REGULATION OF WILD-TYPE AND MUTANT P53 THROUGH DNA-MEDIATED CHARGE TRANSPORT

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ABSTRACT

The global transcription factor p53 controls many cellular processes, including the cellular response to oxidative stress. It had been determined that that dissociation of wild type p53 from its promoter site can occur upon DNA-mediated oxidation. In this work, we use site-directed mutagenesis to construct charge-deficient mutants of p53; the chemistry of DNA-mediated oxidation of p53 was examined using these mutants.

The control point for p53 oxidation through DNA-mediated charge transport (DNA CT) is cysteine 275. Using differential thiol labeling and detection of modified peptides with mass spectrometry, we demonstrated that cysteines 124 and 141 in superstable p53 form a terminal disulfide bond upon DNA-mediated oxidation. This leads to a conformational change that inhibits DNA from binding by p53. The disulfide formed between cysteines 124 and 141 is a result of a series of disulfide bond exchange across the protein from the DNA base stack.

We also investigated the dependence of p53 oxidation on DNA sequences. ESMA analysis of biologically derived p53 recognition sequences with varying quantities of guanine doublets and triplets showed efficient p53 oxidation to depend on the presence of low energy GG or GGG sites. Moreover, consistent results were found with biologically derived promoter sequences. Sequence S100A2, with guanine triplets on the same strand, showed the most oxidation of p53, followed by ODC1 and caspase-1. We confirmed these sequence-specific effects by measuring the change in expression level of the genes after induction of DNA CT *in vivo*. S100A2 mRNA levels decreased after photooxidant and light treatment, reflecting the oxidation and dissociation of p53 from the S100A2

site. However, caspase-1 and ODC1 mRNA levels remained the same, indicating less DNA-mediated p53 oxidation.

The results from this study illustrate how protein oxidation at a distance through DNA CT contributes to cellular signaling. This oxidative signaling can control how p53 regulates gene expression under oxidative stress, and this signaling may be disrupted in cancerous cells.

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