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研究課題名（和文）Breast cancer tumors in deep tissue imaging with bi-modal NIR/MRI probe

研究課題名（英文）Breast cancer tumors in deep tissue imaging with bi-modal NIR/MRI probe

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研究成果の概要（和文）：本研究では、有機化合物を用いたバイモーダルイメージングプローブの開発に成功した。OTN-NIR蛍光色素とGd-DOTAを導入したPLGA-b-PEGベースのポリマーミセルのナノ構造設計により、約20nmサイズのOTN-NIR蛍光およびMRIイメージングプローブのバイモーダル化を実現した。
ハーセプチン抗体もイメージングプローブの表面に結合し、HER2乳がん細胞を選択的に標的とする可能性を示した。

研究成果の学術的意義や社会的意義
The development of a bi-modal NIR/MRI probe for imaging breast cancer enhances diagnostic precision, enables early-accurate tumor detection, and reduces the need for invasive procedures.

研究成果の概要（英文）：The research succeeded to develop bimodal imaging probe from organic compounds. The nanostructure design of polymer micelles based on PLGA-b-PEG introduced with an OTN-NIR fluorescent dye and Gd-DOTA as a bimodal, OTN-NIR fluorescence, and MRI imaging probe with ca. 20 nm size. It demonstrated well bright OTN-NIR fluorescence and MRI contrast enhancement to depict the blood circulation and to observe the organs in live mice. The probe exhibited absorption and emission in
OTN-NIR and T1 contrast enhancement effect in MRI. The imaging results revealed that the Gd-DOTA introduction ratio and locations are important to control the biological response of the probe without losing the imaging contrasts.
The Herceptin antibody was also conjugated on the surface of the imaging probe and showed the potential to selectively target HER2 breast cancer cells.

研究分野：Bioimaging

キーワード：NIR　MRI　Herceptin　IR-1061

１．研究開始当初の背景

Breast cancer has the highest number of cancer patients among Japanese women by region. Therefore, early detection and treatment of breast cancer patients are important to reduce the fatal rate and extend the healthy life expectancy. Tumor mass resection is often guided intraoperatively by anatomical and magnetic resonance imaging (MRI) incorporated into an image-guided surgery platform. In practice, there are some screening methods used for breast cancer imaging such as mammography, ultrasonography, computed tomography, MRI, etc., which have become the gold standard for monitoring treatment and planning surgery of breast cancer. Fluorescence-guided surgery is rapidly emerging as a potential method for surgical guiding because this is a fast, sensitive technique with high resolution and dynamic imaging capacity which can provide not only tumor morphology but also insights of biological processes at the tumor level. It often uses a fluorescent dye to specifically accumulate in tumor tissues, and a specialized system is applied to detect and quantify the tumor via dye characteristics. However, the visible and the shorter near-infrared (NIR) fluorescent probe cannot recognize breast cancer in deep tumors because tissue components tend to absorb and scatter the light, resulting in the loss of signals. Therefore, this research developed a bimodal imaging probe using an over 1000 nm near-infrared (OTN-NIR) fluorescent probe combining with MRI probe that enables bimodal imaging in deeper regions in the bodies. HER2 antibodies were bound on the probe surface for navigation of the receptor on the surface of breast cancer cells.

２．研究の目的

Develop a bimodal organic OTN-NIR /MRI imaging probe with a diameter smaller than 50 nm that can be used for the detection of breast cancer tumors.

３．研究の方法

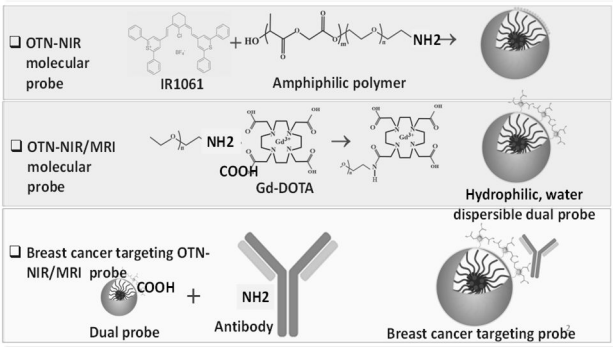

Fig. 1: A schematic synthesis routes of bimodal OTN-NIR/ MRI probes targeting breast cancer.

IR-1061 molecule emits the longest wavelength in OTN-NIR around 1140 nm. Its limitation is highly hydrophobic and quenched in water. Therefore, our group utilized micellar cargos to carry IR-1061 in vivo, as shown in Fig.1. Micelle structure from PEG co-block polymer will be used as cargo to carry hydrophobic IR1061 molecules, Gd-DOTA family as MRI probe will be conjugated to polymer chains by a very stable covalent bond For breast cancer targeting, human epidermal growth factor receptor 2 (HER2), a type of receptor indicates high-grade breast tumors and lymph node involvement as well as high risk of recurrence, will be target by trastuzumab antibody.

４．研究成果

i. Bimodal OTN-NIR/MRI imaging probe characterization:

The probe composed of Gd-DOTA conjugated to poly(lactic–co–glycolic acid)–block–poly(ethylene glycol) copolymer (PLGA–b–PEG) micelles either at the end of PEG or at the border of PLGA and PEG chains following by encapsulating OTN-NIR dye (IR-1061) in the core. This structure exhibited a small diameter of ca. 20 nm (Fig. 2 a). The emission and absorption spectra of the bimodal probe includes the main peak at around 1100 nm and a slight shoulder at around 1200–1300 nm under NIR excitation of 980 nm (Fig. 2 b and c). Besides, the imaging probe absorbed light over a broad wavelength of 740 ± 1100 nm and demonstrated a major absorption peak around 1064 nm and a slight shoulder at 890 nm. The relaxivities, r_1, estimated from the slope of the relaxation rate plot (R1 = 1/T1) as a function of Gd^{3+} concentration ranging within 0.0−0.1 mmol/L (Fig. 2 d). The probe relaxivities are16 $mM^{-1}s^{-1}$ for structure with Gd-DOTA at the end of PEG and 26 $mM^{-1}s^{-1}$ for structure with Gd-DOTA at the border of PLGA/PEG. The relaxivity of the clinical MRI contrast agent GADOVIST is 3.8 $mM^{-1}s^{-1}$, (Fig, 2 d). [1, 2]

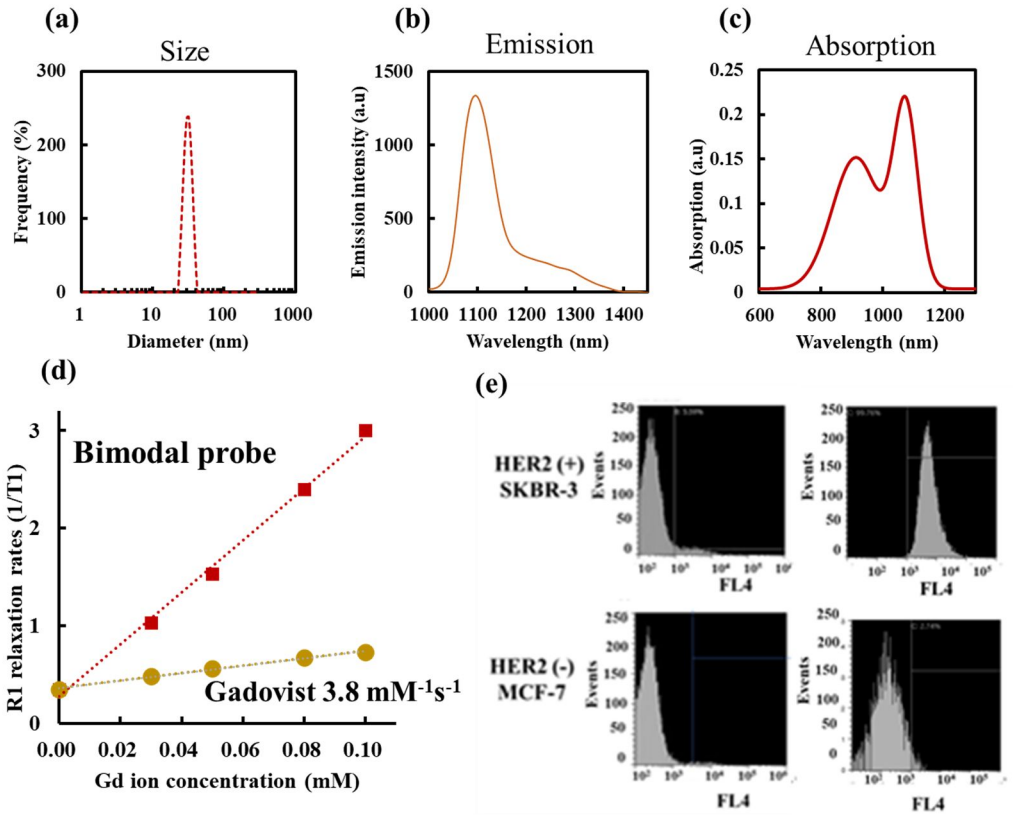

Fig. 2: Figure 2: Bimodal properties of the imaging probe: (a) size distribution, (b) emission, (c) absorption, (d) relaxation rate, and (e) Herceptin binding probe can target the HER2 (+) cells SKBR3.

ii. Conjugation of Herceptin on the bimodal imaging probe

Bimodal probes were composed of PLGA-b-PEG micelles containing Gd-DOTA at the border of PLGA/PEG chains and IR-1061 encapsulated inside the micellar cores. The HER2 targeting antibody (Herceptin) was conjugated on the surface of the imaging probe by means of the EDC/NHS procedure. Herceptin-conjugated probes were used selectively to recognize HER2 receptors over-expressed on breast cancer cells (Fig. 2 e). To accomplish this, Herceptin was covalently conjugated to the free carboxylic group at the end of the PEG shell via amide coupling, facilitated by EDC/NHS. EDC favours formation of amide bonds between the primary amine group of Herceptin and activated carboxyl groups of imaging probes at pH 5.5. the preferred binding site at its Fc region, leaving the antigen-binding sites (Fab) free to interact with HER2 receptors. [3]

iii. Bimodal OTN-NIR/MR in vivo imaging

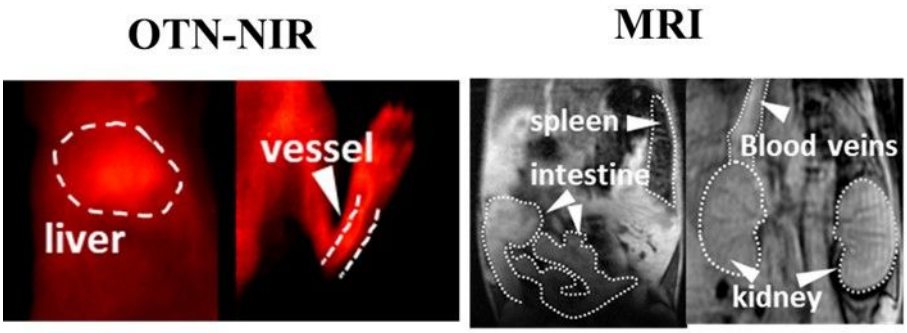

Fig. 3: Bimodal OTN-NIR/MR imaging of mice using imaging probe developed from this project. [2]

The OTN–NIR and MR images of mice using bimodal imaging probe are displayed in Fig. 3. The fluorescence from the structure is very bright to depict the blood vessels and localize the liver. The MR images of the mice receiving bimodal probe exhibit a strong enhancement effect so that different organs such as livers, kidneys, spleens, intestine, and blood vessels can be clearly discerned. Notably, MR images show bright signals in the liver, blood vessels, intestine, and spleen. In the kidney, the signal can be seen only in the posterior area composed of interlobular arteries and

veins inside the kidney; but no signal was observed in the pelvis. This implies that the structure does not penetrate through the glomerular capillary walls and excrete into the urine, but it returns to the circulation system. Ultimately, no signal was observed in the bladder.

Conclusion

The research achievements in developing a bi-modal NIR/MRI probe for deep tissue imaging of breast cancer tumors mark a significant advancement in medical imaging technology. The enhanced precision, non-invasive real-time monitoring, and improved contrast sensitivity of this innovative probe offer substantial benefits for both scientific research and clinical practice, holding promise for improved diagnostics and treatment of breast cancer.

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５．主な発表論文等

〔雑誌論文〕　計7件（うち査読付論文　5件／うち国際共著　5件／うちオープンアクセス　2件）

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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/jbm.b.35327	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 Doan Thi Kim Dung、Umezawa Masakazu、Ikeda Kazuno、Ohnuki Kazunobu、Akatsuka Mizuki、Okubo Kyohei、Kamimura Masao、Yamaguchi Masayuki、Fujii Hirofumi、Soga Kohei	４．巻 6
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〔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

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