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Investigation of MTH1 activity *via* mismatch-based DNA chain elongation

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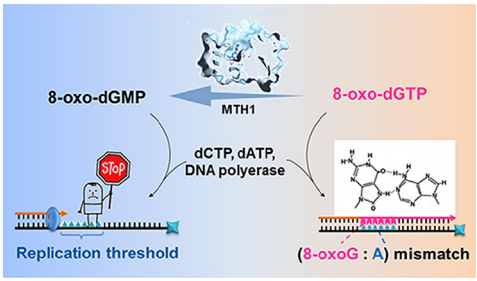
HIGHLIGHTS

- Mismatch-based DNA chain elongation strategy (MB-DCE) is firstly proposed.
- MutT Homolog 1 (MTH1) is critical important for biomedical cancer research.
- An electrochemical method is developed for MTH1 activity based on MB-DCE.
- This method can reveal MTH1 activity in normal and breast cancer cell lines.

GRAPHICAL ABSTRACT



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ABSTRACT

Accumulation and misincorporation of oxidative damaged 8-oxo-7,8-dihydroguanine triphosphates (8-oxo-dGTP) in genomic DNA may cause serious cellular function disorders. MutT Homolog 1 (MTH1), a protein enzyme that can help to prevent 8-oxo-dGTP misincorporation, plays critical roles in oxidative stress neutralization, oncogene-associated tumor malignancy, and anticancer therapies. So, in this work, a simple and function-oriented method is developed for the assay of MTH1 activity. Specifically, a mismatch-based ("8-oxoG: A" mismatch) DNA chain elongation strategy (MB-DCE) is firstly proposed to reveal the misincorporation efficiency of 8-oxo-dGTP. Then, further coupled with the inherent activity of MTH1 to prevent 8-oxo-dGTP misincorporation, a relationship can be established to reveal the activity of MTH1 through MB-DCE. As the method is designed directly towards the cellular function of MTH1, activity of MTH1 in different breast cancer cell lines has been detected, implying the potential application of this assay method for biomedical research and clinical diagnose in the future.

1. Introduction

Dysfunctional redox regulation in cell can produce reactive oxygen species (ROS), which could damage genomic DNA and free deoxynucleoside triphosphates (dNTPs) [1,2]. Among the damaged dNTPs, 8-oxo-7,8-dihydroguanine triphosphates (8-oxo-dGTP) is the most abundant due to the low oxidative potential of guanine [3,4]. 8-oxo-dGTP could be incorporated into DNA by mispairing with adenine (A), forming the "8-oxoG: A" mismatched base pair. This would induce the guanine (G) to thymine (T) transversion mutation, causing cell function disorder and cell death [2,5]. Besides, such mutation often occurs in the coding sequence of

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oncogenes and tumor suppressor genes, revealing the carcinogenic ability of 8-oxoGTP in tumorigenesis [6–9]. So, accumulation of 8-oxo-dGTP in cellular pool may elevate potential risk of cell damage and cancer development [10,11].

MutT Homolog 1 (MTH1) is an 8-oxo-dGTPase that can convert 8-oxo-dGTP to 8-oxo-dGMP. This means MTH1 can reduce the oxidative damages caused by 8-oxo-dGTP misincorporation [12]. Recently, MTH1 has been further validated as a novel and critical component in oncogene-associated tumor malignancy [6] and cancer phenotypic lethal, which renders it as a potential target for anticancer treatment [13–15]. So, the investigation of MTH1 activity is not only essential for evaluating the susceptibility of cancer, but also important for predicting tumor malignancy and evaluating curative effect in clinical practice [13]. Nevertheless, although the current techniques for MTH1 analysis include quantitative reverse transcription-polymerase chain reaction (RT-PCR) at the gene transcription level, western blotting (WB) and enzyme-linked immune-sorbent assay (ELISA) at the protein expressing level [13,14], these methods are used to detect the relative abundance of mRNA or protein. Obviously, the assay of MTH1 activity is more important since it closely relates to its cellular function to prevent 8-oxo-dGTP misincorporation. So far, to our knowledge, there is still no method to investigate MTH1 activity through 8-oxo-dGTP misincorporation. Liquid chromatography–mass spectrometry (LC-MS) may be a potential useful technique, but it cannot be used to determine the 8-oxo-dGTP misincorporation owing to the generation of oxidized bases during MS detection [16,17]. Besides, LC-MS is limited by time-consuming procedures with high cost. So, simple, cost effective and function-oriented method for the assay of MTH1 activity is highly desirable. In this work, we have proposed the mismatch-based ("8-oxoG: A" mismatch) DNA chain elongation strategy (MB-DCE) for the first time. Then, coupled with the inherent activity of MTH1, the MB-DCE can be well developed into a simple and effective electrochemical method to investigate MTH1 activity (Scheme 1).

2. Materials and methods

2.1. Materials and reagents

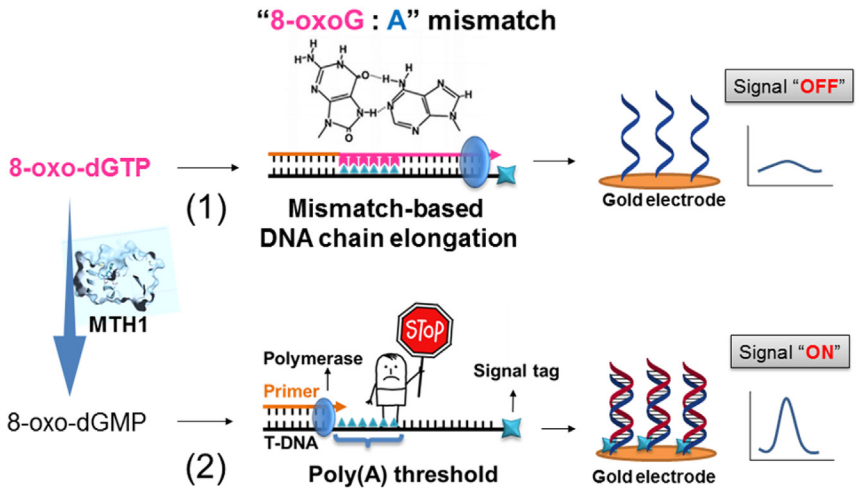
Bst 2.0 DNA polymerase (supplied with 10 × reaction buffer) and human 8-oxoguanine DNA glycosylase 1 (hOGG1) were purchased from New England Biolabs Ltd. 8-oxo-2'-deoxyguanosine-5'-triphosphate (8-oxo-dGTP) was purchased from Bioscience GmbH (Jena, Germany). The MTH1 inhibitor, TH588, was purchased from Selleck Chemicals. dATP (100 mM), dCTP (100 mM), 6-mercapto-1-hexanol (MCH), tris(2-carboxyethyl)phosphine (TCEP), and ethylene diamine tetraacetic acid (EDTA) were from Sigma–Aldrich. The fetal calf serum (FCS) was obtained from Biological Industries (Beth Haemek, Israel). Protein extraction kit (P0027) and BCA protein assay kit (P0010S) were purchased from Beyotime Biotech. Co. Ltd. (Shanghai, China). Other regents were all of analytical grade and were used without further purification. DNA oligonucleotides were synthesized by Takara Biotechnology Co., Ltd. (Dalian, China). Oligonucleotides used in the experiment were listed in supporting information.

Scheme 2. Schematic illustration to show the oligonucleotide sequences immobilized on the gold electrode surface.

Buffer solutions used in the experiments were as follows. Stock solution of DNA oligonucleotides was 10 mM Tris–HCl, 1 mM EDTA, and 10 mM TCEP, pH 8.0. DNA immobilization buffer was 10 mM Tris–HCl, 1 mM TCEP and 1 M NaCl, pH 7.4. DNA hybridization buffer was 10 mM phosphate buffer containing 0.25 M NaCl, pH 7.4. MTH1 reaction buffer was 20 mM Tris–HCl, 4 mM $MgCl_2$, 40 mM NaCl, 0.5% tween 20 and 8 mM DTT, pH 7.4. All solutions were prepared with deionized water, which was purified to a resistance of 18.2 MΩ with a Milli-Q purification system (Bedford, MA).

2.2. Preparation of C-DNA modified gold electrode

DNA modified electrode was prepared with the previously method with slightly modification [18]. Briefly, the gold electrode (3 mm in diameter) was first cleaned with piranha solution (98% H_2SO_4: 30% H_2O_2 = 3: 1, v/v) for 10 min. Then, it was polished to a mirror sheen with alumina powder. After successive sonication in ethanol and deionized water for 5 min, the electrode was immersed in 50% nitric acid for 30 min. After which, it was electrochemically



Scheme 1. Schematic illustration to show principle of the mismatch-based DNA chain elongation (MB-DCE) strategy for the design of an electrochemical assay method to detect MTH1 activity.