REVERSAL OF THE P53 GENE FROM ONCOGENIC TO A NORMAL FUNCTIONING GENE

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The *P53* gene, located on chromosome 17p13.1, encodes the protein P53 and has been coined the guardian of the genome in reference to its ability to prevent genomic mutations. P53 has the ability to suppress unnecessary or detrimental cell proliferation (Pastor-Freed and Rives, 2012). The protein was initially discovered by finding an interaction between SV40 and T-antigens; the unknown protein at the time was bound to a T-antigen of cells infected with SV40 virus. This interaction lead to the expression of a large amount of an unknown protein, which, later would be known as the P53 (Crawford, 1983). This was the discovery of the gene, and it demonstrated that it functioned in the manner of a pathway. This means that the gene requires activation and could have downstream effects as well. P53 earned the name from scientists utilizing SDS-PAGE electrophoresis (Jiang, 2011) and discovering the size of the protein to be 53,000 daltons (Crawford, 1983).

It is imperative to decipher and interpret the full function of *P53* because it is the most highly mutated gene and data indicates that more than half of all tumors have mutations at this particular locus (Vogelstein et al., 2000). In order to effectively understand *P53*, it is necessary to understand its function in relation to other genes. *P53* mutations create invasion, metastases, disruption of cell cycle inhibition, and cellular proliferation (Patricia et al., 2013).

P53 is controlled and regulated by positive and negative feedback loops. P53 acts as a transcription factor when a stress signal is sent through various cellular pathways. There are ten loops that have been identified, and of those, three are positive feedback loops and seven are negative feedback loops. These loops act as a control mechanism similar to a circuit. When acting as a circuit, there are multiple proteins that are activated and influenced by P53. There are seven identified negative feedback loops that down-regulate P53. This is done by interaction with MDM-2, Cop-1, Pirh-2, p73 delta N, cyclin G, Wip-1 and Siah proteins. The three positive

feedbacks loops interact with PTEN-AKT, p14/19 ARF, and Rb leading to an up-regulation of P53 activity (Harris and Levine, 2005).

Then, cell cycle arrest, or cellular senescence and activation of the apoptotic response may occur. The main types of damage that elicit this nuclear response are UV radiation, alkylation of bases, DNA alteration via free radical interaction or oxidative stress reactions, or depurination of DNA (Harris and Levine, 2005). UV radiation increases DNA binding during damage, thus by increasing binding this can induce a multitude of problems (Kapor and Lozano, 1997). The problems induced can translate to skin cancer, cellular mutations, and abnormal progress of cell cycle. Post-translational protein modifications occur to P53, such as methylation, acetylation, sumolation, phosphorylation, or ubiquitination. These post-translational modifications occur normally, but when dysfunction occurs, these changes lead to variability. This variability can lead to inappropriate activation or expression of genes and also misappropriated DNA interaction. The misappropriated DNA influences activity in cells and can alter the function and structure of cells. It is known that once the structure is altered the function of the cell is altered as well. This means a post-translation change occurs and P53 can be modified in a manner that could promote further dysfunction or cancer growth. In order to obtain a full comprehension of P53, it is necessary to understand its implication in cell arrest, apoptosis, and senescence. In regard to cellular senescence, it is not understood which genes are required for activation of cellular senescence. ATM kinase is activated during DNA damage, which assists in DNA repair. ATM kinase will then activate P53 via phosphorylation, which will elicit the necessary nuclear responses that carry out the proper cellular senescence response (Harris and Levine, 2005).

Therefore, it is necessary to understand how cyclin dependent kinases function in regard to mutated and non-mutated forms of P53. CDKs regulate checkpoints during cell cycle, and these CDKs, when activated, can phosphorylate target proteins. This can result in activation or inhibition of cell cycle if there is DNA damage occurring in S phase. This is important in relation to genetic damage; cell cycle or cell growth is occurring and there is an error, it can lead to genetic abnormalities and genetic disorders. The reason that CDKs are important is because CDK inhibitors can become activated and influence the outcome of P53 in response to DNA damage.

The oncogenic cell cycle has the potential to create mutations that can activate CDKs and drive cancerous or abnormal growth. Oncogenic cell cycle allows for mutations to occur by not responding appropriately to DNA damage, and oncogenic cell cycle is not sending the correct signal for cells to grow and divide. The signals that are being sent have the potential to tell a cell to continue to grow, which in this case would facilitate more cancerous growth. The oncogenic cell cycle is not sending the normalized growth signal, which can allow for a mutation to occur. The incorrect growth signal will lead to a mutation or aberrant growth in normal cells. These mutations then have the capability to alter normal growth and function of cells. A mutation is generated by replicating cells that have not been repaired, or by errors in the nucleic acid sequence during DNA synthesis. Abnormal growth can contribute to neoplasms and allow for replication of cells that are cancerous. This demonstrates how a P53 mutation can effect the human body as a whole and begin cancerous growth.

The P21 protein binds to CDKs and acts as an inhibitor of the G1 phase of cell cycle by preventing the interaction between cyclin E-CDK2. This complex phosphorylates the retinoblastoma protein (Rb) and Rb then releases E2F1, which initiates transcription of genes

that promote G1-S phase progression. P53 induces G2 cell cycle arrest and this is thought to occur by 14-3-3 sigma, which is a protein that has an affinity for CDC25C (Wilker et al., 2005). CDC25C phosphatase is important in regard to DNA damage. CDC25C phosphatase enables the kinases termed Chk1 and Cds1 to become activated when DNA damage occurs. Chk1 and Cds1 can phosphorylate CDC25C to inhibit entry into mitosis. This is needed in order to prevent replication of damaged DNA, which would result in misappropriated genetic expression (Hoffman, 2000).

Also, CDC25C promotes cell cycle progression from G2-M. This is completed by the phosphatase removing phosphate groups from various proteins and when the phosphate group is removed, cell cycle can progress to the next phase. The dephosphorylation of Thr 14 and Tyr 14 allow for activation of Cdc25C. Wee1, Mik1, Myt1 are protein kinases that need to have phosphate groups added or removed for phosphorylation or dephosphorylation (Donzell and Draetta, 2003). For example, the proteins involved are condensin proteins (Condensin1, Condensin II, and SMC proteins); these are necessary for formation of mitotic chromosomes. These proteins are important to form a structurally sound chromosome. Mutations can occur during this stage which could generate genetic mutations or multiple copies of a chromosome. P53 acts in an inhibitory fashion by not releasing CDC25C from the cytoplasm, thus not allowing for activation of cyclin B-CDK2 which, is located in the nucleus (Hoffman, 2000). P53 is a transcription factor, however it has an inhibitory ability in G1 phase and G2-M. This occurs because P53 is acting as a checkpoint, it will not allow the release of CDC25C from the cytoplasm when it recognizes there is possible DNA damage. P53 has the ability if the DNA damage is not corrected, to be able to activate apoptosis.

In addition to determining the fate of a cell, there are members that are pro-apoptotic; Bax, Bad, Bak, Bid, Bim, NOXA, and Puma that promote apoptosis (Gross et al., 2003). These are all implicated in the release of cytochrome c and are integral parts of the apoptotic program. These Bcl-2 family members are imperative in determining the fate of life or death on a cellular level. The members of this family contain BH1, BH2, BH3, and BH4 domains, which interact with alpha helical structures. These interactions control mitochondrial-mediated apoptosis. The cellular interactions control mitochondrial-mediated apoptosis by activation and inhibition, and controlling the release of cytochrome c. Cytochrome c release can be promoted or inhibited; this is mediated by interactions of the BH1 and BH2 domains. The role of BH1 and BH2 is to inhibit the apoptotic program and to perform heterodimerization with Bax.

Bim has the ability to translocate to mitochondria when receiving specific apoptotic signals. Bim interacts with Bcl-2 and promotes the formation of heterodimers that will be pro-apoptotic. Bcl-2 and Bcl-Xl can inhibit Bax from becoming activated. The formation of heterodimers or homodimers of Bcl-2 can block the activation ability of Bcl-2 (Basu and Haldar, 1998). Bcl-Xl is a survival factor, which promotes survival of the cell. Finally, Bad has the ability to bind with Bcl-Xl. Thus, Bad has the ability to inhibit a survival promoting Bcl-2 family member. Research currently indicates that Bad is constitutively active, this would be to ensure that the normal apoptotic program can be enacted (Gross et al., 2003). The constitutively active form of Bad, is “always on” therefore, it is always awaiting activation if needed.

Bax when activated, will translocate to mitochondria and homodimerize when cell death occurs. This translocation will allow for Bax to form pores and channels at the outer mitochondrial membrane (Bleicken et al., 2013). The pores and channels that are formed by Bax are needed in order for cytochrome c release to occur. There, Bax acts as a membrane-associated

protein and provides cross-linkage (linkage between proteins) as a heterodimer. Bax can act as heterodimer with itself or other members of the family; the number of apoptotic heterodimers vs. anti-apoptotic heterodimers will determine the cells ability to resist to apoptosis (Basu and Haldar,1998). Bax has the capability to determine the fate of the cell despite survival factors (Gross et al., 2003). Once the heterodimer has been formed, this will activate pro-caspase-9 (Haupt et al., 2003).

PUMA interacts with P21, which will determine if arrest or death occurs. PUMA, when expressed, will promote translocation of Bax. NOXA is believed to act in the same manner as Puma, where it is activated in response to DNA damage and can activate the intrinsic mitochondrial pathway. The activation of NOXA requires P53 and E2F1 to respond to damaged DNA, then it can be activated by transactivation, and then can finally translocate to the mitochondria. NOXA will then shift towards inducing pro-apoptotic effects (Haupt et al., 2003*)*.

Cytochrome c will interact with Apaf-1 forming a structure known as the apoptosome (Schuler and Green, 2001). The structure is composed of Apaf-1, cytochrome c, and pro-caspase-9. Once caspase-9 is cleaved, it goes on to activate effector caspases such as caspase-3 and caspase-7 (Schuler and Green, 2001).

Other caspases, such as caspase-8, have the ability to become activated when the FAS ligand binds to a TNF receptor. This allows for the docking of adaptor proteins FADD and TRADD to initiate the apoptotic cascade and activation of the necessary caspases (Harris and Levine, 2005). Without programmed cell death, cells that may be harmful can not be destroyed properly.

This is known as the death receptor-mediated pathway and it begins when TNF or tumor necrosis factor receptors are stimulated. When stimulated, the death-inducing signaling complex

or (DISC) will form, which is composed of multiple proteins of the death receptor family. When death receptors are bound by extracellular ligands, this causes the adaptor protein named FADD to bind to the receptor. FADD then recruits pro-caspase-8 and pro-caspase-10, which will then join the DISC complex. Caspases-8 and caspase-10 can then homodimerize via allosteric mechanisms. cFLIP can also be recruited into the DISC complex which will inhibit activation of the caspases (Sprick and Walczak, 2004).

Smac/DIABLO is a protein that is found to be associated with mitochondria and has been implicated in the control of apoptosis. This occurs by inactivating the IAP family before cytochrome c can be released from the mitochondria. Smac enters the cytosol when damage has been induced by UV radiation. This is significant because Smac is an important inhibitor and if inhibition is not induced, further damage could occur because of the missing IAPs. Smac/DIABLO can inhibit the active form of caspase-9 in the apoptosome, which is overwhelmed by the missing IAPs that have been inhibited (Adrain et al., 2001). Smac/DIABLO, once released, has the ability to inhibit IAP or other caspase inhibitors (Schuler and Green, 2001). This study also discovered, via single cell analysis, that activation of Bax by P53, in turn, released cytochrome c from mitochondria. The message that can be deduced from this research is that upstream caspases are activated in this manner and P53 can activate cell signaling via a death receptor (Schuler and Green, 2001).

It has been found that effector caspase-16, caspase-17, and caspase-18 are activated by P53, but the mechanism is still unclear (Schuler and Green, 2001). P53 does have the ability, via transcription and non-transcriptional processes, to control expression of CD95/FAS/APO-1 and TRAIL receptors/Killer receptors. TRAIL and Killer receptors are activated by DNA damage in cells that are expressing mutant forms of P53 (Schuler and Green, 2001).

The TRAIL/Killer receptors are only active when cells are undergoing P53- mediated apoptosis. Actinomycin D has the ability to block these receptors and P21, which would allow cell cycle to continue even if arrested. Actinomycin D blocks P21 by down-regulation of P21 and halting proteosomal degradation (Gottfriedi et al., 2003). This is important because P21 is active in S phase and is a possible factor for the genetic aberrations that occur when attempting to regain cell cycle control (Wu et al.,1999).

This indicates that P53-mediated apoptosis occurs through interactions via extracellular signaling and transcriptional control of ligand binding and death receptors that can induce pro-apoptotic responses (Schuler and Green, 2001).

Additionally, it has been found that P53 can be induced via intracellular stress, producing a response that results in inhibition of cell cycle (Vacher et al., 2000). P53 can transactivate genes of the Bcl-2 family that are involved in the apoptotic response. This is significant because Ras is mutated in many cancers that have a P53 mutation. Ras is a downstream target of P53 and once P53 is mutated, P53 transactivates the Ras gene. P21 is implicated in cell cycle arrest and Bax is implicated in the apoptotic response. The missense mutations that occur in P53 does not allow for activation of Bax. P53 aids in the activation of Bax by direct activation, and this occurs when other proteins that inhibit control are not present and the mitochondrium becomes permeable. As mentioned previously, this permeability allows for the apoptotic program to be enacted. P53 will accumulate in the cytosol and when this occurs, P53 can activate pro-apoptotic Bax, which will activate and induce apoptosis (Chipuk et al., 2004). The P53 accumulation in the cytosol utilizes a BH3 domain and activated Bcl-2 in pro-apoptotic form. This is believed to activate Bax and in turn, apoptosis activation will be signaled. (Chipuk et.al., 2004). A study conducted by Campomenosi et al. (2001) discovered that P21 activation can occur even though

P53 is mutated. This indicates that it may be possible to induce cell cycle arrest after P53 has been mutated.

There are alternative pathways where P53 can induce a pro-apoptotic response (Campenosi et al., 2001). P53 can induce caspase activation via ROS (reactive oxygen species), which are chemically reactive molecules that contain oxygen. ROS species are formed by natural metabolism and they can be utilized in cellular signaling and homeostatic processes. It has been discovered that ROS inhibitors are unable to inhibit P53-mediated caspase activation (Schuler & Green, 2001). Therefore, when P53 becomes mutated, the ability to activate caspases becomes exceedingly difficult either via ROS species or cytochrome c release (Schuler and Green, 2001).

The retinoblastoma (RB) protein has been implicated in cell cycle progression and P53-mediated apoptosis. This protein was discovered in ocular cancers. The *RB1*gene becomes mutated and allows specialized ocular cells to grow out of control. Negative feedback loops regulate cell cycle progression of Rb. These regulators are the family of E2F transcription factors and cyclin dependent kinases, which can inhibit cell cycle progression. DNA synthesis will then begin and cell cycle can not be inhibited at this point.

The main function of Rb is to inhibit the progression of cell cycle until the cell is ready to divide. When the cell is ready to divide, Rb will become phosphorylated, which inactivates Rb and this will allow for cell cycle to progress and cells to divide. Therefore, Rb is needed in order to allow for regulated cell cycle progression. This RB protein can be mutated via the *RB1* gene, which is induced by alteration of the normal regulatory processes of CDKs. *RB1* once mutated loses the ability to interact with the CDK complexes and act as a brake or stop mechanism for growth. This means that CDKs are not able to properly arrest cell cycle when needed (Henley and Dick, 2012).

Therefore, it is necessary to explore the function of the Rb protein in relation to P53. Tumors displaying oncogenic Rb can also incur P53 mutations. Once inactivated, P53 is unable to halt tumorigenesis. This can occur by tumor viruses inactivating P53 and once inactivated, the tumor viruses can mutate and transform cells. The tumor virus does this by binding and infecting cells, the cells will then reproduce. The reproduction of infected cells will then generate tumor cells produce tumor mutants. The cells that are produced have altered structure and function and can contribute to aberrant growth (Levine, 2009). E2F and E1A can also induce the apoptotic response when P53 is overexpressed and research indicates that E2F1 is the protein that controls P53 interaction with Rb (Nip et al., 2001). P19 or pARF is a gene which stabilizes P53"(Nip et al., 2001). E2F also has two domains on the amino terminus that allows for interactions with P53. P19 stabilizes P53, however, stabilization is a process independent of cell cycle.

Mutations independent of cell cycle progression were indicated by P53 responding to changes in homeostatic balances via E2F1. Thus, P53 is not responding to changes in transcription control or its ability to promote cell cycle growth. However, these interactions have the ability to hinder P53 in regard to the activation of transcription (Nip et al., 2001).

P21 is a cyclin dependent kinase or CDK inhibitor. P21 regulates cell cycle progression by binding to cyclin/CDK complexes. P21 is controlled and regulated by P53, thus P21 mediates the P53 cell cycle progression arrest response. This protein has PCNA interactions and regulates DNA replication in S phase of cell cycle. PCNA, known as proliferating cell nuclear antigen, increases the process of DNA replication and it is located on chromosome 20 (NCBI, 2015). P21 can also be regulated by DNA damage and can arrest cell cycle progression to allow for the repair of cellular damage before progressing (Xiong, 1992). It is imperative that cell cycle arrest occurs with damage, if cell cycle does not stop, then cell cycle can promote genetic aberrations

(Dolezolva, 2012). Genetic disorders occur when mutations and damage have not been repaired and P21 is necessary for cell cycle arrest. P21 can be expressed without induction via P53. During this expression, cells are unable to differentiate and thus, will inevitably halt proliferation (Dolezolva, 2012). The arrest mechanism via P21 is an important to ensure that cells are being created and differentiated into the correct cell type. For example, if a physiological response dictates production of a red blood cell, it is not beneficial for the cell to produce a muscle cell.

There was a study conducted in Poland, that demonstrated the chemotherapeutic drug named actinomycin D can be utilized to reverse cell cycle. Therefore, transformed cells and untransformed cells have the capability of being reversed during mutated growth processes (Sohn et al., 2009). This indicates that an oncogenic cell cycle could return to normal via chemotherapeutic treatment. However, it was shown that after reintroducing P53, cells can enter into cell cycle and proceed into G1. G2 aberration however, shows that P21 will induce improper DNA synthesis during this phase of cell cycle. The study concluded that endoreplication occurred during the S phase and human diploid fibroblasts were transformed and tumor cells as well. It appears that mutation of the *P53* gene will activate the *P21* gene, which will then induce inappropriate entry into S phase of cell cycle. Thus, mutating P53 and attempting to reinitiate cell cycle does not allow the pre-replicative complexes to form (Sohn et al., 2009).

There has been one successful research study that has indicated P53 can be reversed in relation to G1 and G2 cell cycle arrest. It is imperative to note that during reactivation of P53 in aneuploid cells, which are cells that can be missing a chromosome, there is an accumulation of cells that have undergone aberrations. This indicates that when reversing oncogenic cell cycle there are a multitude of issues. The main issue being that the genetic material in S phase is not correct when reversed from the oncogenic form. This is important to note for future therapies

because during accumulation of aneuploid cells, it is possible to activate genetic disorders. Therefore, these cells could proliferate within the tissue surrounding the effected area and induce further invasive problems. This means cells that have suffered damage could spread and damage distant tissues.

The study was conducted by Bates, Hickman and Vousdan (1999). They utilized a drug named actinomycin D, which is a chemotherapeutic agent that was able to create the reversal of a mutated oncogenic cell cycle. The cells were not successful in reentering the G2 phase because of the P21 protein. However, the study indicates that once cell cycle is oncogenic, it has the ability to be reversed to a degree of normalcy.

Pifthrin-α is the secondary drug that I would utilize; it has the ability to inhibit P53. Sohn et al. (2009) discovered that pifthrin-α will mediate DNA damage induced stress, and will stimulate the apoptotic death program. The drug functions in a manner that does not allow for the activation of the apoptosome and capase function. This specifically effects the apoptosome activation of caspase-3 and caspase-9. The apoptsome-mediated activation is blocked, but the mechanism is not fully understood. It does not interfere with the release of Bax or Bak, which in turn, will not effect the mitochondrial release of cytochrome c that occurs with these two components. This is significant because if you alter cytochrome c release to the mitochondria, it will inhibit the survival promoters that are theorized to help. Thereby, by not inhibiting cytochrome c release, it is feasible to believe that survival promoters can be increased during damage to promote cellular survival. It should also be noted that heat shock protein (HSP) activation can be inhibited by pfithrin-α. This means that the when DNA damage is induced, the HSPs will not be activated to induce cellular death.

The protective properties of pifthrin-α are targeted against gamma radiation (chemotherapy) and it acts as a protectant in cells that are P53 deficient (Sohn et al., 2009). The manner in which the apoptosome is signaled is also prevented when exposed to gamma radiaton. This study indicated that when utilizing pifthrin-α during chemotherapy damage and radiation therapy, it was feasible to incur much less cellular damage. This increases the longevity of the individual and mediates mass cell death.

Finally, this method could mediate unnecessary cell death during patient treatment with radiotherapy or other cancer treatment methods. Therefore, increasing patient life span and cell survival is crucial to the patient during cancer treatments.

Instead of creating a new chemotherapeutic agent that would attempt to block a multitude of transcription factors or alter downstream promoters, it would be in the best interest of a patient to attempt to mutate P53 back into its original form. Restoration of P53 from mutant to normal form could potentially activate tumor suppressor genes that failed to function as a result of P53 mutations. The reversal of P53 could assist in normal functioning of P21 and the return of apoptotic response control, which means that cancer cells could be targeted via the immune response and detection process. Inevitably, this targeting could reverse the process that induced cancerous cell growth.

This theory, if achieved, could have clinical implications in reducing tumorigenesis, and even development of more effective pharmacological agents, instead of utilizing chemotherapeutic drugs. This is clearly more advantageous because of the many negative physiological aspects of chemotherapeutic drugs and radiation treatment. Thus, a reversal of a P53 mutation could potential halt tumor formation and ensure that no more negative cellular responses would occur during the attempt to treat the tumors.

The utilization of gene therapy to mutate the mutant form of P53 back to its original state would be the most reasonable to attempt. It is the most reasonable method because of the mass scale damage that occurs when utilizing chemotherapeutics and radiation therapy. Therefore, creating a new method that would not induce the vast physiological damage that radiation can cause is desirable. One necessary factor to consider is not to invade local tissues with an accumulation of aneuploid cells that can be generated from P53 reactivation. Secondly, it is necessary to create a method that will allow for appropriate DNA synthesis and cell growth during G2 phase.

Initially, the research by Bates, Hickman, and Vousdan (1999) was correct in utilizing human fibroblasts. However, it was noted by Choi et al. (2011) that modulations of these fibroblasts are better substantiated in the extracellular fluid or ECF. Fibroblasts can only complete a finite number of cell replication cycles when the extracellular matrix is utilized for modulation. The medium that should be utilized in order to achieve the most effective results would be to utilize bovine growth serum.

A human cell line containing P53 would need to be incubated for optimal growth at 37°C. In order to induce cell cycle arrest without inducing widespread cell death via apoptosis or necrosis, the temperature of the cells could be changed -30°C. This is the optimal temperature to ensure that complete necrosis of a tissue has occurred. Actinomycin D was utilized previously in the aforementioned study, however it was determined that misappropriated S phase occurred during transformation of the mutant P53. It is necessary to utilize this drug to ensure that DNA is protected and will not be replicated incorrectly. This information indicates that post-treatment with actinomycin and pifthrin-α genetic material was not damaged. This also demonstrates that the DNA is protected and will not be incorrect and induce further aberrations or incorrectly formed cells.

The experiment would involve utilizing pfithrin-α, a compound that is a known inhibitor of P53 (Sohn et al., 2003). This has been utilized in nerve degeneration studies in order to inhibit damage and attempt to promote growth. Thus, it is believed that the same principal could be applied in order to restore cellular normalcy to cancerous cells. The proposed experiment would be to inhibit P53 utilizing the pharmacological agent pfithrin-α, therefore, halting abnormal growth. It would be proposed to increase Bcl-Xl and Bcl-2 in order to promote cell survival. Bcl-Xl and Bcl-2 would be increased by inhibiting P53 (Hemann and Lowe, 2012). The proposed drug would increase Bcl-Xl and Bcl-2 and activate survival promoting genes. The idea behind the methodology would be to increase the amount of survival promoting factors in order to restore cellular normalcy. The goal would be to attempt to increase Bcl-Xl which, has the ability to promote cell survival. This could be accomplished by treating with pfithrin-α, which can increase survival promoting genes and factors such as Bcl-Xl. The manufacturer of the drug has not released the full mechanism by which pfithrin-α functions. However, the research indicates that it acts as protectant against genotoxic and cytoxic stress. Thus, this would create a promotion of cell survival genes. This would, theoretically, halt any aberrations or further genetic or cellular damage that could occur. P53 would activate the apoptotic program during cellular duress or even during surgery or chemotherapy. Thus, inhibiting P53 should create an effect that would allow for a bypass of the death program. Then, by increasing Bcl-Xl this would promote cell survival. Therefore, after stress to the body, it would be feasible to have more normal cells that would have survived a stress such as radiation or chemotherapy. The more healthy cells that are unharmed could make surgery and chemotherapy safer for the patient, and possibly increase longevity.

This study would hopefully return cells to normal cell cycle control. It would also demonstrate controlled DNA synthesis or S phase function and not promote growth of cancerous cells. Thus, restabilizing cells to normal growth would then allow for treatment with a drug to achieve apoptotic death of damaged cells. The study would confirm this via utilizing a TUNEL assay.

A TUNEL assay is important to this study because it would reveal if cell death is occurring. A TUNEL assay is utilized in order to determine if DNA fragmentation has occurred. The assay determines if DNA is undergoing damage (apoptosis) by looking for nicks in the DNA, which is referred to as nick-end labeling. The TUNEL assay could also indicate if the cell death program is being activated by visualization of cells. Cells are stained and then visualized to determine the amount of cell death and if there is something malfunctioning in the apoptotic program (Oberhaus, 2003).

The cells that were mutated should show an indication of collapsing in on themselves, also named nuclear blebbing. It is imperative to conduct a TUNEL assay in order to confirm that apoptosis is not occurring. The TUNEL assay will be used to assess fragmentation which is proof apoptosis is occurring and which would demonstrate if cell cycle is normal or abnormal. Finally, if the DNA lacks fragmentation, it could be assumed that cell cycle has returned to normal.

Second, flow cytometry could be utilized in order to determine the amount of cell death that had occurred. This method is typically utilized to detect proteins or antigens by antibody binding. The cells could then be examined for abnormal molecular markers such as protein specific antigen (PSA) for prostate cancer, BRAF in skin or colorectal cancer, thyroglobulin found in thyroid cancer (Bigbee et al., 2003). There are individual tests to measure for each abnormal marker. One example is to conduct a blood test utilizing monoclonal antibodies to

measure the amount of PSA in the blood. This is necessary because if an inordinate amount of cell loss or healthy cell death is occurring, the drug would not be an effective treatment. One advantage would be able to detect the percentage of dead cells in G1, S, and G2 phases, all throughout cell cycle. The molecular markers could indicate if apoptotic death of damaged cells is occurring or the markers could indicate if normal cells are also being subject to the death program. Vousdan (2012) utilized a DNA profile (DNA/ng amount) for each phase of the cell cycle of as an indicator of any cell death occurring.

Next, whole cell lysates would be obtained, which would be examined using Western blot analysis. The samples would be of treated cells in order to determine if the drug was inducing any aberrations in DNA. These aberrations could be genetic mutations induced by the pharmacological agent. It is necessary to measure for these aberrations to ensure that the expression of protein products of cell cycle are not being altered. The Western blot would demonstrate if P21, P53, and RB were present in the lysates. It is necessary to measure the expression of P21, P53, and RB after drug treatment, as well as, in untreated samples. This technique has the potential to show if there is an over-expression or under-expression of any of theses proteins.

Research on P53 and the ability to reverse the mutation process has vast systemic effects and potential implications for the future. P53 reversal could lead to halting oncogenic progression in aggressive cancers such as Merkel cell carcinoma, lung and bronchial, ovarian, colorectal, and breast cancer. These are main concerns for two reasons. The first being that these are the primary cancers that P53 mutations have been discovered to be a main factor. Second, the cancers listed are aggressive. This means that their growth is invasive, expansive, and rapid. The more invasive, expansive, and rapid a cancer is the more damage a cancer can induce. The

damaged induced by cancer ranges from abnormal cell growth, organ damage, tissue destruction, abnormal cell death, and possibly even death from the products of the aforementioned damage. The technology has the potential to save lives if it can be achieved through these types of studies and if P53 reversal can be achieved, a multitude of damaging effects can be prevented.

For example, P53 reversal can aid in breast cancer as a method of cyclotherapy. This is useful in tumors that lack progesterone receptors, HER2 receptors, and estrogen receptors. This method utilizes activators of P53 and careful selection of these activators can be used to attack aggressive cancer cells, thus limiting the amount of damage to normal tissues. The more normal a tissue can remain, the prognosis will improve. The normalcy of a tissue would also indicate that the cancer would be less likely to spread to other local or distant tissues.

Additionally, when P53 becomes over expressed or mutated, it can induce neurological demyelination. This affects the nervous system, as well as, chemical signaling such as secondary messenger systems. Thus, P53 can be utilized as a potential treatment in multiple sclerosis or potentially in traumatic brain injuries (TBI) in which sheering of axons and demyelination occurs (Li et al., 2008). Mouse models have shown an increase in demyelination when overexpression of P53 was inhibited. TBI, MS, and neuromuscular dysfunction all have the potential to lead to disjointed movements, improper chemical signaling, and demyelination and can have permanent effects as progression proceeds. This is significant because it is necessary, in some neurological cases, to inhibit P53. It is also significant because if demyelination occurs, the likelihood of a vast misappropriated neurological response is quite high.

P53, as a mutated molecular marker, can also facilitate adenocarcinoma detection. This reversal discovery could potentially lead to early treatment of adenocarcinomas. It is known that early treatment mediates the best prognosis for longevity of a patient. Therefore, it is imperative

for future research to be conducted in order to determine if P53 reversal could lead to the inhibition of oncogenic transformation.

Finally, being able to reduce chemotherapeutics utilized with radiation therapy could limit extensive damage to the body. The reversal of P53 has the potential to reduce the amount and even use of chemotherapy. However, it does appear that there would still be a need for chemotherapeutic drugs or pharmacologic alternatives in order to achieve complete reversal. The patient could have a more cost-effective management of disease as well, thus lowering the cost of treatment and aiding many patients both monetarily and physiologically.

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