Perspective

Activatable peptide-based nanoprobes for multimodal imaging in vivo

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Abstract

Multimodal imaging is considered as an important and promising tool for clinical diagnosis and therapy monitoring. Owing to their inherent characteristics such as excellent biocompatibility, biodegradability, and low immunogenicity, peptides have been extensively explored for the construction of multimodal imaging nanoprobes. In particular, by utilizing valuable endogenous triggers, a number of activable peptide-based nanoprobes have been developed for efficient multimodal imaging in vivo. In this Perspective, we will discuss a few valuable endogenous stimuli that can be used for the design of in situ activatable nanoprobes and highlight recent advances in peptide-based nanoprobes for multimodal imaging in vivo. Moreover, some brief and personal opinions on remaining challenges in this field are presented. We envision that this perspective article may inspire for the design of intelligent activatable peptide-based multimodal nanoprobes.

Introduction

Multimodal imaging, which integrates the complementary advantages of different single-modal imaging methods, offers promising opportunities for clinical diagnosis and therapy monitoring [1]. In the last decade, numerous nanoprobes have been extensively explored for efficient multimodal imaging. Due to the on-demand active targeting property and/or their intrinsic passive targeting effect (enhanced permeability and retention effect, EPR effect), these nanoprobes can selectively accumulate at pathological sites, resulting in high multimodal imaging quality in vivo [2]. However, due to the complexity of living entities, there are still huge challenges in the understanding of the absorption, distribution, metabolism and excretion of these nanoprobes in vivo [3]. In addition, problems such as biosafety, scale-up synthesis and reproducibility remain to be resolved, which have become barriers for the clinical translation of these nanoprobes [4]. To overcome these challenges, in situ activatable peptide-based multimodal nanoprobes have been proposed recently and have received considerable attention. Using peptide derivatives as building blocks can endow the multimodal nanoprobes with inherent characteristics such as excellent biocompatibility, biodegradability, and low immunogenicity [5]. Moreover, under the activation of specific endogenous stimuli, these probes can not only intelligently turn on imaging signals from off state to improve the signal-to-noise ratio, but also transform into highly ordered nanostructures in situ to increase their accumulation and retention time (figure 1) [6]. Therefore, these activatable peptide-based nanoprobes are ideal candidates for multimodal imaging in vivo. Herein, we highlight recent advances in activatable peptide-based nanoprobes for multimodal imaging in vivo, and provide some brief and personal opinions on remaining challenges in this field.
Valuable endogenous stimuli for in situ activation

Progresses in molecular and cell biology have elucidated the different microenvironments between pathological and normal tissues [7]. These abnormal pathological microenvironments, including pH difference, overexpressed proteins/enzymes, and high levels of metabolites, have been exploited as powerful triggers for the activation of multimodal imaging probes in vivo [8]. For instance, weakly acidic microenvironment found in tumor and infected tissues can induce protonation of amines or cleavage of acid labile groups, thereby triggering the activation process. Receptors overexpressed on the cell membrane can function as the targeting sites to improve the selectivity of nanoprobes. Overexpressed enzymes (e.g., phosphatases, proteases, esterases, and kinases), abnormal metabolites (e.g., glutathione (GSH) and reactive oxygen species (ROS)) can interact with the corresponding peptide derivatives, thus altering their hydrophilic-hydrophobic balance to trigger their self-assembly and realize multimodal imaging in vivo. While the in situ activation process of most peptide-based nanoprobes relies on a single stimulus, it is worth constructing smart nanoplatforms that can simultaneously respond to multiple stimuli, because a tailored combination of stimuli can enhance the disease selectivity and provide more comprehensive molecular and physiological information.

Single-modal imaging

Molecular imaging has become an indispensable and powerful tool for clinical disease diagnosis and treatment, since it was first proposed by Weissleder in 1999 [9]. The available imaging modalities include fluorescence (FL) imaging, magnetic resonance (MR) imaging, photoacoustic (PA) imaging and radionuclide imaging. By utilizing valuable triggers, numerous peptide-based nanoprobes have been developed for real-time and non-invasive imaging of disease-related biological structures or processes in recent decades. For instance, Xu and co-workers reported supramolecular hydrogels based on enzyme-instructed supramolecular self-assembly (EISA) for imaging different subcellular organelles inside cancer cells [10–12]. Rao and co-workers utilized a biocompatible condensation reaction to construct peptide-based nanoprobes in situ for imaging enzyme activity in vivo [13, 14]. To date, many excellent reviews have summarized the single-modal peptide-based nanoprobes with design principles and biological applications. We refer the reader to some recent reviews including those of Xu et al [15], Wang et al [7], and Liang et al [16].

Dual-modal and multimodal imaging

Fluorescence/Magnetic resonance imaging

FL/MR dual-modal probes can offer high-sensitivity and real-time molecular imaging signals provided by the conjugated fluorophores, as well as anatomical imaging signals with high spatial resolution and excellent tissue penetration depth provided by the modified contrast agents [17]. Therefore, it is considered as a promising tool for clinical diagnosis. Recent studies have shown that it is possible to design activatable peptide-based nanoprobes for tumor imaging by utilizing overexpressed enzymes, including alkaline phosphatase (ALP) and
caspase-3/7, as endogenous stimuli [18, 19]. For example, Ye et al reported an ALP-responsive peptide-based precursor P-CyFF-Gd conjugated with a near-infrared (NIR) dye (merocyanine, Cy5.5) and a contrast agent (DOTA-Gd) for FL and MR imaging, respectively (figure 2(a)). These rational designed peptide-based probes have prequenced fluorescence signal due to their capping groups. However, upon the cleavage of the capping groups via a specific enzyme, their fluorescence signals was turned on immediately (figure 2(b)). Moreover, the enzymatic fragments can self-assemble to form highly ordered nanostructures, resulting in the activation of MR signal in situ (figure 2(c)). In addition to enzymes, abnormal metabolites (e.g. GSH) were also reported as effective triggers for the design of FL/MR dual-modal nanoprobes [20]. These smart probes can be further utilized for imaging-guided tumor therapy.

**Fluorescence/photoacoustic imaging**

PA imaging is an emerging anatomical imaging modality with high spatial resolution and tissue penetration. However, the sensitivity of PA imaging still needs to be improved for in vivo imaging. To address this issue, fluorescence/photoacoustic (FL/PA) dual-modal imaging technique has been developed [21]. In a recent work, an matrix metalloproteinases-2 (MMP-2)-activatable peptide-based probe QSY21-GPLGVRG(125I)Y-Cy5.5 (QC) was designed for FL/PA dual-modal tumor imaging [22]. As shown in figure 3(a), QC was composed of a quencher (QSY21), a peptide substrate of MMP-2 (GPLGVRG) and a NIR dye (Cy5.5). QC can self-assemble to form nanoparticles with a quenched FL signal and a PA signal at 680 nm in aqueous solution. Specific activated by MMP-2, the QC nanoparticles were disassembly into small molecules, thus turning on both fluorescence and ratiometric photoacoustic imaging signal (figures 3(b), (c)). This work provided a novel strategy for non-invasive and quantitative detection of the activity of tumor-related proteases in vivo. Rao and coworkers developed a small-molecule monomer that can be triggered to form polymers by the reducing microenvironment in vivo [23]. Due to this intelligent in situ polymerization process, the designed probe can offer both enhanced FL and PA imaging signals for efficient detection of tumor reducing microenvironment.

**Radionuclide/fluorescence imaging**

Radionuclide/FL imaging is also regarded as an ideal combination for dual-modal in vivo imaging. For example, Liu and coworkers designed a receptor-activatable peptide-based probe, 125I-Rho-FF-Van, consisting of a fluorophore (rhodamine, Rho), a self-assembling dipeptide (Phe-Phe, FF), a tyrosine residue labelled with iodine – 125, and a targeting motif (vancomycin, Van) (figure 4(a)) [24]. Owing to the ligand-receptor interaction between Van and D-Ala-D-Ala moiety overexpressed in the cell walls of Gram positive bacteria, this probe can specifically accumulate on bacterial surface and further self-assemble to form nanoaggregates (figure 4(b)). According to the authors, significantly enhanced fluorescence signal and strong radioactive signal were observed in the methicillin-resistant staphylococcus aureus (MRSA) infection models (figures 4(c), (d)).

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**Figure 2.** (a) The chemical structure of P-CyFF-Gd, and schematic illustration of ALP-mediated fluorogenic reaction and in situ self-assembly of P-CyFF-Gd into NPs that show increased NIR FL and r1 relaxivity. (b) Whole-body fluorescence (down) and bioluminescence (up) imaging of normal mice and orthotopic HepG2/Luc liver tumor xenograft mice. (c) T1-weighted MR imaging of orthotopic HepG2/Luc liver tumor xenograft mice. Reprinted with permission [18]. Copyright 2019, American Chemical Society.
This work provided a smart surface-induced self-assembly strategy for developing activatable peptide-based dual-modal nanoprobes.

Positron emission tomography (PET) imaging, an emerging radionuclide imaging technique, is becoming an indispensable tool for clinical diagnosis. By combining PET with FL, it is possible to further improve the...
diagnostic accuracy. For instance, MMP-activatable peptide-based PET/FL nanoprobes were fabricated for successfully visualizing proteases activity in tumor bearing mice [25].

**Computed tomography/photoacoustic imaging**

Apart from the above-mentioned dual-modal imaging techniques, computed tomography/photoacoustic (CT/PA) imaging has also been proposed for enhanced in vivo imaging. In a recent study, an acid-activatable peptide-based CT/PA nanosystem was developed for tumor imaging [26]. The nanosystem comprised gold nanoparticles (GNPs) to provide CT/PA signals, and acid-responsive peptides to in situ activate the imaging signals. Upon the cleavage of the acid-labile groups in acidic microenvironment, the charge balance of the nanosystem was destroyed, leading to the formation large GNPs aggregates. Therefore, both CT and PA signals were greatly enhanced. In addition, this acid-triggered in situ self-assembly of GNPs could also boost the photothermal therapy (PTT) efficacy towards tumors.

**Multimodal imaging**

Responsive trimodal peptide-based nanoprobes have also been reported for in vivo imaging of abnormal metabolites at the pathological sites. For instance, activatable fluorescence/fluorine magnetic resonance spectroscopic/magnetic resonance imaging (FL/19F MRS/1H NMR) trimodal probes were constructed for noninvasive evaluation of GSH levels in inflammatory livers or tumor sites [27, 28]. By utilizing a smart co-assembly and responsive disassembly strategy, the imaging signal of each modality underwent a smart activate-ON or activate-OFF process in situ, thereby offering rich and complementary anatomical and molecular information for disease diagnosis.

**Outlook**

With the development of hybrid instruments for multimodal imaging, such as PET/CT and PET/MR, activatable peptide-based multimodal nanoprobes have shown increasing application prospects in clinical diagnosis and therapy monitoring. However, some problems and challenges still need to be addressed carefully. Firstly, the biosafety of these peptide-based nanoprobes should be further improved for clinical use. Long-term cytotoxicity and immune response of the peptide probes have not been carefully evaluated in most of current studies. Moreover, it is necessary to optimize the molecular structures of these peptide-based nanoprobes to effectively cross biological barriers (e.g., blood-brain barrier, blood-ocular barrier, etc). Additionally, pathological signals are dynamic; it remains challenging to develop peptide-based multimodal imaging nanoprobes that can reversibly respond to the dynamic signals. Most current multi-modal probes are designed in a trial-and-error fashion, more advanced and rational design strategies are yet to be proposed.

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**Data availability statement**

No new data were created or analysed in this study.

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