The Role p53 in control of cancer

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CERTIFICATE

This research project has been written under my supervision and has been submitted for the award of the BSc. degree in Biology with my approval as a supervisor Dr.Shayan Rashid Abubakr

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DEDICATION

This effort I dedicate to Allah Almighty, my lord, my powerful foundation, my source of inspiration, wisdom, knowledge, and understanding. Throughout this project, he was the source of my energy.

Helen Faris Mustafa

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To begin with, I thank **(Allah)** for His blessing, which made me able to complete and perform this study with success, the lord of the universe, blessing, and peace be on **Muhammad** (Allah's peace and prayers be upon him).Finally, I want to say thanks to all those I forgot them here to mention his/her name, who assisted me even by one useful scientific word directly or indirectly.

ABSTRACT

Cancer is a family of diseases that exhibits uncontrolled cell division and tissue invasiveness (metastasis). The unregulated cell growth and metastasis are caused by mutations in the genes (DNA) of proteins involved in the regulation of cell cycle, and the agents causing DNA damage leading to subsequent transformation of a cell, are called carcinogens .Mutations in the TP53 (p53) gene are present in a large fraction of human tumours, which frequently express mutant p53 proteins at high but heterogeneous levels modification and recruitment of p53 to binding sites in chromatin. As a transcription factor, p53 mediates changes in gene expression that promote apoptosis, senescence or a reversible and protective cell cycle arrest. These mechanisms Mutation or functional inactivation of the tumour suppressor p53 is an almost universal feature of human cancer p53 pathway is triggered by a wide variety of damage signals, which lead to the stabilization, post-translational. The eliminate the damaged cells and suppress tumor genes The name ‘p53’ comes from the apparent molecular mass of the protein. On SDS-PAGE, it runs as a 53-kilodalton (kDa) protein, but, based on calculations from its amino acid residues, p53's molecular mass is actually only 43.7 kDa. This difference in molecular mass is due to the high number of proline residues in the protein, which slows its migration on SDS-PAGE, thus making it appear heavier than it actually is . This effect is observed with p53 from other species too, including humans, rodents, frogs, and fish.

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INTRODUCTION

Mutations in the evolutionarily conserved codons of the p53 tumor suppressor gene are common in diverse types of human cancer. The p53 mutational spectrun differs among cancers of the colon, lung, esophagus, breast, liver, brain, reticulo endothelial tissues, and hemopoietic tissues. Analysis of these mutations can provide clues to the etiology of these diverse tumors and to the function of specific regions of p53. Transitions predominate in colon, brain, and lymphoid malignancies, whereas G:C to T:A trans versions are the most frequent substitutions observed in cancers of the lung and liver. Mutations at A:T base pairs are seen more frequently in esophageal carcinomas than in other solid tumors. Most transitions in colorectal carcinomas, brain tumors, leukemia s, and lymphomas are at CpG dinucleotide mutational hot spots. G to T trans versions in lung, breast, and esophageal carcinomas are dispersed among numerous codons. In liver tumors in persons from geographic areas in which both aflatoxin B1 and hepatitis B virus are cancer risk factors, most mutations are at one nucleotide pair of codon 249. These differences may reflect the etiological contributions of both exogenous and endogenous factors to human carcmo genesis (Hollstein et al,.1991) .

similar to MDM2, MDM4 cannot mediate ubiquitination and inhibits p53 by direct interaction MDM4 also binds MDM2 to form a heterodimer with enhanced ability to ubiquitinate p53 . MDM2 keeps With the better insight about malignant transformation of cells, it has been easy to elucidate that tumor progression is a multistep process which involves several defined events common to cancer cells . Genes, controlling cell cycle events limit cell multiplication by activation of anti-proliferative mechanisms which leads to arrest of cell cycle or apoptosis. Mutations of these genes lead to autonomous cell growth. This, along with metastasis adds more aggression to the armoury of cancer. In the race of finding medical artillery to kill cancer, Science discovered a very crucial gene in

the body, later named as, ‘TP53 gene’. Like the drugs which reduced the ascetic fluid volume were seen as a good anticancer agent and thus purveyed a new technique for targeting cancer, emergence of TP53 gene provided a whole new arsenal to combat cancer. The p53 protein, coded by TP53 gene, is an inducible transcription factor that plays multiple anti-proliferative roles in response to exposure to DNA damaging stress.In physiological context, condition of p53 controls the sensitivity of cells to environmental mutagens. In pathological context, the status of p53 is considered as a key factor in response of cancer cells towards cytotoxic therapies. Thus, p53 functions for the genetic homeostasis of the cells exposed to mutagens (MISHRA et al ,.2013) .

p53 activity is controlled by regulation of its protein levels, a process mediated primarily by the ubiquitin ligase mouse double-minute 2 (MDM2), which targets p53 to the proteasome for degradation . As MDM2 is itself a p53 target gene, induction of its expression by p53 results in a negative feedback loop by which both proteins are returned to basal levels after p53 activation. Another key regulator of p53 is the MDM2-related protein MDM4, also known as MDMX . Although structurally p53 protein levels low in normal, unstressed cells because p53 activity must be constantly suppressed, otherwise the consequent uncontrolled apoptosis and cell cycle arrest will result in death, even in the absence of any stress or damage (Coffill and Lane,2011).

P53 PATHWAY AND ITS REGULATION

p53 plays a significant role in apoptosis, genomic stability and inhibition of angiogenesis. It also executes anticancer function by the virtue of different mechanisms as follows:

Activation of DNA-repair proteins when DNA suffers damage.

Initiation of apoptosis, the Instigates growth arrest by holding the cell cycle at the G1/S regulation point, thus giving ample time to the DNA-repair proteins to fix the DNA damage, and subsequent continuation of the cell cycle .programmed cell death, if in case, the DNA damage proves to be irreparable.p53 exists in inactive state in normal cells due

to the presence of its negative regulator, mdm2. However, on DNA damage (by ionizing radiation, UV radiation, application of cytotoxic drugs or chemotherapeutic agents, and infectious virus) or other stresses such as heat shock, hypoxia, oxidative stress, osmotic shock, ribonucleotide depletion, and deregulated oncogene expression, p53

Two major events causing the activation of p53 can be highlighted as follows:

Drastic increment in the half-life of the p53 protein, leading to a its accumulation in stressed cells.

A conformational change forcing p53 to be activated as a transcription regulator in these cells

TARGETTING P53

More than 50% cancers involve mutations in TP53 gene, this itself underlines the fact that TP53 gene or even p53 protein can provide a link to treat cancer in many cases. Hence, targeting TP53 or p53 is important clinically.

Gene therapy

Loss of potency of TP53 has led to the occurrence of many cancers. Hence, restoration of p53 functioning by replacing the mutant gene with a functional wild-type copy, seemed to be an exciting approach towards the targeting of p53. This exciting approach is utilised and executed by Gene therapy, which in turn, depends upon the efficient delivery of the wild-type TP53 into tumor cells in vivo. Scientists have also proposed various in vitro strategies to restore the tumor suppressing function of p53 in cancer cells.

TP53-gene therapy mediated by Retrovirus

Retroviruses, by the virtue of their unavoidable qualities of getting integrated in a stable form into the genome of infected cells and requiring cell division for the transduction, pose to be an obvious candidate for gene therapy. Retrovirus-mediated gene transfer of the wild-type TP53 gene into both human lung tumor cell lines and xenograft models has

been shown to inhibit the tumor cell growth .So far, no molecule has induced biological response, but it is believed that some may prove to be lead compounds for more biologically active agents, when processed further. A promising target for anti-cancer drugs is the molecular chaperone Hsp90, which interacts with p53 in vivo . TP53-gene therapy mediated by Adenovirus Unlike retrovirus, adenovirus effect is not limited to actively proliferating cells. Hence, these large, double-stranded DNA viruses capable of high transduction efficiency form a second strategy to TP53 gene replacement therapy . Adenoviruses, by secreting certain proteins, compel the host to replicate them. Due to their inability of not getting integrated into the genome, adenoviruses exhibit no risk of insertional mutagenesis. In the 1960s, oral adenoviral vaccines were given to thousands of military recruits without increase in cancer risk p53 inhibitors Pifithrin, a synthetic compound, rescues p53 cells from apoptotic death induced by irradiation and various cytotoxic drugs including doxorubicin, etoposide, paclitaxel and ara-C . Thus, pifithrin may be used to suppress the side effects of radiation therapy or chemotherapy in cancer patients. However, pifithrin could act as an activator of the p53 pathway promoting doxorubicin-induced apoptosis in mouse epidermal JB6 C141 cells (MISHRA et al ,.2013) .

REVIEW OF LITERATURE

The genetic basis of cancer development has only been established recently based on the evidence that familial, epidemiologic, and cytogenetic studies have provided over the last quarter century. The current understanding shows that cancers is a multistage process in which mutations (both inherited and somatic) of cellular genes lead to clonal selection of variant progeny with the most robust and aggressive growth properties. Two classes of genes, are targets for the mutations, i.e. Proto-oncogenes and Tumor Supressor Genes

Proto-oncogenes:

Proto-oncogenes have critical roles in a variety of growth regulatory pathways, and their protein products are distributed throughout many subcellular compartments. The oncogenic variant alleles present in cancers have sustained gain-of-function alterations resulting from point mutations, chromosomal rearrangements, or gene amplifications of the proto oncogene sequences Whereas oncogenic alleles harbour activating mutations, tumor-suppressor genes are defined by their inactivation in human cancer . (Holland, 2003)

Tumor-suppressor genes:

A tumor suppressor gene, called as an anti-oncogene, by Knudson, is a gene that protects a cell from developing cancerous properties. When this gene is mutated to cause a loss or reduction in its function, the cell can progress to cancer, usually in combination with other genetic changes. Some of the epithets used for tumor suppressor genes are the _gatekeeper‘, Caretaker‘ and Landscape(Macleod 2000) .

They are called the gatekeepers because, first, their loss of function is rate-limiting for a particular step in multi-stage tumor genesis; second, they act directly to prevent tumor growth, and third, restoring _gatekeeper‘ function to tumor cells suppresses neoplasia. Ex. Adenomatous Polyposis Coli (APC).Kinzler and Vogelstein subsequently qualified the

_gatekeeper‘ definition of tumor suppressor genes to include all direct inhibitors of cell growth (suppressing proliferation, inducing apoptosis or promoting differentiation). This allows us to define genes such as p53 as a _gatekeeper‘, albeit as a _progression gatekeeper By contrast, _caretaker‘ tumor suppressor genes act indirectly to suppress growth by ensuring the fidelity of the DNA code through effective repair of DNA damage or prevention of genomic instability (such as microsatellite or chromosome instability). As such, a large number of _caretaker‘ tumor suppressor genes are DNA repair genes. Loss of _caretaker‘ function predisposes to cancer by increasing the DNA mutation rate, thereby increasing the chances that _gatekeeper‘ gene function will be lost. Mutation in both alleles of a _caretaker‘ gene requires mutations in both alleles of gatekeeper genes to be functional whereas, gatekeeper mutation does not require caretaker mutation. Mutations in tumor suppressor genes are recessive; that is, as long as the cell contains one normal allele, tumor suppression continues. (Oncogenes, by contrast, behave as dominants; one mutant, or overly-active, allele can predispose the cell to tumor formation) (Macleod 2000) .

Tumor suppressor pathways:

Cells escape growth control by targeting key oncogenes/ tumor suppressor in molecular pathways. These pathways have evolved to integrate positive and negative growth signals according to cellular function and microenvironment during normal development and tissue repair. The RB pathway (RB/p16INK4a/cyclin D1) and the p53 pathway (p19ARF/mdm2/p53) are both frequently targeted in tumor genesis and the mutation occurring in each pathway depends on the tumor type.(p53 and cancer)

P53

the p 53 tumor suppressor gene has been found to be mutated in more than 50% of human cancers, it has attracted the interest of numerous researchers. The capacity of p53 for multiple biological functions can be attributed to its ability to act as a sequence-specific transcription factor to regulate expression of over one hundred different targets, and thus to modulate various cellular processes including apoptosis, cell cycle arrest and DNA repair. The p53 protein with its unique C- and N-terminal structures is rigidly modulated by several important biological processes such as phosphorylation, acetylation and ubiquitination, through which it effectively regulates cell growth and cell death. p 5 3 mutations can lead either to loss or change of p53 binding activity to its downstream targets and may thus induce aberrant cell proliferation, with consequent malignant cellular transformation. Based on p53's critical role in carcinogenesis, scientists have developed multiple effective strategies for treating cancer by enhancing function of wild-type p53 or increasing p53 stability (Bai and Zhu, 2006).

The p53 gene

Human Chromosomal Location: 17p13.1The p53 gene encompasses 20kb of DNA with 11 exons which on transcription gives a 3.0 kb mRNA having 1179bp open reading fra me. On translation, this mRNA produces a 53kDa protein (hence the name p53) (Bai and Zhu, 2006).

The p53 protein and structures

Wild-type p53 protein contains 393 amino acids and is composed of several structural and functional domains (Figure 1): a N-terminus containing an amino-terminal domain and a proline-rich region with multiple copies of the PXXP sequence where X is any amino acid), a central core domain, and a Cterminal region containing an oligo merization domain, a strongly basic carboxyl terminal regulatory domain , a nuclear localization signal sequence and 3 nuclear export signal sequence . The amino-terminal domain is required for transactivation activity and interacts with various transcription factors including acetyltransferases and MDM2 (murine double minute 2, which in humans is identified as Hdm2). The proline-rich region plays a role in p53 stability regulated by MDM2, wherein p53 becomes more susceptible to degradation by MDM2 if this region is deleted . The central core of this protein is made up primarily of the DNA-binding domain required for sequence-specific DNA binding (the consensus sequence contains two copies of the 10-bp motif 5'-PuPuPuC(A/T)-(T/A)GPyPyPy-3', separated by 0-13 bp). The basic C-terminus of p53 also functions as a negative regulatory domain , and has also been implicated in induction of cell death . According to the allosteric model, in which C-terminal tail of p53 was considered as a negative regulator and may regulate the ability of its core DNA binding domain to lock the DNA binding domain as an latent conformation. If the interaction between the Cterminus and the core DNA binding domain is disrupted by posttranslational modification (such as phosphorylation and acetylation), the DNA binding domain will become active, thus induce an enhanced transcriptional activity. The central region of p53 is its most highly conserved region, not only when p53 is compared with its homologues from D r o s o p hila and C a e n o r h a b d itis el e g a n s , but also as compared with its mammalian family members, p63 and p73 . Structural studies of p53 have revealed that the majority of p 53 mutations found in cancers are missense mutations that are mostly located in the central DNA-binding domain, and more than 80% of p 53 mutation studies have focused on residues between. In the p53 family, both p73 and p63 show considerable homology with p53 and have

similar domain structures including an oligo merization domain, with over 60% amino acid identity within the DNA binding region, and all three of these proteins can induce apoptosis. However, at the same time there are many structural and functional differences between p53 and its other two family members. Because space in this review is limited, a description of the differences between p53 and its homologues will not be detailed in this review .

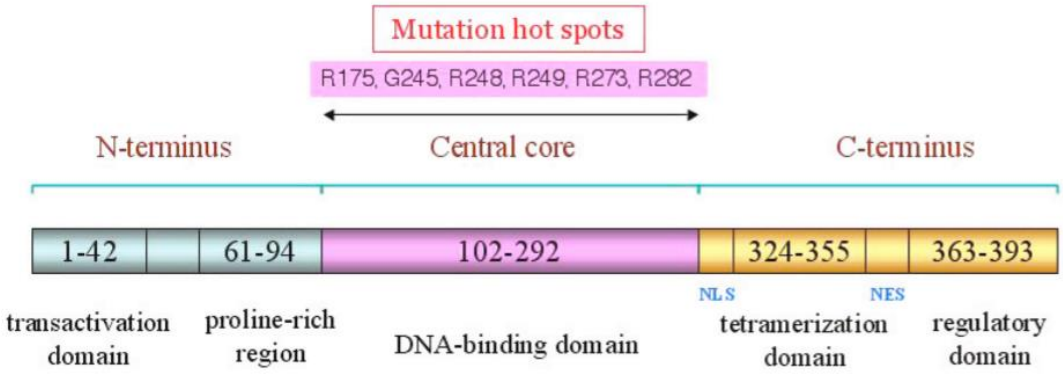

Figure 1: Schematic representation of the p53 structure. p53 contains 393 amino acids, consisting of three functional domains, i.e. an N terminal activation domain, DNA binding domain and C-terminal tetra merization domain. The N-terminal domain includes transactivation subdomain and a PXXP region that is a proline-rich fragment. The central DNA binding domain is required for sequence-specific DNA binding and amino acid residues within this domain are frequently mutated in human cancer cells and tumor tissues. The Arg175, Gly245, Arg248, Arg249, Arg273, and Arg282 are reported to be mutation hot spots in various human cancers. The C-terminal region is considered to perform a regulatory function. Residues on this basic C-terminal domain undergo posttranslational modifications including phosphorylation and acetylation. Numbers indicate residue number. NLS, nuclear localization signal sequence; NES, nuclear export signal sequence (Bai and zhu,.2006).

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 trans dominant tumor suppressor that some level of normal growth to cancerous cells in vitro.

4. p53 is a potent transcription factor and once activated, represses transcription of one set of genes (several of which itare 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 (George,2011).

Activation of p53:

p53 is activated, among others, in response to DNA damage, and many factors interact to signal and modulate this response. There is still controversy over the pathways that lead to the activation of p53. Several mechanisms have been suggested: One idea is that stress-activated protein kinases phosphorylate p53, protecting it from degradation and activating its function as a transcription factor. Indeed, many phosphorylated forms of p53 are found in cells, and by phosphorylation p53 can be released from a latent state, in which it cannot bind DNA. One attractive candidate for p53 activation by phosphorylation is the DNA-dependent protein kinase (DNA-PK). DNA-PK is activated by DNA damage, and one of its substrates is p53[8]. Instead of its phosphorylation, the de phosphorylation of p53 at serine 376 by the ATM-dependent activation of a specific phosphatase might enable DNA binding of p53 and its transcriptional activation. In this process, the so called 14-3-3 proteins bind to the C-terminus of the dephosphorylated p53, and by this possibly activate it. Another pathway towards activation of p53 involves the mdm-2 gene product. MDM-2 can target p53 for nuclear export and degradation; non functional MDM-2 results in accumulation of p53 and activation of p53-dependent transcription. The mdm-2 gene itself is activated for transcription by p53, so this model implies that p53 is constitutively active, driving transcription of the protein (MDM-2) that targets its own degradation. Blocking the p53 degradation pathway would result in the activation of the p53 response. Indeed, it was shown that the ARF tumor suppressor (also called p14ARF) binds to the complex of p53 and MDM-2, by this stabilizing p53, possibly by inducing degradation of MDM-2 . ARF expression itself is regulated by the E2F-1 transcription factor! This connects the Rb pathway to p53: oncogenes like E1A or SV40 T block Rb function, thus activating E2F-1. E2F-1 transcriptional activity leads to the expression of a number of genes required for passage into and through S phase but also to the expression of ARF

which stabilizes p53. This would result in either p53 dependent apoptosis or cell cycle arrest unless p53 itself is inhibited.(S Benchimol,2001).

Cell – cycle regulation

Among various cellular responses induced by p53, most notable are the induction of cell cycle arrest and apoptosis. It appears that the ability of p53 to prevent cell growth is pivotal to its tumor suppressor functions. p53 can induce cell cycle arrest in the G1, G2 and S phases of the cell cycle (Agarwal et al .,2001).

The induction of cell cycle arrest at G1 and G2 by p53 provides additional time for the cell to repair genomic damage before entering the critical stages of DNA synthesis and mitosis. The arrested cells can be released back into the proliferating pool through p53's biochemical functions that facilitate DNA repair including nucleotide excision repair and base excision repair. A cyclin-dependent kinase (CDK) inhibitor, p21waf1/Cip1 is perhaps the best known downstream target of p53 among the various p53 target gene products identified. p21waf1/Cip1 is a primary mediator of p53-dependent G1 cell cycle arrest following DNA damage. In response to cellular stresses, p53 upregulates endogenous p21waf1/Cip1 mRNA and protein levels p21waf1/Cip1 binds cyclin-CDK complexes through the ZRXL motif. Overexpression of p21waf1/Cip1 induces G1 arrest by blocking cyclin E/CDK2-mediated phosphorylation of Rb and release of E2F which functions to induce expression of genes required for S phase entry. This response is also governed by other p53 target gene products, as seen for example, in the increased expression of Gadd45 and 14-3-3δ that participate in p53-driven G2 arrest. Gadd45 binds to CDC2 (i.e. CDK1), preventing cyclin B/CDC2 complex formation and subsequently inhibiting the kinase activity (Zhan et al .,1999).

A scaffold protein 14-3-3δ removes cyclin B/CDC2 from the nucleus to physically separate cyclin B/CDC2 from its target proteins. Overexpression of 14-3-3δ induces G2 arrest.

Induction of apoptosis

As a cellular gatekeeper, one of roles of p53 is to monitor cellular stress and to induce apoptosis as necessary. In tissues where stressors generate severe and irrevocable damage, p53 can initiate apoptosis, thereby eliminating damaged cells. Studies with flies and nematodes have shown that induction of cell death following genotoxic challenges appears to be a function of p53, whereas in higher organisms p53 activity is acquired for cell growth arrest. Apoptotic gene products which are induced by p53 include Bax (Bcl-2-associated X protein), DR5/KILLER (death receptor 5), DRAL, Fas/CD95 (cell-death signaling receptor), PIG3 (p53-inducible gene 3), Puma (p53-upregulated modulator of apoptosis), Noxa (from the Latin word for "harm" or "damage")[66], PIDD (p53induced protein with death domain)[67], PERP (p53 apoptosis effector related to PMP-22), Apaf-1 (apoptotic protease-activating factor-1), Scotin (Bourdon et al., 2012).

p53AIP1 (p53regulated apoptosis-inducing protein 1), and others. The p53 associated apoptotic targets can be divided into several groups based on their functions and their executed pathways (Figure 3). The products of these genes may induce apoptosis through either an extrinsic pathway or an intrinsic pathway, namely the death receptor pathway and the mitochondrial pathway, respectively. The intrinsic apoptotic pathway is engaged when cells are challenged by stress and is dominated by the Bcl-2 family proteins .The Bcl-2 family proteins are composed of three classes: anti apoptotic proteins Bcl-2 and Bcl-XL, pro-apoptotic proteins Bax, Bak and Bcl-Xl , and pro-apoptotic "BH3-only" proteins Bid (BH3-interacting death agonist), Bad, Noxa, and Puma (Haupt et al.,2003).

In the regulation of the intrinsic pathway, pro-apoptotic gene products such as Bax, Bid, Puma, Noxa, and p53AIP1 localize to the mitochondria and promote the loss of mitochondrial membrane potential and release of cytochrome c , resulting in the formation of the apoptosis some complex with Apaf-1 and caspase 9. These apoptosis-related gene products mentioned above are closely associated with p53 function. Bax was the first identified p53-regulated pro-apoptotic Bcl-2 family member , and p53-responsiv (Jeffers et al.,2003).

elements have been unequivocally identified in the b a x gene . Bax is specificlly required for Puma-mediated apoptosis, and it also participates in the death response as an indirect target of p53 through Puma (Jeffers et al.,2003) .

The requirement for Bax in p53-mediated apoptosis appears to be cell-type dependent. Loss of Bax is responsible for nearly half of the accelerated tumor growth in brain tumors that is related to loss of p53 function Bax also accounts for nearly half of p53-dependent apoptosis induced by 5-fluorouracil (5-FU) in colorectal cancer cells (Zhang, 2000).

On the other hand, Bax is dispensable for the apoptosis induced by irradiation in thymocytes and intestinal epithelial cells .The first evidence which suggested that mitochondria might be involved in p53-dependent apoptosis was the observation that Bcl-2 protected cells from p53dependent apoptosis . Several Bcl-2 family proteins and mitochondrial proteins such as Puma, Noxa, p53AIP1, and PIGs are implicated in p53-dependent apoptosis. They are activated in a p53-dependent manner following DNA damage. Puma induces very rapid apoptosis, which occurs within hours following its expression .p53AIP1 can cause mitochondrial membrane potential dissipation by interacting with Bcl-2. p53 also regulates the genes encoding Apaf1, a key component of the apoptosome , and PIG3, which may cause mitochondrial depolarization . Nevertheless, activated p53 can directly or indirectly modulate the expression of its targeted proteins and other proteins that control mitochondrial membrane permeability, and can therefore modulate the release of mitochondrial proteins which further carry out

apoptosis. Another p53-related class of pro-apoptotic gene products is the components of the death receptor-mediated extrinsic pathway. In this cell death pathway, p53 can promote apoptosis through activation of the death receptors located at the plasma membrane, including Fas/CD95 , DR4 andDR5 , and lead to inhibition of the production of IAPs(inhibitor of apoptosis proteins). Both DR5 and DR4 can trigger or induce apoptosis by TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), Fas ligand and che-motherapeutic agents and Fas is indispensable for p53-dependent apoptosis in most tissues . p53 may also induce apoptosis via an endoplasmic reticulum-dependent mechanism by trans activating the expression of Scotin, a protein located in the endoplasmic reticulum and in the nu-clear membrane . It has been suggested that the intrin-sic apoptotic pathway is primarily utilized in p53-mediated apoptosis, whereas the extrinsic pathway is used to aug-ment the apoptotic response .p53 can also promote apoptosis through transcription in- dependent mechanisms (including direct shuttling of p53 to the mitochondrial membrane). p53 probably has direct apoptotic activity in the absence of transcription or protein synthesis under certain conditions and in certain cell types . In mitochondria, p53 directly binds to Bcl-XL/Bcl-2 to displace Bax or BH3 domain-only pro-apoptotic proteins, and thus facilitates Bax-dependent mitochondrial apoptotic changes . p53 can also bind to mitochondrial Bak and induce Bak oligo merization, which facilitates the release of cytochrome c after permeabilization of the mitochondrial membrane (Liu, 2004)

This is a very rapid response (30 min), which precedes the transcriptional response (taking at least 2 h)[18This direct response is tissue-specific and appears to be limited to radiosensitive tissues .While cell cycle arrest can function to inhibit the growth of normal cells, it seems that cells which have attained onco-genic activation are less susceptible to such inhibition .The ability of p53 to induce apoptosis appears to be well correlated with its ability to suppress malignant transforma- tion. Loss of p53-dependent apoptosis accelerates mouse brain tumori genesis . Similarly, the observation that mice harboring

the p53 R172P mutant develop many tumors may be due to lack of p53-induced apoptosis (Zhang et al., 2000) .

These results reveal that regulation of apoptosis is an important and evolutionarily conserved tumor suppressor function of p53(Erster et al ., 2004) .

It appears that transcriptional factors such as c-Myc, JMY (junction-mediating and regulatory protein), ASPP (Apop- totic-Stimulating Protein of p53) family, p63, and p73 can influence the balance between cell cycle arrest and apopto- sis (Yu and Zhang, 2005).

A crucial balance between Puma and p21Waf1/Cip1 has been identified which determines the onset of arrest or death in response to exogenous p53 expression in human colorec- tal cancer cells Growth arrest through activation of p21Waf1/Cip1 is the normal response to p53 expression in these cells. If p21Waf1/Cip1 is disrupted, cells die through apoptosis. However, when Puma is disrupted, apoptosis is prevented. Cell cycle arrest is not only a positive element of the p53 response, but is also a negative element for p53-dependent apoptosis in some situations. Induction of apoptotic genes alone is sometimes not sufficient to induce apoptosis, as the high levels of cell cycle inhibitors may dominant and lead to cell cycle arrest Apoptotic response can be enhanced through abolition of cell cycle arrest (for instance by sup- pression of p21Waf1/Cip1 or 14-3-3δ). Following p53 expression or DNA damage in colorectal can8cer cells, apoptosis is in- hibited through cell cycle arrest mediated by p21Waf1/Cip1 and/or 14-3-3δ . If p21Waf1/Cip1 or 14-3-3δ is removed from these cells, cell death rather than cell cycle arrest may result. When Puma is removed, these cells become resistant to apoptosis . Under certain conditions, cell cycle arrest protects cells from apoptosis. However, under other cir-cumstances, cells undergo apoptosis.

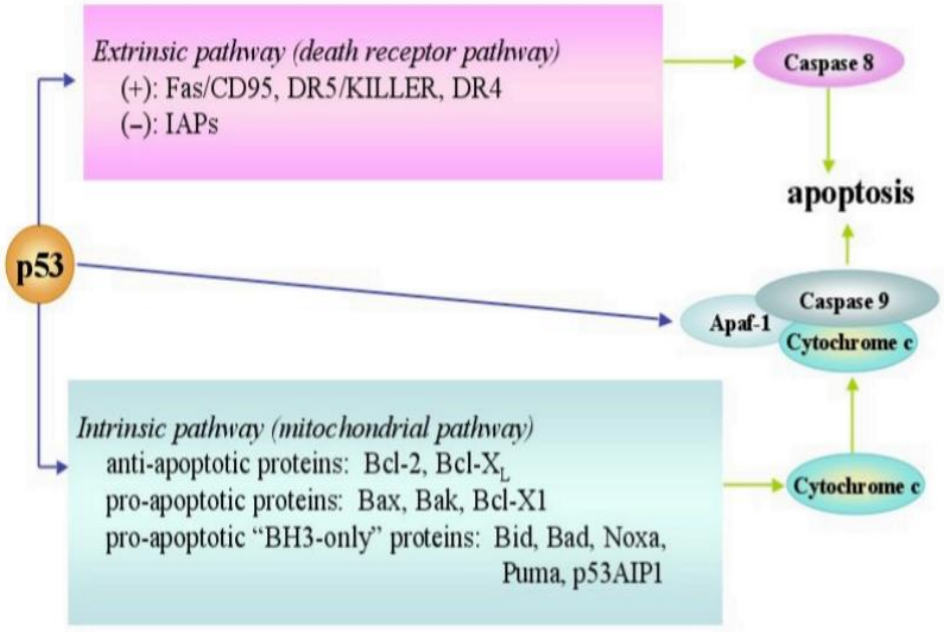

Fig.2: p53-associated genes and pathways involved in apoptotic cell death. p53 induces apoptosis mainly via two pathways: extrinsic andintrinsic pathways. The p53-associated extrinsic pathway is mainly executed by activating caspase 8 to induce apoptosis, whereas the p53-associated intrinsic pathway is executed by influencing mitochondrial proteins, by which activate caspase 9 to induce apoptosis. In addition, p53 may directly activate Apaf-1 to induce apoptosis.(Liu et al ., 2004).

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 r ecessive mutations. Dominant gain-of

function mutationshave also found that lead to uncontrolled cell division. Because these mutations can be expressed in heterozygous conditions, they areoften associated wi th 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 uncont rolled cell growth. Therefore, preventing cell division until damaged DNA is repair ed 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, ce rvix, colon, lung, liver, prostrate, and skin. p53 related cancers are also more agg ressive and have a higher degree of fatalities. P53 was originally viewed as an oncogene, but during t8he past several decades it has come to be understood to be a tumor suppressor gene. During this time, many p53 family transcriptional targets have been identified as having the capacity to modulate various cellular processes including growth arrest apoptosis, , differentiation, and DNA repair. In fact, it has become evident that this small 53-kDa tumor suppressor is a molecular node at the cross roads of an extensive and complex network of stress response pathways. Deregulation of p53 has enormous influence oncarcino genesis as mt p53 can induce an increased epige-netic instability of tumor cells facilitating and accelerating the evolution of the tumor.Understandingthemechanismsofp53'sfunctioniscurrenMany obstacles remain to o ptimize these strategies for use in humans, but, despite these, restoration of p53 fun ction is a promising anti-cancer therapeutic approach.

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