TP53 GENE AND P53 PROTEIN AS TARGETS IN CANCER MANAGEMENT AND THERAPY

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Contents

Summary

The TP53 gene is commonly altered by mutation in many types of human cancers. The p53 protein is an inducible transcription factor that plays multiple, anti-proliferative roles in response to exposure to many forms of stress, including in particular various classes of DNA-damaging agents. Thus, p53 function is essential for the genetic homeostasis of cells exposed to mutagens. In a physiological context, the status of p53 controls the sensitivity of cells to environmental mutagens. In a pathological context, the status of p53 is considered as a key factor in the response to cancer cells to cytotoxic therapies. Thus, control of p53 functions is a very promising target for cancer management.

Given the central importance of TP53 in carcinogenesis, there have already been many attempts at restoring or modulating p53 protein functions through genetic or pharmacological approaches. First, gene therapy experiments using either retroviral or adenoviral vectors have given a proof of principle for the restoration of wild-type TP53 function in cancer cells containing mutant alleles. However, the therapeutic efficiency of such approaches is currently limited by difficulties in targeting cancer cells, in

obtaining high expression levels of the transgene, and in maximizing so-called by-stander effects. Alternative approaches to "replacement" gene therapies are also being developed. For example, the ONYX-015 viral vector is a defective adenovirus that can selectively kill cancer cells with deficient TP53 functions. This vector is currently under clinical evaluation.

The rapid accumulation of knowledge on p53 protein functions has led to the development of a number of pharmacological approaches. One of the most promising makes use of small peptides that target specific regulatory domains of the protein to either activate wild-type p53 or restore function of mutant p53. Other methods address specific biochemical properties of p53, such as sensitivity to redox signals. For example, we have shown that the chemoprotective drug amifostine could activate wild-type p53 and induce preferentially cell cycle arrest in cells containing wild-type alleles. This specific response may contribute to the protective effects of the drug.

It should be kept in mind that the TP53 gene and its product can be used as biomarker for cancer detection, diagnosis, and prognosis. This can be achieved by the analysis of gene or protein status in cancer or pre-cancer lesions, as well as by detection of gene fragments or antibodies in plasma or serum. All these aspects are currently the topic of intense research efforts. However, their outcome on the clinical management of cancer will not be available for several years.

1. Introduction

The TP53 gene was discovered twenty years ago, but it took about ten years before a clear idea of its function eventually emerged in the early 1990s. Experimental studies then demonstrated that this gene encodes a tumor suppressor, and molecular pathological approaches showed that this suppressor was inactive in a majority of human cancers. Since then, the TP53 gene and its product, the p53 protein, have occupied the center stage of the molecular biology of cancer, and have raised great expectations for applications leading to better cancer management or therapy.

TP53 is special among cancer genes in at least three respects. First, most of its alterations in cancers are missense mutations. This is uncommon for suppressor genes, which are classically inactivated by deletions or non-sense mutations. Second, it is altered at a significant frequency (between 20 and 80%) in almost every human cancer, irrespective of the organ site or the histological type. This observation stresses the central role of p53 as one of the basic elements of the cellular growth control machinery. Third, the protein itself is apparently essential for many aspects of normal life. This also contrasts with many tumor suppressors, which encode "vital" proteins. Strikingly, mice deficient in TP53 by homologous recombination show essentially normal development and behavior. However, when they reach 20 or 30 weeks of age, most of them die from multiple, early cancers. Thus, TP53 may be considered as, in the words of M. Oren, the "ultimate tumor suppressor gene", the function of which is essentially to protect cells against the occurrence and development of cancer.

This very special position of p53 in the control of cell proliferation is due to two biological characteristics. First, p53 is an inducible protein at the post-

transcriptional level. It is almost absent, or "latent," in most normal cells and tissues, but becomes stabilized and activated in response to many forms of cellular stress, in particular stress inducing the formation of DNA-damage. Moreover, p53 is capable of regulating many overlapping pathways. P53 is a transcription factor with more than 30 known target genes in pathways such as cell cycle control, apoptosis, DNA repair, differentiation, and senescence. The protein also acts through direct, complex formation with other cellular components, further increasing the range of responses elicited by p53 activation. Overall, p53 appears to sit at the center of a network of signals that connect stress response (in particular to DNA damage) with growth regulation. This special function has earned p53 the nickname of "guardian of the genome". Loss of p53 function thus eliminates a protection system by which cells normally regulate their capacity to proliferate in stressful conditions, and increases the likelihood that such cells may acquire other genetic changes during cancer progression.

Therefore, p53 represents an interesting target for genetic or pharmacological intervention in cancer treatment. Below, we briefly review the implication of TP53 in human cancer, and we describe current approaches for cancer gene therapy, pharmacological modulation of p53 protein, and exploitation of TP53 in cancer detection and monitoring.

2. TP53 Mutations and Human Cancer

The human TP53 gene is located in 20 kb of chromosome band 17p13.1. The gene is composed of 11 exons, the first of which is non-coding. The product of the gene is a 53kD nuclear phosphoprotein, composed of 393 amino acids. The functional molecule is a tetramer and acts as a transcriptional factor. It is involved in cell cycle checkpoints, apotosis, genomic instability, and DNA repair (Figure 1).

The p53 protein is activated in response to genotoxic (DNA-damaging) and non-genotoxic stresses. The stability of the protein is controlled by Mdm2 and by JNK. After activation, p53 regulates genes and proteins involved in cell cycle arrest (in G1, G1/S and G2/M) in replication, transcription, repair, and apoptosis. The p53 protein is constitutively expressed in almost all cell types but has a very rapid turnover and appears to be latent under normal conditions. However, p53 is rapidly converted to an active form in response to a number of physical or chemical DNA-damaging agents such as gamma irradiation, UV rays, oxidizing agents, cytotoxic drugs, and cancer-causing chemicals. Induction of p53 implies nuclear retention, accumulation of the protein as a result of post-translational stabilization, and allosteric conversion to a form with high sequence-specific DNA-binding capacity. This has led to the concept that p53 is specifically activated in response to DNA-damage thus acting as a "guardian" against genotoxic stress.

The p53 protein is a sequence specific transcription factor which binds DNA sequences corresponding to repeats of the consensus motif RRRC(A/T)(T/A)GYYY (where R is a purine and Y pyrimidine). The protein has five structural and functional domains: a N-terminal, transcriptional activation domain, a proline-rich regulatory domain, a sequence-specific DNA-binding domain, an oligomerization domain, and a C-terminal domain involved in the regulation of DNA binding (Figure 2A). In terms of three-

dimensional structure (Figure 2B), the protein is made of a scaffold of beta-sheets that support flexible loops and helixes which are in direct contact with DNA. The position of these loops and helixes is stabilized by the binding of an atom of zinc.
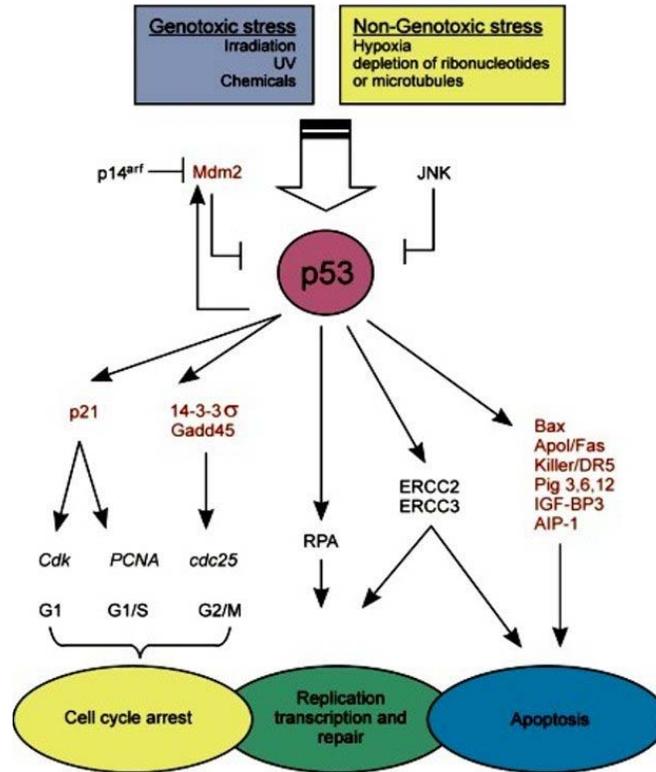
Figure 1: The p53 signaling pathway.
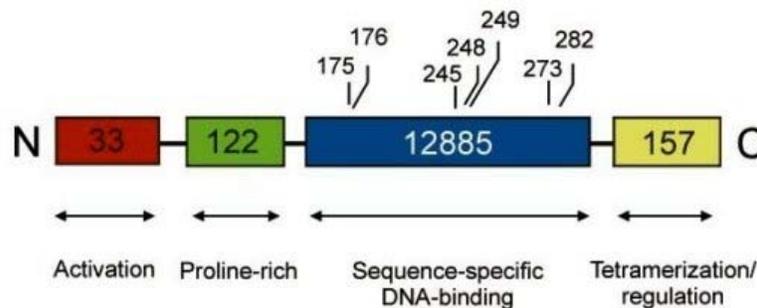
Figure 2: Structure of the p53 protein.

The protein in Figure 2 contains several functional domains, as indicated. The number of mutations detected in the human cancer that falls within each of these domains is given. The most frequently mutated portion is the sequence-specific DNA-binding domain. Within this domain, several residues are "hotspots" for mutation. The three most frequently mutated residues in human cancers are represented using a space-fill

model in which each atom is pictured as a small sphere. The target DNA that p53 binds to is outlined.

In cancer, inactivation of p53 occurs through various mechanisms, including genetic alteration (mutation, deletion), inactivation of the protein by binding to viral or cellular oncoprotein, and sequestration in the cytoplasm. The DNA binding domain contains 93% of all mutations identified to date. This high frequency may be overestimated, since after initial reports that mutations tended to cluster in the central portion of the coding sequence (DNA binding domain), most investigators have limited their analysis to exon 5 to 8. A database of all published mutations is maintained at the International Agency for Research on Cancer. The most frequently mutated residues (Figures 2A and B) are conserved among species and play an important, direct or indirect, role in the contacts between the protein and target DNA. All these mutations result in impaired DNA-binding and loss of transcriptional activity.
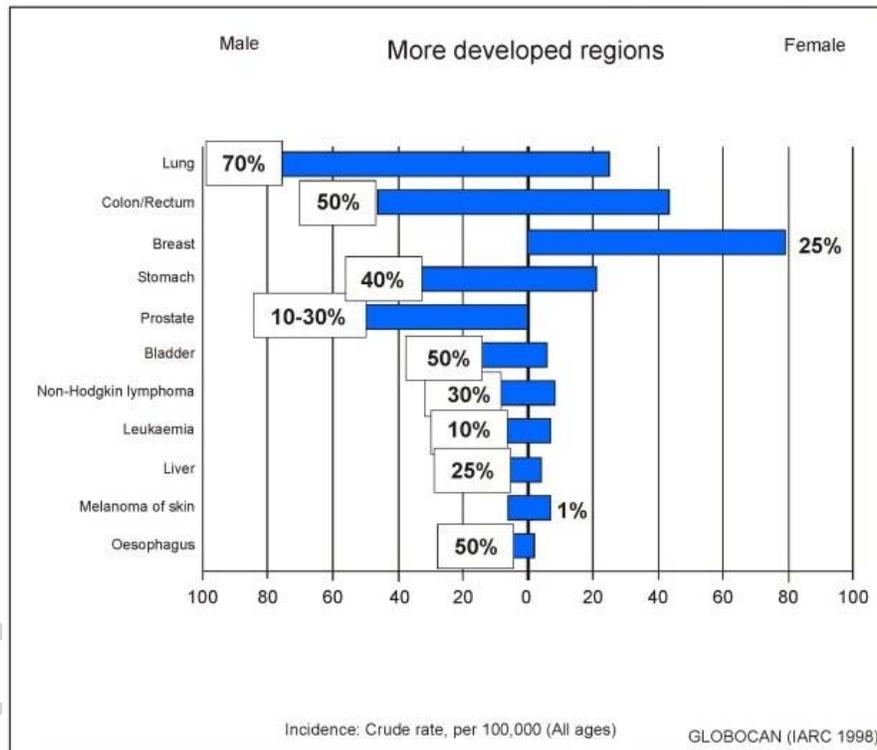
Figure 3: Incidence of cancers in developed countries.

Mutations in the TP53 are found in almost every kind of human tumor. Malignancies in which the mutation prevalence is higher than 50% include skin cancer (except melanoma), late stage cancer of bladder cancers, and carcinomas of the aero-digestive tract. Lymphomas and tumors of the brain, breast, prostate, and liver show an intermediate mutation frequency (15 to 35%). Malignancies with low mutation frequency include leukemia (10%), testicular cancer, and malignant melanoma (both less than 5%). In cancers such as breast and colon, TP53 mutations seem to occur late in

tumorigenesis. In several other cancers (head and neck, lung, skin), mutations occur very early and may even precede tumor development. The nature and type of mutations is often informative of the mutagenic mechanisms that have caused them, making TP53 an interesting gene to study in molecular epidemiology.

Mutations in the p53 protein can have at least three phenotypic effects: loss of function, in which a missense mutation abrogates p53's ability to block cell division or reverse a transformed phenotype; gain of function (or dominant-positive effect), where mutant p53 acquires novel functions as demonstrated with the introduction of a mutant p53 gene into cells lacking wild-type p53 allele, which induces a tumorigenic phenotype; trans-dominant mutation (dominant-negative effect), seen when a mutant p53 allele is introduced into cells bearing a wtp53 allele, resulting in the ability of mutant p53 to drive wtp53 to a mutant conformation overriding of the normal inhibitory function of p53.

3. The p53 Protein—A Sensor of Genotoxic Stress

In most cells, p53 is almost undetectable because it is rapidly degraded by the proteasome. Upon activation, the protein escapes degradation and accumulates in the nucleus. At the same time, it is turned from a latent to an active form by conformational changes which activate its capacity to transactivate target genes. The main factor controlling p53 accumulation is Mdm2, a protein encoded by a gene which is itself a transcriptional target of p53. Mdm2 acts as a ubiquitin ligase to direct p53 out of the nucleus to the proteasome, where it is degraded.

Various types of genotoxic and non-genotoxic stresses can lead to p53 activation, including agents that create single or double-strand breaks in DNA (irradiation, oxidative stress), mutagens—aflatoxins, benzo(a)pyrene, alkylating agents—and inhibitors of topoisomerases. Moreover, damage to the mitotic spindle, ribonucleotide depletion, hypoxia, heat shock, and exposure to nitric oxide can also induce p53. Induction follows a different time-course, depending upon the nature and intensity of the stress.

Induction in response to stress is a multi-step process. It involves phosphorylation of p53 in the N-terminus (e.g. by kinases activated after DNA-damage such as Atm or Chk-2), and dissociation of p53-Mdm2 interactions. Other changes in the protein include acetylation of the C-terminus (by acetyl-transferases of CBP/p300 family), conformational changes in the C-terminus leading to the unmasking of the DNA-binding domain, and changes in oxidation-reduction in the DNA-binding domain. All these changes turn the protein into an active form which binds DNA with high affinity.

Once activated, p53 can trigger several cellular events via two distinct and parallel pathways, transcription-dependent or transcription-independent (Figure 1). Examples of transcription-independent pathways include binding of p53 to components of the DNA replication/repair machinery such as the helicases ERCC2 and ERCC3, or the replication protein RPA. Genes transcriptionally regulated by p53 include cell cycle regulators in G1 and in G2 phases (p21/waf-1, 14-3-3s, GADD45), regulators of

apoptosis (BAX, CD95/FAS, KILLER/DR5, p53AIP1, PIG3, IGF-BP3), and genes involved in cellular responses to stress such as inducible forms of nitric oxide synthase (NOS$_2$) and cyclooxygenase (COX2), which are both repressed by p53. How p53 selects from the set of alternative responses (e.g. choosing between cell cycle arrest or apoptosis) depends upon the nature and the amplitude of the inducting signal, as well as of the cell and tissue type.

An important aspect of the role of p53 in cancer treatment is the fact that the function of p53 is crucial for the cytotoxic response of cancer cells to radio- or chemotherapy. There is evidence that many anti-cancer drugs induce apoptosis through a p53-dependent pathway. However, in clinical terms the presence of a wild-type TP53 gene is not always correlated with good response to treatment, as many other factors can also influence this response. On the other hand, in certain cell types activation of p53 by therapeutic agents may induce cell cycle arrest (and DNA repair) rather than apoptosis, thus resulting in a form of protection of cancer cells against the effects of therapy. Thus activation of p53 may be seen as both a chemo-sensitizer or a chemo-protective mechanism, depending on the cellular context. This is why current, experimental approaches that target p53 for cancer treatment include attempts to activate p53 (and thus induce apoptosis) as well as to inactivate p53 (and thus prevent destruction of normal cells by cytotoxic therapies).

4. Gene Therapy Using TP53

The capacity of wild-type TP53 to arrest the proliferation of cultured cells and induce apoptosis has raised an enormous interest in the possibility that restoring TP53 function in tumor cells may block tumor development. In addition, the finding that the p53 protein is a key factor in determining the response of cancer cells to therapy, has led to the concept that re-introduction of a normal protein may sensitize cells to cytotoxic killing and thus improve therapeutic response. Over the past ten years, several efforts have been made to translate these laboratory findings into clinical applications. One of the most popular approaches to achieve this goal is gene therapy. Below, we summarize the various modalities of TP53-based gene therapy that have been described in the recent literature.

4.1. Replacement Gene Therapy

The function of TP53 is lost in many cancers through mutation or loss of alleles. Therefore it seems reasonable to try to restore TP53 function by replacing the mutant gene with a functional, wild-type copy. The primary requirement to treat cancer with such replacement gene therapies is the necessity for highly efficient delivery of the wild-type TP53 into tumor cells *in vivo*. There must also be sufficient expression of functional p53 protein to mediate tumor suppression either through a direct mechanism involving cell death or growth arrest, or by increasing sensitivity to conventional anti-tumor agents. Other critical success factors include a low level of toxicity towards normal cells and the absence of a host immune response against the gene delivery system. The mechanisms of gene delivery can be subdivided in two broad categories: viral and non-viral.

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Biographical Sketches

Daniela Maurici was born in Bologna, Italy, in 1965. She trained as a cell and molecular biologist under the supervision of Dr N. Baldini at the University of Bologna. After her PhD in 1997, she joined the Group of Molecular Carcinogenesis at the International Agency for Research on Cancer, where she worked on the development of model systems to analyze the effect of drugs modulating p53 protein conformation and activities. She is currently a research fellow at the Joint Research Center, European Union, Ispra, Italy.

Pierre Hainaut was born in Liège, Belgium, in 1958. He is a molecular biologist and is the leader of the Group of Molecular Carcinogenesis at the International Agency for Research on Cancer (WHO). He started to work on TP53 as a postdoctoral fellow in the group of Dr J. Milner, in Cambridge, in 1990. His research addresses the role of metals and redox factors in the control of p53 protein functions. This interest has led him to develop projects aimed at identifying agents that modulate p53 protein activities for therapeutic or preventive uses. Within his activities at WHO, he is also responsible for several molecular epidemiological studies on esophageal cancers and on liver cancers, in which TP53 is an important biomarker. He also supervises the IARC TP53 mutation database, a central resource compiling and annotating all TP53 mutations reported in the world literature.