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Clinical applications of the p53 tumor suppressor gene

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Abstract

The interest on the p53 gene has grown enormously in the last 4 years. It is now known that p53 is directly involved in important cellular functions including regulation of the cell cycle, and that its alteration may be one of the important steps in the initiation of cancer. In this review I will cover briefly basic and clinical aspects related to the p53 gene and protein and explore ways of using the accumulated knowledge for patient diagnosis and monitoring. The literature suggests that it is now appropriate to start assessing the p53 gene status of breast tumors for prognosis. Therapeutic options are at an infancy stage. A new diagnostic approach based on the immune response of cancer patients against mutant proteins is discussed, by using the p53 mutant protein as a model. Although the cancer patient has not as yet benefited directly from the enormous number of investigations on the p53 gene and protein, there is hope that in the long-term these studies will promote the understanding of cancer initiation and progression at the molecular level with a practical return at a later phase.

Keywords: p53 gene; Tumor suppressor genes; Cancer diagnosis; Autoantibodies; Serological tests for cancer

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1. Introduction

In the last 20 years scientists have discovered consistent changes in genes that control cell proliferation and differentiation, and implicated these changes in the initiation and progression of human cancer. Over 100 cancer-related genes (oncogenes and tumor suppressor genes) have been described and their potential for clinical applications has recently been discussed elsewhere [1]. One of the most intensely studied cancer-related genes is the p53 tumor suppressor gene. It has been estimated that in 1993 alone over 1000 scientific papers have been published describing basic and applied findings related to this gene and its protein product [2]. The interest in p53 arises from the realization that the mutations of this gene are the most frequent and consistent genetic alterations in human cancer. Roughly, about 50% of all cancers have a defective p53 gene, the defect usually being a point mutation which is thought to inactivate the physiological function of the wild-type protein. In most cases, not only one allele is mutant but the other allele is deleted or inactivated leading to a complete loss of wild-type p53 protein in the affected cell. This 'homozygous' state is believed to be one of the hallmarks of tumor initiation and progression. Extensive reviews on p53 have recently been published [3–6].

In this review I will briefly describe some basic aspects of the p53 gene and protein and examine clinical applications related to disease diagnosis, monitoring and treatment.

2. Function of p53 protein

The p53 protein was first identified in 1979 by immunoprecipitation analysis. In these experiments, it was found that p53 co-immunoprecipitates with a viral tumorigenic antigen, the Simian Virus 40 (SV-40) large T-antigen. Subsequently, p53, a 53-kDa phosphoprotein localized mostly in the cell nucleus, was found to bind to other viral proteins as well, like the adenovirus type 5 E1B antigen, the Epstein-Barr nuclear antigen 5 and the human papilloma virus 16/18 E6 antigen. The binding of p53 to these viral proteins provided the first clue that p53 may be an important cellular protein which, when inactivated by binding to viral proteins, allows the cell to enter a pathway leading to tumorigenesis induced by these viruses. More recently, many cellular proteins have been found to bind p53. Because these binding proteins are sometimes elevated in cancer, the idea has been proposed that cancer initiation may have been due to wild-type p53 inactivation by these binding proteins. However, the most common mechanism of loss of wild-type p53 function is by point mutation of one allele and deletion of the other allele. This phenomenon has been described in nearly all known malignancies with an overall frequency of roughly 50%. Mutant p53 proteins do not function in the cell as wild-type protein. But what is the normal function of wild-type p53?

Wild-type p53 binds to DNA in a sequence-specific manner. The p53-specific DNA sequences are parts of promoters for a number of genes. Thus, p53 qualifies as a transcription factor. The function of p53 in regulating the expression of other genes is reflected in the following biological phenomena: (a) cell growth arrest in

the G1 phase (this phase is associated with cellular rest, not involving DNA synthesis); (b) cell apoptosis following DNA damage (apoptosis is a pathway which leads to programmed cell death); (c) preservation of genetic stability. These biological functions have recently been combined to form a model for p53 action (Fig. 1). In this model, p53 is described as a 'policeman who guards our genome' [7]. This model implies that p53 induces cell growth arrest following DNA damage so that the cell is given time to either repair DNA or enter the pathway of apoptosis if DNA is unrepairable. If p53 function is lost (through one of the mechanisms described before), cells may divide while bearing damaged DNA and go on to become tumorigenic.

The p53 protein was classified as a tumor suppressor based on the following biological information [8]: (a) wild-type protein can suppress the transformation of cells by activated oncogenes; (b) can suppress the tumorigenic phenotype in-vivo;

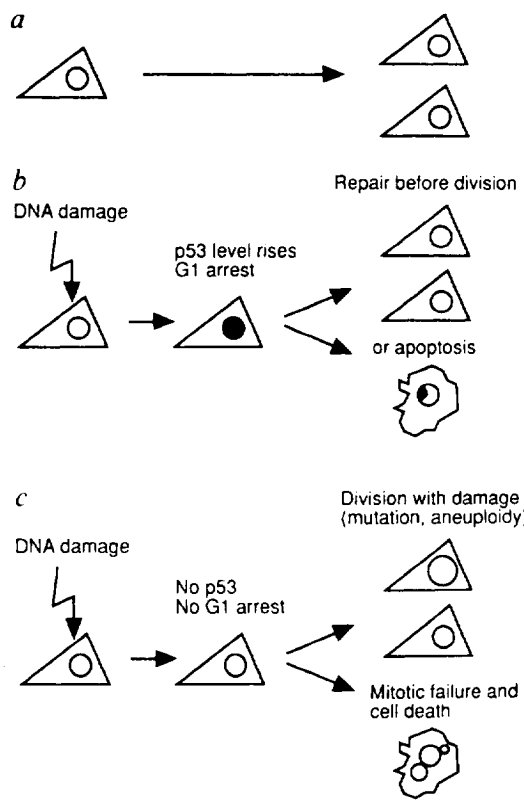

Fig. 1. A model for the function of p53. (a) Normal cell division, for which p53 is not required. (b) In the response of a normal cell to DNA damage, the genome-guarding function of p53 is induced. (c) Cells in which the p53 pathway is inactivated by mutation of p53, or by host (MDM2) or viral oncoproteins, replicate damaged DNA, resulting in mutation, aneuploidy, mitotic failure and cell death. Malignant clones arise from the survivors of this genetic scrambling. Reprinted by permission from Ref. 7.

and (c) can inhibit the growth of malignant cells in-vitro. Moreover, transgenic mice lacking the wild-type p53 gene develop tumors with much higher frequency than heterozygotes or normal animals [9].

Although p53 is now a well-accepted transcription factor, until recently, only a few genes were found to be regulated by its action. These include the muscle creatine kinase gene [10], the GADD 45 gene, whose expression is induced by ionizing radiation and associated with the G1 arrest [11] and the mdm-2 gene, which encodes a cellular protein able to form a stable complex with the p53 protein [12–14].

Mutant p53 does not have the ability to control cell growth, cannot arrest cells in G1 and loses its ability to bind DNA and regulate transcription [8]. However, mutant p53 may acquire other functions which could also be damaging to cells. For example, mutant p53 may inactivate wild-type p53 protein by complexation. Moreover, it could confer a growth advantage to cells even in the absence of wild-type protein, suggesting that mutant p53 has biological functions which are not the result of wild-type p53 inactivation [15]. Others have shown that mutant p53 upregulates genes, such as the multidrug resistance gene, [16,17] which are not upregulated by wild-type protein. This abnormal transcriptional activity may provide a growth advantage to cells expressing mutant p53.

3. How does p53 suppress cell growth?

Until recently, the mechanism of p53-mediated cell cycle arrest in the G1 phase was poorly understood. A few groups have now cloned the gene encoding for a 21-kDa protein which seems to regulate the cell cycle and its concentration is induced by wild-type p53 [18,19]. The same gene was also cloned previously by investigators working in the field of aging [20]. A possible mechanism of cell cycle regulation by p53 is shown in Fig. 2. Wild-type p53 induces the transcription of the Cip1/sid 1/Waf 1 gene (names given for the same gene by three independent groups) which encodes for a 21-kDa protein. This protein apparently binds to enzymes called cyclin-dependent kinases (cdk) which, when bound to another class of proteins called cyclins (cyc), push the cells to escape from G1 and enter the DNA synthetic pathway. The complex of the 21 kDa protein, cdk and cyc does not allow initiation of DNA synthesis and thus, induces arrest of the cell cycle in G1.

4. p53 gene mutations

The p53 gene is located on the short arm of chromosome 17 (17p13) and encodes a 393-aminoacid protein. In cancer, the p53 gene mutations are spread throughout the gene and they are mostly missense mutations leading to aminoacid substitutions in the wild-type protein. Interestingly, the vast majority of the mutations are located in exons 5–8 (the p53 gene has 11 exons in total) which encode for highly conserved regions of the p53 protein [21]. The p53 gene mutations usually lead to the synthesis of a mutant protein which has increased cellular stability. This has an important implication. The mutant p53 protein accumulates in the cell and reaches

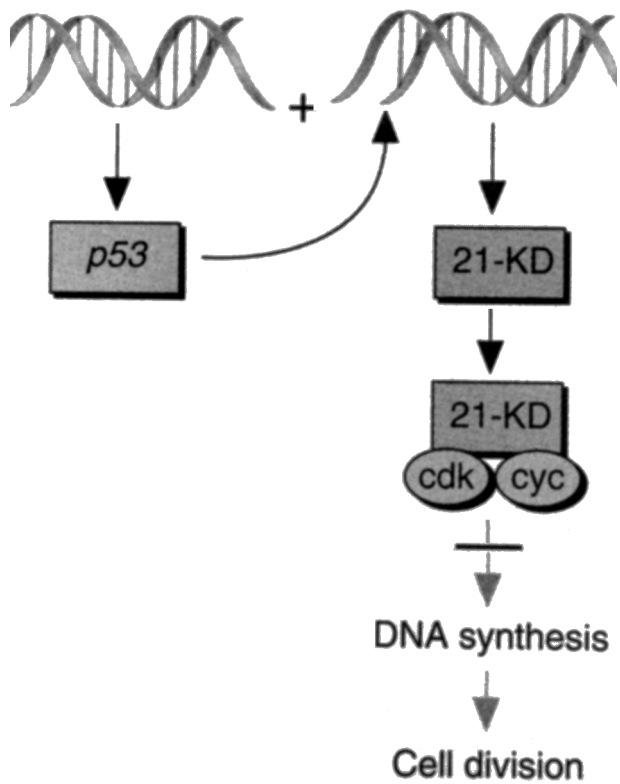

Fig. 2. What p53 does. When the p53 protein is made, it turns on the gene for a 21-kDa protein that blocks Cdk enzymes, and thus cell division. Reprinted by permission from ref. 20.

levels 10–100-fold higher in comparison to the levels of wild-type protein. This mutant p53, irrespective of the location of mutation, can then be conveniently measured by immunological techniques (see below). In the minority of cases (10%) frameshift or chain-terminating (nonsense) mutations lead to truncated or unstable p53 protein which is not usually detected by immunological assays.

It is currently not clear if all p53 gene mutations are pathogenetic events in cancer or they are epiphenomena, appearing at a relatively late stage during cancer progression. The fact that only a fraction of many malignancies contain p53 gene

mutations indicates that p53 gene alterations are not necessary in carcinogenesis. Nor has it been shown by functional assays that all mutant p53 proteins lose the tumor suppressor activity of the wild-type protein. At least some of the p53 gene mutations identified in cancer cell lines may be generated through the culture process. More work needs to be done to clarify if p53 gene mutations are initiating pathogenetic events, and in which cancers, and to study the function of the various mutant p53 proteins in terms of tumor suppressor activity.

5. Study of p53 gene mutations

As already mentioned, most of the p53 gene mutations are spread throughout exons 5–8 of the p53 gene. Thus, the only effective way for their identification at the genetic level is through gene sequencing. The most common approach is to first amplify, using the polymerase chain reaction (PCR), exons 5–8 of the p53 gene (2–4 individual reactions may be necessary). Then, using the simple technique of single-stranded conformational polymorphism (SSCP) it is possible to detect if there is a mutation in the amplified gene area. However, SSCP detects only the presence of the mutation, not its exact localization. Following SSCP, sequencing of the amplified gene areas is effective in finding the exact mutation. In many cases, researchers are bypassing the SSCP step. There are indications that not all p53 gene mutations can be identified by SSCP and it has been already established that in some cases mutations appear outside of exons 5–8. Thus, others prefer to amplify and sequence the p53 mRNA after reverse transcription.

Another effective method for detecting p53 gene mutations is by studying p53 protein levels in tumor cells. It is now widely accepted that tumors with elevated levels of p53 protein frequently have mutant p53 genes [5]. Irrespective of the localization of the mutation in the gene, the mutant protein accumulates, presumably due to increased half-life. Antibodies which react with all p53 mutants have been described and these are used for p53 protein detection. In most cases, p53 protein is detected by immunohistochemistry. More recently, we have developed a highly sensitive and quantitative method for p53, based on monoclonal and polyclonal antibodies and time-resolved fluorometry [22]. This method has many advantages over immunohistochemistry and has been used successfully in clinical studies.

The concordance of results between DNA sequencing and immunological techniques for detecting p53 gene mutations is approximately 90%.

6. Serum antibodies against p53

It has been realized that tumors which contain mutant p53 protein may immunize the host with subsequent production of anti-p53 antibodies which circulate in the patient's serum [23–27]. However, not all tumors containing mutant p53 elicit an immunological response. The most immunogenic tumors appear to be those of colon, ovary, breast and lung [28]. The anti-p53 antibodies can now be measured

with immunological techniques [29,30] and may have some value for diagnosis (see below).

7. Clinical applications

7.1. Genetic screening

The Li-Fraumeni syndrome is a rare autosomal dominant cancer syndrome in which affected relatives develop a diverse set of malignancies, including breast carcinomas, sarcomas and brain tumors. Germline p53 gene mutations have been detected in approximately 50% of Li-Fraumeni families analyzed so far [31]. Except for the Li-Fraumeni syndrome families, germline p53 gene mutations were reported for patients who develop multiple primary cancers [32–34] and patients with a strong family history of cancer affecting multiple tissues.

Identification of the status of the p53 gene in these family members is probably useful because homozygotes and heterozygotes may be closely monitored for early cancer detection so that effective treatment is instituted when the cancer appears. However, this clinical possibility is of very limited applicability since the frequency of this syndrome is very low. The vast majority of cancer patients (> 99%) do not have germline p53 gene mutations.

7.2. Prognosis

Many papers examined the prognostic value of p53 gene mutations in a variety of cancers. These data converge to the conclusion that patients with tumors bearing p53 gene mutations do worse than patients without p53 gene mutations in terms of disease-free and overall survival. The simplistic explanation for this finding is that tumors with p53 gene mutations may have a growth advantage and multiply and spread faster than tumors without p53 gene mutations. The important question here is if the p53 gene status is an independent prognostic indicator which is worth assessing in all patients with malignancy in order to adjust therapy and monitoring strategies. This question has been answered for breast cancer. Two recent papers [35,36] confirmed that p53 status is a strong and independent prognostic indicator in breast cancer. Mutations of the p53 gene, assessed by genetic or immunological techniques, are associated with poor prognosis. We have recently shown that p53 can be measured quantitatively with an immunofluorometric technique in breast tumor extracts prepared for steroid hormone receptor analysis [22]. This technical development could further help in the introduction of p53 testing on a routine basis, for the purpose of breast cancer prognosis. The prognostic value of p53 in other cancers e.g. lung, stomach, ovarian, prostate, cervix, endometrium and bladder has also been examined with the general conclusion that p53 is associated with unfavourable prognosis [37–42].

7.3. Diagnosis-monitoring

Unfortunately, the p53 mutant protein does not seem to be present in serum in measurable amounts [29] and it may not be useful as a diagnostic tool. However, the assay of anti-p53 antibodies in serum may have diagnostic potential as has been

recently suggested by us [28]. This subject is more generally discussed below under a separate heading.

7.4. Therapy

If wild-type protein can arrest the growth of tumor cells, then, its introduction by genetic therapeutic approaches into tumors may have therapeutic potential. This approach is now under intense study. Others are trying to develop drugs that mimic p53 function as a tumor suppressor. The ability of mutant p53 to elicit an immunological response may also eventually lead to the isolation of antibodies capable of inactivating mutant p53. These therapeutic options are now at an infancy stage and only time will show if they have clinical potential.

8. New approaches for the serological diagnosis of cancer — the p53 gene as a model

Early cancer diagnosis with serological testing is still one of the major research priorities among clinical biochemists. Unfortunately, all currently known tumor markers are not useful for such application because they suffer from relatively low specificity. The use of oncogenes and tumor suppressor genes for diagnosis has also been proposed [1]. Some possible biochemical diagnostic strategies are shown in Fig. 3. The detection of cancer cells or their genetic or protein abnormalities in asymptomatic individuals is not a universally applicable approach because such cells would not be generally circulating in the blood of all cancer patients. One could also look for traces of mutant oncogene or tumor suppressor gene proteins in the circulation. Unfortunately, no oncogene or tumor suppressor gene protein has as yet been effectively used for such purposes because there are many problems associated with this approach i.e. the mutant protein may be produced at minute amounts; may not be released into the circulation or released only discontinuously during tumor cell necrosis; may be bound to serum components or antibodies, metabolised or cleared quickly. That is why we and others were not able to find mutant p53 protein in the serum of patients with cancer [29].

Recently, we have proposed a new way of diagnosing cancer based on mutant oncogenic proteins (Fig. 4) [28]. In the proposed model, the tumor is considered to contain cells which have acquired a number of mutant genes and their cognate proteins. We postulate that these proteins may be recognized by the immune system as 'non-self' (break of tolerence) with subsequent production of specific anti-mutant protein antibodies. The detection of these antibodies in the serum may be useful for early diagnosis.

We have recently tested this concept by using the p53 tumor suppressor as a model. Among a dozen different malignancies we found that colon and ovarian cancers could elicit an immunological response against p53 in approximately 20% of patients [28]. The reason why some tumors elicit immunological response and others don't is still unknown. It is also not known at what time during tumor initiation and progression these antibodies appear in the serum at measurable amounts. If they do appear very early, they hold promise of being used for diagnosis. The specificity of this test is very high, approaching 100%.

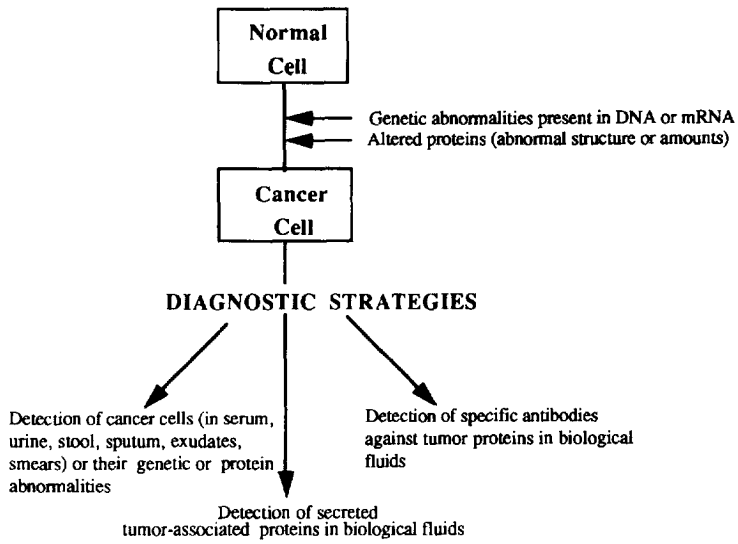

Fig. 3. Diagnostic strategies for cancer.

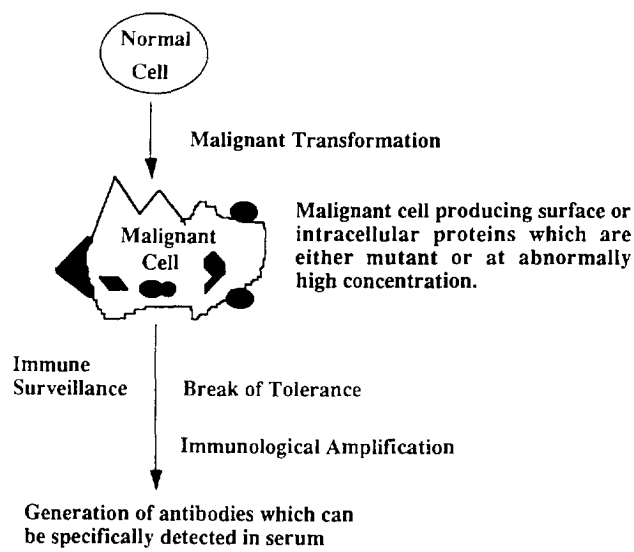

Fig. 4. Concept for the serological diagnosis of cancer. Early during cancer development, alterations in oncogenes and/or tumor suppressor genes may lead to the production of mutant forms or abnormal levels of proteins within tumor cells or on tumor cell membrane (black cartoons). Host's immunological system detects such altered or abnormally abundant proteins and produces antibodies against them. The antibodies are produced in amounts vastly higher than the immunogen (immunological amplification), circulate in the serum for long periods and could be used to spot the cancer initiation event. The immune receptors can be initiated even if the offending immunogen is only transiently released from the tumor. Reprinted from ref. 28.

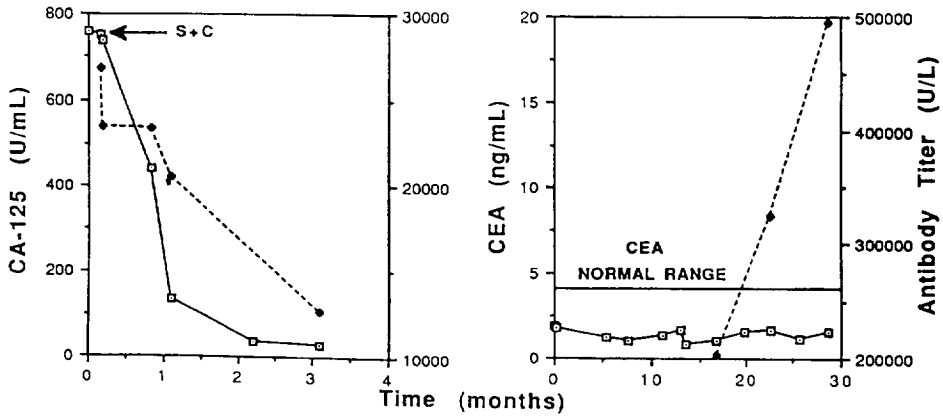

Fig. 5. Monitoring an ovarian (left) and a breast (right) carcinoma patient with serum anti-p53 antibodies (broken line) or with CA-125 or CEA. Note the decrease in both CA-125 and anti-p53 antibody titers after surgery and chemotherapy (S + C). The breast cancer did not produce CEA and the increase in antibody titers were indicative of relapse. Reprinted from ref. 28.

In the case of breast cancer patients we and others have shown that the presence of anti-p53 antibodies in serum is an unfavourable prognostic event [27,28]. It remains to be seen if this is true for ovarian and colon cancers. We have recently reported that the p53 antibody test has some value for patient monitoring post-surgery (Fig. 5) [28].

The concept presented in Fig. 3 could have potential for diagnosis if other immunogenic mutants are identified and combined with the p53 test in order to improve sensitivity. Such approaches are currently under investigation.

9. Conclusions

The alterations of the p53 gene in cancer are highly consistent and prevalent. It remains to be seen if these changes are pathogenetic events or sequelae of cancer. It seems that the p53 gene and its protein product have potential for cancer prognosis and possibly diagnosis of selected classes of cancer. The therapeutic applications are currently under investigation. Only time will show if the enormous literature on this gene and protein will be translated into an application that will significantly benefit the cancer patient.

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