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Role of the p53 gene in DNA damage and human cancer

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Abstract
p53 is a tumor suppressor gene. Its activity stops the formation of tumors. It is also a nuclear transcription factor with a pro-apoptotic function. Most of the human cancers are associated with p53 mutation, but most commonly breast carcinoma, liver, lung and ovarian cancers are implicated by mutations in p53 gene. Mutant p53 acts as the domint- negetive inhibitor toward wild type p53.Basically p53 prevent neoplastic transformation either arrest by cell cycle or by triggering apoptosis. It initiates apoptosis if the damage to the cell is severe, thus p53 has an ability to maintain genomic integrity. Among them, 90% of mutations are detectable within the genomic region encoding the DNA binding domain of p53 and thereby mutant p53 lacks the sequence-specific transactivation ability. Thus, the DNA binding activity of p53 is connected to its tumor suppressive function. The function of p53 gene in cell called as "*Guardian Of The Genome''* may extended to a role in monitoring and repair of DNA damage in addition to direct control of cell growth and death. In the present article, i described the role of the p53 in DNA damage and human cancers.

Keywords: p53, cancer, mutation, apoptosis, cell death

1. Introduction

The p53 protein is the product of the p53 gene, where 'P' stands for protein and '53'stands for weight of the protein (53 kDa). This particular gene located on the short arm of human chromosome 17.It was first discovered in 1979.It is located all normal tissues. Its unstable and degrades very quickly. It is one of the most common mutated gene in cancer.p53 is a nuclear transcription factor and trans activates numerous target genes involved in the cell cycle arrest/or apoptosis. 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. *Lu. et al* elaborated the DNA damage checkpoint and p53 signalling pathways in human tumorigenesis.

All cancer cells contain mutations in combinations of tumor suppressor and oncogenes. The mutation of p53 is one of the most frequent genetic changes seen in cancer cells. Cancers bearing p53 mutations sometimes display the chemo-resistant phenotype, indicating that p53 plays a critical role in the regulation of DNA damage response. Thus, it is quite important to develop a novel strategy to eliminate the negative effect of mutant p53 on wild-type p53 for efficient chemotherapy.

Chemical structure of p53

p53 is composed of three types of functional domain which is-C terminal regulatory domain, DNA binding domain and N-terminal transactivation domain.p53 is a phosphoprotein and made 393 amino acids.

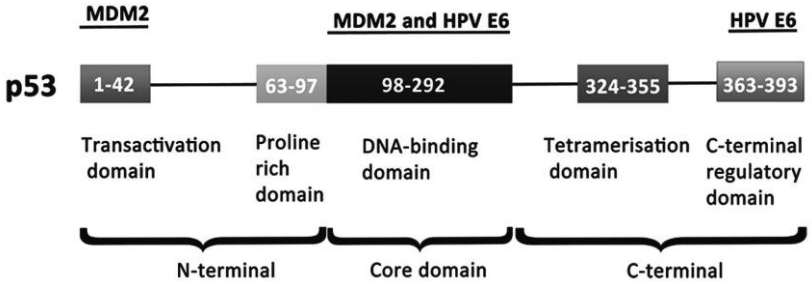

Fig 1: Structure of p53 gene

This protein is a sequence specific transcription factor which binds DNA sequences corresponding to repeats of the consensus motif RRRC(A/T)(T/A)GYYY (R=purine, Y=pyrimidine). The most frequent mutated domain in p53 is DNA binding domain. In cancer, inactivation of p53 occurs in different ways including mutation, deletion etc or binding to cellular oncoprotein.

Mechanism of p53 gene

i) In Normal Cell

p53 protein is a product of p53 gene. It is a kind of housekeeping gene and all the time in very minute amount p53 protein always being produced inside the cell. If inside the cell everything is going good p53 is very much unstable. It produced and degrades very quickly.

ii) Cell Cycle Arrest

When any type of DNA damage occurs in cell cycle, production of p53 grows up.p53 is a type of transcription regulator protein. Transcription regulator are those type of protein which actually sit on promoters and regulate the productivity of the certain gene. Suppression of cell transformation is mediated by specific binding of p53 tetramers to DNA at its recognition motifs in the promoter of wild type p53-activated fragment (WAF1)gene which codes for a universal inhibitor (p21,or CDKI) of the cyclin-dependent kinases, that govern cell cycle progression. When p21 is complexed with CDK2, the cell cannot continue to the next stage of cell division, cell cycle arrest at G1 position and it inturn goes for the apoptosis or programmed cell death and prevent any kind of mutation.

When p21 inhibitor rise, the cyclin /CDK complexes it binds to can no longer phosphorylate Rb proteins. Unphosphorylated Rb proteins binds transcription factors collectively called E2F and thereby prevents E2F mediated transcriptional activation of many genes whose products are required for DNA synthesis. The cell cycle is thus blocked prior to s-phase. Regulation of this G1/S boundary is a critical checkpoint in the cell cycle and is potentially inhibited by p21.

The accumulation evidence demonstrated that certain cancer-derived mutant forms of p53 transactivate various target genes such as the multiple drug resistance gene 1(MDR1), c-myc, proliferating cell nuclear antigen (PCNA), interleukin-6 (IL-6), insulin-like growth factor 1(IGF-1), fibroblast growth factor (FGF) and epidermal growth factor receptor (EGFR).cyclin /CDK inhibition is sufficient for growth suppression of cells, but the p21 inhibitor may also interfere with DNA synthesis directly by binding to PCNA. It is also an essential factor in DNA replication. However, p21 levels can also be increased by a variety of other mechanisms independent of p53 transactivation, suggesting a p53 bypass strategy for therapeutic drugs that would engineer growth arrest in precancerous cells that have lost p53 function.

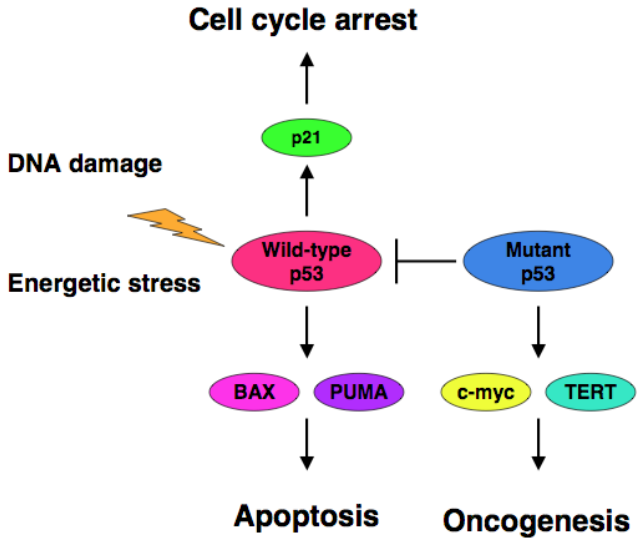

Fig 2

iii) Programmed Cell Death

In response to DNA damage, p53 can trigger exit from the cell cycle and chromosomal disintegration by an active enzymatic process of cell cycle or apoptosis. Wild-type p53 has been demonstrated to up regulate the transcription of a chimeric reporter plasmid utilizing the consensus promoter sequence of BAX approximately 50- fold over mutant p53.Thus it is likely that p53 promotes BAX's apoptotic faculties in vivo as a primary transcription –independent role in apoptosis.

Degradation of p53

i) Mdm2- dependent Ubiquination of p53

Under normal condition, p53 is present at a very low level through the ubiquitin proteasome dependent protein degradation system. A number of studies have shown that mdm 2 is the predominant and critical E3 ubiquitin ligase for p53 and mediates p53 ubiquitination through a RING domain. Mdm 2 ubiquitinates p53 at six key lysine residues located at the c-terminus of the protein, including k370, k372, k373, k381, k382 and k386. Resently, it has been described that MDM2 has a post-ubiquitination function for p53 degradation. *Lai et al.* Found that MDM2 monoubiquitinates p53. More resenlty, it was shown that p53 can also be ubiquitinated in vitro within the DNA binding domain as well. When this domain was removed, the overall ubiquitination and stability of p53 decreased, though these site also not sufficient for degradation.

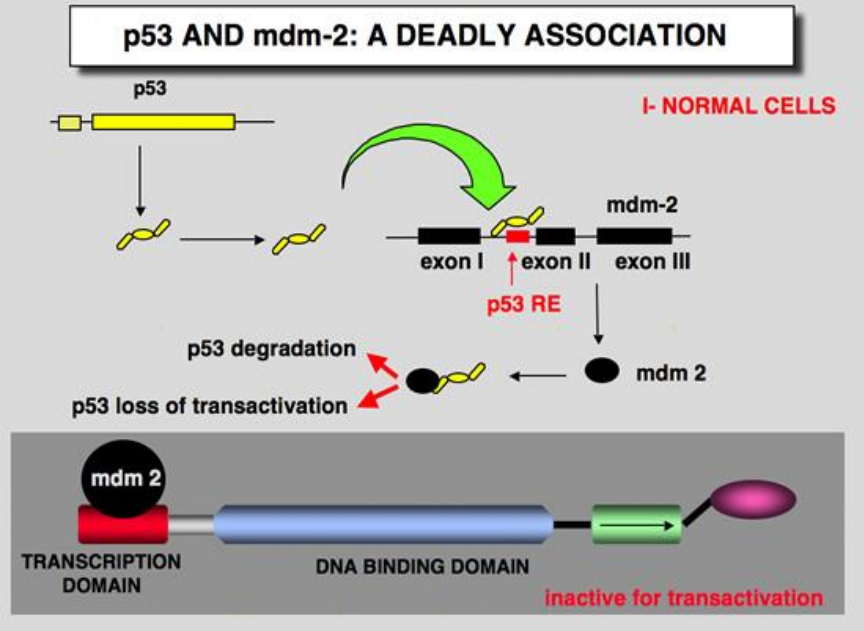

Fig 3: p53-mdm2 association

ii) Mdm2- independent ubiquination of p53

In addition to mdm2, Ring-Finger type E3 ubiquitin protein ligases pirh2 (p53 – induced RING H2 domain protein) and COP1 (constitutive photomorphogenic) also interact with p53 and mediate the ubiquitin / proteasome- dependent degradation manner. Remarkably, p53 was still degraded in the absence of mdm2, suggesting the presence of other mechanisms for p53 for p53 degradation. Indeed, a number of E3 ligases have been described for p53, including pirh2, COP1, ARF-BP1, WW P1, E4F1 AND ubc 13. Like mdm2, pirh2 and COP1 inhibit transcriptional as well as pro-apoptotic function of p53.

p53 Target Genes

P21 is a cyclin dependent kinase inhibitor that is capable of inhibiting all cyclin/CDK complexes it is represents as a p53- target gene products. *Harper et al* discovered p21 as a cdk2 binding protein.p21 tightly binds to active sites of the cdk2 and inhibits its kinase activity and block the phosphorylation of P^{RB} (retinoblastoma susceptibility gene product). Loss of RB gene function also is found in more common cancers that arise later in life (eg:- carcinomas of lung, breast and bladder). In response to cellular stresses, p53 induces G1 cell cycle arrest through the up-regulation of p21.

The key target of mdm2 is the tumor suppressor. It participates in a negative auto-regulatory feedback loop, which controls p53 expression level. Following DNA damage, phosphorylation of mdm2 leads to changes in protein function and stabilization of p53.

Since p53 dependent apoptosis is mediated by mitochondrial dysfunction, p53 inducible mitochondria proteins are of particular interest. *Selvakumaran et al* found that BAX (Bcl-2 associated X protein) is an immediate early p53-responsive gene. In mammalian cells, the majority of apoptosis, BAX is induced to undergo conformational change followed by formation of membrane-inserted homo-oligomers that results in the release of cytochrome C and other pro-apoptotic factors from the mitochondria, often referred to as mitochondrial outer membrane permeabilization, leading to activation of caspases. *Oda et al* identified $p53^{AIP1}$ as one of the p53 target gene products. It inhibits the proliferation og PC-3M cells, arrests cell at S/G2-M phase, decreases the abilities of invasion and migration and promotes cell apoptosis. PUMA has been identified as one of p53 target gene products. Increase in PUMA levels induces apoptosis through mitochondrial dysfunction.

Mutation in p53 gene

1) Germ-line mutation

Germ-line mutations in the p53 tumor suppressor gene have been observed in patients with Li-Fraumeni syndrome, brain tumors, second malignancices and breast cancer. The inability of the germ-line p53 mutants to block the growth of malignant cells can explain why patients with these germ-line mutations have an increased risk for cancer. Germ-line p53 mutations were initially reported in patients with the rare Li-Fraumeni syndrome, a family cancer syndrome and detected in pedigrees with a limited familial history and few available samples, the genetic analysis is sometimes impossible to perform.

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. 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 dinuleotides at positions 175,248,273 and 282.

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.

3) Viral inactivation of p53 protein

Infection with viruses introduces foreign DNA into the cell, p53, along with other proteins, is responsible for the cells

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. For example-SV40 is believed to suppress the transcriptional properties of the tumor-suppressing p53 in humans through the SV40 small T-antigen. P53 is responsible for initiating regulated cell death or cell cycle arrest when a cell is damaged. In vitro, the interaction of TP53 with cancer associated / HPV viral proteins lead to ubiquitiniation and degradation of TP53 giving a possible model for cell growth regulation.

P53 in cancer therapy

The therapeutic efficiency of anti-cancer agents depends strongly on their ability to trigger apoptosis in target cells. Since p53 plays a pivotal role in the regulation of cell fate in response to DNA damage, the therapeutic strategies which activate p53-mediated pro-apoptotic pathway and/ or eliminate the dominant-negative effect of mutant p53 on wild-type p53 should be required. 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.

P53 inhibition for cancer therapy

The inhibition of p53 can protect normal cells during genotoxic chemotherapy or radiation therapy. The small molecule pifithrin-alpha can block p53-dependent transcriptional activity and protect mice from the lethal side effects associated with anticancer treatment. Avoiding dose-limiting genotoxic stress to normal cells during chemotherapy or radiotherapy for cancer, it will thus allow a higher dose to be used for patients who are not sufficiently responsive to conventional chemotherapy.

Inhibiting the p53-MDM 2 interaction as a new therapeutic strategy

MDM2 binds to the NH_2-terminal region of p53 and inhibits its transcriptional as well as pro-apoptotic function. It also facilitates proteosomal degradation pathway of p53. *Vassilev et al*. Discovered the first potent and selective low molecular weight inhibitor of p53-MDM2 binding termed Nutlin. According to their results, Nutlin bound to MDM2 in the p53-binding pocket and blocked the interaction between MDM2 and p53., which resulted in the stabilization of p53 and also activation of p53- mediated pro-apoptotic pathway in cancer cells bearing wild-type p53. 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

The tumor suppressor gene is likely the most commonly mutated tumor suppressor gene in human cancer. Its functions include modulation of both cell cycle arrest and apoptosis. 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 in normal cells thereby diminishing therapy-related, p53- dependent toxicity and the other one is utilize a peptide derived from the c-terminus of p53 to activated wild type or mutant p53 proteins. 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 survical.

p53- based immunotherapy

Tumor-associated antigen- specific cytotoxic T lymphocyte can mediate immune response of host against cancer in vivo. Pre-clinical data 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. 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. 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. 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

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 concentration may have a prognostic value in early diagnosis of lung cancer. A detailed immunochemical analysis shows that protein p53 comprises a range of dominants against which specific antibody may be produced. Those antibodies can recognize both wild and mutated forms of p53. Statistical analysis showed that anti-p53 antibodies could be regarded as a specific biomarker of cancer process.

Conclusion

p53 is a tumor suppressor gene and it's located on the short arm of human chromosome 17. In normal cells, the p53 protein level is low. DNA damage and other stress signals may trigger the increase of p53 proteins, which have three major functions: growth arrest, DNA repair and apoptosis. 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 are the most common. Extensive mutation searches demonstrated that over 50% of human cancers carry the loss of function mutations in p53 gene.

Several therapeutic strategies have been employed in the attempt to restore p53 function to cancerous cell. 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. The development of novel strategies to re-activate mutant p53 is required to provide clues to effectively treat malignant cancers bearing p53 mutations.

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