

Title	Development and evaluation of cancer-targeted pre-operative and intra-operative dual-imaging probes based on metal nanoparticles(Abstract_要旨)
Author(s)	Ding, Ning
Citation	Kyoto University (京都大学)
Issue Date	2019-03-25
URL	https://doi.org/10.14989/doctor.k21713
Right	学位規則第9条第2項により要約公開; 許諾条件により要約は2020-03-25に公開
Type	Thesis or Dissertation
Textversion	none

京都大学	博士（薬科学）	氏名	DING NING
論文題目	Development and evaluation of cancer-targeted pre-operative and intra-operative dual-imaging probes based on metal nanoparticles (金属ナノ粒子を基盤とするがん標的術前・術中デュアルイメージングプローブの開発と評価)		
<p>Surgery remains one of the main treatments for many types of solid tumors. In order to remove as much tumor tissue as possible, both highly precise pre- and intra-operative diagnoses are considered necessary. However, since different imaging probes are currently employed for pre- and intra-operative diagnoses, this might cause information divergence and difficulties in the localization of tumors during surgery. Therefore, dual-imaging probes (radio- and fluorescence-labeled probes are the most commonly used) not only provide complementary information for the diagnosis, but also bridge the gap between surgical planning and image-guided resection with a single, molecularly targeted agent.</p> <p>In recent years, photoacoustic imaging (PAI) has emerged as a new type of biomedical diagnostic method. PAI noninvasively detects ultrasonic waves thermoelastically generated from optical absorbers irradiated with a pulsed laser through the PA effect, and these ultrasonic waves are scattered much less than photons, making it a promising intra-operative imaging method. In this research, metal nanoparticles (gold nanorods (AuNRs) and iron oxide nanoparticles (IONPs)) were selected as intense PA signal emitters. It is easy to chemically modify the surface of AuNRs and IONPs with signal emitters for pre-operative diagnosis by other imaging modalities. Furthermore, IONPs themselves are clinically used as biocompatible magnetic resonance imaging (MRI) probes.</p> <p>Therefore, this study was aimed at the development and evaluation of cancer-targeted pre-operative and intra-operative dual-imaging probes based on metal nanoparticles. Human epidermal growth factor receptor 2 (HER2) was chosen as the target because of its close association with a poor prognosis and high expression in various cancers including breast, ovarian, and gastric cancer.</p> <p>Chapter 1. Preparation and investigation of the feasibility of using radiolabeled anti-HER2 monoclonal antibody (trastuzumab)-conjugated AuNRs as PAI and SPECT probes</p> <p>AuNRs, efficient PA signal generators, were chosen as the platforms. AuNRs conjugated with different amounts of trastuzumab on the surface of nanorods (Tra-AuNRs) were prepared. Furthermore, Tra-AuNRs were labeled with indium-111 (¹¹¹In) for single photon emission computed tomography (SPECT) because SPECT is widely used in clinical pre-operative diagnosis. As the uptake of Tra-AuNRs by HER2 high-expressing tumor cells markedly increased with the number of trastuzumab molecules conjugated with AuNRs, AuNRs conjugated with high dose of trastuzumab were used in further experiments. In an <i>in vivo</i> biodistribution study using mice inoculated with HER2 high- and low-expressing tumor cells, ¹¹¹In-labeled Tra-AuNRs were accumulated in HER2-positive tumors HER2-specifically at 96 h post-injection. In a SPECT imaging study, HER2-positive tumors were visualized using ¹¹¹In-labeled Tra-AuNRs. However, the blood clearance of Tra-AuNRs was not fast enough to obtain PA images with a high tumor-to-blood ratio during the first few hours following administration.</p> <p>Chapter 2. Preparation and investigation of the feasibility of using anti-HER2 scFv-IONPs as PAI and MRI probes</p> <p>Although Tra-AuNRs demonstrated partial HER2-specific tumor accumulation <i>in vivo</i>, it was difficult to perform earlier post-injection imaging. Thus, in order to overcome this problem, a study using IONPs approved clinically as a platform for PA/MR dual imaging was conducted. In our previous study, IONPs conjugated with anti-HER2 single-chain Fv (scFv) demonstrated potential as an HER2-targeted PAI probe. In this research, the feasibility of using scFv-IONPs as PA/MR dual-imaging probes was investigated.</p>			

The thiol residue of scFv was reacted with the maleimide group on the surface of IONPs to ensure the affinity of scFv for HER2. The scFv-IONPs exhibited a high proton relaxivity (r_2) as an indicator of sensitivity in MRI. In an *in vitro* MRI study using scFv-IONPs, a significant decrease in MR signals was observed in HER2 high-expressing tumor cells, which was inhibited by co-treatment with excess trastuzumab. In an *in vivo* MRI study, scFv-IONPs exhibited significant MR signal reduction in HER2 high-expressing tumors at 24 h after injection, which was blocked by the co-injection of overabundant trastuzumab. Moreover, Berlin blue staining of resected tumors confirmed the tumor uptake of scFv-IONPs in HER2 high-expressing tumors. These results indicate that scFv-IONPs accumulated in HER2-specific tumors with an earlier timing post-injection compared with Tra-AuNR. The potential for using scFv-IONPs as robust PA/MR dual-imaging probes targeting HER2 was demonstrated. However, many more IONPs were needed to achieve PAI of tumors compared with MRI, indicating the need to improve the imaging sensitivity of probes, especially when used as a PAI probe.

Chapter 3. Preparation and investigation of the feasibility of using trastuzumab-conjugated liposomes encapsulated with IONPs as PA and MR dual-imaging probes

Since the density of PAI probes might be a key factor determining the sensitivity of PA signals, in this study, PAI probes with IONPs inside liposomes at a high concentration were designed and prepared. The liposomes encapsulated with IONPs (Lipo-IONPs) were conjugated with different amounts of trastuzumab on their surface. Lipo-IONPs showed significantly higher PA and MR signals compared with dispersed IONPs at the same concentration. In an *in vitro* cellular uptake study using iodine-125 (^{125}I)-labeled Tra-Lipo-IONPs, the HER2-specific binding of Tra-Lipo-IONPs was proved, and the higher binding of Tra-Lipo-IONPs to HER2 high-expressing tumor cells as the number of trastuzumab conjugated to liposomes increased. In the *in vitro* MRI and PAI study using Tra-Lipo-IONPs, HER2 high-expressing tumor cells were clearly visualized. Furthermore, in the *in vivo* biodistribution study, Tra-Lipo-IONPs accumulated in HER2 high-expressing tumors at 1 h post-injection with higher tumor-to-blood ratios compared with Tra-AuNRs and trastuzumab-conjugated IONPs. Therefore, Tra-Lipo-IONPs shows potential as a sensitive PA/MR dual-imaging probe targeting HER2.

In conclusion, in this study, cancer-targeted probes that can be applied to sensitive intra-operative PAI and pre-operative SPECT or MRI based on metal nanoparticles were developed. These data provide useful information for the development of imaging probes applicable for pre- and intra-operative diagnoses and precise cancer therapy.

(論文審査の結果の要旨)

がんの摘出手術において高い治療効果を得るためには、高精度ながんの術前・術中診断が必須であり、現在それに有効なイメージングプローブの開発が高い注目を集めている。最近、新たな術中診断技術として光音響イメージング法に関心が集まっていることから、著者は、光音響シグナル素子として金属ナノ粒子である金ナノロッド (AuNRs) および酸化鉄ナノ粒子 (IONPs) に着目し、これらを基盤とするプローブ開発を行った。なお、標的分子にはがんの悪性度に関与する Human epidermal growth factor receptor 2 (HER2) を選択した。

第一章では、AuNRs に抗 HER2 モノクローナル抗体 (Trastuzumab) を結合させた Tra-AuNRs を作製し、さらに放射性同位元素であるインジウム-111 (^{111}In) にて標識したプローブを合成し、光音響/核医学デュアルイメージングプローブとしての有効性を評価した。得られた ^{111}In -Tra-AuNRs を用いて、Trastuzumab の導入数のがんへの集積性に与える影響を検討し、がんへの HER2 特異的な集積性を示す最適条件を見出した。さらに、SPECT 撮像実験において、 ^{111}In -Tra-AuNRs は HER2 陽性がんを明瞭に描出した。しかしながら、血液からのクリアランスが遅く、良好なコントラストを得るためには、投与後長時間を要した。

そこで第二章では、酸化鉄ナノ粒子に抗 HER2 単鎖抗体を結合させたプローブ (scFv-IONPs) を設計、合成し、光音響/磁気共鳴 (MR) デュアルイメージングプローブとしての有用性を評価した。scFv-IONPs は、高いプロトン緩和能を示し、インビボ MR 撮像実験においても HER2 陽性がんの明瞭な描出に成功した。これまで所属研究室では、scFv-IONPs の光音響イメージングプローブとしての有用性が示されていることから、所期の通り、光音響/MR デュアルイメージングプローブの開発に成功した。ただし、MR 撮像と比較して光音響イメージングでは高用量のプローブを投与する必要があったことから、特に光音響イメージングプローブとしての感度を改善する必要が考えられた。

そこで次に第三章では、光音響シグナルの高感度化を達成するために、光音響シグナル素子の存在密度を高めることが有効ではないかと考え、IONPs をリポソームに内包させたプローブ (Lipo-IONPs) を合成し、その表面に Trastuzumab を導入することで HER2 標的光音響/MR デュアルイメージングプローブとしての有用性を評価した。その結果、IONPs の濃度が同じ条件において、Lipo-IONPs は IONPs と比較して感度の改善を認めた。また、HER2 特異的ながん細胞への集積性を示した。さらに、担がんマウスにおいて高い腫瘍対血液比を認め、高感度光音響/MR デュアルイメージングプローブとしての有用性を示した。

以上、本研究は金属ナノ粒子を基盤とするがん標的術前・術中デュアルイメージングプローブの開発に成果を収めたものであり、これらの知見は今後、術前・術中診断用プローブの開発研究に有益な情報を提供するものと考えられる。

よって、本論文は博士 (薬科学) の学位論文として価値あるものと認める。また、平成 31 年 2 月 15 日、論文内容とそれに関連した事項について試問を行った結果、合格と認めた。

なお、本論文は京都大学学位規程第 14 条第 2 項に該当するものと判断し、公表に際しては、当分の間当該論文の全文に代えてその内容を要約したものとすることを認める。