This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2003)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2003 Term

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The Role of p53 in the Redox Modulation in the DNA Binding

by

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For 77: 222, Spring 2003

March 13, 2003
Paper III

Abbreviations

PDTC: pyrrolidine dithiocarbamate
DMP: dimercaptopropanol
GSH: glutathione
GPx: glutathione peroxidase
COX: cyclooxygenase
MPB: 3-(maleimidopropioryl) biocytin
NAC : N-acetylcysteine
DTT: dithiotheitol
EDTA: ethylenediaminetetracetic acid
NOS: nitric oxide synthase
NEM: N-ethylmaleimide
ECL: enhanced chemiluminescence

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Abstract

The tumor suppressor protein p53 is a multifunctional protein involved in the suppression of cell transformation by oncogenes, redox modulation in the DNA binding, and transcriptional regulation of specific target genes. This report will highlight the redox state of p53 in the regulation of antioxidant-induced apoptosis, and DNA binding process. Antioxidants can exert their effects through altering the cellular redox potential of p53. Oxidation disruptes p53 conformation and inhibits DNA binding. The reduced state of p53 is required for DNA binding and control transcription of downstream genes. Also, these redox effects are mediated by the binding of zinc. Moreover, pyrrolidine dithiocarbamate (PDTC) is a thiol compound widely used to study the redox regulation of p53 and the activation of its transcription factors.

Introduction

P53 was found in most cell lines derived from tumors of epithelial and mesodermal origin, as illustrated in Table 1. The exceptional lines in which p53 could not be detected are the HeLa and EJ cell lines, thus rapid growth in culture is not sufficient to accentuate p53 [1].

Table 1. The occurrence of p53 in human tumor cell lines and normal cell cultures [1].

Cells	Type of tumor	p53
Lines		
Daudi	Burkitt lymphoma	+
Bristol 7	Lymphocyte transformed by EBV	+
NALM 1	Chronic myelogenous leukemia	+
BT 20	Primary mammary carcinoma	+
SK BR3	Metastatic mammary carcinoma	+
T47D	Metastatic mammary carcinoma	+
Hs 578T	Primary mammary carcinosarcoma	+
Tera 1	Teratocarcinoma	+
Tera 2	Teratocarcinoma	+
MG	Teratocarcinoma	+
BeWo	Choriocarcinoma	+
Jar	Choriocarcinoma	+
HeLa D98	Cervical carcinoma	–
C33I	Cervical carcinoma	+
EJ	Bladder carcinoma	–

The p53 protein is a cellular 53-kDa nuclear phosphoprotein bound to the large transforming antigen of the SV40 DNA virus (SV40T antigen), as shown in Figure 1. It induces the transcription of several genes such as mdm2 (murine double minute) oncogene, adenomavirus E1b and human papillomavirus E6 in the upstream regulatory region [2].

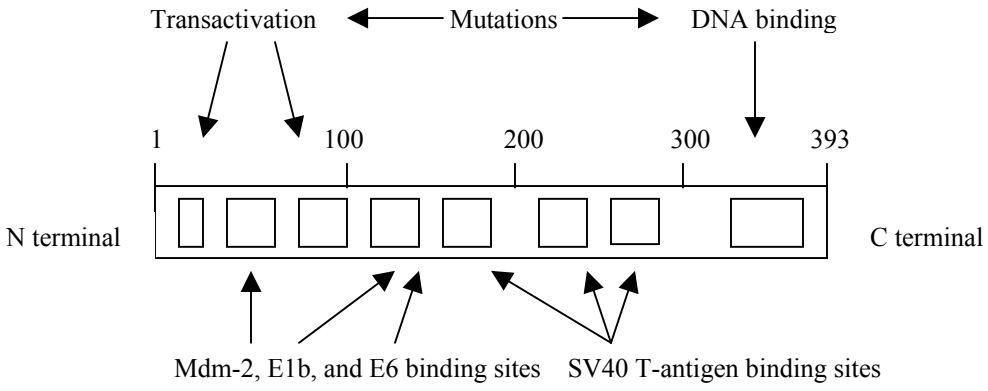

Figure1. The p53 protein [2].

P53 mutations are detectable when two copies of alleles (heterozygous) are different on chromosome 17p13. In the survey of 1312 patients with p53 mutations, 83% are missense mutations, 10% are delections and insertions, 6% are nonsense mutations, and 1% are silent mutations. Hence, the function of p53 protein is sensitive to subtle changes in the amino acid sequence of the molecule [2].

The structure of the DNA-binding domain was elucidated by x-ray crystallography. This domain is made up of an array of two beta-sheets supporting large loop-helix structures directly involved in contacting DNA. These loops are bridged together by a divalent zinc atom on three cysteins (residues 176, 238, and 242) and one histone (residue 179) [3].

The purpose of this report is to summerize the antioxidant action and the effectors in the p53 signalling pathways, with particular emphasis on how redox and metal factors cooperate the DNA-binding capacity of the p53 protein.

Antioxidant action

Antioxodant-induced apoptosis requires the p53 gene and there are two antioxidant pathways, as shown in Figure 2. p53-dependent mechanism reflects changes in the redox potential, and non-p53 apoptosis mechanism involves radical species. In the redox pathway, sulfur-containing antioxidants such as N-acetylcysteine (NAC) and dimercaptopropanol (DMP) alter intracellular thiol levels, elevate p53 protein, and induce apoptosis. NAC induces the expression of p53 protein through enhanced p53 mRNA translational efficiency. In the radical pathway, chain-breaking antioxidants alter cell cycle related proteins such p21 and Rb, and create cell cycle

perturbations [4]. p53 may exert a direct block on DNA replication and act as an anti-helicase or bind to the essential initiation protein RP-A. Also, it induces the cyclin-kinase inhibitor, p21 cip1, which directly blocks kinase avticity of cyclin E-Cdk2 in G1, cyclin A-Cdk2 at G1/S and cyclin A-cdc2 in S phase. Thus, p53 can cause G1 and S phase cell cycle arrest [5].

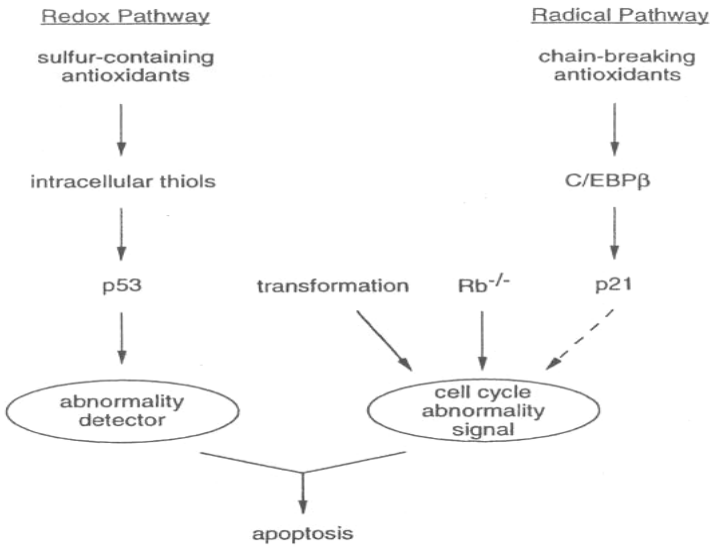

Figure 2. Two antioxidant pathways for apoptosis [4].

DNA binding

Redox state of p53 plays a role in sequence-specific DNA binding. Human p53 encodes ten cysteine residues all within the DNA binding domain of the protein. Seven of ten cysteine residues expose thiol groups on the surface of the protein. During oxidation, disulfide bonds would be formed and the conformation of p53 protein would be altered [6].

Figure 3 shows p53 binds preferentially to double-stranded damaged DNA and to mismatched DNA at the site of the mismatch, so oxidized and reduced p53 bind equally well to mismatched DNA. Oxidative stress can be initiated by H_2O_2 or O_2. Ref-1, DTT (dithiothreitol), GSH (glutathione) and NAC (N-acetylcysteine) can maintain the reduced state of p53. However, p53 must be in a reduced state in order to bind to specific consensus DNA and control transcription of adjacent genes such as WAF1, bax and GADD45. The lack of binding of oxidized p53 to sequence-specific DNA means oxidized p53 is not able to transactivate target genes [7].

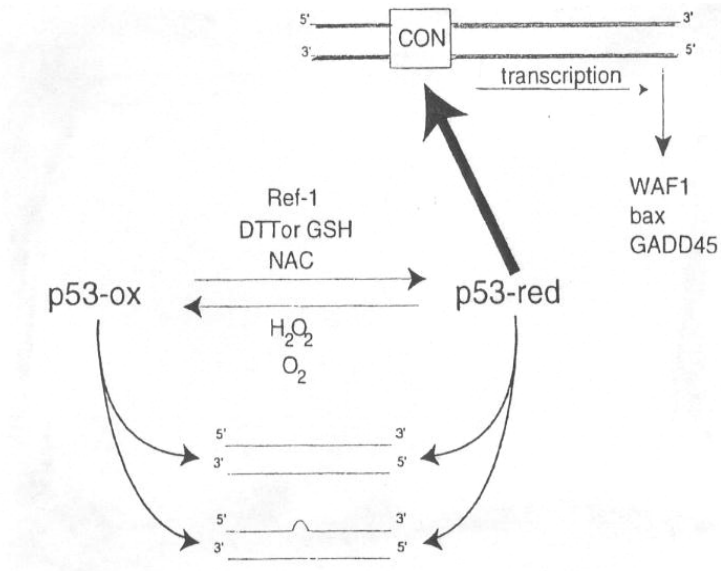

Figure 3. The redox state of p53 on DNA binding [7].

Regulation

Redox regulation of p53 involves two clusters of cysteine residues in the central domain of the protein. One cluster of cysteines (residues 121, 132, 138 and 272) is located near the loop-sheet-helix region of p53 that makes contact with the consensus DNA sequences. This cluster doesn't involve the interaction of p53 with zinc. The other cluster contains three cysteins (residues 173, 235 and 239) responsible for the coordination of a zinc ion [7].

Zinc binding is crucial for the stabilization of p53 in the folded form, as shown in Figure 4. Unfolded form (pAb240) is unable to bind to DNA with high affinity. Addition of Zn^{2+} causes a conformational change to the folded form (pAb1620) and acquires DNA-binding ability. Metal chelators such as ethylenediaminetetracetic acid (EDTA) or orthophenanthroline result in unfolded form of p53. This effect is companied by loss of DNA-binding activity. Also, Cadmium (Cd^{2+}) can abrogate DNA binding activity of p53 [8].

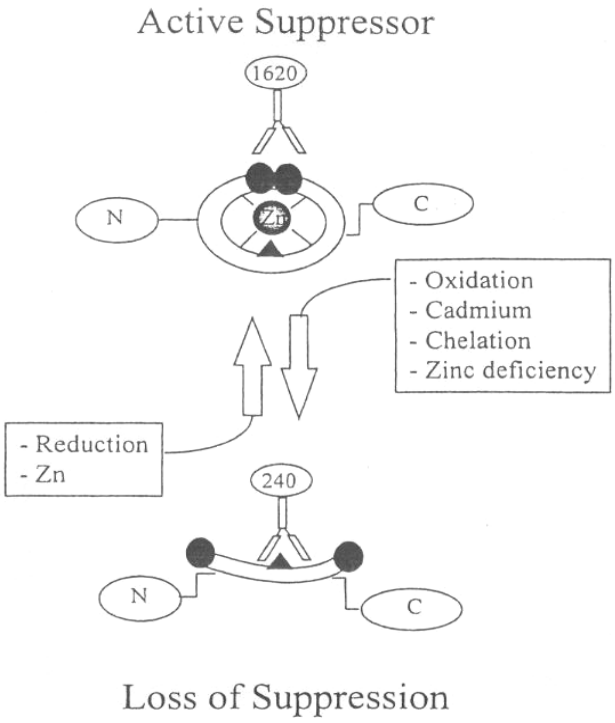

Figure 4. Role of metals and oxidation-reduction in the control of p53 conformation [8].

Redox effectors

Once activated, p53 can act as a transcription factor to regulate other reactive oxygen species, as shown in Table 2. COXs (cyclooxygenases) are the key enzymes in the conversion of

arachidonic acid to prostaglandins and other eicosanoids. GPx (glutathione peroxidase) is a primary antioxidant enzyme that scavenges hydrogen peroxide or organic hydroperoxides. NOS (nitric oxide synthase) is the enzyme that catalyzes the formation of NO (nitric oxide). Nitric oxide is a putative endogenous mutagen which induces p53 protein accumulation and cause oxidative DNA damage. PIG 3 encodes an NADPH quinone oxidoreductase homologue, PIG 6 is a proline oxidase homologue, and PIG 12 is a homologue of microsomal glutathione transferase [8].

Table 2. Redox effectors regulated by p53 [8].

Factor	Activity	Mode of regulation
COX2	Inducible cyclooxygenase	Transcriptional repression
GPx	Glutathione peroxidase	Transcriptional activation
NOS2/iNOS	Inducible nitric oxide synthase	Transcriptional repression
Pig-12	Glutathione Transferase homologue	Transcriptional activation
Pig-3	Quinone oxidase homologue	Transcriptional activation
Pig-6	Proline oxidase homologue	Transcriptional activation

Detection

Pyrrolidine dithiocarbamate (PDTC) is a thiol compound widely used to detect the redox regulation of p53. PDTC contains two thiol moieties that can chelate metal ions and may exert antioxidant or pro-oxidant effects. Figure 6 shows the experimental design for the detection of cysteine oxidation on p53. First, endogenous protein free sulfhydryl groups were blocked by lysing the cells in the presence of N-ethylmaleimide (NEM). NEM is a reagent that forms nonreducible thioether bonds with free sulfhydryl groups. Second, proteins were treated with dithiotheitol (DTT) to free disulfide-linked cysteine residues, and newly formed sulfhydryl groups were covalently modified with 3-(maleimidopropioryl)biocytin (MPB). MPB is a

biotinconjugated maleimide that forms a nonreducible thioether bond. Third, MPB-modified p53 was immunoprecipitated with an antibody mixture (pAb421 and pAb240) followed by SDS-PAGE analysis. Finally, after electroblotting onto polyvinylidene difluoride membrane, MPB-modified p53 was detected with peroxidase-conjugated avidin followed by enhanced chemiluminescence (ECL) kit. On the other hand, immunoprecipitation efficiency was assessed by duplicate immunoprecipitations followed by standard p53 Western analysis [9]. Because PDTC treatment is based on the oxidation of cysteine residues on p53, PDTC-mediated oxidation of p53 can be used for assess the relationship between the oxidation of p53 and the reduction of downstream effector genes by using appropriate antibodies.

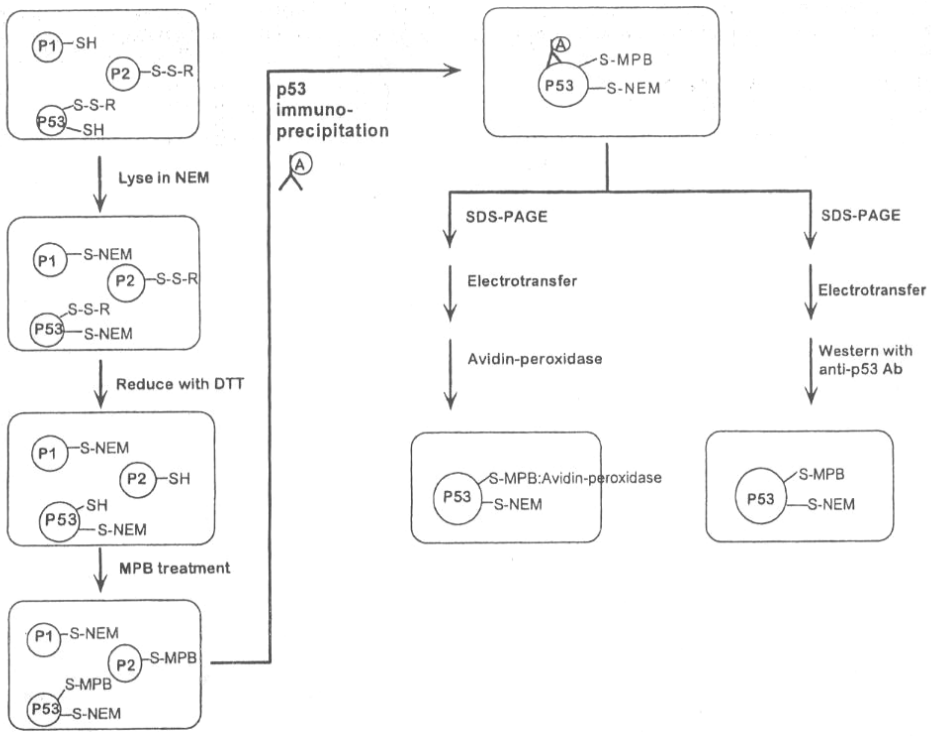

Figure 5. Experimental design to selectively detect p53 cysteine disulfide bonds [9].

P1 and P2 represent cellular proteins, other than p53, which contain free sulfhydryl groups (-SH) and disulfide bonds (-S-S-R), respectively. P53 protein is shown with two cysteine residues, one in a disulfide linkage and the

other with a free sulfhydryl group. The circle A represents protein A-Sepharose. The upside-down Y-shaped line represents the p53-specific antibodies used for immunoprecipitation [9].

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