

**Appendix D - UNIVERSITY HONORS PROGRAM
SENIOR PROJECT - APPROVAL**

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PROJECT TITLE: p53's Role in Tumor Suppression

I have reviewed this completed senior honors thesis with this student and certify that it is a project commensurate with honors level undergraduate research in this field.

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p53's Role in Tumor Suppression

Abstract

The p53 protein is involved in many varied cellular processes including DNA repair, recombination, differentiation, and senescence (Bates and Vousden, 1999). This review concerns with the tumor suppression functions of the protein through cell arrest and apoptosis. The most well known function of p53 is its transcriptional activity. The protein has a sequence-specific DNA binding domain which mediates this process. This binding causes the transactivation of genes like Bax, p21, and MDM2 which are important players in the tumor suppression function of the protein. This review will deal with how p53 is recruited, the role of p53 in causing cell cycle arrest and apoptosis, and how the decision of which pathway to follow is made. Some historical and background information will be provided and the discussion will be concluded with some possible uses of p53 in the clinical treatment of cancer. Most of the experiments cited in this paper used either mice or human tumor cell lines, which were deficient in wild type p53, in the hopes that some common mammalian model may emerge that can provide insights into the role p53 plays in tumor suppression.

Introduction

In recent study by the CDC and the National Centers for Health Statistics, it was estimated that 541,859 deaths in America were contributed to malignant neoplasms (National Centers for Health Statistics, 2001). This means that approximately twenty-three percent of all recorded deaths were the result of cancer, making it the number two killer of Americans. It is no wonder why so much energy has been focused toward how

cancer is caused and how we can treat this killer. An emerging player in the study of cancer is a protein known as p53. This protein has gotten much attention because it has been estimated that between twenty and fifty percent off all cancer cases have a mutated p53 gene. The protein that this gene codes for has many functions within the cell and its properties could make it a very effective player in cancer treatment.

I. History of p53

The p53 protein was first identified as a protein that precipitated out of cells along with the SV40 large T antigen (Maxwell et al, 1995). It was a phosphoprotein with a molecular weight of 53,000. The protein was initially thought to have oncogenic properties because it could immortalize cells in culture and could cooperate with the activated *ras* oncogene to transform cells in culture (Maxwell et al, 1999). It was also initially found that over expression of the p53 protein enhanced the transformed phenotype of tumor cells. Because of these early findings, it was thought that p53 would actually cause cancer. Upon further investigation, however, it was found that all of the p53 discovered had actually been mutated versions of the wild type p53 tumor suppressor. The structure of p53 consists of three domains (Fig. 3.). The N-terminal domain is responsible for the transactivation of p53 by other proteins (Choisy-Rossi et al, 1998). The central domain is responsible for the sequence specific DNA binding of p53. The C-terminal domain is responsible for the oligomerization of p53 and the possible negative regulation of p53. The theories of the role of the C-terminal domain are discussed below.

II. Background

To fully understand the effects of p53 on the cell and its functions we must discuss the processes that serve as the backdrop to the protein's function. The p53 protein helps regulate cycles that are continuously occurring within the cell. Completely comprehending p53's effects requires understanding of the cell cycle, the apoptotic pathway, and how p53 behaves when there is no cellular stress.

A. Cell Cycle

Since p53 is a known tumor suppresser, one must realize how these tumors begin to form. Tumors form when cells lose the ability to control the normal process of cellular growth and division. This regulated growth and division cycle is most commonly known as the cell cycle (Fig. 1.). The cell cycle consists of four stages. The mitotic, or M, stage is the point in the cell cycle in which the cell divides. The following phase is one of two phases in the cell cycle in which the cell's organelles and cytoplasm grow. This first growth phase, G1, is the longest of the four phases and can be extended by arresting the cell in senescence. Muscle and neuronal cells can be placed in senescence permanently. The replication or synthesis of a new copy of the cells DNA characterizes the next stage, synthesis, or S phase. The second growth phase, G2, is responsible for the growth and division of organelular components of the cell. Another mitotic division and another cell cycle follow this final stage. (Voet and Voet, 1995)

The above process needs tight regulation to avoid over-replication and tumor formation. The progression from G1 to S and G2 to M provide the checkpoints for most of the cell cycle's progression (Bird, 1996). This regulation comes in the form of cyclin-dependent kinases (CDK). The CDK are composed of the catalytic kinase unit and a

cyclin cofactor and are only active when the cofactor is present and the kinase is in its proper phosphorylation state (Voet and Voet, 1995). The prototypical example CDK and its activity is the cyclin B-p34 complex. Once the CDK is active, it will phosphorylate and activate specific proteins which will propel the cell cycle forward. This paper will be concerned mostly with the G1 to S transition. Cyclin E-cdk2 complex and the cyclin D₁,₂, or₃-cdk4, 5, or 6 complexes are responsible for regulation of this progression (Bird, 1996). These complexes phosphorylate Retinoblastoma protein (Rb) which will in turn release E2F. E2F is a transcription factor that transcribes proteins such as dihydrofolate reductase, thymidine kinase, and DNA polymerase α , which are required to begin S phase (Bird, 1996). Release of E2F along with activation other proteins via phosphorylation will cause the cell to progress into S phase. p53's activity at this checkpoint will be discussed below in greater detail.

B. Apoptosis

A key function of p53 is apoptosis (Fig. 2.). Some general information about the apoptotic pathway should be mentioned to help us understand what role p53 has in this process. Apoptosis seems to rely on proteins known as caspases (Hengartner, 2000). The proteolytic activity of these caspases is critical to the initiation of apoptosis. Caspases are present in the cell in inactivated forms called procaspases (Hengartner, 2000). These procaspases are activated in one of two ways. The first activation pathway occurs via a death receptor (Bates and Vousden, 1999). The death receptor molecule is a receptor protein that resides on the condemned cell's membrane. For the receptor to become activated, it must bind to an extracellular molecule that is released when death needs to be promoted. The cell death receptor complex has a proteolytic activity, which

causes a procaspase 8 molecule to be cleaved and thus activated (Hengartner, 2000). The second method of activating caspases relies on signals from the mitochondria. Cellular signals, including those of p53, cause the mitochondria to lose membrane potential and release cytochrome c into the cytoplasm (Bates and Vousden, 1999; Hengartner, 2000). The free cytochrome c binds to a different caspase molecule, procaspase 9 and its cofactor, Apaf-1, to form the activated caspase 9 (Soengas et al, 1999). Several of the activated caspase 9 molecules come together possibly along with other proteins to form an apoptosome (Hengartner, 2000). Caspase 8 and the apoptosome are able to cleave themselves and other procaspases like caspase 3, 6, and 7 (Hengartner, 2000). This results in a caspase cascade which enhances the apoptotic pathway.

These caspases are the primary proteins involved in proteolytic cleavage of substrates in apoptosis. The cleavage of specific proteins by caspases can either activate cell destruction enzymes like CAD and PAK-2, or destroy existing proteins like Lamin, fodrin, or gelsolin (Hengartner, 2001). CAD is a DNase which will clip DNA in to thousands of fragments. PAK-2 causes the blebbing seen in dying cells. Cleavage of Lamin results in the shrinking and budding of the nucleus. Fodrin and gelsolin are cytoskeletal elements whose destruction leads to the cells loss of shape. These effects along with others are the main causes of cell death and are characteristic of the apoptosis pathway.

C. p53 in Normal/Healthy Cells

This report deals with the function of p53 in cells that are under some kind of stress, but what happens to p53 in normal cells. In a healthy cell p53 is present in the nucleus of the cell in very small amounts. The p53 protein is bound by MDM2, a protein

transcribed by p53 in a negative feedback loop. MDM2 is an E3 ligase which means that it can attach ubiquitin molecules to proteins, including p53. This ubiquitination results in the expulsion of the complex from the nucleus to the cytoplasm and flags the protein for degradation via proteasome. Therefore, the activity of MDM2 keeps the cellular concentrations of p53 low in the cell. (Vousden, 2000) Although most of the time p53 must be shuttled outside of the nucleus by MDM2, recent studies have shown that p53 can also be degraded inside of the nucleus. This nuclear degradation is proposed to have a faster effect and tighter control of the active p53. Nuclear degradation would act as a “quick off switch” (Shirangi et al, 2001)

III. Recruitment of p53

In normal cells, it has been shown that p53 is removed from the nucleus and degraded by proteasomes as a result of the interaction with MDM2. Therefore, in order for p53 to become activated and arrest or destroy the cell, the interaction with the MDM2 protein must be abolished. There are several cellular pathways involved in this process. These pathways are what signals cell damage and recruits the activities of p53 by stabilizing p53's presence in the nucleus.

A. Phosphorylation

MDM2 binds to p53 at the N-terminal region of the p53 protein (Bai and Merchant 2001). If the p53 protein is phosphorylated at or near the N-terminal end, then this could cause steric interference or a conformational change that would not allow MDM2 to bind to p53 and would therefore limit the removal of p53 from the nucleus (Rich et al, 2000). The lack of MDM2 interaction would result in more p53 in the nucleus and an increase in the transcriptional activity of the protein.

One specific example of this pathway occurs when there is a double strand break in the DNA. When a double strand break occurs in the DNA, a protein called Ataxia Telangiectasia Mutated (ATM) binds to the break (Rich et al, 2000). Binding to the DNA activates the protein kinase activity of ATM, which phosphorylates p53 preferentially at Serine 15 in the amino terminal end. This phosphorylation changes p53's conformation and inhibits the interaction of p53 with MDM2 (Rich et al, 2000). Phosphorylation, then, results in the loss of interaction of p53 with MDM2 and an upregulation of p53 activity (Fig. 4.).

B. Removal of MDM2

Another way the MDM2/p53 interaction can be inhibited is through the removal of MDM2 by other proteins. The best example of this type of interaction is the ARF protein. The ARF protein is able to bind to MDM2 which disrupts the MDM2/p53 interaction by sequestering and degrading MDM2 (Bai and Merchant, 2001). ARF binds to MDM2 in a region separate from the p53 binding domain, therefore, the ARF protein does not directly disrupt the interaction between p53 and MDM2 (Bai and Merchant 2001). ARF is activated by the over expression of oncogenes such as Myc, Ras, and E2F1 (Vousden 2000). These oncogenes serve as signals to the cell that there is abnormal proliferation and will result in the activation of ARF and eventually p53.

ZBP-89 is another protein that inhibits the actions of MDM2 on p53. ZBP-89, however, binds to p53 instead of MDM2 and does not inhibit the binding of p53 to MDM2. The effect of ZBP-89 is that although MDM2 is still bound to p53, it can no longer transport it out of the nucleus or target it for degradation. It is thought that the ZBP-89 protein can either sterically mask the degradation sites on p53 or recruit p300 to

acetylate p53 (Bai and Merchant 2001). Acetylation by p300 may act in a similar manner as phosphorylation in protein stabilization. In either case p53 remains in the nucleus where it can act to arrest or destroy the cell. (Bai and Merchant, 2001)

C. p53 Binding

Once the p53 is present and stable in the nucleus, the protein must bind to the specific sequences of its target genes. Two thoughts exist on how p53 accomplishes this task. The classical view of this process is that the C-terminal end of the protein regulates the binding of p53 to DNA target sequences. The C-terminal end negatively controls DNA binding, but this inhibition can be alleviated by acetylation, phosphorylation, or protease cleavage. Once modified the p53 can then bind to DNA and begin the transcription process by recruiting the transcription machinery of the cell. (Espinosa and Emerson, 2001)

The most recent view states that p53 has intrinsic chromatin binding activity and the C-terminal domain of p53 actually serves as a regulatory element. This model suggests that the p53 protein binds to DNA in its chromatin complex without the need for modification (Espinosa and Emerson, 2001). Recently it has been shown that p53 actually binds to a coactivator CBP/p300 (Livengood et al, 2002). Interruption of this binding leads to reduced p53 transcriptional activity. Once p53 binds to the DNA, it recruits p300 to acetylate the nucleosomes immediately up and downstream of p53. This results in the spreading of the open conformation of DNA to the TATA box where transcription can begin in earnest. In either case, the presence of p53 results in the

transcription of genes needed in the regulation of the cell cycle. (Espinosa and Emerson, 2001)

IV. Functions of p53 in Tumor Suppression

As previously discussed p53 can undertake activities such as DNA repair, recombination, and differentiation. The main body of research, however, has been in the tumor suppressive function of the protein. Once p53 is active and present in the nucleus, it can function to suppress tumor growth in one of two ways (Fig. 5.). The protein can stop uncontrolled cell growth by arresting the cell cycle thus allowing for DNA repair, or it can trigger the cell to undergo apoptosis. Either pathway prohibits the cell from endangering the organism further.

A. Arrest

One main function of the tumor suppression activities of p53 is the arrest of the cell so that damaged DNA is not synthesized. The transcription of the gene p21 is the primary method by which p53 stops the cell cycle when slight DNA damage is present. The p21 protein is a potent cyclin dependent kinase inhibitor. Expression of the p21 protein results in the cessation of the cell cycle between the G1 and S phases. The arresting capabilities of p21 are so efficient that the protein has been implicated in the terminal differentiation of cell lines such as muscle and gut epithelium (Bird, 1996; El-Deiry, 1998). Upon the p53 mediated transcription of p21, the protein interacts with the cyclin dependent kinase (Cdk) complex in order to inhibit the Cdk's activity. By inactivating the Cdk, the E2F1 transcription factor remains bound to the Retinoblastoma (Rb) protein. If the Rb protein is not phosphorylated and thus inactivated by the Cdk, then the cell cycle cannot progress into S phase.

There are several possible ways in which p21 can inhibit the Cdk complex. The p21 protein possesses tyrosine phosphatase activity which can remove phosphate groups from the Cdk molecule (Bird, 1996). The Cdk molecule must be phosphorylated at conserved residues to become active (Chellappan et al, 1998). Without the correct phosphorylation status, the Cdk molecule will be inactive and progression through the cell cycle would cease. The p21 protein could possibly dephosphorylate the Cdk at specific sites that would reduce its activity and thus arrest the cell cycle.

In a similar manner, p21 could interrupt the interaction of Cdk to the Cdk-activating kinase, an enzyme responsible for the phosphorylation and resulting activation of the Cdk. Experiments have shown that p21 blocks the phosphorylation of Cdk by the Cdk-activating kinase (Aprelikova et al, 1995). Because there was no direct interaction found between p21 and the activator, it is believed that p21 covers the sites of the Cdk molecule that need to be phosphorylated (Aprelikova et al, 1995). As described above, unless the Cdk is in its correct phosphorylation state, then it will remain inactive.

The p21 protein could also interact with the Cdk complex by interfering with the cyclin binding to the Cdk molecule. By altering the interaction of the cyclin and the Cdk molecule, the Cdk molecule will remain inactive. This hypothesis is supported by the fact that the cyclin-Cdk inhibitory domain of p21 contains distinct cyclin- and Cdk-interacting regions (El-Deiry 1998). A similar molecule, p27, has features similar to those of p21. The Cdk complex with p27 bound has an altered kinase formation resulting in steric interference of ATP binding in the active site (El-Deiry 1998).

A second way that p21 can arrest the cell cycle of the cell is by its association with Proliferating Cell Nuclear Antigen (PCNA). PCNA associates with DNA

polymerase δ to increase the polymerase's processivity (Voet and Voet, 1995). PCNA forms a trimeric ring around the DNA molecule in order to keep the polymerase attached and continue replication (Voet and Voet, 1995). The crystal structure of p21-PCNA-DNA suggests that the p21 protein masks the sites on PCNA that allow for binding to proteins such as DNA polymerase δ (El-Deiry, 1998). This activity of p21 will cause the cell to stop the replication of its DNA, and keep the cell in the G1 phase of the cell cycle.

Whether by interacting with the Cdk complex or with the PCNA, p21 arrests the cell cycle in the G1 phase. This arrest will allow the cell to repair or replace the damaged DNA so that the mutation rate due to damage is minimized. This p53-mediated pathway can only work effectively when DNA damage is minimal. More extensive damage results in more drastic measures.

B. Death

If the DNA damage is extensive enough, p53 will cause the cell to undergo programmed cell death. The p53 protein can utilize two pathways to sensitize the cell to apoptosis. The first pathway utilizes the mitochondria and its constituents. As discussed above, cytochrome c is an important component in beginning the caspase cascade. The second pathway involves the upregulation of death receptors which also results in a caspase cascade. In either case, genes transcribed by p53 will cause the cell to undergo programmed cell death.

i. Mitochondrial Interactions

The first pathway that will be considered is the mitochondrial mechanism which results in apoptosis. The mitochondria are well known for being the powerhouses of the cell. Besides providing the ATP the cells uses for energy, the mitochondria houses

proteins whose release results in rapid cell death. Proteins such as cytochrome c, Smac/DIABLO, AIF, and an array of procaspases are located within the mitochondria (Hengartner, 2000). Release of these proteins from the mitochondria will likely result in the apoptotic destruction of the cell (Fig. 2.). p53 transcribes genes whose activities result in the release of some of these proteins.

The most effective and thus deadly of the proteins which the mitochondria carry is the cytochrome c molecule. Cytochrome c usually functions as an electron carrier on the outer surface of the inner mitochondrial membrane (Voet and Voet, 1995). Once cytochrome c is released into the cytosol, however, it complexes with procaspase 9 and Apaf-1 to form an active caspase (Soengas et al, 1999). Several of these active caspase molecules will converge to form a larger structure called an apoptosome whose activity results in the cleavage of key cellular components to induce apoptosis (Hengartner, 2000). The ability to shuttle cytochrome c out of the mitochondria, as is evident, is a crucial step in triggering the apoptotic pathway.

Smac/DIABLO and AIF are two other proteins housed in the mitochondria that have a direct effect in the apoptotic activities of the cell. Smac/DIABLO is a protein whose activity inhibits the activities of the inhibitors of apoptosis proteins (IAP), a powerful inhibitor of activated caspases (Hengartner, 2000). Random, unintentional activation of a caspase could quickly activate other caspases to begin the caspase cascade. IAPs protect against this possibility. When the mitochondria is disturbed enough, it will release the Smac/DIABLO protein to ensure the IAP will now inhibit the apoptotic cascade. The AIF is another protein whose presence results in apoptosis, yet in a caspase independent pathway that has yet to be fully described (Hengartner, 2000).

The activity of p53 is involved in the release of these mitochondrial proteins into the cytosol. p53 alters the functions of the cell membrane through several of its target genes. Noxa, Bax, p53AIP1 and p53DINP1 are targets of p53 whose activities disrupt the membrane permeability and lead to the release of some or all of the proteins mentioned above.

a. Bcl-2 Family

The p53 protein transcribes two genes, Bax and Noxa, who are members of a family of proteins called Bcl-2 proteins. The Bcl-2 family derives its name from B-cell lymphoma, a condition in which the gene is involved. This family is composed of three subcategories: the antiapoptotic group 1 and the proapoptotic groups 2 and 3 (Fig. 6.). The Bcl-2 family is related by their homologies over four possible sequences. The Bcl-2 family of proteins are the molecules that are primarily responsible for the integrity of the mitochondrial membrane. (Hengartner, 2000)

The antiapoptotic members of group 1 are responsible for suppressing the mitochondrial disruption signals that the other two groups convey. Members of this group, including Bcl-2 and Bcl-XL, possess sequence homology within all four BH domains. The group 1 family also possesses a hydrophobic transmembrane domain on the C-terminal end of the protein which anchors the protein into the surface of the mitochondria. (Hengartner, 2000)

The proapoptotic members of group 2, including Bax and Bak, possess homologies in the BH1-BH3 domains (Hengartner, 2000). This group is missing the BH4 homology, but it does have a transmembrane domain on their C-terminal ends. This allows the group 2 members to insert themselves into the mitochondrial membrane once

they are activated. The group 3 members have homology only in the BH3 domain, so are often referred to as BH3 domain only molecules. Proteins such as Noxa, Bid, and Bik are members of this group (Oda E, et al, 2000). The group 2 and 3 members are able to exist both in the cytosol and in association with the mitochondria's membrane.

The way these proteins interact with each other determines the fate of the mitochondria and also the cell. It has been found that the ratio between the antiapoptotic and multidomain proapoptotic members helps determine the susceptibility of the cell to the death signal (Cheng et al, 2001). A greater concentration of group 1 members will inhibit the release of cytochrome c and increase the likelihood of survival for the cell. A greater concentration of group 2 members, however, will result in the loss of cytochrome c and other possible proteins which will initiate the caspase cascade.

The proapoptotic group 2 members are believed to be located in the cytosol in an inactive form. Upon interaction with the BH3 domain only members, the group 2 members become activated and localize to the mitochondria (Fig. 7.). Once in the mitochondria, the group 2 members homooligomerize and act in a variety of ways to disrupt the mitochondrial membrane. The group 1 members limit the activational properties of the BH3 domain only members by sequestering them in mitochondrial complexes. If the antiapoptotic members bind all of the BH3 members, then the activation of the proapoptotic members cannot occur. (Cheng et al, 2001)

Several theories exist on how the Bcl-2 family members disrupt the mitochondrial membrane (Fig. 8.). The first hypothesis asserts Bcl-2 family members are able to form pores in the mitochondria's membrane which can allow the transport of cytochrome c into the cytoplasm. Two main lines of evidence support this belief. The Bcl-X_L is

structurally similar to the pore forming subunit of the diphtheria toxin. In vitro, Bcl-2 family members can insert themselves in synthetic lipid bilayers, oligomerize, and form channels which allow the passage of particles. These channels could, conceivably, allow the passage of cytochrome c and activate the caspase cascade. (Hengartner, 2000)

Another theory suggests that the Bcl-2 family of proteins could interact with other proteins already present on the surface of the mitochondrial membrane. It has been shown that the Bcl-2 family is able to associate with other proteins. These interactions could interact with membrane channel proteins already present in the outer membrane of the mitochondria. This could alter the conformation and allow the release of the cytochrome c protein. The activity of the Voltage Dependent Anion Channel (VDAC) is shown to be regulated by Bcl-2 family members. The pore of the VDAC is too small to allow for the cytochrome c to pass, so the channel will have to undergo significant conformational changes in order for this event to occur. (Hengartner, 2000)

The final theory suggests that the Bcl-2 family members cause the rupture of the cell membrane through the loss of mitochondrial membrane potential. This could happen via the intrinsic pore forming abilities and the association with other proteins discussed above. Channel formation by the Bcl-2 family members could result in the alteration of the mitochondria physiology possibly through the ion exchange or oxidative phosphorylation perturbations. This loss of homeostasis would result in organelle swelling and rupture of the outer membrane. The rupture would cause the release of cytochrome c and other mitochondrial proapoptotic proteins mentioned above. The interaction with the VDAC protein could also result in the loss of membrane potential. The VDAC is a subunit of the permeability transition pore (PTP), whose opening results

in loss of membrane potential and organellar swelling. Opening of the PTP corresponds to a release of cytochrome c and rupture of the organelle's membrane. In either case, the contents of the mitochondria are released and apoptosis shortly follows. (Hengartner, 2000)

The p53 targets of Bax and Noxa are key players in the regulation of mitochondria membrane integrity. Both Bax and Noxa are proapoptotic proteins whose expression leads to a higher probability of apoptosis occurring. Not all of p53's target genes are members of the Bcl-2 family, however, and apoptosis could occur by other mechanisms.

b. Non Bcl-2 genes

Bax and Noxa are not the only targets of p53 whose expression result in increased apoptotic activity. The protein p53DINP1 contains a p53 binding sequence and is expressed in response to an upregulation of p53 (Okamura et al, 2001). The role of this protein is to promote phosphorylation of p53 by an unknown serine kinase. Phosphorylation of p53 at Serine 46 results in the increased affinity of p53 to the p53AIP1 promoter (Okamura et al, 2001; Oda K. et al, 2001). Once p53AIP1 is transcribed, the protein is localized to the mitochondria where it disrupts the mitochondrial membrane potential (Oda K. et al, 2001). Similar to the actions of the Bcl-2 group, the loss of membrane potential results in the rupture of the mitochondria's outer membrane and loss of cytochrome c. The p53 protein, itself, can affect the membrane potential of the mitochondria by localizing to the mitochondria and disrupting the membrane potential through an as yet undetermined mechanism (Marchenko et al, 2000).

ii. Death Receptors

Other than the mitochondrial pathway, p53 can induce apoptosis by upregulating the transcription of cell death receptors and their adaptor molecules. A correlation has been shown between the activity of p53 and the expression of death receptors such as DR5 and Fas (Bates and Vousden, 1999). Although no p53 promoter sites have been found on these receptor's genes, a positive correlation is present and p53's activity is thought to be involved in some way. Active p53 can also transcribe other proteins needed by the death receptors to link them to the caspases. Pidd is one of these adaptor molecules that p53 can transcribe (Lin et al, 2000).

Death receptors are proteins that activate a caspase molecule upon binding of a ligand (Fig. 2.). This may seem relatively simple, but quite a bit of coordination goes into this process. First the death receptor must translocate itself from the Golgi to the cell membrane (Bennett et al, 1998). Experiments have shown that p53 helps in this translocation process. Once the receptor is on the surface of the cell it can bind to a death ligand such as TRAIL. Binding of the ligand causes the association of several death receptors and recruitment of an adaptor molecule such as Pidd or Fadd. These adaptor molecules have domains which associate with both the death receptor and the caspase (Lin et al, 2000). Caspase cleavage will result from this process. How cleavage occurs is unclear, yet it is hypothesized that procaspases have slight intrinsic protease activity (Hengartner, 2000). When several procaspases are in close proximity (as in the case of the death receptors), spontaneous cleavage will occur and the caspase cascade will begin.

Whether by transcribing genes or interacting with the cell itself, p53s presence greatly enhances the cells predisposition to undergo apoptosis. By utilizing the two distinct pathways of mitochondrial interactions and death receptors, p53 allows the cell

more than one way to destroy itself. This ensures that the cell will undergo apoptosis if such drastic measures are needed.

So p53 acts as a tumor suppressor by arresting the cell cycle if slight DNA damage is present and causing apoptosis when DNA damage is more severe. These mechanisms help to guarantee the fidelity of the genetic code as the cell is replicated. These activities limit the frequency of tumor development and enhance the overall survival of the organism.

Decision Between Arrest and Death

It has been shown that p53 has two responses to cell damage: arrest of the cell cycle and programmed cell death. The question remains, however, how does the cell choose which response will be initiated? Do other cellular factors control arrest or death, or can the protein make the decision itself? Two models have been proposed to explain the possible decision making process.

A. Dumb Model

One model suggests that p53 reacts the same way each time it is activated by cellular stress (Vousden, 2000). In this “dumb” model, the protein will transcribe the same genes whenever it is activated. The decision will rely on the cell to make the decision between arrest and death. Signals for arrest and programmed cell death are sent from p53 and the cell must either inhibit the death signal to arrest the cell cycle without killing the cell, or amplify the death signal above threshold to trigger apoptosis (Vousden, 2000). The anti-apoptotic members of the Bcl-2 family, Bcl-X_L and Bcl-2, have been shown to suppress the apoptotic activity of Noxa (Cheng et al, 2001). Similar anti-apoptotic proteins or survival factors could bring the apoptotic signal below threshold and

save the cell from death. The opposite scenario could be that other p53 independent pathways could produce their own signals which would amplify p53s signals, thus triggering the death pathway (Vousden, 2000). An example of this pathway involved p53 and another tumor suppressor E2F-1. Neither of these proteins acting alone can induce apoptosis efficiently, but when p53 is active and E2F-1 is deregulated, apoptosis occurs (Bates and Vousden, 1999). The singular activation of all p53 targets along with the reliance on other cellular signals characterizes the dumb model.

B. Smart Model

The second model used to describe the decision making process involves p53 being expressed in various concentrations and various active states. In this model, p53, itself, is responsible for affecting which outcome is produced. One theory suggests that the arresting genes have promoters that bind p53 with greater affinity than the apoptotic promoters. This would mean that enough active p53 would have to be present to bind first to the arresting genes and then still have enough free protein left over to bind to the apoptotic genes. If the cell was damaged or stressed to a small degree, then the p53 protein would only be present in small amounts, thus transcribing arresting genes which would allow time for the removal of the stress. If too much stress is present, then the levels of p53 would be greater so that it would bind to both the arresting and the apoptotic genes. This may be the case since it has been noted that cells going through the apoptotic pathway have also been arrested. (Vousden, 2000)

Besides the amount of p53 produced, the state of the protein can also help determine which genes would be produced. One apoptotic target gene of p53, p53AIP1, is dependent on the phosphorylation status of p53. If p53 is phosphorylated at Serine 46,

then the levels of p53AIP1 are greatly increased (Oda K. et al, 2000). p53 can also be modified by acetylation, sumoylation, and glycosylation (Vousden, 2000). These modifications can lead to alterations in the conformation of p53 causing a change in the affinities for certain promoters. These alterations in p53 could possibly switch the transcriptional activity from arrest to apoptosis, or vice versa.

Clinical Implications

Due to the role of p53 in cellular arrest and destruction and the high frequency of p53 mutations in human cancers, the loss of p53 may be a key component in the formation of tumors. Loss of the DNA damage inducible checkpoint, for example, could lead to tumor formation due to the propagation of damaged DNA through the replication cycle. Replicating damaged DNA results in higher mutation rates for the replicated cell. Loss of the p53's apoptotic activities would allow cells which are growing without regulation to continue to grow uncontrollably and without a way to check that growth. In either case, the loss of p53 could quickly lead to the loss of genomic integrity and the eventual formation of tumors. (Wallace-Brodeur and Lowe, 1999)

Since p53 mutations are so closely linked with cancer, scientists have been trying to find away to use p53's functional status as a tool to direct their prognosis and treatment. The nature of p53 suggests that tumor cells with mutant p53 should be inherently more aggressive than tumors bearing the wild-type protein. Physicians have begun trying to find a correlation between the status of p53 and the prognosis of the patient. Not all tumor types show this a correlation, and some cancers are even treated more effectively when a mutant p53 is present. The status of p53, for example, provides the best indicator for the recurrence in breast cancer. (Wallace-Brodeur and Lowe, 1999)

Physicians are also able to use p53 as a prognostic indicator because of its ability to make tumors resistant to chemotherapies. Most chemotherapeutic drugs use cytotoxic agents that directly or indirectly damage DNA. These drugs use the cells own apoptotic systems to trigger cell death in cells that have been treated. If there is loss of p53's apoptotic activity, then the cell may not react nearly as effectively in beginning apoptosis. p53 is linked to drug resistance in several tumor types including; non-Hodgkin's lymphoma, acute myeloid leukemia, myelodysplastic syndrome, and chronic lymphocytic leukemia. Loss of p53 in these tumor types is generally associated with poor prognosis and shorter survival times. This correlation is not universal. One cell line, p21 deficient HCT116 colon carcinoma cells, actually shows increased sensitivity due to mutated p53. No matter what sign p53 mutations may give, the hope is that physicians and scientists may rely of p53 to give some prognostic information to the patient's condition. (Wallace-Brodeur and Lowe, 1999)

Another, more ambitious, thought is that p53 status could be used to determine the course of treatment for the patient. For example, if a patient is harboring a p53 mutant, then insertion of a functional p53 gene or protein could confer sensitivity to the cytotoxic drugs used in chemotherapy. Another option is to use drugs that circumvent the p53 pathway in order to cause the tumor cells to die. An example of this type of treatment involves using microtubule inhibitors to kill the cell. A third option involved using the body's own immune system to destroy the cell. By creating antibodies that recognize mutant p53, the immune cells in the body can target the tumor genes and destroy them. Success has been approach has been seen in mouse dendritic cells. The final option involves using a virus to replicate and destroy cells that are deficient in p53

activity. The ONYX-015 virus inserts itself into all the cells in the region, but is only able to replicate in cells lacking p53 activity. ONYX-015's replicative cycle causes lysis in the tumor cells while leaving the healthy cells unaffected. All of these new therapies hold promise for the effective treatment of human cancers. (Wallace-Brodeur and Lowe, 1999)

Summary and Conclusions

As discussed above, p53 is a transcription factor whose activity suppresses tumor growth and formation. This tumor suppression occurs through arrest or apoptotic pathways. The p21 protein is the major effector for the arrest pathway, while the apoptotic pathway is activated by the p53-mediated transcription of various genes involved in mitochondrial interactions and cell death receptors. The decision between arrest and death is made by the activity status of the protein or the activities of other proteins in the cell. In either case, the cell is protected against the replication of dysfunctional DNA and the possibility of tumor formation.

As the high mutation rates of p53 in various human cancers suggest, p53 plays a major part in tumor suppression. By studying p53, scientists will be better able to understand how our own bodies fight to prevent harmful mutations that could deregulate the growth of the cell. The study of p53 will provide more specialized and effective ways to treat human cancers. While a cure for cancer is still decades away, studies of p53 and other tumor suppressors will provide valuable information which will lay the groundwork for better cancer therapies and possibly a cure.

Fig. 1. Cell Cycle (Voet and Voet, 1995)

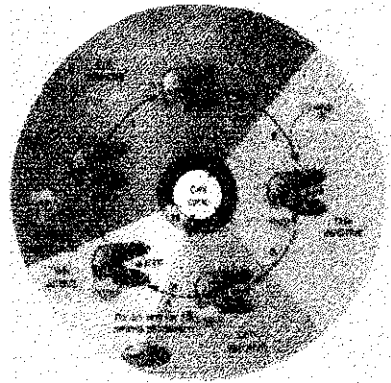


Fig. 2. Apoptotic pathways (Hengartner, 2000)

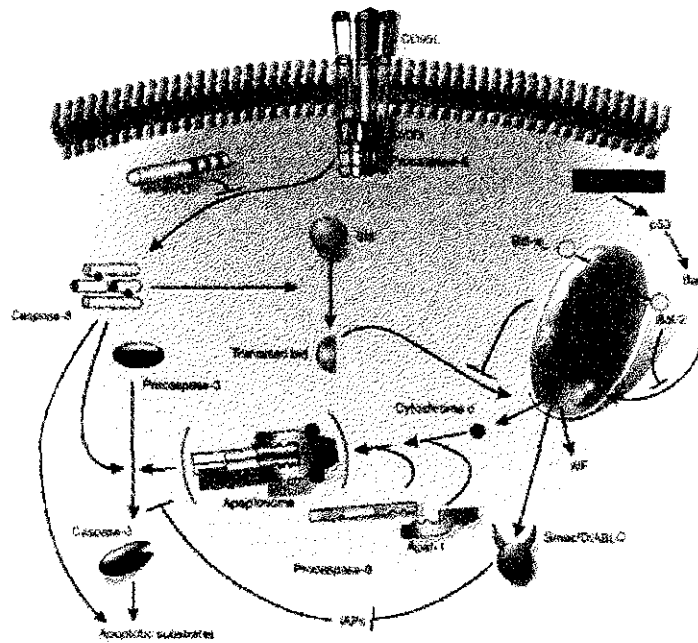


Fig. 3. Structure of p53 (Choisy-Rossi et al, 1998)

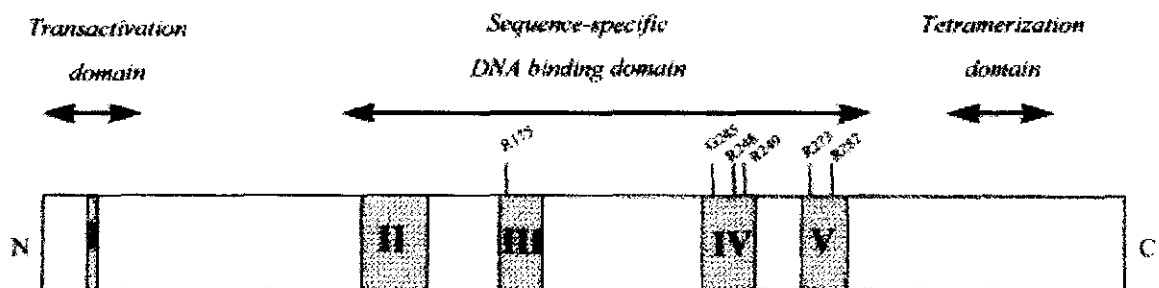


Fig. 4. Activation of p53 via phosphorylation (Rich et al, 2000)

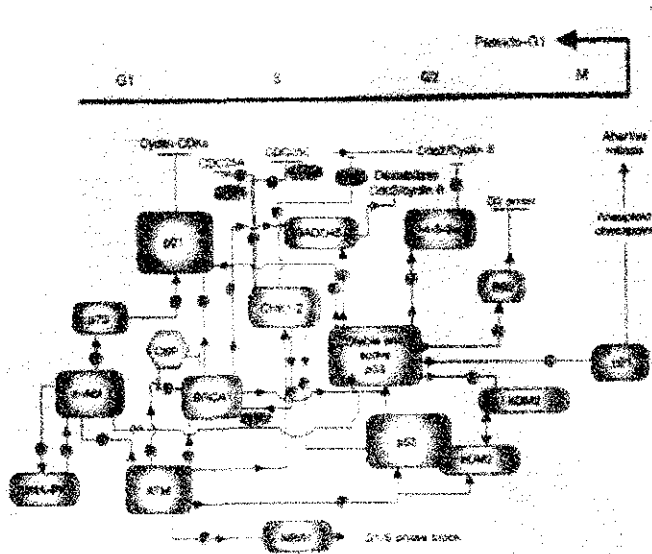


Fig. 5. Activities of p53 in tumor suppression (

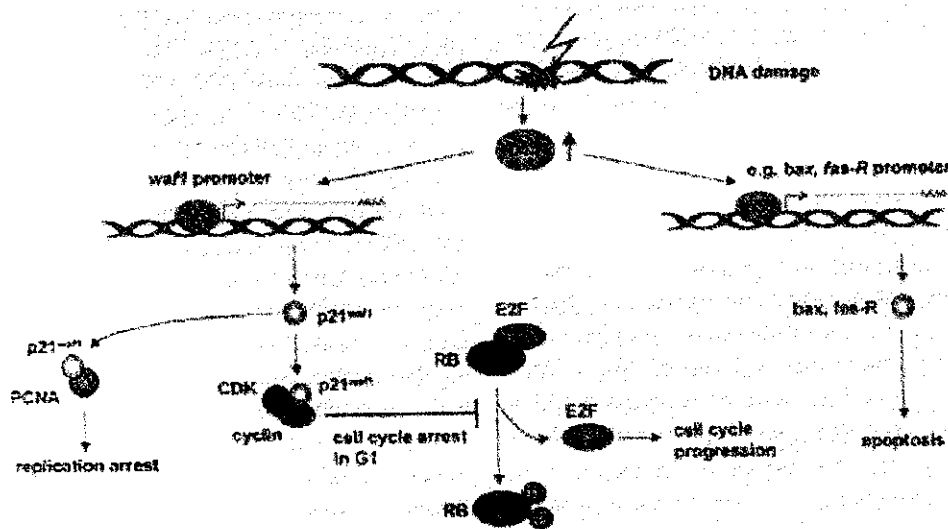


Fig. 6. Bcl-2 Family (Hengartner, 2000)

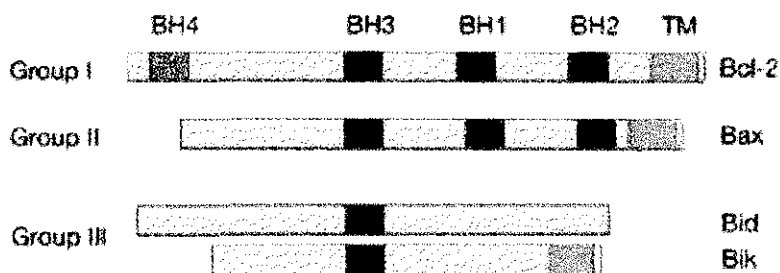


Fig. 7. Activities of Bcl-2 Proteins (Hengartner, 2000)

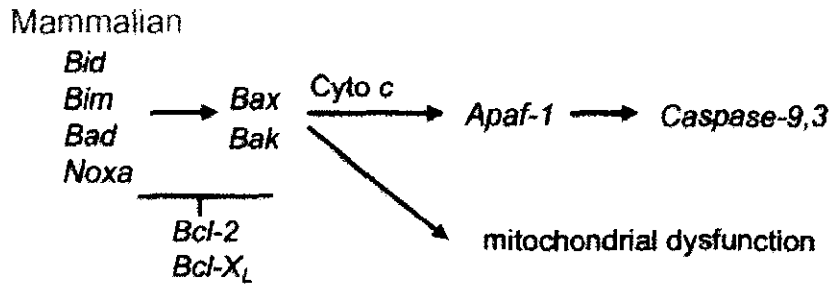
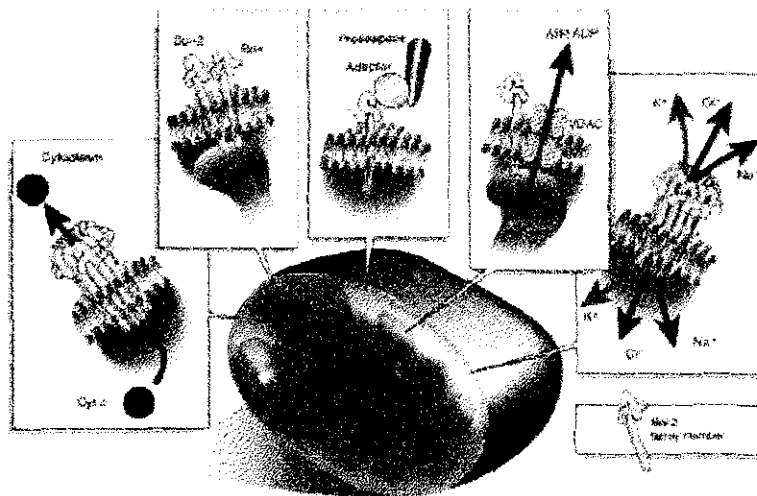


Fig. 8. Interactions of Bcl-2 Proteins with the Mitochondria Membrane (Hengartner, 2000)



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