P53 HOW CRUCIAL IS ITS ROLE IN CANCER?

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ABSTRACT

The p53 gene located in human chromosome 17, suspends the cell cycle when there is DNA damage. If there is a mutation in p53, the cell cycle continues unrestrained and reproduces the damaged DNA, leading to uncontrolled cell proliferation and cancer tumors. The p53 protein is a transcription factor and its pivotal role in maintaining genomic integrity has earned it the nickname "guardian of the genome". p53 gene is mutated in about 50% of human cancers of breast, colon, lung, liver, prostate, bladder, and skin. Since the loss of p53 function is so prevalent in human cancer, this protein is an ideal candidate for cancer therapy. Several gene therapeutic strategies have been employed in the attempt to restore p53 function to cancerous cells.

Keywords: p53gene, p53 protein, Tumor suppressor gene, Mutation, Cancer therapy.

INTRODUCTION

p53 is the most commonly mutated gene in human cancers and more than 50% of human cancers contain p53 mutations Arnold Levine, David Lane and William Old discovered the p53 gene in 1979. It was first thought to be an oncogene, but10 years later team lead by Bert Vogelstein and Ray White, then studying colon cancer, showed p53 to be a tumor suppressor gene. In the past decade, the roles of p53 in human cancers have been investigated extensively in many aspects and intervention to restore wild-type p53 activities is an attractive approach for cancer therapy.

p53 gene is not reactive in cells where DNA is undamaged. When there is DNA damage, the gene suspends the cell cycle until the damage can be repaired. If there is a mutation in p53, the cell cycle continues unrestrained and reproduces the damaged DNA, leading to uncontrolled cell proliferation and cancer tumors. Cancer results as the cell with damaged DNA divides, the damaged DNA is replicated and each daughter cell's cycle is also unrestrained. Lu,et al(2008) elaborated the DNA damage checkpoint and p53 signaling pathways in human tumorigenesis. Moore et al . (2007). Revealed aging-associated truncated form of p53 which interacts with wild-type p53 and alters p53 stability, localization, and activity.

All cancer cells contain mutations in combinations of tumor suppressors and oncogenes. The removal of functional p53, from a cell allows for the accumulation of even more DNA damage and the division of cells that contain damaged DNA. The mutation of p53 is one of the most frequent genetic changes seen in cancer cells. In addition to mutations that arise during the growth and development of individuals (sporadic mutations), there are forms of cancer associated with the inheritance of a damaged version of p53. In addition, several viruses have evolved ways of inactivating the p53 protein.

The p53 gene

Human Chromosomal Location: 17p13.1

The p53 gene encompasses 20kb of DNA with 11 exons which on transcription gives a 3.0 kb mRNA having 1179bp open reading frame. On translation, this mRNA produces a 53kDa protein (hence the name p53)
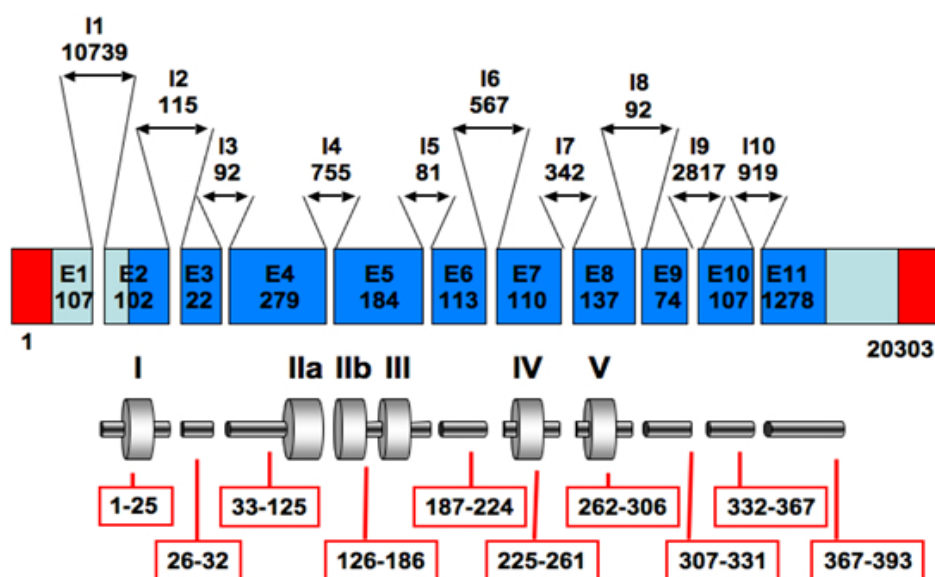

Fig. 1: The p53 gene

The p53 protein (p53)

Synoname: TP53 (tumor Protein 53)

The p53 protein is a 393-residue polypeptide and form N- terminal to C-terminal. It contains five functional domains.

1. N-terminal domain (residues 1-43) that is involved in transcriptional activation.

2. a Proline rich domain (63-97) Mdm2 binds to both these domains.

3. The large, central core domain (residues 100-300) that involves in DNA binding, and is the location of almost all oncogenic p53 mutations.

4. The oligomerization domain(residues 320-360) contains nuclear localization signals and it is involved in p53 tetramerization.

5. The basic C-terminal domain (residues 364-393) is a negative regulatory domain that can inhibit sequence- specific DNA binding by the core domain.
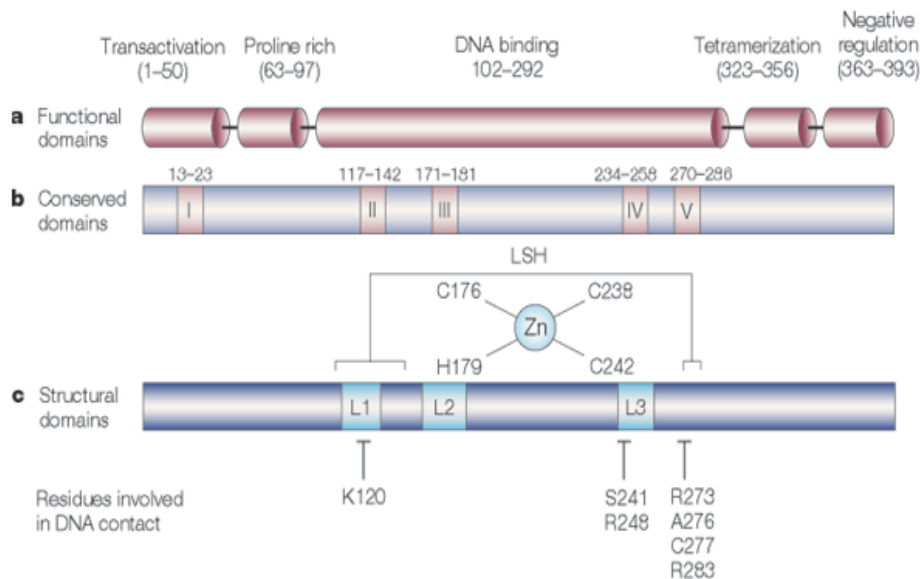

Fig. 2: p53 protein- TP53 (tumor Protein 53)

Features of p53 protein

Cofactor: Binds 1 zinc per subunit.

Subunit: Binds DNA as a homotetramer.

Sub cellular location: Cytoplasmic and nuclear. Agents that damage DNA induce p53 to become very stable by a post-translational mechanism, allowing its concentration in the nucleus to increase dramatically.

Cellular functions of p53

1. Suppresses progression through the cell cycle in response to DNA damage , thereby allowing DNA repair to occur before replicating the genome; hence, p53 prevents the transmission of damaged genetic information from one cell generation to the next. It does this by binding to a transcription factor called E2F. This prevents E2F from binding to the promoters of proto-oncogenes such as *c-myc* and *c-fos*. Transcription of *c-myc* and *c-fos* is needed for mitosis to blocking the transcription factor needed to turn on these genes prevents cell division.

2. Initiates apoptosis if the damage to the cell is severe and works as an emergency brake on cancer development by killing cells that attempt to proliferate in oxygen-deficient regions of tumors.

3. Often act as tumor suppressor: Mutations in p53 can cause cells to become *oncogenically transformed,* and transfection studies have shown that p53 act as a potent transdominant tumor suppressor that some level of normal growth to cancerous cells *in vitro*.

4. p53 is a potent transcription factor and once activated, it represses transcription of one set of genes (several of which are involved in stimulating cell growth) while stimulating expression of other genes involved in cell cycle control. Among them, p21 is one of the most important. The product of the p21 gene is a negative regulator of cyclin-dependant kinases, enzymes that are critical in the progression of the cell cycle and ultimately the cell division. By stimulating the transcription of the cell cycle and ultimately cell division. By stimulating the transcription of the p21 gene, p53 prevents cell proliferation. This stoppage gives the cell the opportunity to make repairs, if possible. If substantial DNA damage has occurred can help to trigger cell death.

5. The function of p53 is critical to the way that many cancer treatments kill cells since radiotherapy and chemotherapy act in part by triggering cell suicide in response to DNA damage. This successful response to therapy is greatly reduced in tumors where p53 is mutant so these tumors are often particularly difficult to treat.

p53- Activating Signals

Under normal conditions, p53 is latent. It does not interfere with cell cycle progression and cell survival. p53 is not essential for the normal performance of cells within the body. A variety of conditions can lead to rapid induction of p53 activity, which represents 5 types of stress, that are likely to favor the emergence of cancer-bound cells. Such conditions include direct DNA damage as well as damage to components involved in the proper handling and segregation of the cellular genetic material (eg. The mitotic spindle, ribonucleotide depletion, hypoxia, heat shock, and exposure to nitric oxide (NO). Accumulation of genomic aberrations is a key carcinogenic mechanism; the rapid induction of p53 activity in response to genomic damage thus serves to ensure that cells carrying such

damage are effectively taken care of. Furthermore, p53 may also contribute directly or indirectly, to particular DNA repair processes. In addition, p53 activity is triggered by a variety of oncogenic proteins, including Myc, Ras, adenovirus E1A, and β-catenin. p53 activation may also involve a change in subcellular localization; whereas latent p53 may often be cytoplasmic, at least during part of the cell cycle, exposure to stress results in its accumulation in the nucleus, where it is expected to exert its biochemical activities.

The p53-Mdm2 Loop (Fig. 3)

A key player in the regulation of p53 is the Mdm2 protein. Mdm2 is the product of an oncogene, whose excess activity facilitates several types of human cancer. Mdm2 exhibits a unique relationship with p53. On the other hand, the Mdm2 protein binds to p53 and inactivates it. The binding occurs right within the p53

transactivation domain, interfering with recruitment of basal transcription machinery components.

Mdm2 can actively repress transcription when tethered to p53. Importantly, Mdm2 binding can also lead to complete elimination of p53 through proteolytic degradation. On the other hand, p53 binds specifically to the *mdm2* gene and stimulates its transcription. This duality defines a negative feed back loop, which probably serves to keep p53 in tight check and to terminate the p53 signal once the triggering stress has been effectively dealt with. In the absence of functional Mdm2 protein, p53 becomes strongly deregulated. In certain human cancers; excessive Mdm2 expression, achieved through *mdm2* gene amplification or other mechanisms, can lead to constitutive inhibition of p53 and thereby promote cancer without a need to alter the p53 gene itself. Excess Mdm2 can also promote cancer independently of p53.
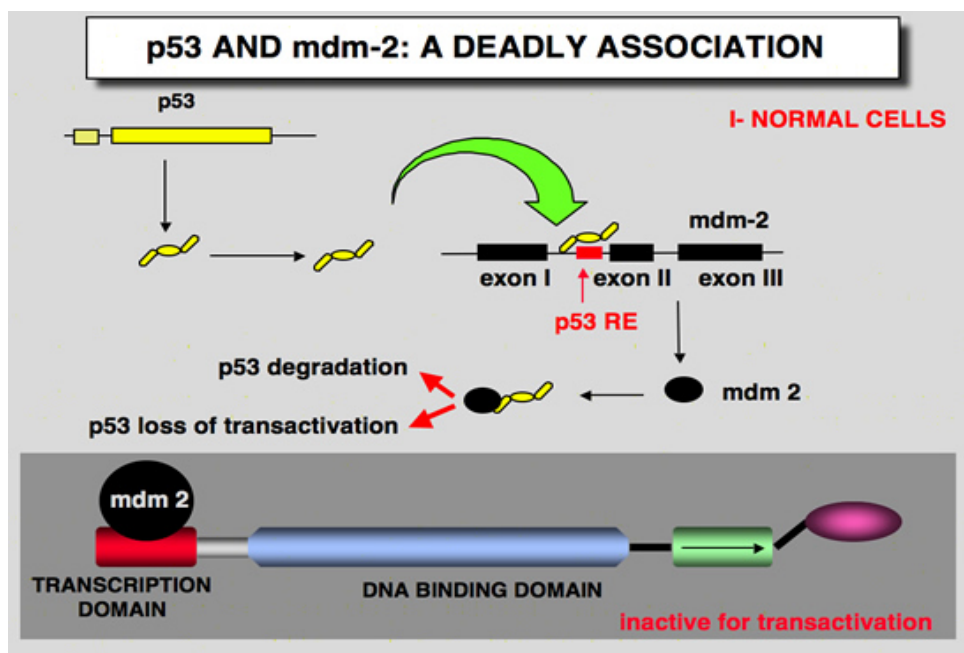

Fig. 3: p53 and mdm-2 association

Covalent Modifications of p53 (Fig. 4)

The rapid stabilization and activation of the p53 protein upon stress also involves stress-induced covalent modifications of p53. p53 becomes phosphorylated on multiple sites *in vivo* in response to various types of stress, and many stree activated kinases can phosphorylate p53 *in vitro.* A potential outcome of such phosphorylation might be the stabilization of p53 through inhibition of p53 ubiquitination and degradation. In the case of p53 , several candidate sites within its Mdm2-binding domain have been identified which are modified in response to DNA damage and whose phosphorylation reduces the affinity of p53 for Mdm2.

(a) Expression of Mdm2 is activated by p53.

(b) Binding of p53 by Mdm2 can trigger the degradation of p53 via the ubiquitin system.

(c) Phosphorylation of p53 at Ser15, Thr18 or Ser20 will disrupt its binding with Mdm2. In normal cells, these three residues are not phosphorylated, and p53 is maintained at low level by Mdm2.

(d) DNA damage may activate protein kinase (such as ATM, DNA-PK, or CHK2) to phosphorylate p53 at one of these three residues, thereby increasing p53 level. Since Mdm2 expression

is activated by p53, the increase of p53 also increases Mdm2, but they have no effect while p53 is phosphorylated. After the DNA damage is repaired, the ATM kinase is no longer active. p53 will be quickly dephosphorylated and destroyed by the accumulated Mdm2.

For example, DNA- damaging agents activate phosphorylation at serine (Ser) 15, likely by a family of protein kinases including ATM and ATR, and Ser20 by the Chk2 kinase. (Chk2 is a protein kinase that phosphorylates serine 20. Defects in the Chk2 gene cause a predisposition to cancer.) These phosphorylation events are believed to contribute to p53 stabilization by preventing the binding of Mdm2 and rendering p53 more resistant to Mdm2.

In addition to potentially regulating Mdm2 binding , phosphorylation was also shown to modulate the transcriptional activity of p53. For example,phosphorylation at Ser15 stimulates p53 interaction with its transcriptional co-activators p300 and CBP, and a mutation that eliminates this phosphorylation leads to p53 transcriptional defects.

Another potential mechanism that may play a critical role in p53 activation is acetylation. Multiple lysine (K) residues in p53 are acetylated by p300 and its family member CBP or by P/CAF.
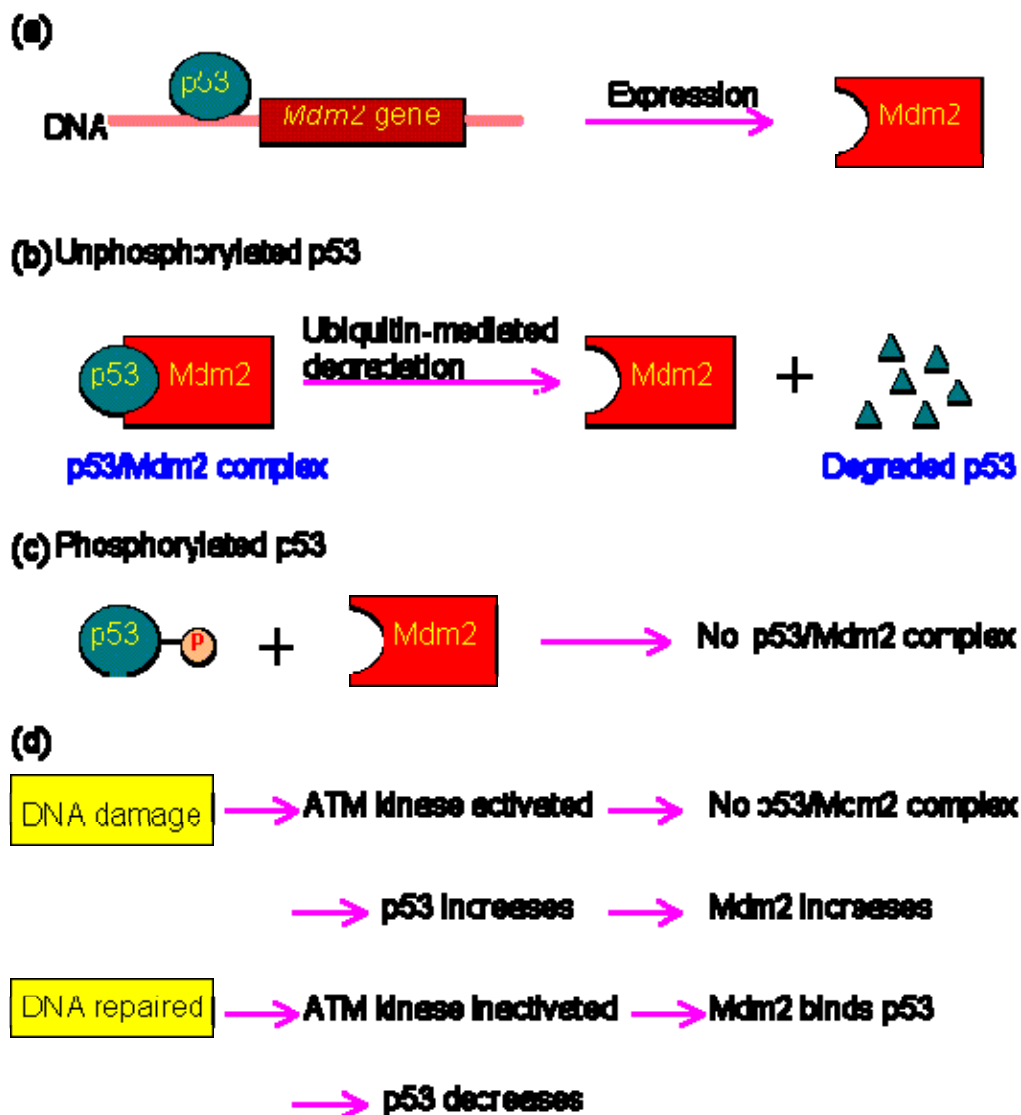
Fig. 4: Covalent Modifications of p53

p53 and Oncogenic stress

Oncogenic stress, such as the deregulated expression of oncoproteins like Myc, Ras, adenovirus E1A, and β-catenin activates p53 response. Excess activity of these oncoproteins leads to massive induction of ARF protein that arises through translation of an alternative reading frame derived from the INK4A tumor suppressor gene. This induction is primarily because of enhanced transcription, some of which is mediated through the E2F transcription factor.

The induced ARF protein then binds to Mdm2, and inhibits the p53 biquitin ligase activity of Mdm2. Because the ubiquitin ligase activity of Mdm2 appears to be essential for the degradation of p53, it is possible that by directly binding and inactivating Mdm2, p14ARF bypasses the need for phosphorylation in p53 activation. Loss of the p14ARF gene causes Mdm2 to increase in concentration leading to a decrease in the levels of p53.

The inhibitory effects of p53 are not triggered when Myc or Ras proteins are recruited as part of a properly orchestrated growth response, initiated by the binding of a growth factor to its receptor, or else such cells would not be able to execute a mitogenic response. When a cell is exposed to a growth factor, one arm of the response drives the neutralization of p53 concurrently with the activation of Myc, Ras, and E2F by the other arm.

p53- dependent Apoptosis

p53 trancriptionally activates genes leading to cell cycle arrest or cell death(apoptosis). p21WAF1/CIP1 is a G_1cyclin/cyclin-dependant protein kinase inhibitor, which blocks the activity of a G_1cyclin- dependent protein kinase. This results in cell cycle arrest. p53-binding sites in the regulatory region of the gene directly activate transcription of the *Bax* gene, which is located in mitochondria. When over induced, they induce apoptosis.

There are several potential mediators of p53-induced apoptosis. The *bax* protein is an apoptosis inducing member of the Bcl-2 protein family. p53-binding sites in the regulatory region of the gene directly activate transcription of the *bax* gene. Bax is located in mitochondria. When over expressed, Bax induces apoptosis.
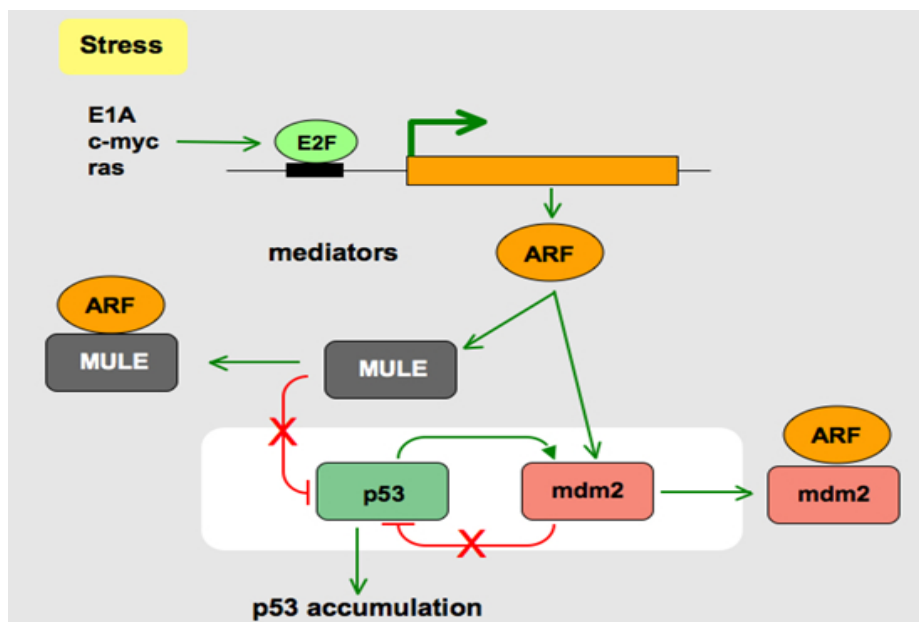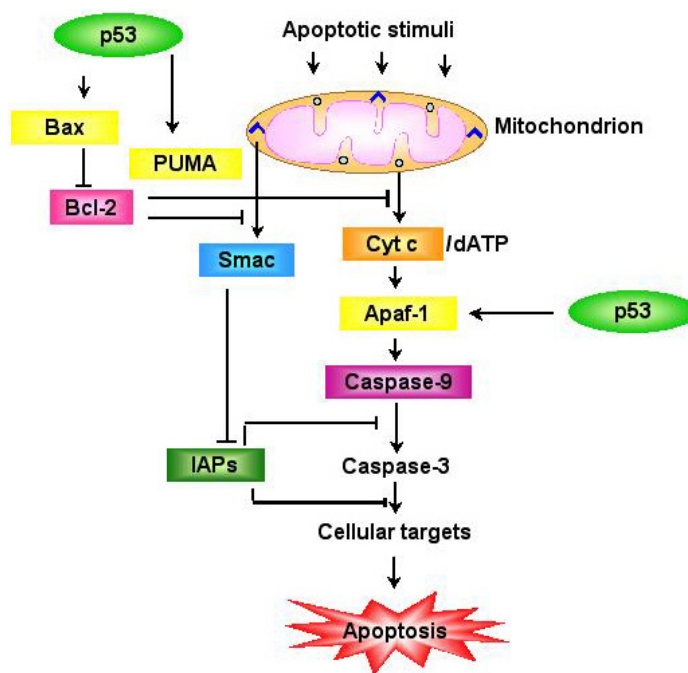
Fig.5: p53and oncogenic stress

Fig. 6: p53 dependent apoptosis

Mutations in p53 gene

1. Germinal Mutation

Associated with the rare familial Li-Fraumeni syndrome, a dominantly inherited disease as affected individuals are predisposed to develop sarcomas, osteosarcomas, leukemia and breast cancer at unusually early ages.

2. Somatic Mutation

The p53 gene is mutated in about 50% of human cancers, and the non mutated allele is generally lost; the frequency and the type of mutation may vary from one tumor type to another; and the mutations tend to cluster in central DNA-binding domain. Among the cancers involved are breast, colon, lung, liver, prostate, bladder and skin. These mutations are missense(80%), non-sense(7.5%), deletions, insertions or splicing mutations (12.5%); there are some hot spots for mutations at CpG dinucleotides at positions 175, 248, 273 and 282. Mutational events are related to carcinogens that affect p53 are ultraviolet radiation and cigarette smoke. Mutations are often dominant negatives, since p53 acts as a tetramer.

Alterations in the p53 gene have several different effects on the activity of the gene, depending on the location of alteration. A mutation in the promoter region can result in the decrease or absence of p53 in the cell. Mutations that occur in the protein-coding region of the gene can have impact in the expression of the gene (or activity of protein).

In addition, some sarcomas **amplify** another gene, called **mdm-2**, which produces a protein that binds to p53 and inactivates it, much the way the DNA tumor viruses do. The elimination of functional p53 from the cells clears the way for cell division even in the presence of DNA damage. In the absence of p53, genetic instability as evidenced by increased mutations and aneuploidy are likely to increase. The increase in genetic damage leads to the accumulation of defective tumor suppressors and oncogenes. The tumor derived mutant p53 proteins that have been tested thus far do not **bind to DNA** in the same manner as wild-type p53.
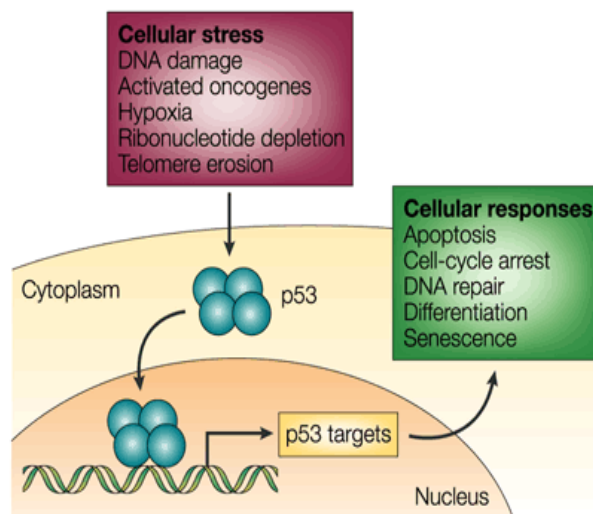
3. Viral Inactivation of p53 protein

Infection with viruses introduces foreign DNA into the cells. P53, along with other proteins, is responsible for the cell's response to the presence of foreign DNA, which include shutting down cell division and cell death. To avoid these responses, several different tissues have ways of inactivating the p53 protein. An example of this is Simian Virus 40 (SV40). Upon infection with SV40, viral proteins are produced with in the cell cytoplasm. One of the proteins produced is termed the Large T antigen. A function of this protein is the binding and inactivation of the p53 protein. Other viruses such as Hepatitis and Human Papillomavirus produce similar proteins.

In vitro, the interaction of TP53 with cancer associated/HPV (56) viral proteins lead to ubiquitination and degradation of TP53 giving a possible model for cell growth regulation. This complex formation requires an additional factor, E6-AP, which stably associates with TP53 in the presence of E6. C-terminus interacts with TAF1, when TAF1 is part of TFIID complex.

P53 in cancer therapy

Several gene therapeutic strategies have been employed in the attempt to restore p53 function to cancerous cells. These approaches include introduction of wild type p53 into the cells with mutant p53; the use of small molecules to stabilize mutant p53 in wild type, active conformation; and the introduction of agents to prevent degradation of p53 by proteins that normally targets it. In addition, because mutant p53 has oncogenic gain of function activity, several approaches have been investigated to selectively target and kill cells harboring mutant p53 and the introduction of gene that, in the absence of functional p53, produces toxic product. Many obstacles remain to optimize these strategies for use in humans, but, despite these, restoration of p53 function is a promising anti-cancer therapeutic approach.

Cellular stress
DNA damage
Activated oncogenes
Hypoxia
Ribonucleotide depletion
Telomere erosion

Cytoplasm   p53

Cellular responses
Apoptosis
Cell-cycle arrest
DNA repair
Differentiation
Senescence

p53 targets

Nucleus

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Tumor suppressing gene therapy

Use of tumor suppresses genes as anticancer therapeutics has been investigated rigorously in both experimental and clinical researches. Transfer of various tumor suppressor genes directly to cancer cells has been demonstrated to suppressor tumor growth via induction of apoptosis and cell cycle arrest and, in some cases, with evidence for bystander effect. Various studies have shown that combination of tumor suppressor gene therapy with conventional anticancer therapy can yield synergistic therapeutic benefits. Clinical trials with p53 gene, have demonstrated that the treatment is well tolerated, and; favorable clinical responses, have been observed in a subset of patients with advanced diseases or with cancers resistant to conventional therapy. Yet, current gene replacement approaches in cancer gene therapy must be improved if they are to have a boarders clinical impact. Efficient systemic gene delivery systems will be required ultimately for the treatment of metastatic disease.

Inhibiting the p53-MDM2 interaction: an important target for cancer therapy.

Inhibiting the p53- MDM2 interaction is a promising approach for activating p53, because this association is well characterized at the structural and biological levels. MDM2 inhibits p53 transcriptional

activity, favors its nuclear export and stimulates its degradation, so inhibiting the p53-MDM2 interaction with synthetic molecules should lead to p53-mediated cell-cycle arrest or apoptosis in p53-positive stressed cells.

Turning p53 on or off: either way may treat cancer.

Two recent strategies have been proposed to exploit p53's unique death-regulating activity in opposite directions and improve cancer treatment. One approach seeks to inhibit p53 in normal cells there by diminishing therapy-related, p53-dependent toxicity. The other utilizes a peptide derived from the C-terminus of p53 to activate wild type or mutant p53 proteins, triggering apoptosis with selectivity for transformed cells. These novel approaches hold promise for targeting p53 in cancer therapy and may shed light on mechanisms underlying the role of p53 in cancer cell survival.

Viral-mediated gene transfer for cancer treatment.

Pre-clinical data has suggested that cancer growth can be arrested or reversed by treatment with gene transfer vectors that carry a single growth inhibitory or pro-apoptotic gene or a gene that can recruit immune responses against the tumor. Many of these gene transfer vectors are modified viruses that retain the capability of the

viruses for efficient gene delivery but are safer than the native viruses due to modifications that eliminate or alter one or more essential viral functions. The field of viral-based gene transfer vectors for the treatment of cancer has now entered the final stage of clinical testing prior to possible product approvals. Three viral vectors are currently undergoing this Phase III or Phase II/Phase III clinical testing for cancer treatment. All three of these vectors are based on adenovirus, a common human virus that in its native state can cause cold or flu-like symptoms. In two of these vectors, genes essential for viral replication have been replaced with the wild-type p53 tumor suppressor gene, a gene that is deleted or mutated in over 50% of human cancers and which, when transferred into tumor cells, can induce cell death. The three vectors represent two of the approaches now being taken to develop viral-based gene transfer vectors for cancer treatment. Additional approaches include the transfer of the genes capable of converting non-toxic prodrugs into toxic forms, using anti-angiogenic gene transfer to block the transfer to block the formation of tumor blood vessels, inhibiting the activity of oncogenes through blocks to transcription or translation, stimulating the body's own immune system with immunomodulatory genes, and "cancer vaccination" with genes for tumor antigens.

Anti-p53 antibodies as biomarkers of cancer process

Change in expression and mutations of gene p53 cause variations of cellular p53 protein concentration. Higher cellular protein p53 levels are associated with increased protein transfer to the extracellular liquid and to blood. It has been observed that increased blood serum protein p53 concentrations may have a prognostic value in early diagnosis of lung cancer. The results of a number of studies confirm that accumulation of a mutated form of protein p53, and presumably also large quantities of wild forms of that protein in the cells, may be a factor that triggers the production of anti-p53 antibodies. Statistical analysis showed that anti-p53 antibodies could be regarded as a specific biomarker of cancer process. The prevalence of anti-p53 antibodies correlated with the degree of cancer malignancy. The increased incidence of anti-p53 antibodies was also associated with the higher frequency of mutations in gene p53.

CONCLUSION

p53, located in human chromosome 17, is a gene with tumor suppressor activities. This protein contains 393 amino acids and a single amino acid substitution lead to loss of function of the gene. Mutations at amino acids 175, 248, and 273 can lead to loss of function and changes at 273 (13%) are the most common. All these act as recessive mutations. Dominant gain-of-function mutations have also found that lead to uncontrolled cell division. Because these mutations can be expressed in heterozygous conditions, they are often associated with cancers. This genetic function of this gene is to prevent cell division of cells with damaged DNA. Damaged DNA could contain genetic changes that promote uncontrolled cell growth. Therefore, preventing cell division until damaged DNA is repaired is one mechanism of preventing the onset of cancer. About 50% of human cancers can be associated with a p53 mutation including cancers of the bladder, breast, cervix, colon, lung, liver, prostrate, and skin. p53 related cancers are also more aggressive and have a higher degree of fatalities.

Several gene therapeutic strategies have been employed in the attempt to restore p53 function to cancerous cells. These approaches include introduction of wild-type p53 into p53 mutant cells ; the use of small molecules to stabilize mutant p53 in a wild-type, active conformation; and the introduction of agents to prevent degradation of p53 by proteins that normally target it. In addition, because mutant p53 has gain of function activity, several approaches

have been investigated to selectively target and kill cells harboring mutant p53. These include the introduction of mutant viruses that cause cell death only in cells with mutant p53 and the introduction of a gene that, in the absence of functional p53, produces a toxic product. Many obstacles remain to optimize these strategies for use in humans, but, despite these, restoration of p53 function is a promising anti-cancer therapeutic approach.

REFERENCES

1. White,R. 1998. Tumor suppressor pathways. *Cell* 92: 591-592
2. Harris, C.C. 1993.p53: at the crossroads of molecular carcinogenesis and risk assessment. *Science* 262 : 1980-1981
3. Ko, L., and Prives, C. 1996. p53: puzzle and paradigm. *Genes and develop.* 10: 1432-1433,
4. Lu, X., Nguyen T., Zhang, X., and Donehower, L. A. (2008). The Wip1 phosphatase and Mdm2: Cracking the "Wip" on p53 stability. *Cell Cycle* 7(2):164-168.
5. Lu, X., Nguyen T., Moon S., Darlington, Y., Sommer, M., and Donehower, L. A. (2008). The Type 2C Phosphatase Wip1: An oncogenic regulator of tumor suppressor and DNA damage response pathways. *Cancer and Metastasis Reviews* 27(2):123-35.
6. Levine, A. 1997p53, the cellular gatekeeper for growth and division. *Cell* 88: 323-331,.
7. Prives, C. 1998.Signalling to p53: breaking the MDM2-p53 checkpoint. *Cell* 96: 5-8,
8. Twyman, R.M. 2003. *Advances Molecular Biology.2nd* edn., pp.258-260. Viva Books, New Delhi.
9. Lodish, H., Zipursky, S.L. and Baltimore, D.2000. *Molecular Cell biology*. 4th edn., pp.1076-1078. Freeman and Company, New York.
10. Lewin, B. 2004. *Genes VIII.* Pp.917-930. Prentice Hall, New Jersy.
11. Vogestein, B., Lane, D. and Levine, A.J. 2000 Surfing the p53 network. *Nature,* 408: 307-310.
12. Blagosklonny, M.V. 2002 p53: An ubiquitous target of anticancer drugs. *International Journal of Cancer,* 98: 161-166.
13. McCormick, F. Cancer gene therapy: fringe or cutting edge. *Nat. Rev. Cancer,* 1 : 130-141, 2001.
14. http:// www. ncbi.nlm.nlm.gov/ disease/ p53.html.
15. http://p53.genome.ad.jp/documents/ about_p53.html.
16. http://www3.cancer.gov/ intra/ LHC/ p53ref.htm.
17. http://student.biology.arizona.edu/honors2002/ group07/p53apop.html.
18. http://www.bioscience.org/news/ science/ p53.htm.
19. http://users.rcn.com/jkimball.ma.ultranet/Biology   pages/T/ tumor suppressorgenes.html.
20. http://www.web-books.com/MoBio/Free/Ch4Hp53.htm
21. http://www.celldeath.de/encyclo/misc/p53.htm
22. http://oregonstate.edu/instruction/bb492/lectures/p53Lectu re.html.
23. www.chemheritage.org/Educational services/pharma/chemo/readings/p53.htm.
24. http://en.wikipedia.org/wiki/p53_gene.
25. http://www.actabp.pl/pdf/2_2005/321.pdf.
26. http://www.weizmann.ac.il/Biology/open_day_2002/book/m oshe_oren.pdf.
27. http://www.suite101.com/article.cfm/new_cancer_treatment/ 111979.
28. http://www.rcsb.org/pdb/molecules/pdb/molecules/pdb31_ 1.html.
29. http://www.jci.org/cgi/content/full/104/3/223.
30. http://www.cancerquest.org/index.cfm/413
31. http://www.mskcc.org/mskcc/html/10861.cfm.