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

令和　５ 年　 ６ 月 １３ 日現在

機関番号：８２６２６
研究種目：若手研究
研究期間：2021～2022
課題番号：２１Ｋ１４５０８

研究課題名（和文）Light-activatable nanoparticles to treat triple negative breast cancers

研究課題名（英文）Light-activatable nanoparticles to treat triple negative breast cancers

研究代表者
于　躍（YU, YUE）

国立研究開発法人産業技術総合研究所・生命工学領域・研究員

研究者番号：９０８８１６３１

交付決定額（研究期間全体）：（直接経費）　　3,600,000円

研究成果の概要（和文）：3つの共通薬物標的を持たないトリプルネガティブ乳がん（TNBC）には、ほとんどの抗がん剤が有効ではない。2022年度は新しい高分子材料である共有結合性有機フレームワーク（COF）の合理的設計と修飾に基づき、光応答性ナノ粒子を開発した。COFナノ粒子は、Transient receptor potential vanilloid 1（TRPV1）チャンネルを光熱的に活性化することで細胞内のCa2+流入を促進し、結果としてTNBCの生存に不可欠なWntシグナル伝達経路を阻害することができることを明らかとした。この結果は、COFナノ粒子がTNBC治療のための新規薬剤としての有用性を示す。

研究成果の学術的意義や社会的意義
本研究は、光活性化COFナノ粒子を用いたTNBCに対する新しい方向性を提供するものである。生体毒性を有する化学薬品を使用する現行の治療法とは異なり、開発したCOFナノ粒子は、制御可能な方法でがんを治療する安全で効果的な方法を提供する。この新しい戦略は、TNBCを治療するための新しい技術を提案するだけでなく、ナノバイオ医薬品に関する新しい洞察をもたらし、様々な形態の癌に対する改良型ナノメディカルの将来の開発の指針となる可能性がある。

研究成果の概要（英文）：As indicated by the name of “triple negative” that comes from the lack of three common drug targets, most cancer drugs are not effective for triple negative breast cancers (TNBC). In this this study, we developed a light-responsive nanoparticle (NP) based on rational design and modification of covalent organic framework (COF) which is a new class of polymeric materials. The COF NPs are able to initiate cellular Ca2+ influx through photothermal activation of transient receptor potential vanilloid 1 (TRPV1) channels, and consequently inhibit Wnt signaling pathway that is essential for TNBC survival. The results indicate COF NPs are potentially useful as a novel photo-agent for TNBC treatment.

研究分野：ナノバイオサイエンス

キーワード：cancer　nanoparticle　phototherapy　calcium ion　Wnt signaling

１．研究開始当初の背景

Triple negative breast cancer (TNBC) is one of the most challenging cancers to treat. It is called as triple negative because it doesn't have three markers associated with other types of breast cancer, which are important for prognosis and treatment. Wnt signaling might be a potential therapeutic target because it is particularly activated in TNBC. However, disruption of Wnt signaling in non-cancerous cells also influences normal cellular functions. Therefore, traditional Wnt interfering agents with insufficient targeting ability may cause severe side effect.

Nanomaterials have potentials to manipulate cellular activities by specific targeting photothermal sensitive Ca^{2+} channels. Transient receptor potential vanilloid 1 (TRPV1) is a calcium-permeable ion channel best known for its ability to control many cellular processes via Ca^{2+} modulation. TRPV1 channels are activated by heat (>43 degrees C) and are overexpressed in numbers of tumor types including TNBC. Emerging evidence indicates intracellular Ca^{2+} overload resulted from TRPV1 activation compromises Wnt signaling. Therefore, developing TRPV1-targeting nanoparticles (NPs) that covert light energy to heat for Ca^{2+} influx induction could be a promising therapeutic option for treating TNBC. Furthermore, the light-guided activation of TRPV1 channels can limit Wnt-mediated therapy only on TNBC without harming normal tissues.

２．研究の目的

TNBC is an aggressive disease that grows quickly and metastasizes early. There are no effective therapies for TNBC, so new strategies are needed. The goal of this project is to meet this need using light-activatable NPs that can optically control TNBC-associated signaling transductions on demand. Specific goals of this study include: (1) design and functionalization of NPs for TRPV1 targeting, (2) elucidate the molecular mechanism of action of these NPs by studying their interaction with TNBC cells, and (3) validate whether they can safely prevent the growth of TNBC under light irradiation.

３．研究の方法

This study started from synthesis of light-harvesting NPs, followed by surface functionalization for TRPV1 targeting. Live cell imaging was conducted to investigate NPs' effect on TRPV1 activation triggered by near infrared (NIR) light (785 nm). Treatment conditions (such as NPs concentration, light intensity, irradiation time, etc.) were optimized to avoid over-heating the cells while initiating Ca^{2+} influx. The subsequent impacts on Wnt signaling was studied using TNBC cells (MDA-MB-231). After finding out mechanism of interaction between light-driven NPs and Wnt signaling, the therapeutic performance of NPs was determined by examining the viability of TNBC cells after the treatment.

４．研究成果

(1) Construction of light-activatable COF NPs

Due to the pre-designable optical properties, covalent organic framework (COF) holds a great promise in optical control of cellular functions. Several nanostructured COF containing light-absorbing units were synthesized by solvothermal method, and evaluated for light-to-heat conversion efficiency (785 nm laser, 5 min). Among them, a porphyrin-based COF NPs showing best performance was selected for further modification with TRPV1 antibody (**図** 1). The resulted nanoconjugates showed good water dispersibility with an average size around 140 nm.

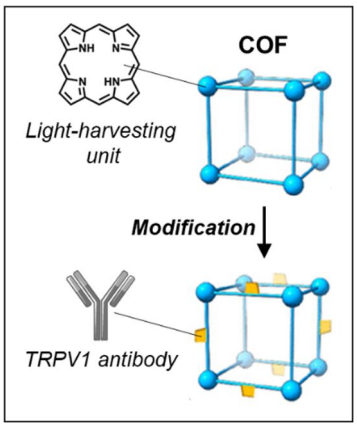

図１．COF NPs の合成と機能化

(2) Targeted Ca^{2+} influx by light irradiation

TRPV1-enriched and -compromised cancer cells were established for comparing the antibody-mediated targeting efficiency of COF NPs. To examine whether light-induced COF NPs is able to activate TRPV1 Ca^{2+} channels, a Ca^{2+} indicator plasmid (GCaMP6) was transfected into TNBC cells (MDA-MB-231). The stably established cells were subsequently incubated with COF NPs, and subjected to live cell imaging for

in situ Ca^{2+} detection. The results showed a robust increase in fluorescence signal after NIR light irradiation (**図** 2), suggesting light-driven COF NPs are capable of inducing Ca^{2+} influx in TNBC cells.

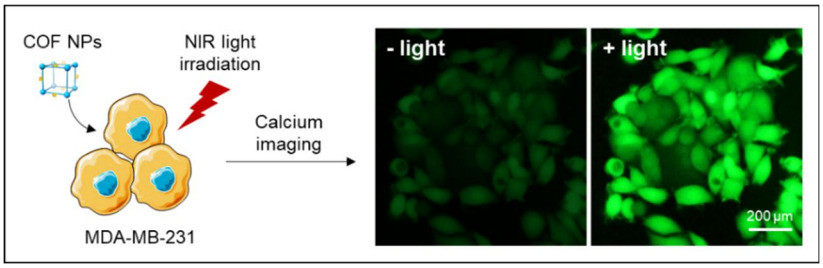

図２．光照射 COF NPs による細胞内 Ca^{2+}流入

(3) Molecular analysis of Wnt signaling in TNBC

β-catenin, the main effector of Wnt signaling, was investigated using COF NPs-treated TNBC cells. Western blotting and luciferase reporter assays showed that, light-induced cellular Ca^{2+} influx repressed the activity of β-catenin, and attenuate its nucleus translocation. Consequently, its downstream Wnt target oncogenes responsible for TNBC growth were inhibited after this treatment (**図** 3).

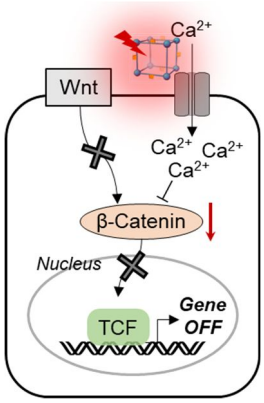

図３．光照射 COF NPs による Wnt シグナル阻害のメカニズム

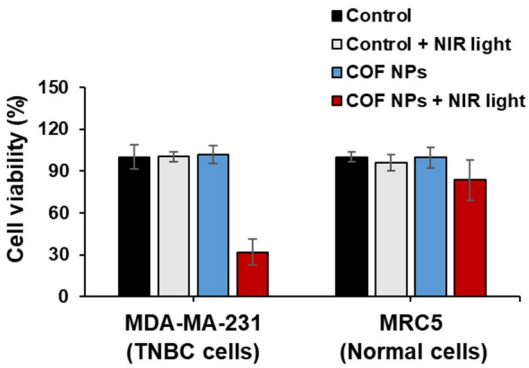

図４．光照射した COF NPs 処理後の細胞生存率の評価

(4) Therapeutic evaluation of COF NPs for TNBC

To investigate the anticancer effect of COF NPs, TNBC cells were incubated with COF NPs for 6 hours, and exposed to NIR light for 5 min. Cell viability was evaluated by WST-8 assay after further incubation of 24 hours. In addition to TNBC cells, normal fibroblasts (MRC5) was also used to confirm the safety of COF NPs for non-cancerous cells. As shown in **図** 4, COF NPs-treated TNBC cells showed significant reduction in viability upon NIR light irradiation, whereas normal cells showed negligible decrease. Of note, COF NPs alone (without light) did not affect the survival of both cells, suggesting COF NPs has low toxicity. Collectively, above results indicate light-induced COF NPs is able to kill TNBC through interfering Wnt signaling.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　0件）

１．著者名 Zhang Yuwei、Li Ziping、Sun Qikun、Li Zhongping、Zhi Yongfeng、Nie Riming、Xia Hong、Yu Yue、Liu Xiaoming	４．巻 42
２．論文標題 Light‐Emitting Conjugated Organic Polymer as an Efficient Fluorescent Probe for Cu2+ Ions Detection and Cell Imaging	５．発行年 2021年
３．雑誌名 Macromolecular Rapid Communications	６．最初と最後の頁 2100469～2100469
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/marc.202100469	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 Li Kejuan、You Fengming、Zhang Qin、Yuan Ruijiao、Yuan Qianghua、Fu Xi、Ren Yifeng、Wang Qian、Li Xiaohong、Zhang Zhenya、Shichiri Mototada、Yu Yue	４．巻 12
２．論文標題 Chemical and Biological Evidence of the Efficacy of Shengxian Decoction for Treating Human Lung Adenocarcinoma	５．発行年 2022年
３．雑誌名 Frontiers in Oncology	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fonc.2022.849579	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 Wan Jieqiong、Shi Wei、Li Yan、Yu Yue、Wu Xiaohan、Li Zhongping、Lee Seung Yong、Lee Kyu Hyoung	４．巻 43
２．論文標題 Excellent Crystallinity and Stability Covalent Organic Frameworks with High Emission and Anions Sensing	５．発行年 2022年
３．雑誌名 Macromolecular Rapid Communications	６．最初と最後の頁 2200393～2200393
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/marc.202200393	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 Yu Yue、Zhang Guoxin、Li Zhongping、Wang Jia、Liu Yang、Bhardwaj Rahul、Wadhwa Renu、Nagao Yuki、Shichiri Mototada、Gao Ran	４．巻 -
２．論文標題 Designed fabrication of active tumor targeting covalent organic framework nanotherapeutics via a simple post-synthetic strategy	５．発行年 2023年
３．雑誌名 Nano Research	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/s12274-022-5265-7	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

〔学会発表〕　計4件（うち招待講演　2件／うち国際学会　0件）

１．発表者名
Yue Yu

２．発表標題
Cancer Targeted Delivery of Withaferin A Using a Nanostructured Covalent Organic Framework

３．学会等名
12th ISAJ Annual Symposium（招待講演）

４．発表年
2021年

１．発表者名
Yue Yu, Zhongping Li, Ran Gao

２．発表標題
Targeted Delivery Using Covalent Organic Frameworks Improves the Efficacy of Anticancer Drugs

３．学会等名
The International Workshop on Agricultural Waste Reclamation and Utilization (AWRU2021)

４．発表年
2021年

１．発表者名
Yue Yu

２．発表標題
Covalent Organic Framework: A versatile tool for targeted cancer therapy

３．学会等名
関西バイオ医療研究会第１６回講演会（招待講演）

４．発表年
2022年

１．発表者名
Yue Yu

２．発表標題
Targeted Drug Delivery Using Highly Tumor Specific Framework Nanoparticles

３．学会等名
第２０回 産総研・産技連 LS-BT合同研究発表会

４．発表年
2022年

〔図書〕　計0件

〔出願〕　計1件

産業財産権の名称 ドラッグデリバリープラットフォーム化合物及びその製造方法並びに医薬組成物	発明者 于躍、七里元督	権利者 国立研究開発法人産業技術総合研究所
産業財産権の種類、番号 特許、2021-131345	出願年 2021年	国内・外国の別 国内

〔取得〕　計0件

〔その他〕

―

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