DNA-mediated oxidation of transcription factor p53

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Kathryn Nicole Schaefer

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ABSTRACT

Transcription factor p53 is the most commonly altered gene in human cancer. As a redox-active protein in direct contact with DNA, p53 can directly sense oxidative stress through DNA-mediated charge transport. Electron hole transport occurs with a shallow distance dependence over long distances through the π-stacked DNA bases, leading to the oxidation and dissociation of DNA-bound p53. The extent of p53 dissociation depends upon the redox potential of the response element DNA in direct contact with each p53 monomer. The DNA sequence dependence of p53 oxidative dissociation was examined by electrophoretic mobility shift assays using radiolabeled oligonucleotides containing both synthetic and human p53 response elements with an appended anthraquinone photooxidant. Greater p53 dissociation is observed from DNA sequences containing low redox potential purine regions, particularly guanine triplets, within the p53 response element. Using denaturing polyacrylamide gel electrophoresis of irradiated anthraquinone-modified DNA, the DNA damage sites, which correspond to locations of preferred electron hole localization, were determined. The resulting DNA damage preferentially localizes to guanine doublets and triplets within the response element. Oxidative DNA damage is inhibited in the presence of p53, however, only at DNA sites within the response element, and therefore in direct contact with p53. From these data, predictions about the sensitivity of human p53-binding sites to oxidative stress, as well as possible biological implications, have been made. On the basis of our data, the guanine pattern within the purine region of each p53-binding site determines the response of p53 to DNA-mediated oxidation, yielding for some sequences the oxidative dissociation of

p53 from a distance and thereby providing another potential role for DNA charge transport chemistry within the cell.

To determine whether the change in p53 response element occupancy observed *in vitro* also correlates *in cellulo*, chromatin immunoprecipition (ChIP) and quantitative PCR (qPCR) were used to directly quantify p53 binding to certain response elements in HCT116N cells. The HCT116N cells containing a wild type p53 were treated with the photooxidant $[Rh(phi)2bpy]^{3+}$, Nutlin-3 to upregulate p53, and subsequently irradiated to induce oxidative genomic stress. To covalently tether p53 interacting with DNA, the cells were fixed with disuccinimidyl glutarate and formaldehyde. The nuclei of the harvested cells were isolated, sonicated, and immunoprecipitated using magnetic beads conjugated with a monoclonal p53 antibody. The purified immounoprecipiated DNA was then quantified via qPCR and genomic sequencing. Overall, the ChIP results were significantly varied over ten experimental trials, but one trend is observed overall: greater variation of p53 occupancy is observed in response elements from which oxidative dissociation would be expected, while significantly less change in p53 occupancy occurs for response elements from which oxidative dissociation would not be anticipated.

The chemical oxidation of transcription factor p53 via DNA CT was also investigated with respect to the protein at the amino acid level. Transcription factor p53 plays a critical role in the cellular response to stress stimuli, which may be modulated through the redox modulation of conserved cysteine residues within the DNA-binding domain. Residues within p53 that enable oxidative dissociation are herein investigated. Of the 8 mutants studied by electrophoretic mobility shift assay (EMSA), only the C275S mutation significantly decreased the protein affinity (K_D) for the Gadd45 response

element. EMSA assays of p53 oxidative dissociation promoted by photoexcitation of anthraquinone-tethered Gadd45 oligonucleotides were used to determine the influence of p53 mutations on oxidative dissociation; mutation to C275S severely attenuates oxidative dissociation while C277S substantially attenuates dissociation. Differential thiol labeling was used to determine the oxidation states of cysteine residues within p53 after DNA-mediated oxidation. Reduced cysteines were iodoacetamide labeled, while oxidized cysteines participating in disulfide bonds were $^{13}C_2D_2$-iodoacetamide labeled. Intensities of respective iodoacetamide-modified peptide fragments were analyzed using a QTRAP 6500 LC-MS/MS system, quantified with Skyline, and directly compared. A distinct shift in peptide labeling toward $^{13}C_2D_2$-iodoacetamide labeled cysteines is observed in oxidized samples as compared to the respective controls. All of the observable cysteine residues trend toward the heavy label under conditions of DNA CT, indicating the formation of multiple disulfide bonds potentially among the C124, C135, C141, C182, C275, and C277. Based on these data it is proposed that disulfide formation involving C275 is critical for inducing oxidative dissociation of p53 from DNA.

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