Detection of Glutathione in Oral Squamous Cell Carcinoma Cells With a Fluorescent Probe During the Course of Oxidative Stress and Apoptosis

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Purpose: To detect glutathione (GSH) in oral squamous carcinoma cells (OSCCs) with a GSH selective fluorescent probe during the course of oxidative stress and apoptosis.

Materials and Methods: A novel GSH probe was applied to assess GSH in human tongue squamous cell carcinoma cells (cal-27). The cellular GSH and reactive oxygen species (ROS) levels were assessed with a GSH probe and DCF-DA (2,7-dichlorofluorescin diacetate) probe. The mitochondrial GSH and ROS levels were assessed with a GSH probe, DCF-DA probe, and Mitotracker Red CM-H_2XRos probe (Invitrogen, Carlsbad, CA). To further study whether oxidative stress would induce apoptosis of OSCCs, we then applied a GSH probe and annexin V–fluorescein isothiocyanate probe to assess cellular GSH levels and eversion of phosphatidylserine, and the cellular GSH levels and mitochondrial membrane potential ($\Delta\Psi m$) were assessed with a GSH probe and JC-1 probe during the course of oxidative stress and apoptosis induced by hydrogen peroxide and ethacrynic acid. The fluorescence was observed under laser confocal fluorescence microscopy.

Results: The intensity of fluorescence that represented intracellular alteration of GSH levels, cellular ROS formation, mitochondrial ROS formation, and apoptosis occurrence, respectively, could be visualized under laser confocal fluorescence microscopy.

Conclusions: The GSH selective fluorescent probe can evaluate cellular GSH levels sensitively during the course of oxidative stress and apoptosis of OSCCs induced by exogenous hydrogen peroxide, which could be enhanced by depletion of mitochondrial GSH.

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Oral squamous cell carcinoma is the most common head and neck malignant neoplasm, with high recurrence and metastasis rates. Chemotherapy is one of the main therapeutic strategies and suffers more and more resistance.[1] Reactive oxygen species (ROS) are the most plentiful free radicals with unpaired electrons in mammalian cells.[2] ROS formation is the fundamental instrument of chemotherapy agents and ionizing radiation, based on the rationale that persistent oxidative stress can cause the collapse of the intracellular antioxidant system and apoptosis of tumor cells.[3,4] The intracellular oxidation-reduction (redox) state remains homeostasis, which is tightly regulated by intracellular antioxidant systems.[5] Glutathione (L-γ-glutamyl-L-cysteinyl-glycine, GSH) is the most abundant non-protein thiol and redox buffer in mammalian cells to provide the first-line defense of ROS.[6,7] GSH can not only directly interact with ROS or act as a substrate for different enzymes to eliminate endogenous and exogenous compounds, but also conjugate with xenobiotics such as chemotherapy agents directly.[8-10] So there is an intimate relationship between high GSH levels and anticancer drug resistance.[11,12]

Most ROS are generated from the mitochondrial respiratory chain.[3] Mitochondrial DNA, lipids, and proteins are susceptible to be damaged by oxidative stress because they are closer to the region where ROS are produced.[13] Therefore, mitochondria play a considerable role in apoptosis induced by oxidative stress, which is executed through an external or internal pathway and depends on mitochondrial dysfunction.[14,15] ROS can activate apoptosis through a mitochondrial pathway by opening of the mitochondrial permeability transition pore.[8] GSH is synthesized in cytoplasm and distributed into intracellular organelles mainly including mitochondria, nucleus, and endoplasmic reticulum.[9] Mitochondrial glutathione (mtGSH) plays a pivotal role in retaining mitochondrial redox homeostasis and helping cells escape apoptosis.[12,13] Oxidative damage to mitochondria can be prevented by mtGSH chiefly through the role of mitochondrial S-transferases (GSH S-transferase).[13] Thus, mitochondrial dysfunction, mitochondrial permeability transition pore opening and subsequent apoptotic cascade all result from inhibition of mtGSH.[16,17]

During the past several years, a few fluorescent probes have been reported for quantitative detection of GSH.[18] We have already devised a colorimetric and ratiometric fluorescent probe to detect GSH in living HeLa cells.[19] In this research, the GSH selective probe was applied to investigate the relationship between depletion of GSH and apoptosis of oral squamous carcinoma cells (OSCCs). Hydrogen peroxide (H_2O_2), the representative of ROS, is always applied to model oxidative stress.[20] Ethacrynic acid (EA), an effective inhibitor of GSH S-transferase P1-1, can deplete more mtGSH than cytoplasmic GSH and has been proved to enhance the toxic effect of chemotherapeutic agents such as cisplatin.[17,21-23] Variation of fluorescence intensity that represented intracellular alteration of GSH levels, cellular ROS formation, mitochondrial ROS formation, and apoptosis occurrence, respectively, could be visualized under laser confocal fluorescence microscopy. The level of GSH and cellular and mitochondrial ROS formation, as well as apoptosis occurrence, could be evaluated by observing the variation of fluorescence intensity under laser confocal fluorescence microscopy.

The purpose of this study was to determine whether the GSH selective fluorescent probe could be applied to detect alteration of cellular GSH levels sensitively during the course of oxidative stress and apoptosis of OSCCs induced by exogenous H_2O_2, as well as enhanced by depletion of mtGSH. The intact process of oxidative stress and apoptosis of OSCCs by depletion of GSH and mtGSH could be visualized. It proved that depletion of cellular GSH and mtGSH would facilitate apoptosis during the course of oxidative stress through the study, which showed a promising way to avoid resistance to therapy by monitoring GSH levels of patients who received chemotherapy or radiotherapy with the probe.

Materials and Methods

REAGENTS

The GSH fluorescent probe was provided by Professor Baocun Zhu (School of Resources and Environment, University of Jinan, Jinan, China). The following products were used for this study: Dulbecco's modified eagle medium high-glucose culture medium, fetal bovine serum, streptomycin-penicillin, and 0.25% trypsin (HyClone; GE Healthcare, Buckinghamshire, UK); *N*-acetyl-L-cysteine (NAC), H_2O_2, EA, and dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO); Mitotracker Red CM-H_2XRos (Invitrogen, Carlsbad, CA); ROS Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China); and mitochondrial membrane potential assay kit with JC-1 and annexin V–fluorescein isothiocyanate (FITC) Apoptosis Detection Kit (BIOBOX, Nanjing, China).

CELL CULTURE

Human tongue squamous cell carcinoma cell lines (cal-27) were provided by Professor Wantao Cheng