Design and application of a genetically-encoded probe for peroxiredoxin-2 oxidation in human cells

By

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Technical Summary

Hydrogen peroxide (H_2O_2) is a well-known oxidant species commonly produced in eukaryotic organisms as a result of cellular metabolism that plays a central role in numerous processes in cells, and dysregulation of this species can result in a number of different disease states in human cells. In the case of cancer, elevated metabolism is believed to result in higher rates of H_2O_2 production in these cells, as well as more susceptibility to H_2O_2-induced apoptosis than normal cells. To this end, researchers have identified several therapeutic compounds that are believed to kill cancer cells via the intracellular elevation of one or more oxidants. However, due to the limitations of current tools for detection of these species, little is known about which therapeutic compounds induce toxicity via elevation of specific oxidants, which would aid in the identification of susceptible tumors to these treatments.

Currently, the main limitation of genetically-encoded tools for detection of H_2O_2 in these applications is the low sensitivity to H_2O_2. Most genetically-encoded probes for this species (e.g. HyPer) used in human cells utilize responsive domains with reaction rate coefficients nearly two orders of magnitude lower than other, more reactive peroxidases in the cell, such as peroxiredoxins (Prxs). In this regard, several studies have demonstrated that Prxs should react with the majority of intracellular H_2O_2 on the basis of a high reaction rate coefficient with H_2O_2 and intracellular abundance. In light of these studies, research in the field of redox biology has shifted to focus more on Prxs' role as natural sensors of H_2O_2 fluctuations in human cells.

In the first part of this thesis work, the relative reactivity of various molecular species inside HeLa cells towards H_2O_2 was evaluated, and it was determined that the reactivity of Prxs dwarfed that of other intracellular species in these cells. The feasibility more sensitive genetically-encoded probe for H_2O_2 in human cells based on human Prx2 was then explored by construction of a FRET-based sensor composed of Prx2 flanked by two fluorescent proteins. This probe was then extensively characterized both in purified form as well as in the cytosol of human cells in response to various intracellular perturbations and determined that the probe responded both specifically and sensitively to H_2O_2. The response of the probe was then measured in response to the redox-therapeutics auranofin and piperlongumine, which both resulted in an elevation in the signal from the Prx-based probe but only minimal (in the case of auranofin) or no change (in case of piperlongumine) in the signal from HyPer.

After construction of the Prx2-based probe for H_2O_2, this tool was then used to screen two different libraries of small molecule compounds for those that act through H_2O_2-mediated mechanisms. In both high-throughput screens, several small-molecule compounds were identified that altered the signal from the fluorescent probe, and a subset of these compounds

appeared to act through H_2O_2-mediated mechanisms of action. In particular, small molecule enhancer of rapamycin 3 (also known as SMER3) was identified as a compound of interest that appears to raise H_2O_2 in the mitochondria of several human cancer cell lines. Among the different human cancer cell lines tested, the HT-29 colorectal cancer cell line was determined to have the highest susceptibility towards this therapeutic compound (compared to HeLa and A549 cells), and also contained significantly elevated levels of oxidized Prx3 prior to treatment with the therapeutic compared to the other two cell lines, which suggests that Prx oxidation status before treatment with the therapeutic compound could predict sensitivity to the H_2O_2-mediated treatment

In this last part of this thesis, Prx2 and Prx3 oxidation status were then measured in patient-derived tumor samples as a means to identify specific tumors with altered Prx oxidation status and potentially higher susceptibility to the H_2O_2-mediated therapeutics identified in the high-throughput screens. The initial experiments with patient-derived xenograft models of gastrointestinal stromal tumors suggested that the cell lysate samples obtained from these tumors contained large amounts of lysis-induced Prx oxidation. The subsequent experiments with these tumors revealed that introduction of alkylating agents to block the free thiols groups in the cell immediately after removal of the tumor from the rest of the tissue significantly reduced the amount of lysis-induced oxidation observed. With this knowledge, an optimized protocol for extraction of intracellular protein from these tumors and preservation of Prx oxidation state was devised.

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