[Frontiers in Bioscience 8, s715-717, May 1, 2003]

DYSFUNCTION OF *p53* IN PHOTOCARCINOGENESIS

Celine M Gervin, Andrea McCulla, Mandy Williams and Allal Ouhtit

Department of Oncology, Cancer Research Centre, The Queen's University Belfast, Belfast BT9 7AB, Northern Ireland

TABLE OF CONTENTS

1. ABSTRACT

The tumor suppressor protein p53 plays a critical role in the orchestration of the cellular responses to a variety of genotoxic and cytotoxic stresses. Mutations or functional inactivation of p53 seriously compromise these cellular processes and foster tumor development. *p53* is the most frequently mutated gene in human cancers and over 90% of human non-melanoma skin cancers (NMSC) harbour *p53* mutation. It plays a vital role in the control of the immediate and adaptive responses to ultraviolet radiation (UV) and the onset of NMSC. During the process of photocarcinogenesis, UV-specific *p53* mutations occur early in the keratinocytes resulting in the loss of the wild type p53 function and continued UV exposure leads to clonal expansion of p53-mutated keratinocytes and promotion of skin tumors. Precisely how clones of keratinocytes containing such mutations, in an apparently normal epidermis, progress to a malignant carcinoma is unknown. Further examination of the functional significance of these UV-*p53* mutations in affecting the immediate and adaptive responses of the skin to UV is critical to the development of effective prevention and therapeutic strategies for human skin cancer. The purpose of this article is to provide an overview of accumulating evidence pointing towards a critical role for p53 mutation in photocarcinogenesis.

2. INTRODUCTION

The incidence of skin cancers exceeds that of all other human cancers combined (>50%) and is dramatically increasing, possibly due to depletion of the ozone layer (1-2). UV radiation, in particular UV-B, induces a plethora of effects ranging from erythema, burns, immune suppression, increased photo aging and eventual skin cancer, the most common being non-melanoma skin cancers (NMSC) including basal and squamous cell carcinoma (BCC, SCC). The absorption of UV photons by the DNA of epidermal cells and the rearrangement of electrons lead to the formation of photoproducts at adjacent pyrimidine sites (3). These photoproducts can be removed in normal cells by nucleotide excision repair mechanism mediated by p53 (4). The photodamage induces an elevation of p53 expression in the skin, blocking the cell cycle at G_1-S phase, thereby permitting the repair of the damage and/or induction of apoptosis to eliminate cells containing severely damaged DNA (5-6). However, DNA photoproducts become carcinogenic when they persist in a chronic UV-scenario and are passed on during cellular replication as C to T or CC to TT mutations, known as UV fingerprints (7). The most significant mutations occur in tumor suppressor genes, *p53* being the key UV-responsive gene. Mutations in *p53* generally result in inactivation of its tumor suppressor function and are thought to initiate the process of NMSC development (8). Several factors underscore the importance of p53 in tumor suppression: (***i***) *p53* is the most frequently mutated gene identified in human cancer; (***ii***) Li-Fraumeni syndrome is a genetic disease often attributed to a germline mutation in *p53* (9); (***iii***) Mice that lack *p53* develop normally, but are remarkably predisposed to developing lymphoma and a broad spectrum of other cancers (10). However, some mutant forms of *p53* also confer a `gain-of-function' phenotype, manifested by augmented cell growth and tumorigenic potential. These tumor-promoting

functions may significantly contribute to the initiation and/or progression of neoplasm.

3. THE TUMOR SUPPRESSOR *P53* GENE

Photocarcinogenesis often involves a dysfunction of one or more tumor suppressor genes including *p53*. Over 90% of human NMSC harbour a mutation in *p53* (7) and loss of its function fosters tumor development by increasing genetic instability.

3.1. P53 Structure and function

The human *p53* gene is localized on chromosome 17p13 and contains 11 exons. It encodes a 53,000 molecular weight protein containing 393 amino acids (11) with a number of well-characterized functional domains. The transcriptional activation domain is located within the amino-terminal 73 amino acids (12) and two residues within this domain, leu22 and tryp23 are required for interaction of the activation domain with TATA-box binding protein associated factors (TAFs) (13). A sequence-specific DNA binding domain is located within the central, conserved portion of the protein and encompasses amino acids 102-292. It is within this DNA binding domain that the majority of missense mutations have been detected in tumors of cancer patients (14). Among these sites are a number of mutational hot spots that occur with unusually high frequency, impairing sequence specific DNA binding by p53, therefore abolishing its function (15). The carboxyl terminus contains a tetramerization domain as well as a regulatory region that controls the ability of the protein to allosterically switch from a latent form to one that is active for sequence-specific DNA binding (16).

The stable p53 protein is activated by phosphorylation (17), dephosphorylation and acetylation (18) yielding a potent sequence-specific DNA-binding transcription factor that modulates multiple cellular functions, including gene transcription, DNA synthesis and repair, cell cycle arrest, senescence, and apoptosis. A major characteristic of p53 is its interaction with specific DNA elements, and the interactions of cellular proteins with its C-terminus result ultimately in the stimulation of the DNA binding activity of latent p53. The wide range of p53's biological effects can, in part, be explained by its positive transcriptional activation of a number of downstream target genes including $p21^{WAFI}$, *GADD45, bax, Fas/APO1, KILLER/DR5, IGF-BP3* or transcriptional repression of *Bcl-2* and survivin (19). Defining all the players that function as upstream regulators and downstream mediators of the p53 signaling pathway and their mechanisms of action remains a significant challenge.

3.2. UV-*p53* fingerprints, a key player in photocarcinogenesis

Significant progress has been made towards understanding the mechanisms of NMSC, a complex process involving at least two distinct, mutagenic (20) and immune suppressive pathways, most likely triggered by UV-induced specific DNA damage. Exposure to sunlight and absorption of UV photons by the DNA of epidermal cells leads to the formation of photoproducts at adjacent pyrimidine sites (3). These photoproducts are "cyclobutane dimers" or "pyrimidine-pyrimidone (6-4) photoproducts" and can be removed in normal cells by nucleotide excision repair process mediated by p53 (4). The photodamage induces an elevation of p53 expression in the skin and arrest the cell cycle at G_1-S phase to permit the repair of the damage and/or induce apoptosis to eliminate severely damaged cells (5). However, these photoproducts may also interfere with both DNA-binding and transcriptional activities resulting in a defect of p53-dependent DNA repair and apoptosis mechanisms. Consequently, these photoproducts become carcinogenic mutations when they persist following chronic UV exposure and are transformed to C to T (70%) or CC to TT (10%) mutations (6). Nine hotspot *p53* mutations were identified in human skin tumors at codons 152, 177, 179, 196, 245, 247/248, 273, 277 and 281/282 (7, 21). Some of these mutations have been detected at the early stages of skin photocarcinogenesis (in normal exposed skin) and are thought to be involved in the initiation process (7). Following subsequent UV exposure, cells containing *p53* mutations can expand preferentially in a clonal fashion at the expense of the normal surrounding keratinocytes; these keratinocytes, containing wild type p53, die by apoptosis, leading to the appearance of *p53* mutated clones in the epidermis. Recently, Brash *et al*, have found that clonal expansion requires sustained UVB, enabling the *p53*-mutant keratinocyte to colonize adjacent epidermal proliferating units without incurring additional mutations (22). Sun-exposed human skin contains thousands of clones of *p53*-mutant keratinocytes, which are histologically normal; yet contain the same kinds of *p53* mutations observed in BCC and SCC (8). Ananthaswamy *et al* have reported that application of sunscreens to the skin reduced the frequency of *p53* mutation and slowed the development of NMSC, suggesting that p53 mutations can be used as a surrogate early biologic endpoint for photoprotection against skin cancer (23).

4. CONCLUSION AND PERSPECTIVES

UV-induced *p53* mutations arise very early and well before the appearance of NMSC, and represent a key component of the multi-step process of photo-carcinogenesis. Sun-exposed human skin contains thousands of clones of *p53*-mutant keratinocytes but how these clones, in an apparently normal epidermis, progress to a malignant carcinoma is unknown. Further examination of the molecular and cellular mechanisms involved in the early responses to UV will provide a framework for better understanding the functional significance of UV-*p53* mutations in skin pathogenesis. Our current work is focused on identification of UV-specific *p53* mutation capable of deregulating p53-mediated early responses to UV, especially the cell cycle and apoptosis programmes. Furthermore, characterization of their downstream targets and signaling pathways could assist in the development of novel clinical strategies to hinder NMSC development.

5. ACKNOWLEDGMENTS

This study was supported by Belfast City Hospital Trust.

6. REFERENCES

1. Gloster H. M. & D. G. Brodland: The epidemiology of skin cancer. *Dermatol Sur* 22, 217-26 (1996)
2. Armstrong B. K. & A. Kricker: Epidemiology of sun exposure and skin cancer. *Cancer Surveys* 26, 133-53 (1996)
3. Brash D. E: UV mutagenic photoproducts in Escherichia coli and human cells: a molecular genetics perspective on human skin cancer. *Photochem Photobiol* 48, 59-66 (1988)
4. Ford J. M. & P. C. Hanawalt: Expression of wild-type p53 is required for efficient global genomic nucleotide excision repair in UV-irradiated human fibroblasts. *J Biol Chem* 272, 28073-28080 (1997)
5. Ouhtit A, K. Muller, D. Davis, S. E. Ullrich, D. McConkey & H. N. Ananthaswamy: Temporal events in skin injury and the early adaptive responses in ultraviolet-irradiated mouse skin. *Am J Pathol* 156, 201-207 (2000)
6. Ziegler A, A. S. Jonason, D. J. Leffell, J. A. Simon, HW Sharma, J. Kimmelman, L. Remington, T. Jacks & D. E. Brash: Sunburn and p53 in the onset of skin cancer. *Nature* 372, 773-6 (1994)
7. Brash DE, J. A. Rudolph, J. A. Simon, A. Lin, G. J. McKenna, H. P. Baden, A. J. Halperin & J. Ponten: A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc Natl Acad Sci U S A* 88,10124-8 (1991)
8. Leffell D. J & D. E. Brash: Sunlight and skin cancer. *Sci Am* 275, 52-3, 56-9 (1996)
9. Malkin D & S. H. Friend. Correction: a Li-Fraumeni syndrome p53 mutation. *Science* 259, 878 (1993)
10. Donehower L. A, M. Harvey, B. L. Slagle, M. J. McArthur, C. A. Montgomery Jr, J. S. Butel & A Bradley: Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* 356, 215-21 (1992)
11. Harlow E, N. M. Williamson, R. Ralston, D. M. Helfman & T. E. Adams: Molecular cloning and *in vitro* expression of a cDNA clone for human cellular tumor antigen p53. *Mol Cell Biol* 5, 1601-10 (1985)
12. Field S & S. J. Jang: Phosphorylation of p53 tumour suppressor gene. Science 249: 1046-1049 (1990)
13. Thut C. J, J. L. Chen, R. Klemm & R. Tjian: p53 transcriptional activation mediated by coactivators TAFII40 and TAFII60. *Science* 267, 100-4 (1995)
14. Hollstein M, D. Sidransky, B. Vogelstein & C. C. Harris: p53 mutations in human cancers. *Science* 253, 49-53 (1991)
15. Vogelstein B & K. W. Kinzler. p53 function and dysfunction. *Cell* 70, 523-6 (1992)
16. Sturzbecher HW, R. Brain, C. Addison, K. Rudge, M. Remm, M. Grimaldi, E. Keenan & J. R. Jenkins: A C-terminal alpha-helix plus basic region motif is the major structural determinant of p53 tetramerization. *Oncogene* 7, 1513-23 (1992)
17. Hao M, A. M. Lowy, M. Kapoor, A. Deffie, G. Liu & G. Lozano: Mutation of phosphoserine 389 affects p53 function *in vivo*. *J Biol Chem* 271, 29380-5 (1996)
18. Gu W & R. G. Roeder. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell*. 90, 595-606 (1997)
19. Hoffman W. H, S. Biade, J. T. Zilfou, J. Chen & M. Murphy: Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. *J Biol Chem* 277, 3247-57 (2002)
20. Soehnge H, A. Ouhtit & H. N. Ananthaswamy: Mechanisms of induction of skin cancer by UV radiation. *Front Biosci* 2, D538-D551 (1997)
21. Daya-Grosjean L, N. Dumaz & A. Sarasin. The specificity of p53 mutation spectra in sunlight induced human cancers. *J Photochem Photobiol* B. 28, 115-24 (1995)
22. Zhang W, E. Remenyik, D. Zelterman, D. E. Brash & N. M. Wikonkal: Escaping the stem cell compartment: sustained UVB exposure allows p53-mutant keratinocytes to colonize adjacent epidermal proliferating units without incurring additional mutations. *Proc Natl Acad Sci U S A* 98, 13948-53 (2001)
23. Ananthaswamy H. N, S. M. Loughlin, P. Cox, R. L. Evans, S. E. Ullrich, M. L. Kripke: Sunlight and skin cancer: inhibition of p53 mutations in UV-irradiated mouse skin by sunscreens. *Nat Med* 3, 510-4 (1997)

Key Words: Sunlight, UV-induced p53 mutations, p53-mutant Keratinocytes, non-melanoma skin caners, Review

Send correspondence to: Allal Ouhtit, Ph D, Photobiology Group, Department of Oncology, Cancer Research Centre, The Queens University Belfast, Belfast City Hospital, Lisburn Road, Belfast BT9 7AB, Northern Ireland, Tel; +44 (0)28 9026 3911, Fax; +44 (0)28 9026 3744, E-mail: a.ouhtit@qub.ac.uk