Biomaterials 133 (2017) 60–69

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Imaging lysosomal highly reactive oxygen species and lighting up cancer cells and tumors enabled by a Si-rhodamine-based near-infrared fluorescent probe

Hongxing Zhang [a, 1], Jing Liu [a, 1], Chenlu Liu [a], Pengcheng Yu [a], Minjia Sun [a], Xiaohan Yan [a], Jian-Ping Guo [b, **], Wei Guo [a, *]

[a] School of Chemistry and Chemical Engineering and Institute of Biotechnology, Shanxi University, Taiyuan 030006, China
[b] State Key Laboratory of Solid Waste Reuse for Building Materials, Beijing 100041, China

ARTICLE INFO

Article history:
Received 11 February 2017
Received in revised form
10 April 2017
Accepted 13 April 2017
Available online 14 April 2017

Keywords:
Fluorescent probes
Highly reactive oxygen species
Lysosomes
Cancer cells
Tumors

ABSTRACT

Lysosomes have recently been regarded as the attractive pharmacological targets for selectively killing of cancer cells *via* lysosomal cell death (LCD) pathway that is closely associated with reactive oxygen species (ROS). However, the details on the ROS-induced LCD of cancer cells are still poorly understood, partially due to the absence of a lysosome-targetable, robust, and biocompatible imaging tool for ROS. In this work, we brought forward a Si-rhodamine-based fluorescent probe, named **PSiR**, which could selectively and sensitively image the pathologically more relavent highly reactive oxygen species (hROS: HClO, HO•, and $ONOO^-$) in lysosomes of cancer cells. Compared with many of the existing hROS fluorescent probes, its superiorities are mainly embodied in the high stability against autoxidation and photoxidation, near-infrared exitation and emission, fast fluorescence off–on response, and specific lysosomal localization. Its practicality has been demonstrated by the real-time imaging of hROS generation in lysosomes of human non-small-cell lung cancer cells stimulated by anticancer drug β-lapachone. Moreover, the probe was sensitive enough for basal hROS in cancer cells, allowing its further imaging applications to discriminate not only cancer cells from normal cells, but also tumors from healthy tissues. Overall, our results strongly indicated that **PSiR** is a very promising imaging tool for the studies of ROS-related LCD of cancer cells, screening of new anticancer drugs, and early diagnosis of cancers.

1. Introduction

Lysosomes, featured with numerous hydrolases, including protein-degrading cathepsin enzymes, and a high proton concentration (pH < 5.5), are responsible for the controlled recycling of cellular and extracellular components [1,2]. Compared with those in normal cells, the lysosomes in cancer cells are more numerous and larger, and have greater cathepsin activity. Oncogene-driven transformation alters lysosomal membranes of cancer cells and sensitizes them to lysosomal membrane permeabilization (LMP), which promotes tumor invasion and progression by releasing cathepsins into the extracellular space [3,4]. Ironically, sensitized lysosomal membranes in cancer cells have increased susceptibility for leakage, and, depending on the extent of LMP and the amount of active cathepsins released into the cytoplasm, a variety of death morphologies from classic apoptosis to necrosis can be triggered [5,6]. As such, lysosomes have recently been regarded as the attractive pharmacological targets for selectively killing of cancer cells *via* lysosomal cell death (LCD) pathway [7–9], a new therapeutic strategy that is promising to overcome the problem of multidrug resistance. Among various stimuli that can induce LMP, reactive oxygen species (ROS) was most frequently implicated [3–11], which might contribute to LMP by causing lipid peroxidation, damaging lysosomal membrane proteins, activating lysosomal Ca^{2+} channels, and altering the activity of lysosomal enzymes. In fact, almost all nonsurgical anticancer treatments, including chemotherapy, photodynamic therapy, and radiotherapy, share a common ROS-related treatment mechanism [12–14]. However, the details on the ROS-induced LCD of cancer cells are still poorly understood. On the other hand, among various ROS, superoxide ($O_2^{\bullet -}$)

* Corresponding author.
** Corresponding author.
E-mail address: guow@sxu.edu.cn (W. Guo).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biomaterials.2017.04.023
0142-9612/

is first produced in mitochondrial respiratory chain, which is further converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). Both $O_2^{\bullet -}$ and H_2O_2 are not strong oxidants and thus not particularly toxic *in vivo*. However, they could be converted into hypochlorous acid (HClO), hydroxyl radicals (HO•), and peroxynitrite ($ONOO^-$) by myeloperoxidase (MPO)-mediated reaction of H_2O_2 and Cl^-, iron (II)-promoted breakdown of H_2O_2, and radical coupling of nitric oxide (NO•) and $O_2^{\bullet -}$, respectively. By comparison, HClO, HO•, and $ONOO^-$ all are the highly reactive oxygen species (hROS) [15], which can directly oxidize a large variety of cellular components including lipid, DNA, protein and enzyme, ultimately leading to cell death. In this sense, real-time imaging of hROS generation in lysosomes of cancer cells is very important not only for elucidating the LCD-related anticancer mechanisms but also for evaluating new anticancer drugs. Moreover, such tool is also expected to be able to discriminate cancer cells from normal cells because the former commonly have higher ROS level to drive tumor development [12–14].

Various efforts have been made to develop fluorescent probes for detecting hROS in living cells [16–31]. But, most of them mainly focus on a single hROS in order to uncover its unique pathophysiological roles. In fact, from the standpoint of disease diagnosis, it would be more desirable to fabricate such a fluorescent probe that can respond to a collection of hROS because these hROS all can contribute to serious intracellular oxidative stress. In this regard, the reduced xanthene dyes, such as dihydrorhodamine and dihydrofluorescein, are the earliest exploited and most versatile indicators for cellular oxidative stress to date [32–35]. However, these "dihydro" dyes are unstable and easily suffer from autoxidation and photoxidation. To overcome the limitation, Nagano's group exploited the 4-aminophenyl ether-based fluorescein (for APF) and rhodamine (for MitoAR) for hROS detection [36,37]. Although improved in autoxidation and photoxidation, the two probes have excitation and emission wavelengths in the visible region, thus limiting their possible *in vivo* imaging applications. Considering the high tissue penetration and low phototoxicity of near-infrared (NIR) light as well as small background autofluorescence of biomacromolecules in NIR region, Murthy's and Nagano's groups further exploited NIR fluorescent probes hydrocyanines [38] and Cy5–Cy7 dyad [39] for hROS, respectively. However, the formers easily suffer from autoxidation and photoxidation [40], and the latter is cell membrane-impermeable. Moreover, these fluorescent probes cannot specifically target lysosomes, thus unsuitable for studying the ROS-related LCD in cancer cells. Herein, we bring forward the first lysosome-targetable NIR fluorescent probe, named **PSiR**, for the real-time tracking of hROS generation in lysosomes of cancer cells based on the Si-rhodamine dye platform (Scheme 1). The advantages of the probe include the high resistance to autoxidation and photoxidation, NIR excitation and emission, fast, selective, and sensitive fluorescence response for hROS, and specific lysosomal localization. Its practicality was confirmed by the real-time and long-term imaging of hROS generation in lysosomes of human non-small-cell lung cancer cells stimulated by anticancer drug β-lapachone. Moreover, the probe is sensitive for basal hROS in cancer cells, enabling its further imaging application to discriminate not only cancer cells from normal cells but also tumors form healthy tissues.

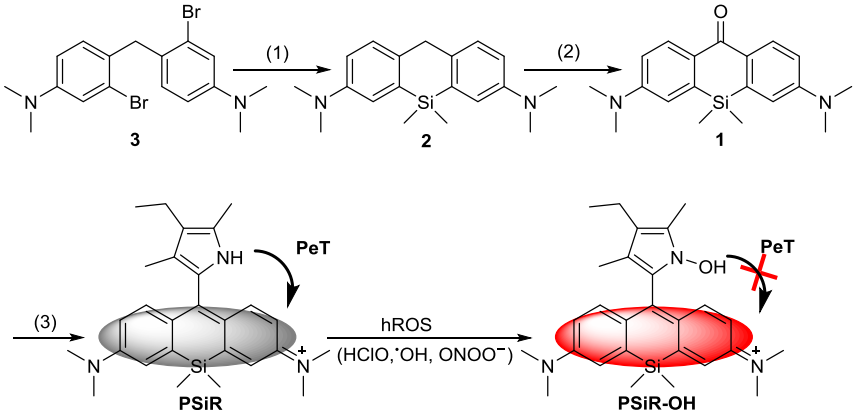
2. Materials and methods

2.1. General information

LysoTracker Green DND–26 and MitoTracker Green FM were purchased from Invitrogen (USA). SIN-1, NOC9, TEMPO, FeTMPyP, and ABH were purchased from J&K (Shanghai, China). β-Lapachone and dicoumarol were purchased from Nanjing Natural Selection Biotechnology Co., Ltd., Other common reagents and solvents were purchased from local commercial sources and were of the highest grade. All cell lines were provided by Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education (China). Solvents were dried according to standard procedures. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC). Flash chromatography was performed using silica gel 60 (200–300 mesh). Absorption spectra were taken on a Varian Carry 4000 spectrophotometer. Fluorescence spectra were taken on Hitachi F-7000 fluorescence spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively, and the following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer. The imaging assays of cells and tissue sections were performed in Olympus FluoView™ FV1000 confocal microscope, unless otherwise mentioned. The imaging assays of living body were performed in Bruker In-Vivo FX Pro small animal optical imaging system with an excitation filter 630 nm and an emission filter 700 nm. The synthetic details for **PSiR** were shown in Supporting Information.

2.2. Preparation of the test solution

For *in vitro* assays, $O_2^{\bullet -}$ was prepared by adding KO_2 (7.1 mg) and 18-Crown-6 (1 equiv) to dry dimethyl sulfoxide (5 mL) and stirring



Scheme 1. Synthesis and proposed sensing mechanisms of **PSiR** for hROS. (1) i: *n*-BuLi, THF; ii: $Si(CH_3)_2Cl_2$. (2) $KMnO_4$, acetone. (3) i: trifluoromethanesulfonic anhydride (Tf_2O); ii: 2,4-dimethyl-3-ethylpyrrole.