Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Rapid and sensitive fluorescent imaging of tiny tumors *in vivo* and in clinical specimens

Mako Kamiya[1,3] and Yasuteru Urano[1,2,4]

Fluorescence-guided diagnostics is one of the most powerful techniques for real-time *in situ* tumor detection. Here, we introduce two categories of fluorescence probes used for tumor imaging (always-on probes and activatable probes) and briefly summarize recent advances in tumor-targeted fluorescence imaging probes and their clinical/preclinical applications, including our recent work on rational design of activatable fluorescence probes for tumors expressing aminopeptidases and glycosidases. These probes enable rapid and sensitive detection of tiny tumors as small as <1 mm in diameter, both *in vivo* and in clinical specimens.

Addresses
[1] Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[2] Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[3] PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan
[4] CREST, Japan Agency for Medical Research and Development, 1-7-1 Otemachi, Chiyoda-ku, Tokyo 100-0004, Japan

Corresponding author: Urano, Yasuteru (uranokun@m.u-tokyo.ac.jp)

Current Opinion in Chemical Biology 2016, **33**:9–15

This review comes from a themed issue on **Molecular imaging**

Edited by **Ji-Xin Cheng** and **Yasuteru Urano**

http://dx.doi.org/10.1016/j.cbpa.2016.04.004

Introduction

Successful surgical treatment of cancers requires rapid and accurate detection of cancerous tissues with high specificity and sensitivity in order to enable complete resection or ablation. Although the accuracy of preoperative diagnosis by using imaging technologies such as positron emission tomography (PET), X-ray computed tomography (CT), and magnetic resonance imaging (MRI) has been improving, the ability of the unaided human eye to detect millimeter-sized tiny foci, such as metastases or invading cells, or to distinguish accurately the borders between cancer and normal tissues during surgery or endoscopy is limited. Since residual tumors can lead to local recurrence, the ability to completely remove tumors is a crucial factor in determining patient outcome [1,2]. Thus, better techniques for intraoperative visualization of tumors are urgently needed.

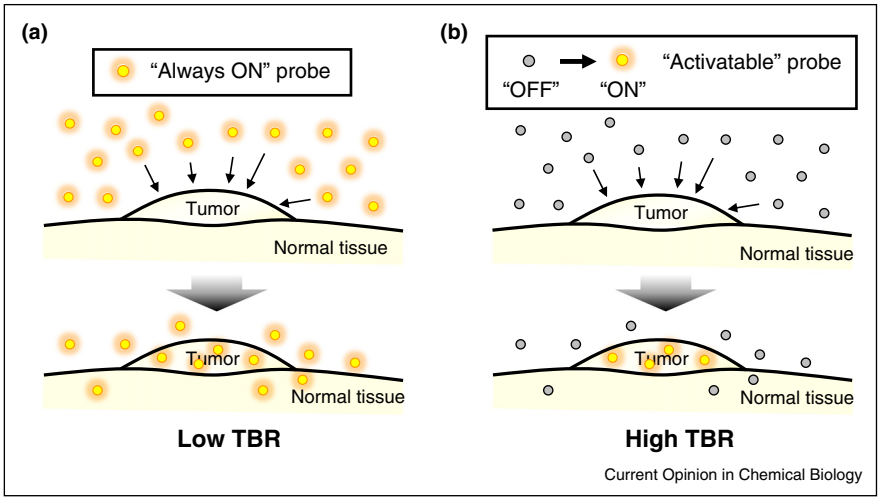
Over the past few decades, the emergence of various types of fluorescence probes has led to increasing interest in fluorescence imaging of tumors as an aid to optically guided surgery and endoscopy, because of its high sensitivity, low cost, portability, real-time capabilities, and absence of ionizing radiation [3,4]. There are two major categories of fluorescent probes that have been used for tumor imaging: one is 'always-on' and the other is 'activatable' (Figure 1) [5,6]. Since always-on probes emit a constant fluorescence signal regardless of where they are situated, we have to wait until a sufficient amount of imaging probe is accumulated at the tumor site and excess non-targeted probe is excreted from the body to obtain a sufficiently high target-to-background signal ratio (TBR). By contrast, since the fluorescence signal of an activatable probe is activated only when it reaches and recognizes a tumor site, these probes provide a rapid tumor-specific signal with high sensitivity and high TBR. In this review article, we briefly introduce representative fluorescent probes currently used for tumor imaging, together with some recent clinical/preclinical applications, and then we describe our recent preclinical work using our activatable fluorescence probes, which target enzyme activities over-expressed in tumor cells, thereby enabling rapid and sensitive detection of even tiny tumors, both *in vivo* and in clinical specimens.

Always-on fluorescent probes for tumor imaging

Always-on fluorescent probes are generally composed of fluorescent reporting units (fluorophores) linked to tumor-targeting moieties such as antibodies, peptides, or tumor-specific ligands. Various kinds of always-on fluorescent probes have been developed, and their ability to visualize tumors has generally been evaluated in terms of targeting ability, pharmacokinetics, and TBR, mostly in cultured cells or in mouse models. Frangioni *et al.* reported that fluorescent probes based on ZW800-1, a zwitterionic near-infrared (NIR) fluorophore, exhibit excellent TBRs in *in vivo* mouse models due to their zwitterionic character (Figure 2a) [7,8•]. Kobayashi *et al.* successfully demonstrated photoimmunotherapy (PIT) using a conjugate of a NIR phthalocyanine dye, IR700, and tumor-targeting antibodies both to visualize tumor sites and to induce tumor cell death under NIR light irradiation (Figure 2b) [9••].

In recent years, pioneering first-in-human studies of always-on fluorescent probes have been reported by

Figure 1



Two categories of fluorescent probes used for tumor imaging: **(a)** tumor imaging with 'always-on' probes. To obtain sufficient target-to-background ratios (TBRs), it is necessary to wait until a sufficient amount of probe is accumulated at the tumor site and excess non-targeted probe is washed out. **(b)** Tumor imaging with 'activatable' probes. The fluorescence signal of activatable probes is activated only at tumor sites, affording high TBRs.

two groups. Ntziachristos *et al.* reported intraoperative fluorescence imaging of ovarian cancer in human patients by using a folate receptor-α (FR-α)-targeted fluorescent agent, a conjugate of folate and fluorescein isothiocyanate (folate-FITC). In this pilot study, the fluorescence signal was detectable intraoperatively (2–8 h after iv injection) in all patients with malignant tumor expressing FR-α, but not in patients with malignant tumor not expressing FR-α or in those with benign tumor (Figure 2c) [10••]. More recently, Hardwick *et al.* demonstrated a colorectal cancer-targeted probe (GE-137), which consists of a 26-amino-acid cyclic peptide targeted to human tyrosine kinase c-Met coupled to a fluorescent cyanine dye. Fluorescence colonoscopy in colorectal cancer patients after intravenous administration of GE-137 visualized not only neoplastic polyps that were discernible by conventional white-light imaging, but also an additional nine polyps that were missed by conventional methods and by the unaided human eye (Figure 2d) [11••]. Thus, sufficient TBRs could be achieved by optimizing the timing of observation after iv injection of always-on probes. These first-in-human studies strongly suggest that molecular imaging using fluorescent probes is indeed a promising modality to enable the detection of small polyps or lesions.

Activatable fluorescence probes for tumor imaging

By contrast to always-on probes, activatable fluorescent probes generally exhibit suppressed fluorescence, but are activated upon recognition by or reaction with a tumor-specific receptor or enzymatic activity. For example, 5-aminolevulinic acid (5-ALA) has been clinically used as an activatable fluorophore/photosensitizer [12] for diagnosis of malignancies including ovarian carcinoma metastases [13], malignant gliomas [14] and bladder carcinoma [15]. However, false-positive rates were high [16], and further, it takes several hours to a day for a sufficient amount of fluorescent protoporphyrin IX (PpIX) to be biosynthesized from 5-ALA and accumulated at the tumor site.

Various strategies have been used to design activatable fluorescent probes whose fluorescence signals are rapidly turned on by molecular stimuli at tumor sites, including quenching through Förster resonance energy transfer (FRET), photoinduced electron transfer (PeT), intramolecular spirocyclization, and self-quenching. These probes are activated in the tumor environment, by pH, by enzymatic activity, or by ligand binding. Weissleder *et al.* reported self-quenched near-infrared fluorescence probes, the ProSense series, which tend to be accumulated at tumor sites via enhanced permeability retention (EPR) effects and undergo fluorescence activation by tumor-residing proteases [17,18]. Bogyo *et al.* developed FRET-based fluorescently quenched activity-based probes (qABPs) for detecting enzymatic activities at tumor sites, and demonstrated their practical applicability by topical applying qABPs to human clinical samples or to fresh-frozen tissues from patients (Figure 3a) [19•,20,21]. Suzuki *et al.* designed and tested a novel benzothiazolyl-phenol-based sialic acid derivative (BTP-Neu5Ac) as a fluorescent sialidase substrate to visualize human colon cancers *ex vivo* (Figure 3b) [22].