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Original Contribution

Catalase enrichment using recombinant adenovirus protects αTN4-1 cells from H_2O_2

Wanchao Ma [a,1], Irene Nunes [b,1,2], C.S. Hamish Young [c], Abraham Spector [a,*]

[a] *Department of Ophthalmology, Columbia University, 630 West 168th Street, New York, NY, USA*
[b] *Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY, USA*
[c] *Department of Microbiology, Columbia University, New York, NY, USA*

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Abstract

Since oxidative stress has been implicated in the development of numerous diseases including cataract, this laboratory has created and investigated the stress response of murine immortal lens epithelial cell lines (αTN4-1) conditioned to withstand lethal peroxide concentrations. Two of a group of antioxidative defense (AOD) enzymes found in such cells to have markedly enhanced activity are catalase (CAT) and GSH *S*-transferase α2 (GST). In order to determine if enrichment of one or both of these AODs is sufficient to protect αTN4-1 cells from lethal H_2O_2 levels, these cells were infected with adenovirus vectors capable of expressing these AODs at a high level. With this system, gene enrichment and increased enzyme activity were observed with both CAT and GST vectors. The percentage of cells infected ranged from about 50 to 90% depending on the multiplicity of infection (MOI). CAT but not GST protected the cells from H_2O_2 stress. The CAT activity was increased from 15- to 150-fold and even at the lower levels protected the cells from H_2O_2 concentrations as high as 200 μM or more (H_2O_2 levels which rapidly kill nonenriched cells). Even when only about 50% of the cell population is infected as judged by GFP infection, the entire population appeared to be protected based on cell viability. The CAT enrichment appears to protect other intracellular defense systems such as GSH from being depleted in contrast to nonenriched cell populations where GSH is rapidly exhausted. The overall results suggest that enriching the cellular CAT gene level with an appropriate recombinant viral vector may be sufficient to protect in vivo systems from peroxide stress.

Keywords: Catalase; Recombinant adenovirus; H_2O_2; αTN4-1 cells; GSH *S*-transferase; Cataract; Lens; Oxidative stress; Free radical

Introduction

Oxidative stress is believed to be involved in a broad range of diseases involving numerous tissues with different functions and characteristics including skin, kidney, heart, brain, and eye [1]. The lens of the eye is an attractive tissue for studying the impact of oxidative stress since it is relatively simple, being avascular, encapsulated, and composed of only epithelial cells which terminally differentiate into fibers [2,3]. Oxidative stress-related disease may cause a loss of transparency (cataract), cell death, breakdown of membrane integrity, and disorganization of the uniform packing of epithelial cells and fibers. At the molecular level, many changes in biological parameters have been observed including the oxidation of thiols [4,5], cross-linking of proteins [6], oxidation of membrane lipid [7,8], and depletion in reduced GSH and other antioxidative defenses (AODs) [9,10,12]. Examination of the sequence of events leading to cataract formation in response to oxidative stress suggests a complex pattern with changes in the concentration of numerous components, some of which, such as c-*Fos* and GSH, have high swings in levels and then return to baseline concentrations well before there is any apparent loss of transparency [9,13,14].

The rapid changes in cellular components, the biological heterogeneity of the epithelial cell population, and the small number of cells per lens stimulated us to seek a more uniform system in which to investigate the response to oxidative stress. Immortal lens epithelial cells provide an attractive system for

Abbreviations: AOD, antioxidative defense; CAT, catalase; EMC, encephalomyocarditis; ffu, fluorescent focus units; GFP, green fluorescent protein; GSH, glutathione; GST, glutathione *S*-transferase; IRES, internal ribosome entry site; MOI, multiplicity of infection.

* Corresponding author. Fax: +1 (212) 305-6205.
E-mail address: as42@columbia.edu (A. Spector).
[1] Contributed equally to this work.
[2] Present address: Merck Research Laboratories, RY80B-100, PO Box 2000, 126 Lincoln Avenue, Rahway, NJ 07065, USA.

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this purpose. They retain most characteristics of lens epithelial cells and can be easily grown and maintained. Investigation of the impact of oxidative stress upon the murine lens epithelial cell line, αTN4-1 was, therefore, initiated. However, because of the rapid and complex change in cell biology in response to stress and the short time available before cell death when the stress was at levels reported to cause cataract, it was apparent that another approach was required [15]. We, therefore, took preparations of αTN4-1 and gradually conditioned them over a period of 6–7 months to survive peroxide conditions that have been reported to contribute to human cataract formation [15]. Examination of the differential gene expression of such preparations indicated that approximately 950 of 12,422 genes had a significant change in expression [16]. This population contained about 28 genes that could be defined as AOD genes, including many that were enhanced by either H_2O_2 or tertiary butyl hydroperoxide (TBOOH) conditioning, [16,17]. Examination of the enzyme activity of some of these AOD gene products indicated that catalase and a few of the members of the GSH-*S*-transferase (GST) family had a large increase in activity.

Is overexpression of all of these AOD genes required for the αTN4-1 cells to survive H_2O_2? To attempt to answer this question, two genes, CAT and GST, were enriched in the αTN4-1 cell line utilizing recombinant virus technology [18–20]. Recombinant replication deficient adenoviruses are attractive vectors for gene transfer since they infect most cell types, do not require cell division, and have a high infection level and efficiency of gene transfer. Therefore, vectors containing CAT cDNA or GST cDNA were constructed with green or red fluorescent marker genes (GFP or RFP, respectively).

The work demonstrates that the vectors successfully enriched the αTN4-1 cells, producing significant increases in enzyme activity. CAT cDNA enrichment resulted in resistance to H_2O_2 stress, suggesting that this approach may protect the lens from peroxide stress-related cataract formation.

Materials and methods

Construction and preparation of adenovirus vectors

Four individual adenovirus vectors were designed for this investigation. Two of the four vectors expressed either the enhanced green fluorescent protein (GFP) or red fluorescent protein (RFP). The GFP or RFP transgenes are located in the E1 region of the virus genome and are under the transcriptional control of the murine cytomegalovirus immediate-early promoter and under the translational control of the encephalomyocarditis (EMC) virus internal ribosome entry site (IRES). These two viruses, termed vGFP and vRFP, respectively, were used as controls in coinfection experiments. The other two vectors expressed either the murine catalase protein, whose coding sequence was located upstream of the IRES and the GFP coding sequences, or the GSH-*S*-transferase-α2 protein, whose coding sequence was placed upstream of the IRES and RFP coding sequence. Thus each vector expresses a bicistronic mRNA encoding an experimental protein and a marker protein. These two viruses are referred to as vCAT-GFP and vGST-RFP, respectively. All viruses were constructed using the technique of in vivo site-specific recombination developed by Ng et al. [18]. Briefly, the shuttle plasmid pDC516 was modified to contain the sequences encoding either GFP or RFP, each controlled by the IRES of EMC virus. These two plasmid constructs were used to construct virus expressing the fluorescent marker only. The coding sequences for catalase and GST were then cloned upstream of the IRES in the GFP vector and the RFP vector, respectively. Each of the four plasmid constructs was cotransfected with the large genome-containing plasmid pBHG,frt, Delta E1, E3, FLP into 293 cells, where site-specific recombination between the frt sites pBHG and pDC516, mediated by the flp recombinase encoded by pBHG, resulted in full-length adenovirus genomes containing the genes of choice. After transfection, cytopathic effects were observed as the result of virus replication. The structure of the recombinant genomes was confirmed by restriction enzyme digestion of viral DNA isolated from infected cells [19]. Larger scale preparations of virus were then prepared using CsCl purification of virus from 5 infected 15-cm plates of 293 cells. Purified virus was dialyzed against four changes of dialysis buffer (10 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 3% sucrose), stored in aliquots at −80°C, and titrated by standard fluorescent focus assay on 293 cells as described [20]. Virus titers were in the range of 10^9–10^{10} fluorescent focus units (ffu) per milliliter. In our experience, the ratio of physical particles to ffu is in the range of 20 to 50 to 1. For infections of the lens cell line, the virus was diluted in lens cell culture medium to give the appropriate multiplicities of infection (MOI).

Infection of α-TN4-1 cells

Cells were grown in 60-mm plates and were usually infected with pairs of viruses each at a MOI of 30 ffu per cell. Unless stated otherwise, all infections were set up with all four pairs of the four different viruses to control for possible effects of expression of each transgene upon heterologous gene expression. Medium (see below) was removed from the cells and an inoculum of 0.3 ml of virus mixture was added. Plates were incubated at 37°C in a cell culture incubator, with periodic shaking for 90 min to disperse the inocula. The plates were then overlaid with fresh medium before further manipulation.

Cell infection and exposure to H_2O_2

αTN4-1 cells were cultured in DMEM culture medium. The standard medium contained DMEM (Invitrogen, Cat. No. 11965), 100 U penicillin, and 100 μg streptomycin per milliliter of medium (Gibco BRL), and a final concentration of 10% fetal bovine serum (Hyclone, Logan, UT; Cat. No. 5430070-02). Cells (1×10^6) were infected with different combinations of adenovirus, vGFP, vGFP/vRFP, vGFP + vGST-RFP, vCAT-GFP + vRFP, vCAT-GFP + vGST-RFP, at MOIs varying from 3 to 30. Infected cultures were replated 24 and 48 h following infection at approximately 1.5×10^5 cells and the following morning subjected to peroxide stress. At 24 and 48 h following exposure to H_2O_2, fluorescent-positive cells