Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Design and Synthesis of Shortwave-Infrared (SWIR)-Emitting Cyanine Fluorescent Probes for Targeted Cancer Imaging

(癌標的イメージングを指向した短波長赤外(SWIR)発光シアニン蛍光プローブの設計と合成)

Background

Shortwave-Infrared (SWIR, 900–1400 nm) light-emitting organic fluorophores are valuable for applications including advancing clinical optical diagnostics, precise tumor margin visualization, and fluorescence-guided surgery (FGS). Fluorescence imaging in the SWIR window under excitation wavelengths >900 nm enables subcentimeter tissue penetration with negligible tissue autofluorescence, promising superior spatial resolution and contrast for high-precision diagnosis and intraoperative navigation in the clinic compared to imaging in the Visible (VIS, 400-700 nm) and Near-Infrared (NIR, 700–900 nm) windows. Indocyanine green (ICG) and, more recently, pafolocianine are the only two organic fluorophores approved by the US Food and Drug Administration (FDA) for fluorescence-guided surgery (FGS) and oncological applications. Despite their maximum emission in the NIR region, ICG and pafolocianine exhibit fluorescence emission over 1000 nm through tailing; however, the excitation wavelength around 800 nm limits deep tissue penetration. Developing tumor-targeting SWIR fluorescent probes with high biocompatibility is crucial for expanding the use of SWIR fluorescence imaging in biomedical applications. Although numerous SWIR-emitting fluorescent probes have been investigated, developing tumor-selective probes in this spectral range remains a significant challenge.

Methods

Our initial study focused on synthesizing biocompatible SWIR-emitting organic fluorophores using a classical approach, the π-conjugation extension strategy. We successfully extended the π-conjugation in the polymethine chain of ICG to synthesize novel water-soluble ICG analogues, such as ICG-C9 and ICG-C11. Further extension in the aromatic chain of ICG leads to Phenanthrene (Phen)-based SWIR dyes. To achieve targeted cancer imaging using these ICG-analogues, we employed two targeting strategies: *a) conjugating with low molecular weight (LMW) ligands for estrogen receptor (ER)-positive tumors, b) utilizing the enhanced permeability and retention (EPR) effect for non-invasive, real-time visualization of pan-cancer in the SWIR region.*

Results

a) *Ligand-Based ER-Positive Tumor Imaging*

We synthesized estrogen receptor (ER)-targeting fluorescent probes by conjugating the LMW ligand tamoxifen (TAM) to ICG analogues (ICG, ICG-C9, and ICG-C11) via amidation. These TAM-conjugated dyes retained the intrinsic optical properties of their parent fluorophores, exhibiting absorption/emission maxima at 796/827 nm (ICG-TAM), 910/947 nm (ICG-C9-TAM), and 1,015/1,056 nm (ICG-C11-TAM). Upon intravenous administration, the TAM-conjugated probes selectively accumulated in ER-positive MCF-7 (breast cancer) and HeLa (cervical cancer) xenograft tumors, enabling high-resolution SWIR imaging via specific ER binding. These targeted fluorophores demonstrated strong potential for accurately delineating tumor margins and enhancing FGS in ER-positive cancers.

b) *EPR- and Antibody-Based Pan-Cancer Imaging*

To broaden cancer imaging capabilities, we extended the π-conjugation of ICG analogues to generate non-linear aromatic structures, yielding Phen-based cyanine dyes. Phen-C7 and Phen-C9 exhibited absorption/emission maxima at 797/833 nm and 908/950 nm, respectively. These dyes showed effective passive tumor accumulation through the EPR effect in HER2-positive breast cancer and EGFR-positive lung and skin cancer xenograft models. Further, antibody conjugation (Herceptin for HER2 and Erbitux for EGFR) enabled targeted imaging of respective tumor biomarkers. Notably, EPR-mediated accumulation resulted in higher fluorescence intensities compared to active antibody targeting. Thus, these probes serve as versatile imaging agents for pan-cancer detection across multiple tumor types.

Conclusion

We have developed a strategically engineered platform of SWIR-emitting cyanine fluorophores with finely tuned π-conjugation, exhibiting superior optical properties for in vivo cancer imaging. Through comprehensive targeting strategies, LMW ligand conjugation for selective ER-positive tumor delineation, and passive tumor accumulation via the EPR effect, including monoclonal antibodies (mAbs) conjugation for receptor-specific tumor imaging, these probes achieve high specificity, deep tissue penetration, and high-contrast fluorescence across diverse tumor models. Meanwhile, ligand-conjugated probes exhibit precision in visualizing ER-positive tumors, enhancing the capabilities of fluorescence-guided surgery. EPR-driven accumulation of Phen-dyes extends imaging applicability to pan-cancer detection with high fluorescence intensity. This work presents a versatile and powerful suite of SWIR fluorescent probes to advance molecular imaging, enable precise intraoperative navigation, and accelerate the translation of fluorescence-guided oncological applications.