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研究課題名（和文）Label-free intracellular dynamics investigation of carrier-free nanoparticle-based drug delivery systems

研究課題名（英文）Label-free intracellular dynamics investigation of carrier-free nanoparticle-based drug delivery systems

研究代表者

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研究成果の概要（和文）：この研究では、金属有機構造体（MOF）で表面コートした貴金属ナノワイヤーを用いて、がん細胞内での抗がん剤前駆体代謝の増強ラマン検出を目指した。これを単一細胞内視鏡アプローチと組み合わせ、高い空間分解能を有する高分子選択性単一細胞エンドスコピーを開発した。本研究では、薬前駆体イリノテカンがエステラーゼ酵素によって代謝され、抗がん特性を有するSN-38が放出される様子を生きたがん細胞内でリアルタイム検出することに成功した。このアプローチは、細胞内分子代謝過程の位置・時間の追跡を可能にするため、細胞内代謝の理解に重要な貢献をすると考えられる。

研究成果の学術的意義や社会的意義
生きた細胞内での薬物分子の動態と分布を理解することは、新しい治療法の設計と発見にとって重要である。我々が開発した単一細胞解析アプローチは、内在性生体分子からの干渉なしに、複雑な細胞内環境でSN-38薬物分子を選択的に追跡および監視することを可能にした。この方法は、広範囲の薬物分子の代謝のリアルタイム監視に応用でき、薬物分子設計のさらなる開発と改善に貢献できる。

研究成果の概要（英文）：In this work, metal organic framework coated nanowire endoscopy was developed and applied for detection of anticancer drug metabolism inside cancer cells. The developed technique combines the high spatiotemporal resolution and non-invasiveness of the endoscopic approach, and high-selectivity of metal organic framework coating. This enables a real-time localization of SN-38 released from irinotecan anticancer drug by esterase enzyme-mediate cleavage inside HeLa cancer cells. We were able observed the release of SN-38 inside cytoplasm, followed by their diffusion to the nucleus where SN-38 molecules interact with topoisomerase and DNA complexes to inhibit DNA replication. This approach shows potential for specifically tracking drug molecules and exploring their intracellular metabolism.

研究分野：Nanomaterials

キーワード：Raman spectroscopy　SERS　Drug delivery　Single-cell endoscopy　Nanoparticles

１．研究開始当初の背景

To address the limitations of conventional nanocarrier drug delivery systems (DDSs), such as low drug loading capacity and the long-term side effects from carrier materials, researchers have explored alternative carrier-free drug nanoparticles (NPs) made entirely of hydrophobic drugs or prodrugs (Bull. Chem. Soc. Jpn. 2016, 89, 540). Various strategies have been developed to optimize the intrinsic physicochemical properties and external modifications of NPs to enhance drug delivery efficiency. However, their clinical translation remains uncertain due to insufficient information on their interactions with biological systems, which is essential for optimizing dosing strategies and minimizing off-target and toxic effects. Understanding the intracellular fate of these DDSs is critical for their clinical application. Traditionally, intracellular metabolism has been assessed by modifying drugs or NPs with fluorophores and using fluorescence microscopy to observe their behavior. However, this method is limited by low contrast, autofluorescence, and photobleaching, which hinder continuous monitoring. Additionally, the intrinsic differences between fluorescent dyes and drug compounds mean fluorophores do not accurately represent NP behavior (Science 2020, 368, 1386). Therefore, label-free analysis is essential to verify the intracellular actions of NPs and drug compounds.

２．研究の目的

This work aims to 1) develop an approach based on Raman spectroscopy for the selective detection of target compounds and 2) unveil the intracellular dynamics (localization, permeation, and enzyme-mediated drug activation) of drug molecules by utilizing the developed approach. With the chemical fingerprinting capability of Raman spectroscopy, the method is an ideal label-free platform as the vibrational modes of the NPs/prodrug/drug/DNA-drug complexes are expected to be remarkably affected by molecular changes and interactions with target biomolecules. The approach introduced is expected to provide information about the chemical state and the action mechanism of DDSs, which is crucial information for optimizing the preparation protocol for advanced DDSs.

３．研究の方法

This work applied the silver-nanowire (AgNW)-based single-cell surface-enhanced Raman spectroscopy (SERS) endoscopy (Fig. 1) technique developed by the host laboratory (Uji-I Laboratory, Hokkaido University) to studying drug intracellular dynamics. This internal organ endoscope-like approach involves the mechanical insertion of AgNW probe into the selected subcellular sites (e.g., nucleus, cytosol, membrane) of a living cell from where the Raman signal of molecules in the proximity of the AgNW is collected without severely damaging the cells. The research was conducted in 3 main steps. 1) Synthesis and optimization of AgNW probe. 2) evaluation of probe selectivity against target compounds. 3) Intracellular detection and monitoring of anticancer drug molecules.

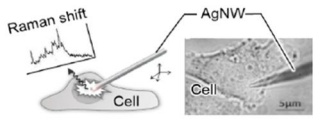

Fig. 1 Single-cell SERS endoscopy

4．研究成果

Silver nanowire with a width of 100-150 nm was synthesized by the polyol method. To enhance AgNW's SERS sensitivity for the intracellular detection of target molecules, the smooth surface was roughened by the Galvanic replacement reaction with $HAuCl_4$. The improvement of SERS performance was evaluated by functionalizing the AgNW and gold-etched AgNW (AuAgNW) probe with commonly used Raman reporter 4-mercaptobenzoic acid (4-MBA). Compared to the smooth AgNW, the modified probe displayed an enhancement factor of 10^5 in the SERS signal of 4-MBA. For the coating with MOF, zeolitic imidazolate framework-8 (ZIF-8) was chosen due to its biocompatibility and high structural stability in water. AuAgNW probe was coated by mixing the wire with ZIF-8 precursors in the ice bath. STEM and SEM measurements confirmed the 10-20 nm coating of ZIF-8 on AuAgNW. The shell structure was characterized by EDS and XRD analysis. The mixing time of AuAgNW with ZIF-8 precursors was found to affect the thickness of the final ZIF-8@AuAgNW probe. To prevent cell damage during probe insertion, the mixing time was adjusted to obtain the probe with a size of <200 nm.

The molecular selectivity of the modified AuAgNW@ZIF-8 probe was evaluated using glutathione (GSH) peptide, 4-MBA, and 6-(4-(phenyldiazenyl) phenoxy) hexane-1-thiol (ABT) in water solution. While the SERS fingerprint spectrum of GSH, 4-MBA, and ABT can be detected by a non-coated AuAgNW probe, only the SERS signal of 4-MBS and ABT can be observed by AuAgNW@ZIF-8 probe. The hydrophilicity of GSH peptides could lead to the low affinity of the molecules with the hydrophobic ZIF-8 pore, resulting in the exclusion of GSH molecules from SERS detection. Moreover, the signal of ABT, which has much higher hydrophobicity compared to 4-MBA, can be observed at a much faster rate. Considering that the molecular size of ABT is comparatively larger than that of 4-MBA, the results indicated that the synthesized AuAgNW@ZIF-8 probe has high selectivity against hydrophobic molecules. The feasibility of the probe for intracellular detection of SN-38, metabolites of irinotecan anticancer drugs, was also confirmed. No signal of irinotecan was observed by AuAgNW@ZIF-8, owing to the low hydrophobicity of irinotecan, which inhibits molecular adsorption by ZIF-8. On the contrary, a distinct SN-38 signal can be observed after esterase enzymes were added to the irinotecan solution. This confirms the endoscopic probe's sensing capability towards SN-38 released from irinotecan by enzyme-mediated hydrolysis. The detection limit of the probe against SN-38 was found to be 10^{-7} M for the developed method.

Intracellular metabolism of irinotecan was observed in living cells using single-cell SERS endoscopy with the developed AuAgNW@ZIF-8 probe. The intracellular release of SN-38 from irinotecan was first observed after 3h in the cytoplasm of HeLa cancer cells, while no signal was observed in the nucleus up until 5h. The results can be explained by the fact that hydrolysis enzymes are mainly distributed in the cytoplasm rather than the nucleus compartment. Interestingly, the signal of SN-38 cannot be detected in both cytoplasm and nucleus after 24h. Considering that cell death can be observed after

24h of incubation with irinotecan, the disappearance of the signal could be associated with the interaction of the SN-38 molecule with the topoisomerase-DNA complex. Overall, it was shown that the monitoring of drug metabolism could be performed using the proposed SERS endoscopy techniques. The methods developed here can be applied to monitor a wide range of drug molecules and explore their intracellular metabolism.

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〔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