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Tetramethylrhodamine is an essential scaffold of azide probe in detecting cellular acrolein

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ABSTRACT

Tetramethylrhodamine (TAMRA)-phenyl azide is a chemical probe used to detect intracellular acrolein directly in live cells. Herein, we demonstrated that TAMRA is the optimum fluorophore for the probe. TAMRA-phenyl azide was used to reveal that high levels of acrolein are generated in a variety of breast cancer cells, regardless of the tumor subtype. These findings corroborate the analysis presented in our previous report, in which TAMRA-phenyl azide was used to label breast cancer tissues resected from breast cancer patients. Because high levels of acrolein were generated in all cancer cell types, we believe that acrolein detection may be useful as a general method for labeling cancerous tissues.

1. Introduction

Peroxidative modification of unsaturated phospholipids and glycolipids, triggered by reactive oxygen species, occurs in cells during oxidative stress to generate prominent lipid hydroperoxides.[1,2] The lipid hydroperoxides can then undergo reductive degradation in biological systems to produce numerous types of aldehydes as lipid peroxidation end products. Acrolein, one possible product, is the most reactive α,β-unsaturated aldehyde and is considered to be a toxic messenger that disseminates and augments the initial free radical events.[3–5] An elevated intracellular level of acrolein has long been associated with numerous oxidative stress-related diseases, including cancer and Alzheimer's.[6–13] Consequently, acrolein holds great potential as a key biomarker in oxidative stress-related diseases. Direct measurements of acrolein in biological samples are important for therapeutic and diagnostic applications.[14]

Traditionally, acrolein has been detected using fluorometric analysis after derivatization with 3-aminophenol[15,16] or 2,4-dinitrophenyl hydrazine.[17] More recently, a fluorescence-based method involving a two-step tethering approach on microbeads was developed to detect acrolein in human plasma.[18] Mass spectroscopy techniques have been used to detect acrolein or its metabolite (i.e., 3-hydroxypropyl mercapturic acid) in biological samples.[19,20] Alternatively, the acrolein-lysine adduct, 3-formyl-3,4-dehydropiperidine, may be detected using a monoclonal antibody[21] or 4-nitrophthalonitrile[22] to estimate the acrolein level in the biosystem. Despite this progress, an efficient method that can directly detect acrolein in live cells is not yet available.

2. Results and discussion

Previously, we described the 1,3-dipolar cycloaddition between phenyl azide **1** and acrolein, which proceeded under physiological conditions, even without a catalyst, to give the 4-formyl-1,2,3-triazoline derivative **2** (Fig. 1A).[23] The reaction is highly chemoselective to the acrolein, and any clicked products could not be observed by the reaction with α- or β- substituted acrolein (e.g., methacrolein, crotonaldehyde, *trans*-2-octenal) under the physiological conditions.[23] By attaching the TAMRA fluorophore (see Fig. 1D) to the phenyl azide **1**, the reaction could be used selectively and sensitively to detect acrolein in live cells (Fig. 1B).[23,24] The procedure required two simple steps: first, the cells were treated with TAMRA-phenyl azide **3**, and second, the total fluorescence intensity, which was proportional to the acrolein concentration generated by the cells, was measured. We demonstrated the feasibility of using this method to determine the acrolein levels in various human cell lines and found that cancer cells have higher levels of acrolein compared to normal cells (Fig. 2A shows the representative

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