

## P53 REGULATION AND FUNCTION IN NORMAL CELLS AND TUMORS

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**Abstract** Mechanisms to protect organisms from the consequences of DNA damage include the tumor suppressor p53 pathway. p53 protein binds specifically to a DNA consensus sequence to induce growth inhibitory genes or nonspecifically to damaged sites leading to DNA repair or apoptosis. While p53 protein is susceptible to post-translational modifications and binding to other proteins, few of the modifications or associations have been demonstrated in the context of the cell. We used a novel, sensitive DNA binding assay to examine p53 proteins in lysates prepared from cells responding to DNA damage. Non-transformed progenitor keratinocytes exhibited rapid and sustained induction of activated p53 protein binding to the consensus sequence, correlated with sustained induction of a downstream target gene, the cyclin-dependent kinase inhibitor p21. Binding to a mismatched DNA probe was transient and correlated with total p53 protein in the lysate, suggesting that most or all of the endogenous p53 proteins induced after damage were capable of mismatched DNA binding. Squamous cell carcinoma (SCC) derivatives showed defects in induction p53 protein after DNA damage and disproportional losses in DNA binding, compared to total p53 protein steady state levels, along with increased MDM2/p53 association. Maximum induction of endogenous p53 protein binding to the mismatched probe correlated with transcription-independent induction of apoptosis. We suggest a model in which activated forms of p53 carry out transcription-dependent functions in growth arrest and DNA repair which may vary by cell type, while most or all wild type forms of p53 are capable of binding to damaged DNA. p53 protein loss of binding to damaged DNA causes failure of cells to trigger apoptosis and may lead to resistance to chemotherapy. Implications for new directions in therapeutics include functional molecular profiling of individual patient tumors for p53 DNA binding to predict treatment response better than mutational analysis alone and targeted activation of p53 DNA damage binding in tumor cells for increasing their sensitivity to chemotherapy.

**Key words:** p53 protein, DNA binding, apoptosis, carcinogenesis

**Resumen** *Regulación y función de p53 en células normales y tumorales.* Entre los mecanismos que protegen a los organismos contra las consecuencias del daño al ADN se incluye la vía del supresor de tumor p53. La proteína p53 se une específicamente al ADN sobre una secuencia consenso para activar genes inhibidores de crecimiento o inespecíficamente a sitios dañados para inducir la reparación del ADN o la apoptosis. Mientras que la proteína p53 es susceptible a modificaciones post-traduccionales y a la unión a otras proteínas, pocas de estas modificaciones y asociaciones han sido demostradas en el contexto celular. Hemos empleado un nuevo y sensible ensayo de unión al ADN para detectar proteínas p53 en lisados de células respondiendo al daño al ADN. Keratinocitos progenitores no transformados presentaron una inducción rápida y sostenida de la unión de la proteína p53 activada, a la secuencia consenso correlacionada con una inducción sostenida de un gen blanco río abajo, el inhibidor de la kinasa dependiente de ciclina p21. La unión a una sonda de ADN con bases mal apareadas fue temporaria y correlacionaba con la cantidad total de p53 en el lisado, sugiriendo que la mayoría o la totalidad de las proteínas p53 endógenas inducidas después del daño, eran capaces de unirse al ADN con bases desapareadas. Derivados de células de carcinoma escamoso (SCC) presentaron defectos en la inducción de la proteína p53 después del daño al ADN y una pérdida desproporcionada de la unión al ADN, comparada con los niveles normales de proteína p53 total, junto a un aumento de la asociación MDM2/p53. La máxima inducción de la unión de la proteína p53 endógena a la sonda con bases mal apareadas correlacionó con una inducción de la apoptosis independiente de la transcripción. Nosotros sugerimos un modelo donde las formas activadas de p53 llevan a cabo funciones dependientes de la transcripción en el arresto del crecimiento y reparación del ADN, que puede variar con el tipo celular y donde la mayoría o todas las formas salvajes de p53 sean capaces de unirse al ADN dañado. La pérdida de la unión al ADN dañado por aporte de la proteína p53 causa fallas en las células para disparar la apoptosis y puede conducir a la resistencia a quimioterapia. En consecuencia, para considerar nuevos caminos terapéuticos se incluye la caracterización funcional molecular de tumores de pacientes individuales para la actividad de unión al ADN de p53 para predecir la respuesta al tratamiento más que al mero análisis mutacional y la activación dirigida de unión de p53 al ADN dañado en células tumorales para el incremento de su sensibilidad a quimioterapia.

The cells of the human body are programmed to respond to a variety of physical, chemical, and pathological agents including ultraviolet light, g-irradiation, and chemical carcinogens. Mechanisms to protect the integrity of inherited genetic information from the consequences of such exposure include the pathway of the tumor suppressor p53 protein, a guardian of the genome. p53 is a DNA binding protein; attention has focused on its specific binding to a consensus sequence within promoter regions of growth inhibitory genes. However, p53 also binds sequence-independently to damaged sites in DNA and is postulated to have a role in DNA repair or apoptosis. Mutations of p53 have been found in more than 50% of human cancers<sup>1</sup>. However, loss of p53 function has been estimated to occur in almost 80% of human cancers, due not only to mutation but also to defects in activating events or inactivation by the products of tumor viruses or cellular oncogenes.

We became focused on wild type p53 protein loss of function due to observations that chemically-transformed mouse keratinocytes exhibit abnormal p53 mRNA and protein expression levels and G1 checkpoint deficiencies at the stage of malignant conversion<sup>2</sup>. The potential causes of this loss of function may stem from deficiencies in post-translational modifications or in cooperating p53 binding proteins. For example, activation of wild type p53 can occur by phosphorylation of serine residues in the N-terminal transcriptional activation domain of p53 by JNK (Jun N-terminal kinases) and ATM (Ataxia-Telangiectasia Mutant) kinases or in the C-terminal regulatory domain by casein kinase II<sup>3</sup>. Acetylation of lysines in the C-terminus by p300 or pCAF (p300/Creb Binding Protein associating factor) also activates p53 for transcriptional function<sup>4</sup>.

Proteins that modulate p53 protein function include the ref-1 C-terminal binding protein and the MDM2 (HDM2 in humans) N-terminal binding protein. MDM2 prevents sequence-specific DNA binding and transactivation and targets p53 protein for ubiquitination and degradation<sup>5</sup>. The p53 protein is also subject to conformational variation due to redox states in the cell<sup>6</sup> and inhibition of DNA binding by poly ADP-ribosylation due to PARP (Poly-ADP ribose polymerase)<sup>7</sup>. However, few of the multiple p53 protein post-translational modifications have been demonstrated within the context of the cell responding to DNA damage.

We set out to characterize the defects in the p53 pathway during carcinogenesis and to evaluate the biological significance of p53 protein binding to non-specific damage sites in DNA. Studies of recombinant proteins in active or latent states for sequence-specific DNA binding, carried out by means of electrophoretic mobility shift assays (EMSA), indicated that while only activated forms bound the consensus site, both active and latent forms bound to a nonspecific DNA sequence containing insertion deletion mismatches. Further,

competition studies suggested that the p53 forms that bound to the mismatched probe were qualitatively different from the forms activated for sequence-specific binding. In order to examine the p53 response pathway in cells, we developed a DNA binding assay (DNA affinity immunoblotting assay – DAI). DAI is at least fourfold more sensitive than EMSA and permits retrieval, identification, and quantification of proteins bound to the consensus or the mismatched DNA probe in cell lysates prepared at various times after treatment of cells with a DNA damaging agent. Non-transformed progenitor keratinocytes exhibited rapid and sustained induction of activated p53 protein binding to the consensus sequence, correlated with sustained induction of a downstream target gene, the cyclin-dependent kinase inhibitor p21. Binding to the mismatched DNA probe was transient and correlated with total p53 protein in the lysate, suggesting that most or all of the endogenous p53 proteins induced after exposure of cells to g-irradiation were capable of mismatched DNA binding, consistent with the results for recombinant proteins. The squamous cell carcinoma (SCC) derivatives showed defects in induction of p53 protein after DNA damage. A poorly differentiated SCC showed dramatic disproportional losses in consensus DNA binding compared to total p53 protein. These correlated with increased MDM2/p53 association and increased PARP (Poly-ADP ribose polymerase) protein steady state levels. Comparison of total p53 protein and subsets of p53 proteins with specific phosphorylations at serine15 and serine389 consistently indicated that most or all endogenous p53 forms were capable of mismatched DNA binding after UVB, g-irradiation, or hydroxyurea treatment yet suggested the possibility of certain p53 forms having greater specificity for binding damaged DNA. Maximum induction of endogenous p53 protein binding to DNA occurred for the mismatched probe (in response to hydroxyurea, 40 fold, compared to 11 fold for the consensus probe) and correlated with transcription-independent induction of apoptosis.

We suggest a model in which activated forms of p53 carry out transcription-dependent functions in growth arrest and DNA repair which may vary by cell type, DNA damaging agent, and specific p53 post-translational modifications and p53 interacting proteins; however, since cells more generally may require a means to trigger apoptosis in response to overwhelming DNA damage, and this mechanism must maintain its functional capacity under stress, most or all forms of p53 are capable of binding to damaged DNA. It is the threshold of this p53 binding to damaged DNA which triggers apoptosis even if the transcriptional capacity of the cell is compromised.

Implications of the current findings and conceptual model for new directions in therapeutics include the following: 1) functional molecular profiling of individual patient tumors for p53 DNA binding may better predict

treatment response than mutational analysis alone; 2) if a broader spectrum of p53 conformational forms is capable of triggering apoptosis than transcription, therapeutics designed to activate p53 DNA damage binding may be more feasible and equally or more effective than activation of sequence-specific binding; 3) novel therapeutics may be based on small molecule p53 mimics which activate a p53 downstream cascade to apoptosis; and 4) p53 modified forms may be designed based on maximum apoptotic effect and used in localized or systemic p53 gene therapy in tumors unresponsive to conventional chemotherapy.

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Se necesita que investigadores de renombre aparezcan regularmente en los medios, y de esta manera conformen modelos, demostrando por su presencia lo que es un científico, cómo piensa y actúa, y explicando lo que es la investigación en sí. Existen buenos ejemplos de este tipo en deportes, en política, en industria, en derecho, y en medicina. La ciencia los necesita también.

Michael Crichton

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