine: The Next Generation	5．発行年 2020年
3．雑誌名 Journal of the American Chemical Society	6．最初と最後の頁 538～559
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2．論文標題 Transient stealth coating of liver sinusoidal wall by anchoring two-armed PEG for retargeting nanomedicines	5．発行年 2020年
3．雑誌名 Science Advances	6．最初と最後の頁 eabb8133
掲載論文のDOI（デジタルオブジェクト識別子） 10.1126/sciadv.abb8133	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 －

1．著者名 Li Junjie、Anraku Yasutaka、Kataoka Kazunori	4．巻 132
2．論文標題 Self‐Boosting Catalytic Nanoreactors Integrated with Triggerable Crosslinking Membrane Networks for Initiation of Immunogenic Cell Death by Pyroptosis	5．発行年 2020年
3．雑誌名 Angewandte Chemie	6．最初と最後の頁 13628～13632
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/ange.202004180	査読の有無 有
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2．論文標題 Dual-Sensitive Nanomicelles Enhancing Systemic Delivery of Therapeutically Active Antibodies Specifically into the Brain	5．発行年 2020年
3．雑誌名 ACS Nano	6．最初と最後の頁 6729～6742
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acsnano.9b09991	査読の有無 有
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〔学会発表〕　計2件（うち招待講演　1件／うち国際学会　0件）

1．発表者名 Junjie Li
2．発表標題 Glucose oxidase (GOD) as cancer therapeutics—GOD of Small Things
3．学会等名 第29回次世代医工学研究会プログラム/The 29th Next Generation Medical Engineering Conference
4．発表年 2022年～2023年

1．発表者名 Junjie Li
2．発表標題 Strategies to advance functionality of polymeric nanomedicine
3．学会等名 九州大学 先導物質化学研究所 特別講演会（招待講演）
4．発表年 2022年～2023年

〔図書〕　計0件

〔出願〕　計2件

産業財産権の名称	発明者	権利者
Non-fouling or super-stealth vesicle	Junjie Li, Kazunori Kataoka	同左
産業財産権の種類、番号	**出願年**	**国内・外国の別**
特許、2022-169220	2022年	国内

産業財産権の名称	発明者	権利者
Non-fouling or super-stealth vesicle	Junjie Li, Kazunori Kataoka	同左
産業財産権の種類、番号	**出願年**	**国内・外国の別**
特許、-	2022年	外国

〔取得〕　計0件

〔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