University of Veterinary Medicine Budapest

Department of Anatomy and Histology

Anti-cancer mechanism of p53 gene and its
application for treatment

Sihyun Han

Supervisor: Dr. Rácz Bence, professor

Department of Anatomy and Histology

2023

Abstract

The p53 protein is a transcriptional active factor and occupies a pivotal part of the mechanism of action related to cancer suppression through multi-area regulation. mdm2 is a well-known p53 regulator that maintains the expression of p53 at an appropriate level by the Negative feedback system.

Representative anticancer mechanisms of p53 include cell cycle arrest, DNA repair, apoptosis promotion, and angiogenesis inhibition.

 The current treatment method is to restore the function of p53, which seems to be able to effectively remove cancer cells. However, failure to control the expression of p53 can lead to problems and side effects of p53 are feared. The expression of p53 in cancer cells can cause abnormalities due to interactions with other signaling systems, and excessive expression of p53 can promote cell aging. Cell necrosis caused by apoptosis in normal cells during chemotherapy is also due to the expression of p53.

Cancer treatment using p53 at the current stage should proceed as a treatment method specifically targeting the expression of p53 and should play an auxiliary role in parallel with other anticancer drugs. In the long run, through specific research on the mechanism of p53's action, it is necessary to maintain p53 at an appropriate level and apply a treatment method suitable for the cause of each cancer.

While extensive research has focused on p53 in humans, veterinary medicine has lagged behind. However, studying p53 in animals, especially species with natural cancer resistance like elephants, can offer valuable insights for both animal health and human medicine. Furthermore, the connection between p53 in dogs and its association with cancer is explored. These findings highlight the importance of bridging the gap in veterinary research to benefit animals and advance our understanding of p53's broader role in biology and cancer treatment.

Összefoglaló

A p53 fehérje egy aktív transzkripciós faktor, és a rák elnyomásával kapcsolatos hatásmechanizmusban több terület szabályozásán keresztül kulcsfontosságú szerepet tölt be. Az mdm2 egy jól ismert p53 szabályozó, amely a negatív visszacsatolási rendszer révén megfelelő szinten tartja a p53 expresszióját. A p53 reprezentatív rákellenes mechanizmusai közé tartozik a sejtciklus megállítása, a DNS-javítás, az apoptózis elősegítése és az angiogenezis gátlása.

Az egyik rákellenes kezelési módszer a p53 funkciójának helyreállítása, amely úgy tűnik, hogy képes hatékonyan eltávolítani a rákos sejteket. A p53 expresszió szabályozásának elmulasztása azonban problémákhoz vezethet, és a p53 mellékhatásaitól tartanak. A p53 expressziója a rákos sejtekben rendellenességeket okozhat más jelzőrendszerekkel való kölcsönhatások miatt, és a p53 túlzott expressziója elősegítheti a sejtek öregedését. A kemoterápia során a normál sejtekben az apoptózis által okozott sejtelhalás szintén a p53 expressziójának köszönhető. A p53-mal történő rákkezelésnek a jelenlegi szakaszban a p53 kifejeződését kifejezetten célzottan célzó kezelési módszerként kell folytatódnia, és más rákellenes gyógyszerekkel párhuzamosan kiegészítő szerepet kell játszania. Hosszú távon a p53 hatásmechanizmusának specifikus kutatásával a p53-at megfelelő szinten kell tartani, és az egyes rákbetegségek okának megfelelő kezelési módszert kell alkalmazni.

Míg emberekben a p53-mal kapcsolatos kutatások kiterjedtek, az állatorvoslás e téren lemaradt. A p53 tanulmányozása állatokon, különösen az olyan természetes rákellenállással rendelkező fajokon, mint az elefántok, azonban értékes ismereteket nyújthat mind az állategészségügy, mind a humán gyógyászat számára. Továbbá a kutyák p53-jának és a rákos megbetegedésekkel való kapcsolatának összefüggései is tanulságosak lehetnek. Ezek az eredmények rávilágítanak az állatorvosi kutatásban meglévő szakadék áthidalásának fontosságára az állatok javára, valamint a p53 biológiában és a rák kezelésében betöltött szélesebb körű szerepének megértésére.

Contents

1. List of abbreviations

Apaf-1 : Apoptotic protease activating factor 1

ATM : Ataxia-Telangiectasia mutated

ATR : Ataxia-Telangiectasia Rad3-related

BAX : Bcl-2 Associated X-protein

Bcl : B cell lymphoma

Bid : BH3-interacting domain death agonist

CDB3 : Cytoplasmic Domain of Band 3

Cdk : Cyclin-dependent kinase

Chk1/2 : Checkpoint Kinase ½

CI : Confidence Interval

Cytc : Cytochrome c

DDB2 : DNA Damage Binding protein

DR5 : Death Receptor 5

EF2 : Elongation factor

Gadd : Growth Arrest and DNA Damage

HIF-1 : Hypoxia Inducible Factor 1

HRE : Hypoxia Responsive Element

MDM2 : Mouse Double Minute 2

miRNA : microRNA

MDR1 : Multi drug resistance 1

MI-219 : mdm2 inhibitor

PBL : Peripheral Blood Lymphocyte

PCNA : Proliferating cell nuclear antigen

PRIMA-1 : Proline-rich membrane anchor 1

Puma : p53 upregulated modulator of apoptosis

TP53 : Tumor Protein 53

TRAIL : TNF-related apoptosis-inducing ligand

VHL : Von Hippel-Lindau

WT p53 : Wild Type p53

2. Introduction

The p53 protein is a major transcription associated with cancer suppression and is expressed by the TP53 gene present in the 17q11 chromosome site of the human body. [10] The first p53 was found in transgenic cells by sv40 virus. [21,26] In the early days of discovery, p53 existed in large amounts in the form of binding to the T antigen, which was recognized as a cancer-causing protein. However, the high expression rate in cancer cells was due to p53 of Mutant type. [3] As research on p53 progressed, it was found that WT p53 does not have the ability to transform and conversely performs the function of cancer suppression. [3, 21]

Although many other factors exist as cancer-related regulators, p53 has important implications for cancer research and treatment. P53 plays an important role in maintaining the cancer suppression signaling system as a "guardian of genes." [21] p53 is composed of 393 amino acids, and the amino acid (109-292) forms the DNA binding site. Since this site recognizes about 300 genes, p53 can be involved in various signaling processes. [10] For example, p53 stops the cell cycle, providing time for intracellular DNA to recover from chemical and physical stress, and inhibiting the replication of damaged DNA by interfering with the action of DNA polymerase. [15] If the damage is severe, it causes apoptosis to prevent the transmission of wrong genetic information. [17,24] In addition, it causes aging of cells and suppresses neonatal angiogenesis and energy metabolism of cancer cells. [15, 21] In the event of an abnormality in the action of p53 related to various regulatory mechanisms, it is a major cause of cancer. Therefore, it can be said that restoring the function of p53 also has a great effect on cell normalization. In particular, the area where mutations occur in the DNA binding site of p53 is called Hot spot, and the 175, 245, 248, 249, 273, and 282 amino acids are included. [10, 11] The study of p53 can be said to be of high utility in treating cancer cells, with mutant of p53 found in more than 50% of human cancers. [21,22,23] and long-term studies will reveal many of the mechanisms of action, making it easy to apply.

In this article, we will first look at the process of controlling the expression of p53, and then look at the representative anticancer mechanism in which activated p53 is involved and treatment methods using it. Using the control factor or signal system of p53, it can be said that cancer treatment can be easily performed through p53 function recovery in cancers related to p53 control abnormalities. However, there are problems in the cancer treatment process using p53. This is because the expression of p53 does not always involve successful results. [10] Therefore, considering the side effect of p53, overcoming measures to solve

problems occurring in the treatment process of p53 will be discussed. Additionally, we will delve into studies related to p53 within the veterinary field, highlighting their significance not only in potentially benefiting humans as well but also in animal clinical contexts expanding our understanding of p53's broader implications.

3. Principles of controlling p53 Expression

The cancer inhibitory activity of p53 depends on the quantitative level of the p53 protein. The expression rate of [1, 2, 17] p53 remains at a certain level, but p53 in normal cells exists at a low concentration due to mdm2. [20] mdm2 is a well-known regulator of p53 that binds to the transcriptional active site located in the 1-42 amino acids in front of the DNA binding site of p53. [15] The mdm2 regulator is transcribed by p53, but inversely, it acts to suppress the activity of p53. As a result, the negative feedback process in which p53 decreases by mdm2 and the mdm2 transcription activity of p53 decreases, and the amount of p53 recovers again is repeated **(Fig. 1.)**. [1,15] Considering the importance of p53's range of activity and ability to regulate, there are various p53 regulators in addition to mdm2. If regulators are found in cancer treatment, the scope of treatment can be expanded.

mdm2 combined with p53 inhibits the activity of p53 by using three main paths. The first path is to transform the distal region so that proteosome can recognize it as Ubiquitin ligase. [20,24] As a result, p53 is degraded and loses its ability to control. [10, 17] The second is to release p53 out of the nucleus. [15] The p53 that has moved to the cytoplasm loses its chance to bind to DNA and becomes neutralized. Finally, mdm2 inhibits the transcriptional activiting ability of p53. p53 has a polymerized site in the 324-355 amino acids on the C-terminal side of the amino acid. [10] In the steady state, p53 acts as a transcription factor by polymerization of four units. [1] In the presence of a large amount of mutant p53, a p53 polymer having no ability to activate is produced. [11] mdm2 binds to the p53 polymer to prevent p53 from adhering to the target DNA site for transcriptional activity. [1] MDMX, a homologous body of mdm2, also inhibits the transcription activity of p53, and MDMX binds to mdm2 and promotes Ubiquitination without being directly involved in the p53 decomposition process. [15] In addition to the feedback by p53, there is a mechanism to control the activity of mdm2. ARF expressed by oncogen isolates mdm2 from the nucleosome. [10] Therefore, when there is a possibility of developing cancer, the expression of p53 increases. [20,24]

The control process of p53 indicates that there is a possibility of cancer even if there is an abnormality in the system that controls p53 as well as the problem of p53 itself. Therefore,

if Mutant p53 is present in cancer cells, recovery to WT p53 is required, but if p53 is normal, it is necessary to find an error in the upper stage of controlling p53 such as the inhibition path of mdm2 and proceed with treatment.
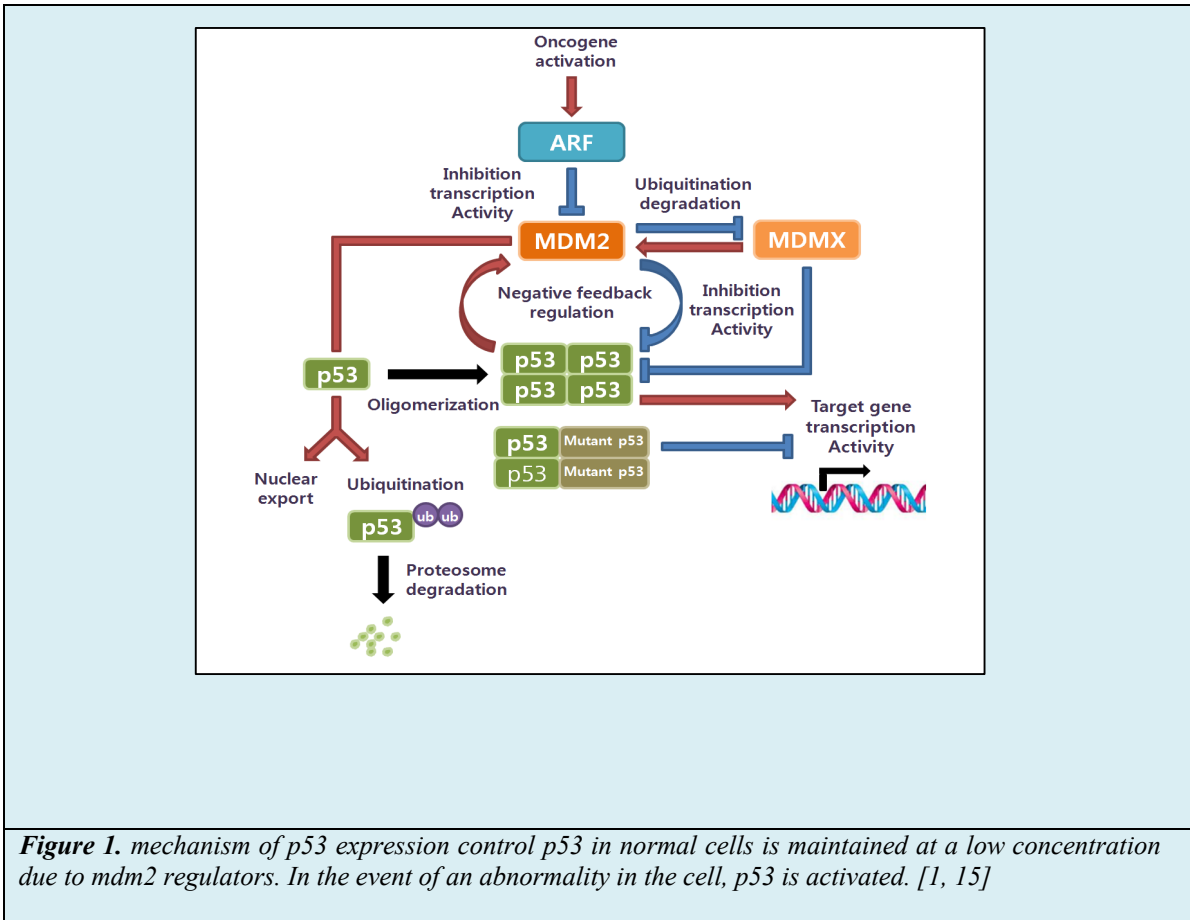

Figure 1. mechanism of p53 expression control p53 in normal cells is maintained at a low concentration due to mdm2 regulators. In the event of an abnormality in the cell, p53 is activated. [1, 15]

3. Anti-cancer mechanism of p53

3.1 Cell cycle arrest and DNA repair of p53

Damage to DNA can cause errors in genetic information. Incorrect genetic information needs immediate repair. If repairs are not carried out, genetic information will continue to be damaged. As a result, the expression of mutations accumulates and abnormalities occur in the cell system, increasing the possibility of progressing to cancer. If cells replicate DNA or are just before replication stages, there is also a problem of increasing homogeneous cells with damaged DNA.

 p53 directly stimulates DNA repair pathways and indirectly stops the cell cycle when DNA in cells is damaged by radiation, ultraviolet rays, and genetic toxic substances or when cells are stressed, preventing abnormal cell proliferation and providing time for cells to normalize. [10,26] p53 can effectively cope with the recovery process by affecting both paths. **(Fig. 2.)**
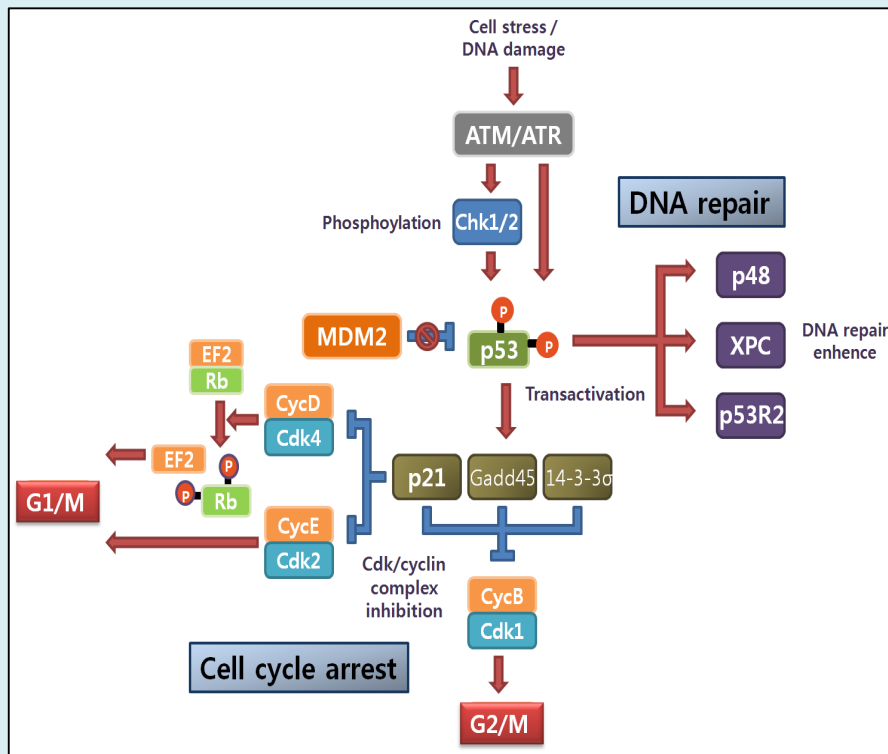
Figure 2. *Cell cycle arrest, DNA repair of p53. Stabilized p53 induces cell normalization by inhibiting the cell cycle-regulating complex or promoting transcription of proteins that increase DNA repair. [10, 15, 26]*

Cell cycle regulation is initiated by ATMs and ATRs that recognize DNA damage signals. These proteins phosphorylate p53 via chk1/2 or act directly as kinases. [10, 15] Phosphorylation of p53 means stability because it inhibits the interaction with mdm2. [2] The stabilized p53 does not decompose but induces transcription of p21, Gadd45, and 14-3-3σ acting on the cell cycle control complex. [15] The p21 protein transcribed by p53 inhibits the action of two types of Cdk/cyclin complexes, one of which is a complex of Cdk2 and cyclinE, and the other of which is a complex of Cdk4 and cyclinB. [11] The Cdk4 and cyclinB complex act to advance the cell cycle by expressing EF2 through phosphorylation of Rb. [15] As a result, cell cycle progression stops in phase G_1. And WAF1 expressed by p53 may bind to PCNA, a subunit of the DNA polymerase δ, to prevent DNA from being replicated. [8] In addition, it plays a role of preventing the cycle progression from the G_2 stage to the M stage by suppressing the action of the Cdk1 cyclinB composite along with Gadd45, 14-3-3σ. [15] The DNA repair process is accomplished by p53 activating the transcription of proteins involved in the repair. Phosphorylated p53 promotes the transcription of p48 repair protein expressed in the DDB2 gene, Xeroderma pigmentosum group C protein (XPC) that recognizes and binds the base position of damaged DNA, and
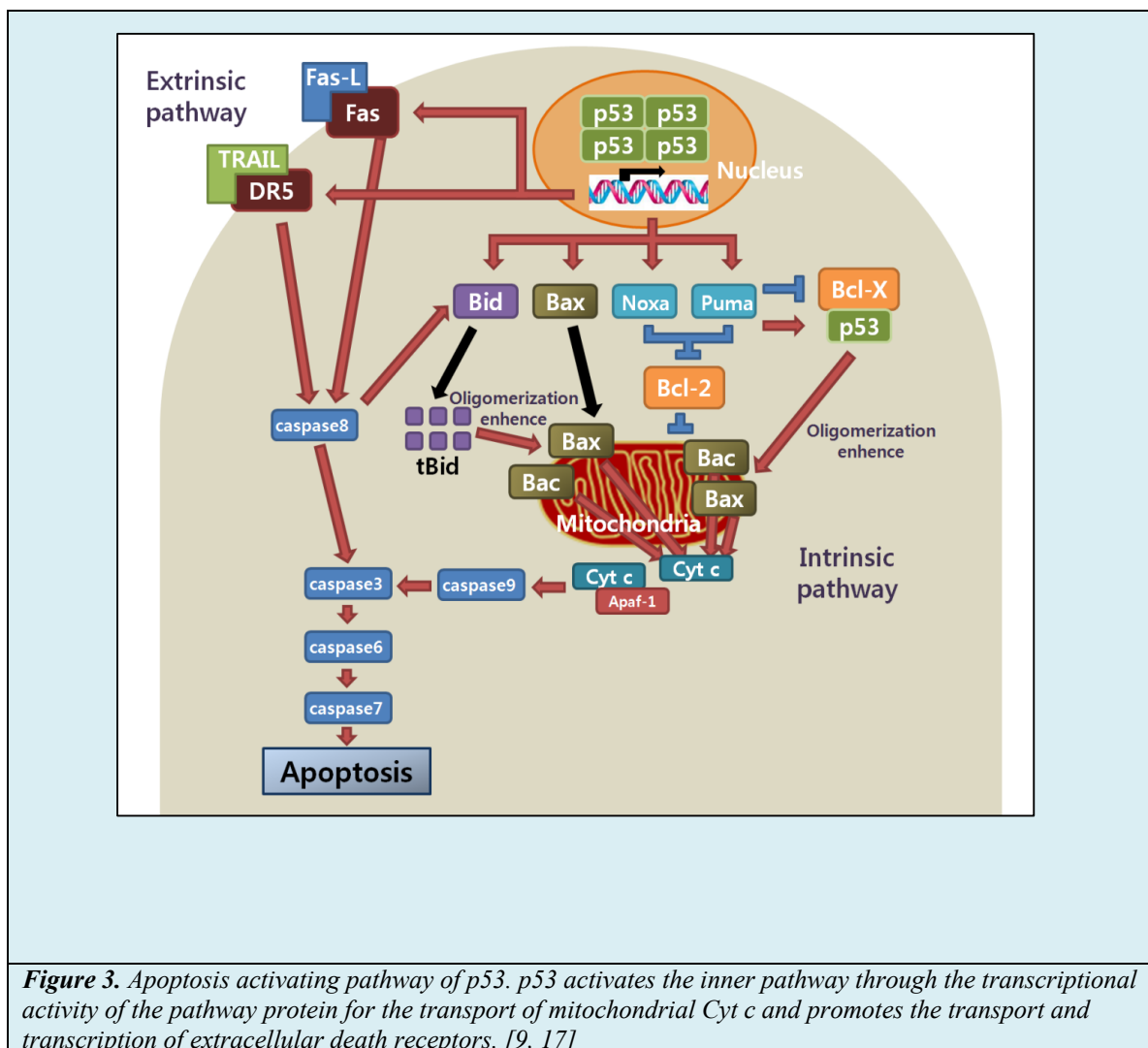
9

p53R2 that provides nucleotides to damaged DNA. [12, 15, 18]

3.2 Apoptosis promoting effect of p53

When a problem occurs, the easiest way to deal with it is to reject it. Cells kill cells that have abnormalities through the apoptosis process. Eliminating cells that are no longer recoverable is an effective means of preventing cancer. The sacrifice of a small number of cells allows other cells to escape the threat of cancer. But Apoptosis is a very careful process. If cell death does not proceed according to the appropriate time and cell condition, it can develop into cancer, but on the contrary, if it goes too far, even viable cells can die meaninglessly. The p53 protein can control both the cell cycle arrest and apoptosis described above. Therefore, when an intracellular abnormality occurs, you face a problem of choice. Although this part has not been revealed in detail yet, according to research, the degree of expression by phosophyllation and acetylation of p53 depends on the type of stress, exposure time, and cell characteristics. [2, 17, 26] In the related mechanism of action, there is an apoptosis selection process by factors that form a transcription complex along with p53 **(Fig. 3.)**. When the cell cycle arrest process is performed, p53 binds to the transcription active site (TAD) together with the Miz-1 protein to induce transcription of p21. [10] However, if the Myc protein expressed in the cancer gene is present, Myc binds to the transcription active site together with Miz-1, interferes with the cell cycle rest of p53. [10, 17]

Apoptosis by p53 is divided into an intrinsic pathway in the mitochondria and an extrinsic pathway associated with the death receptor in the cell membrane, depending on where the reaction takes place. [17,24] A p53-dependent reaction occurs in the extrinsic pathway by the protein transcribed by p53 and a p53-dependent reaction occurs in the intrinsic pathway as well as a p53-independent reaction in which p53 acts directly. [21] The first step in the intrinsic pathway is the synthesis of Bax, Noxa, Puma, and Bid proteins by p53 active factors in the nucleus. [10] Bax and Bac form mitochondrial outer membrane pathways through oligomerization, allowing Cytc to be released into the cytoplasm. [6, 10] Cytc released forms Apaf-1 and Aposome, activates capase-9 and sequentially activates capase-3, 6, and 7 to lead to apoptosis. [6] Noxa and Puma promote Apoptosis by inhibiting anti-cellular apoptic factor Bcl-2. [10] Puma inactivates the action of Bcl-xL, an inhibitor that binds to p53 independently of the cytoplasm. [6,17,24] Unresisted p53 moves to the mitochondria and helps the oligomerization of Bax and Bac. [6,17,24] The extrinsic pathway is the process by which the membrane protein synthesized by p53 moves to the cell surface and induces an apoptosis reaction. There are Fas, DR5 receptors, and these membrane proteins are called death

receptors and activate the apoptosis reaction by bound ligands. Overexpression of p53 increases the transport of Fas to the cell membrane and the Fas receptor binds to the FasL ligand present in the T cell. [17,24] DR5 is produced by DNA damage and binds to the TRAIL ligand. [6] TRAIL is expressed in the spleen, small intestine, and thymus. The binding of the ligand induces the caspase-8 active pathway of the receptor, which results in apoptosis through caspase-3. [17] Another action of activated caspase-8 is to cleave the Bid transcribed by p53 so that the activated tBid helps the oligomerization of Bax and Bac. [6]

Figure 3. *Apoptosis activating pathway of p53. p53 activates the inner pathway through the transcriptional activity of the pathway protein for the transport of mitochondrial Cyt c and promotes the transport and transcription of extracellular death receptors. [9, 17]*

3.3 Angiogenesis inhibitory effect of p53

Living cells use energy. Therefore, sufficient oxygen and nutrients must be supplied to maintain cell survival. As cells gradually grow and the number increases through division, it is necessary to open a passage for efficient transport of substances. Angiogenesis is the process of taking endothelial cells from existing blood vessels and creating new blood vessels. This is also done when blood vessels are created, or vascular damage is present in the early stages of occurrence. Under normal conditions, angiogenesis is a beneficial activity. But in

the case of cancer cells, it is different. Cancer cells lose control of the cell cycle and are constantly divided. At this time, a lot of energy is consumed, and new blood vessels are forced to be generated in order to receive a supply of substances. Because p53 is responsible for some of the mechanisms that inhibit angiogenesis, it can inhibit the growth and proliferation of cancer cells.

p53 affects the part that regulates the new angiogenesis inducing substance and indirectly inhibits it. Vascular endothelial growth factor (VEGF) is a signaling factor that activates vascular production. [10] HIF-1 is a hypoxic angiogenic expression factor and consists of two subunits, α and β. α and β form a complex, bind to the HRE portion of the VEGF gene, and then induce transcription of VEGF. (10) The generated VEGF binds to the VEGF receptor present in vascular endothelial cells to promote the migration and production of vascular cells. [10] Normally, HIF-1α is easily decomposed as it is recognized by VHL with a hydroxyl group attached by Prolyl-4-hydroxylase. [10] p53 was found to have the transcriptional activity of Prolyl-4-hydroxylase, which induces the decomposition of HIF-1α. [7] In addition, p53 acts to enhance the ubiquinization of HIF-1α by mdm2 molecules. [15] Other studies have also shown that miRNA-107 overexpressed by p53 inhibits the expression of HIF-1. [13] Therefore, p53 affects the inhibition of expression of HIF factors in various ways **(Fig. 4.)**.

Figure 4. Angiogenesis suppression mechanism. (10, 16)
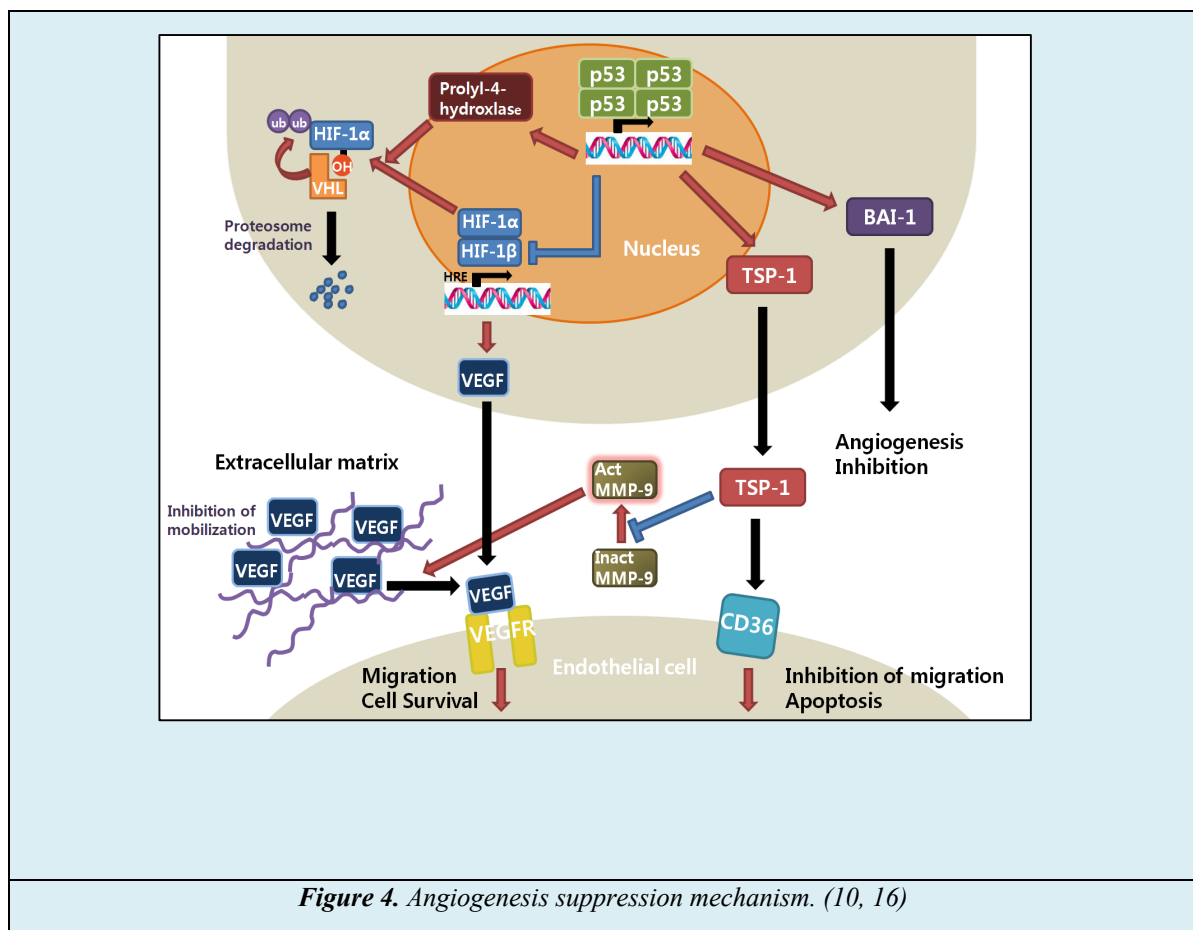
Another angiogenesis inhibitory action of p53 is to express angiogenesis inhibitory proteins such as TSP (Trombospondin)-1 and BAI (Brain-specific angiogenesis inhibitor)-1 through transcriptional activity. [15, 16] TSP-1 protein transcribed by p53 is a typical angiogenesis inhibitor and binds to CD36 receptors in vascular endothelial cells. [16] Activation of CD36 receptors causes apoptosis in cells or inhibits migration and inactivates matrix metalloproteinase (MMP). [16] MMP-9 acts to release VEGF combined with extracellular matrix. [16] As a result, VEGF is isolated to the Extracellular matrix and angiogenic signaling due to VEGF is inhibited. (16)

4. Side effect of p53

In cancer treatment, the genetic treatment method using p53 has less toxicity or side effects than chemical or radiation treatment. However, it is difficult to know what side effects will occur when expanding to the in vivo range for actual treatment in a single cell-level study. Considering the various regulatory mechanisms of p53, interactions with other systems in vivo may have unpredictable effects beyond treatment purposes. If the side effects of p53 occur, the damage will be great and recovery will be difficult. Until now, studies have been conducted focusing on the anticancer mechanism of p53, and not many studies have been conducted on the side effects of p53. Recently, side effects of p53 have been reported in liver cancer, and an increase in the expression of p53 may stimulate other circuits associated with the occurrence of cancer and promote tumor formation. Increasing the expression of WT p53 in stage 3 hepatocellular carcinoma activates the Notch1-Snail pathway, increasing the infiltration and metastasis of cancer cells. [4]

The currently known problem with p53 is the side effect of p53 when it is overexpressed. Another problem may arise if the appropriate level of p53 expression is not maintained in the process of recovering the function of p53. Among the side effects of p53, the current focus is on cell aging. When telomere is shortened or DNA is damaged, the Ras and Raf pathways are activated when oncogene is expressed, stabilizing p53 and aging may proceed through the expression of p21. [15] Therefore, p53 expression in cancer cells can have an effect of suppressing cancer, but if over expressed, side effects that promote aging will occur. [5] This is not just for cancer cells. If cancer cells are not selectively targeted in the process of p53 gene therapy, the expression of p53 in normal cells may also increase, so caution should be taken.

The expression of p53 in cancer treatment using radiation or genotoxic drugs increases

apoptosis in cells. [10] In particular, lymphocytes, bone marrow, and intestinal epithelial cells are sensitive to p53, so normal cells easily die during cancer treatment. Therefore, in this case, it is necessary to lower the expression of p53 in normal cells. Therefore, it will be considered that the control of p53 expression in the treatment stage requires different prescriptions by dividing cancer cells and normal cells.

5. A Study on the Treatment of Cancer Using p53 and Its Future Plan

5.1 Importance of Vector

Existing chemotherapy-centered cancer treatment methods often cause side effects and complications such as decreased immune function or vomiting, diarrhea, and weight loss due to damage to normal cells. The advantage of the treatment method using p53 is that cancer cells can be selectively treated without affecting normal cells. For effective treatment, it can be said that the role of vectors to introduce genes or therapeutic substances into cancer cells is important. The priority task in cancer treatment using p53 is to first increase the cancer cell specificity of the vector. Here, specificity refers to the ability to distinguish between cancer cells and normal cells, but before that, it presupposes the ability to reach the target point without decomposing or discharging a vector into a living body. In addition to specificity, stability is also an important issue. In general, viruses are often used as vectors. Viruses can transmit genes more efficiently than other bacteria, plasmids, or synthetic vectors, but there is a high risk of mutation or cell destruction because they multiply by inserting their genetic material into the host cell's DNA. To overcome this, the adenovirus is used as a vector. Unlike other viruses, adenovirus can carry a relatively large amount of DNA and does not insert genes within host cells. [21] Currently, stability in the human body is secured by using a type that has been artificially suppressed. [20] However, the potential risk of the virus vector itself accumulated in the body cannot be overlooked. Adenovirus has a limited number of cells that can be applied and no gene is inserted, so it is also necessary to overcome the short-living disadvantage. [20] For stable treatment, various types of vectors safer than adenovirus need to be discovered and cancer cell specificity needs to be improved.

5.2 Treatment of cancer by restoring p53 function

If cancer progresses beyond the expression of p53, the simplest treatment is to introduce the Tp53 gene into cancer cells to express normal p53. [19] In order to increase the activity of p53, a method of applying a slight genetic modification to the introduced p53 using recombinant technology is used. For example, p53 with the amino end removed exhibits a

14

high expression rate because ubiquitination by mdm2 does not occur well. [20] In malignant glioma, p53 attached with IFV hemagglutinin-2 protein is used to facilitate transport into nucleus in cancer cells. [20] However, in most cancer cells, mutant p53 is present in large amounts, which can inhibit the transcriptional activity of p53 expressed in the introduced gene. [1] And mutant p53 itself can also affect cancer cells. The function of mutant p53 in cancer cells has not yet been revealed, so future research is needed. What is known to date is the ability to change the phenotype of cells or to increase the resistance of cancer cells in other chemotherapy. [19, 20] If mutant p53 is present, cancer cells are treated by injecting small molecules to restore it. CDB3 molecules bind to the core domain of p53 and function as chaperone to help distorted p53 recover its proper folding structure. [20, 21] Through this, apoptosis is induced by activating the transaction of p53 in cancer cells. [19] CP-31398 also helps shape change from mutant p53 to WT p53 and reduce ubiquitination without phosphorylation of p53, causing cell cycle arrest and apoptosis. [19, 21] PRIMA-1 also induces a normal shape change of p53, and unlike CDB3 and CP-31398, it has a specificity that is activated only in the presence of mutant p53. [19, 20, 21] P53R3 specifically binds to the DNA binding site, the 175th and 273rd amino acids of Mutant p53, helping to bind p53 well to the target promotor. WR-1065 is a substance derived from amifosine that relieves radioactivity during exposure [21] The mechanisms acting on Mutant p53 have not yet been identified. WR-1065 reactivates p53 and protects normal cells during chemical and radiation treatment **(Fig. 5.)**. [20]

Even when p53 expression is normal, the activity of p53 may be suppressed, which is a case where an abnormality occurs during p53 control. [20] In this case, the function is normalized as a method of inhibiting the action of regulators, and the interaction is suppressed by blocking the binding of mdm2 and p53. A typical mdm2 inhibitor includes Nutlin-3, which binds to mdm2 molecules to increase the transcription activity of p53 and promotes the suspension of the cell cycle of normal cells to help cell recovery in chemotherapy. [10, 15, 21] MI-219 also binds to mdm2 [15], and HLI-98 is a factor that interferes with ubiquitination of mdm2 molecules, and both substances cause apoptosis by p53 in cancer cells. [21]

Contrary to the functional activity of p53 in cancer treatment, there are cases in which a method of suppressing p53 is required. As described above, p53 inhibitors are used to suppress apoptosis in normal cells during chemotherapy. PFT (pifithrin)-α is a substance that reversibly interferes with the transcription activity of p53, and can inhibit apoptosis. [21] Using PFT-α can reduce side effects such as fatigue and hair loss in the process of

chemotherapy. [10]

The cancer treatment currently under development is to restore the function of p53 by introducing p53 or by inhibiting interaction with mdm2 molecules, which is too comprehensive compared to the complex regulation process of p53. In some cases, the symptoms of cancer may be improved by p53, but since the occurrence of cancer has a complex cause, it can be said that there is a limit to treatment only with the recovery of p53 function. One alternative is to use it supplementary in parallel with other treatments. Nutlin-3 has a synergistic effect with anticancer drugs such as doxorubicin and chlorambucil, and [15] combination with other drugs can also reduce the dosage of drugs.
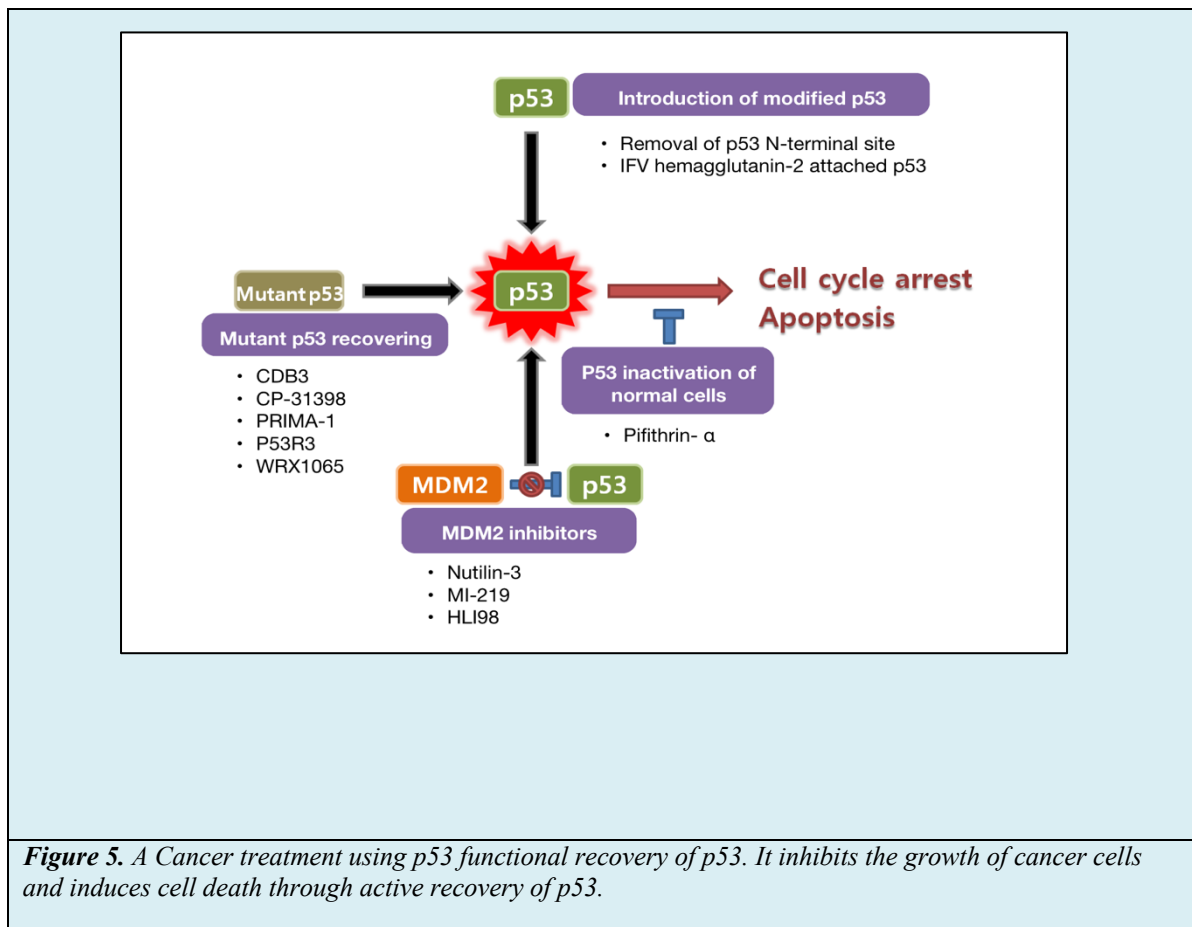

Figure 5. A Cancer treatment using p53 functional recovery of p53. It inhibits the growth of cancer cells and induces cell death through active recovery of p53.

5.3 A Specific Virus Treatment Method Using the Expression of p53

For the treatment method using p53 to be effective, it is necessary to move away from the existing treatment method and approach from other directions. In this regard, there is a method of using the presence or absence of p53 as a specific target for drugs. The Onyx-015 virus used here is a variant of the adenovirus, and the E1b gene portion of the virus's early genes is knock-out. [10] In the case of adenovirus, the expression of E1a activates Rb to promote the transcriptional activity of EF2 and inhibits the action of p53 through the

expression of E1b, resulting in the progression of the cell cycle. [10, 20] This allows the virus to proliferate and exit the cell. The Onyx-015 virus cannot inhibit the action of p53 due to the deficiency of the E1b gene. [10] Therefore, in the case of normal cells in which p53 is expressed, proliferation is suppressed by suspension of the cell cycle, but in the case of cancer cells, p53 is not expressed, so only cancer cells are selectively destroyed through proliferation and release. [21] Another virus is 01/PEME. This virus contains an artificial E2F inhibitor gene with a p53-dependent promotor. Therefore, virus proliferation in normal cells in which p53 is expressed is further suppressed. [20] Another characteristic of 01/PEME virus is to promote destruction of cancer cells by increasing the expression of E3, which artificially promotes virus release **(Fig. 6.)**. [20]
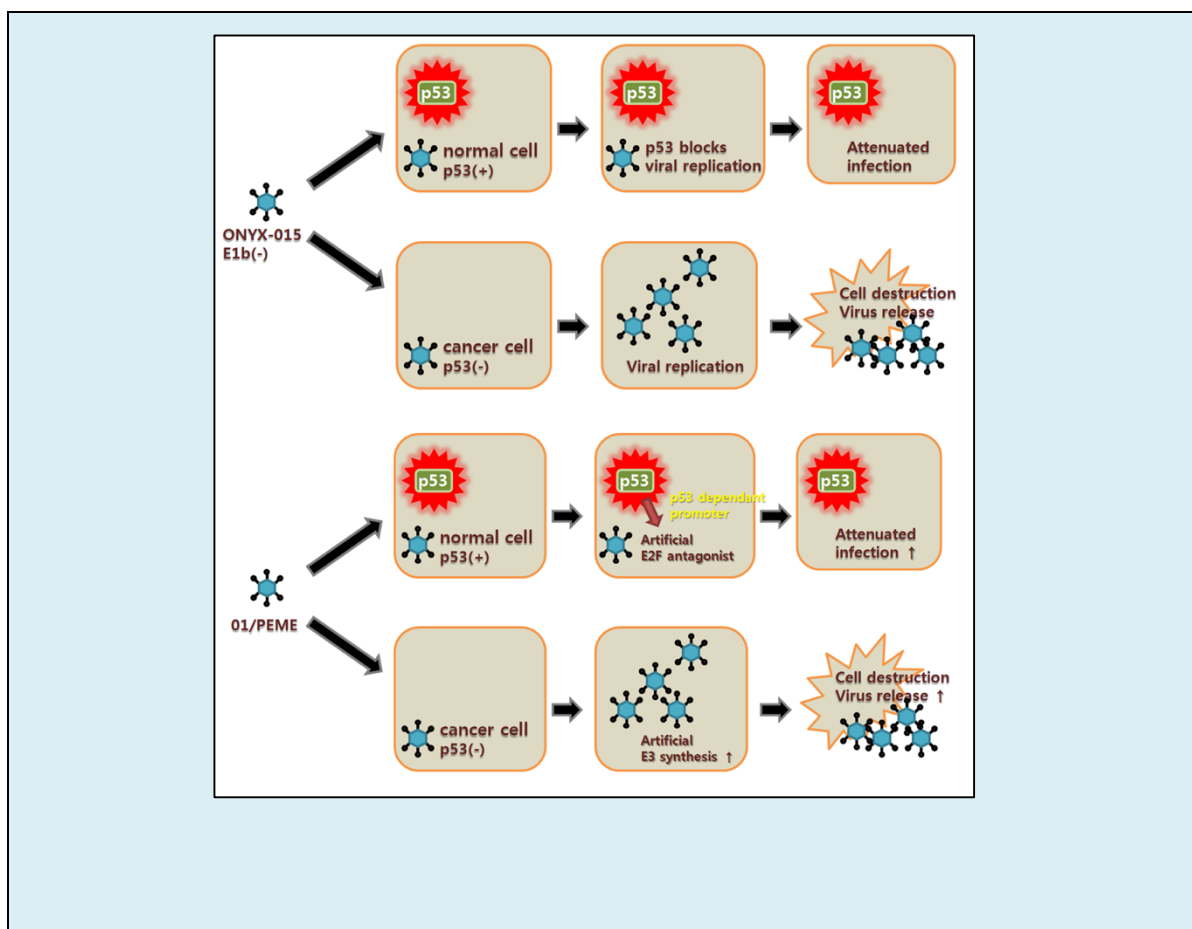

Figure 6. *A specific virus treatment method using the expression of p53. The ONYX-015 virus, in which the E1b gene portion of the adenovirus is missing, proliferation and extracellular release are determined by the presence or absence of p53. 01/PEME virus is an increase in proliferation inhibition in normal cells and release activity in cancer cells through artificial genetic modification. [10, 20]*

When looking at the above methods, it is considered safe in terms of side effects to not touch the expression of p53 in the treatment process. Therefore, treatment plan that applies the specific expression of p53 should be proposed. It is a method of improving the action of

existing anticancer drugs that could not distinguish normal cells from cancer cells by linking the expression of p53 with the inhibitory action of anticancer drugs. The MDR1 gene is a multidrug-resistant gene that combines with anticancer drugs such as Doxorubicin, Vinblastine, and Imatinib to synthesize P-glycoprotein, which releases drugs out of cells. [14] Recombine the MDR1 gene that artificially binds the p53-dependent promotor to Adenovirus. When vectors are introduced, no reaction will occur in cancer cells, but in normal cells where p53 exists, the drug-releasing resistance will be activated. Although experimental evidence is required, anticancer drugs administered under the assumption that P-glycoprotein does not affect surrounding cancer cells will be specifically effective only in cancer cells, and drug action will be suppressed in normal cells to reduce side effects. Chronic resistance due to the continuous expression of the MDR1 gene can also be considered, but due to the nature of Adenovirus, it is temporarily expressed without being inserted into the genome, so I think it is possible to take the drug according to the timing of taking the anticancer drug. And if it is additionally inserted into the gene portion of the ONYX-015 virus or 01/PEME virus

described above, cancer cells can be destroyed more effectively **(Fig. 7.)**.

Figure 7. Hypothesis of selective activation of anticancer drug using p53. Using Adenovirus, a recombinant MDR1 gene with p53-dependent promotor, by p53 expression, can promote extracellular release from normal cells due to drug resistance, thereby improving drug specific action.
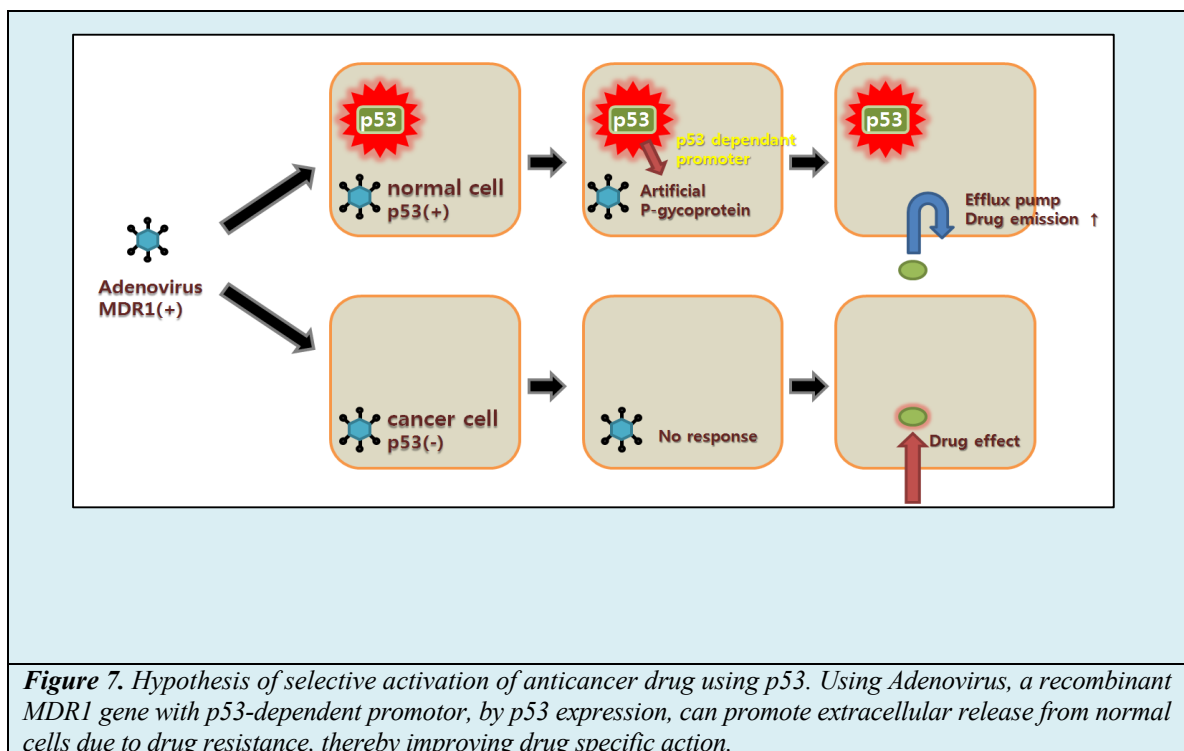
6. Veterinary aspect of p53

6.1 The Current State of Veterinary Medicine and p53

Once again, P53, commonly known as the "guardian of the genome," stands as a central protein derived from the TP53 gene. It has crucial function in cell cycle advancement regulation, maintenance of genomic stability, and coordinating cellular reactions to DNA damage and external stressors. Consequently, TP53 gene mutation is frequently associated with onset of diverse cancers, making it a focal point for investigation in human cancer research.

Given the well-established importance of p53 in human health, the scientific community has invested substantial resources and efforts in unraveling its mechanisms and exploring therapeutic strategies. These endeavors have led to significant breakthroughs in understanding how p53 functions and how its malfunction contributes to cancer progression.

Research on p53, from its underlying mechanisms to therapeutic applications, has been ongoing since 1979. However, the situation differs when it comes to veterinary medicine. While p53 is a highly conserved gene across species, and animals also face cancer and environmental challenges, the research on p53 in veterinary contexts remains limited. Most studies involving animals are oriented towards advancing human medical knowledge, neglecting the unique adaptations and requirements of various animal species.

Closing this research gap is essential not only for the well-being of animals but also for the advancement of veterinary medicine and our understanding of p53's broader role in biology. By conducting more comprehensive research that addresses the specific needs and challenges faced by animals, we can gain insights into the intricate interplay between p53 and various environmental stressors. This, in turn, may open new avenues for the development of targeted therapies and preventive strategies to combat cancer and other health issues in animals.

6.2 Veterinary research of p53 for human health

Investigating the complex ways animals naturally combat cancer can provide us with valuable knowledge that goes beyond specific species. This research is essential for not only improving cancer prevention and treatment methods but also addressing the specific difficulties encountered by particular groups of people, including those with a high risk of cancer, like individuals with Li-Fraumeni Syndrome (LFS), and the growing aging population. [34]

At the core of this exploration is p53, a protein commonly found to be mutated in human cancers. These mutations can lead to aggressive tumor development, invasion into surrounding tissues, metastasis to distant organs, and evasion of the immune system's defenses. Given the crucial role of p53 in cancer, researchers have been investigating its potential in disease risk

prediction, personalized treatment strategies, and prognosis assessment.

What makes this research particularly fascinating is the discovery of TP53 mutations in wild animals that closely resemble the mutations found in human cancers. These p53 variations in the animal kingdom have evolved as a natural defense mechanism against cancer and the environmental challenges they face in their habitats.

By studying these p53 variations in wild animals, scientists aim to gain deeper insights into the functioning of p53 across species. This knowledge could provide innovative approaches for targeting p53 in therapeutic contexts, ultimately benefiting not only humans but also the diverse animal species that share our planet. [34, 35]

How does large animals overcome cancer despite of their high number of cells? A comprehensive examination of necropsy data encompassing mammalian species was conducted to assess cancer mortality in relation to body size and life span. The study spanned from the striped grass mouse (weighing 51g with a maximum life span of 4.5 years) to the elephant (weighing 4800kg with a maximum life span of 65 years). [34] **(Fig. 8.)** Surprisingly, cancer risk did not exhibit an increase with mammalian body size and maximum life span among the 36 species analyzed. This phenomenon, known as 'Peto's Paradox,' highlighted that cancer mortality did not escalate with body size and/or maximum life span across mammals. [34, 36] Despite their substantial body size and extended life span, elephants maintain resistance to cancer, demonstrating a lower estimated cancer mortality. In an attempt to unveil the secret of elephants' cancer resistance, the African and Asian elephant genomes underwent analysis for potential mechanisms. [34, 36] Elephants have a single TP53 gene and 19 TP53 retrogenes compared to one copy in most mammals including human. This expansion occurred alongside the evolution of large body sizes and long lifespans in the Proboscidean lineage (elephants and their extinct relatives). TP53 retrogenes (copies derived from reverse transcription) evade MDM2-mediated ubiquitination by disrupting the interaction between TP53RTGs and MDM2. Simultaneously, the stabilization of p53 occurs as it forms a TP53RTG/p53 dimer, preventing degradation by MDM2. This mechanism enhances the sensitivity of elephant cells to DNA damage and apoptosis (programmed cell death) rather than DNA repair in elephants. [34, 35, 36] Therefore, by increasing the copy number of TP53 may have helped elephants to overcome the increased risk of cancer associated with having more cells and living longer, and that this may be a novel mechanism for resolving Peto's paradox. Furthermore, research comparing cellular response to DNA damage among elephants(n=8), healthy human controls(n=11) and cancer-prone patients with Li-Fraumeni syndrome

(LFS)(n=10) with their peripheral blood lymphocytes was performed. [34] After subjecting lymphocytes to 2 Gy of ionizing radiation, distinct levels of apoptosis were revealed. Notably, cells from patients with LFS displayed markedly reduced apoptosis(2.71%, 95% CI), compared with healthy human PBLs(7.17%; 95% CI) and elephant PBLs (14.64$; 95% CI) [34] **(Fig. 9.)** Both elephant and human PBLs demonstrated p53 and p21 protein expression following ionizing radiation exposure, with higher p21 protein expression observed in elephant PBLs compared with human PBLs.
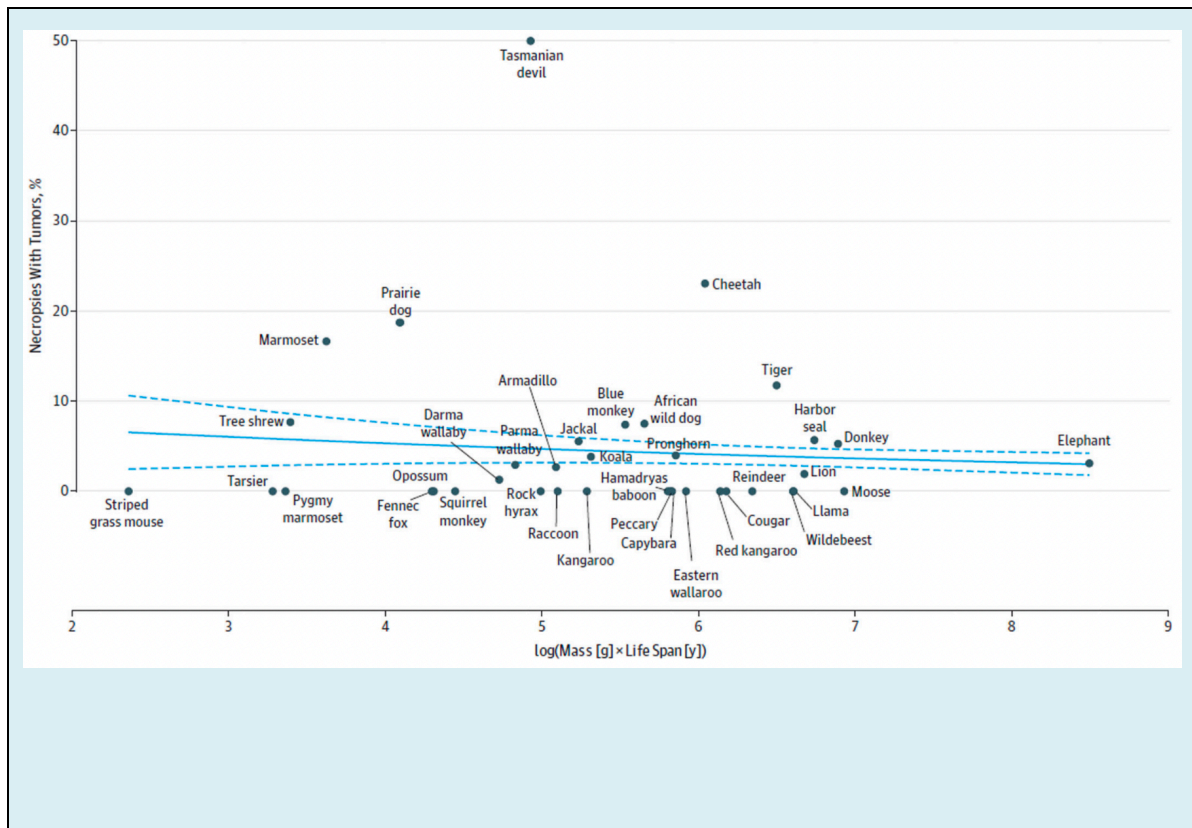

Figure 8. Cancer mortality across species according to body size and life span. Logistic regression shows incidence of cancer is not associated with mass and life span (model fit shown as blue line, 95%CIs(Confidence Interval) shown as dashed line.) *Note. From Abegglen LM, Caulin AF, Chan A, Lee K, Robinson R, Campbell MS, Kiso WK, Schmitt DL, Waddell PJ, Bhaskara S, Jensen ST, Maley CC, Schiffman JD (2015) Potential Mechanisms for Cancer Resistance in Elephants and Comparative Cellular Response to DNA Damage in Humans. JAMA 314:1850–1860.*
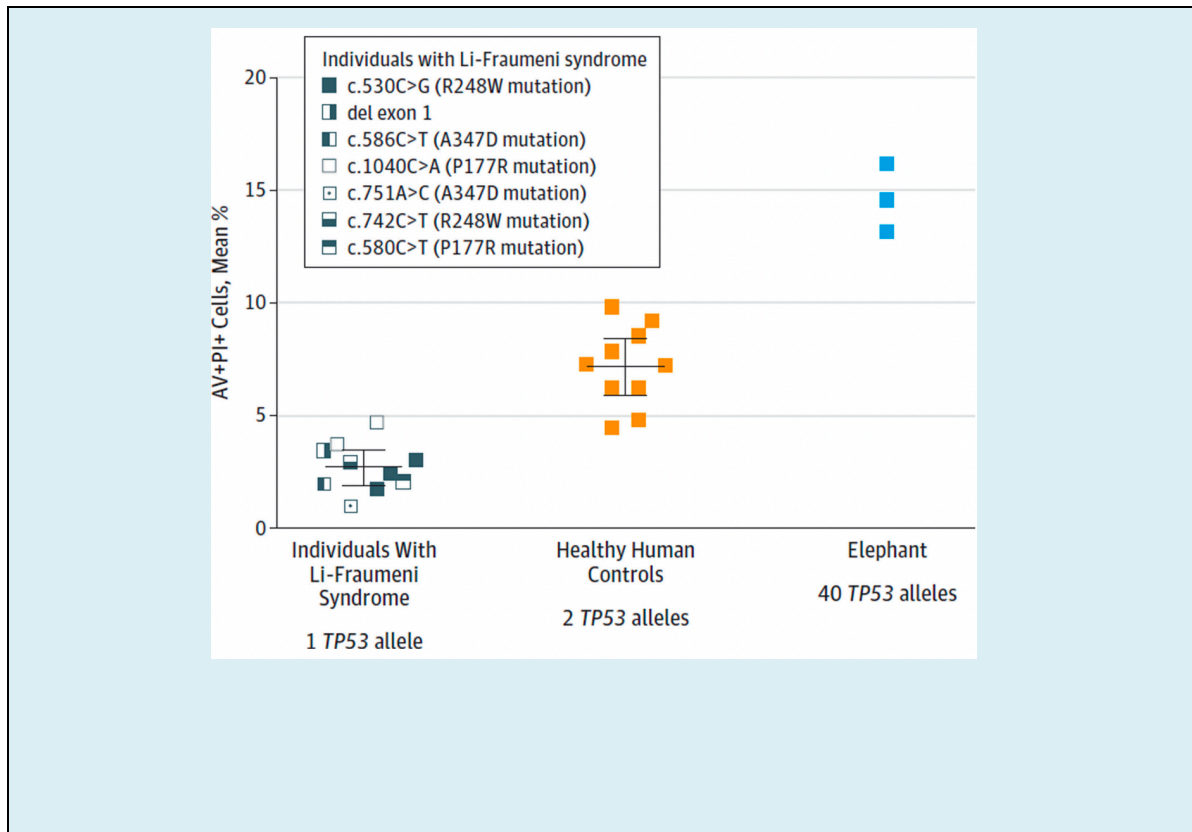
Figure 9. Relation between Number of Copies of TP53 and Apoptosis Response. The extent of ionizing radiation-induced apoptosis exhibited a direct correlation with the number of additional TP53 copies displaying an inverse relationship with cancer risk. Lymphocytes obtained from healthy individuals demonstrated a significantly higher rate of apoptosis when compared to those from patients with Li-Fraumeni Syndrome (LFS) (P< .001). Moreover, lymphocytes from elephants exhibited a markedly elevated rate of apoptosis in comparison to those from healthy controls (P< .001, determined through a two-sided t-test). *Note. From Abegglen LM, Caulin AF, Chan A, Lee K, Robinson R, Campbell MS, Kiso WK, Schmitt DL, Waddell PJ, Bhaskara S, Jensen ST, Maley CC, Schiffman JD (2015) Potential Mechanisms for Cancer Resistance in Elephants and Comparative Cellular Response to DNA Damage in Humans. JAMA 314:1850–1860.*
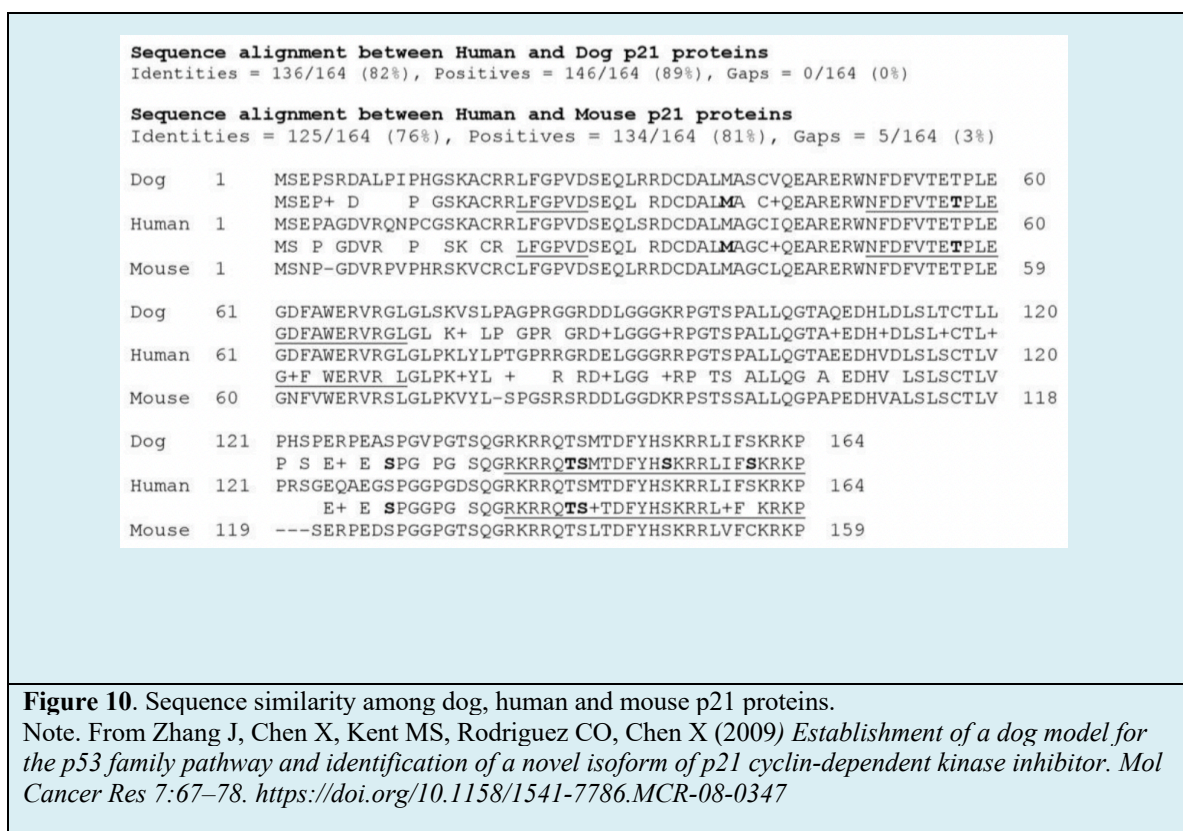
Researchers have recognized the value of using companion dogs as a resource in cancer research due to the similarities in genes and physiology between dogs and humans. Dogs with spontaneously occurring neoplasms can serve as valuable models to study cancer etiology in humans, potentially advancing our understanding of cancer prevention and treatment strategies. In this context, the research aimed to develop a canine model for the p53 family pathway. [37] It was observed that canine p53 responds to DNA damage and Mdm2 inhibition, leading to the activation of p53 target genes such as p21 and MDM2, similar to their human counterparts **(Fig. 10.)**. Additionally, p63 and p73, two other members of the p53 family, were expressed in canine cells under conditions of DNA damage. These findings suggest that canine p53 family members can be upregulated under stress conditions. In the context of cancer, melanomas in both humans and dogs are often highly resistant to radiation and chemotherapy. Surprisingly, in this study, it was discovered that out of the five melanoma cell lines examined, only one lacked a functional p53, while the others seemed to possess a functional p53 pathway. [37]

Notably, the p53-deficient cell line displayed significantly higher levels of p63 and p73 compared to other melanoma cell lines, indicating a potential role of these proteins in the inhibition of cell growth in response to DNA damage. This led to the categorization of the melanoma cell lines into two groups: those with functional wild-type p53 and low p63/p73 expression and those without functional p53 but high p63/p73 expression. These findings suggest that targeting the p53 pathway, possibly using Mdm2 inhibitors like nutlin-3, could be explored in canine spontaneous tumor models to manage certain cancers in humans. Another intriguing discovery in this study was the identification of two isoforms of canine p21, both inducible by DNA damage agents or nutlin-3 treatment. [37] The mapping of the canine p21 gene revealed that specific amino acid sequences played a crucial role in the expression of these isoforms. Interestingly, only a few amino acids differed between dog and human p21. This observation raises the possibility of similar mechanisms generating various p21 isoforms in both species, which could have different biochemical and biological functions. Understanding these mechanisms could provide insights into the generation and functions of various human p21 isoforms, potentially advancing our knowledge of cancer biology and therapy.

Figure 10. Sequence similarity among dog, human and mouse p21 proteins.
Note. From Zhang J, Chen X, Kent MS, Rodriguez CO, Chen X (2009) *Establishment of a dog model for the p53 family pathway and identification of a novel isoform of p21 cyclin-dependent kinase inhibitor. Mol Cancer Res 7:67–78. https://doi.org/10.1158/1541-7786.MCR-08-0347*

6.3 Veterinary research of p53 in animals

While research on veterinary cancer treatment involving p53 is relatively limited, there are

more studies that explores the mutation of the p53 gene and its correlation with clinical outcomes in companion animals.

One research focused on identifying mutations in the p53 gene in dogs with untreated high-grade lymphoma, revealing that 16% of the dogs exhibited p53 gene mutations. [38] This percentage aligns with previous studies on dogs with lymphoma, indicating a relatively frequent occurrence of p53 mutations in canine lymphoma compared to feline lymphoma. [38] The study employed PCR-SSCP analysis, known for its high sensitivity in detecting mutations, with primers designed for optimal sensitivity. The mutations observed in canine tumors were similar in location and type to those in human tumors, particularly in conserved p53 gene domains, and included point mutations and single nucleotide insertions. The presence of p53 mutations was associated with a significantly lower response to chemotherapy and shorter overall survival in dogs with lymphoma, suggesting a potential link between p53 inactivation and drug resistance. While the study did not delve into the functional consequences of these mutations, it highlighted the importance of further research to elucidate the relationship between p53 dysfunction and clinical drug resistance, ultimately offering valuable insights for lymphoma treatment in dogs. Additionally, the study indicated that p53 mutation could serve as a prognostic factor for lymphoma in dogs, emphasizing the need for larger-scale studies to confirm the precise correlation between mutation incidence and specific subtypes of lymphoma in these animals.

Apocrine sweat gland carcinomas (ASGCs) are infrequent malignant skin tumors found in both dogs and humans. While existing research has primarily concentrated on the clinical and epidemiological aspects of ASGCs, their underlying causes remain poorly understood. Researchers explored the involvement of the p53 gene and ultraviolet radiation (UV) in the pathogenesis of apocrine sweat gland carcinomas (ASGCs) in dogs. [39] The study revealed that 13 out of 40 canine ASGCs displayed p53 gene mutations, including unique mutations not previously reported in any tumor. These mutations were characterized by transitions and double transitions consistent with UV radiation-induced alterations. Notably, one mutation at codon 90 was observed in six cases, suggesting a potential link to the development of ASGCs. Additionally, mutations at codon 157, codon 178, and other locations were identified, with some of these mutations associated with other human cancers. The findings indicated that both UVA and UVB radiation might contribute to ASGC formation, with p53 gene mutations playing a role. These results not only shed light on the pathogenesis of ASGCs in dogs but also uncovered novel mutations in the p53 gene with potential implications for cancer research.

7. Discussion

The current treatment method, which mainly involves the recovery of p53, clearly has limitations considering the fact that a complex cause plays a role in cancer development. Therefore, the treatment range should not be limited to p53, but should be used supplementary through parallel with other anticancer treatments. In order to utilize the anticancer mechanism of p53, it is important to identify the specific path of the subphases. By unraveling the knots of the complex signal system nets one by one, drugs targeting regulatory factors at each stage can be developed and disturbances with other signal systems can be prevented. Finally, a customized treatment method suitable for the patient's symptoms can be applied case by case.

Studies on the side effects of p53 that have not been noted so far should not be overlooked. It is necessary to minimize the overexpression of p53 and continue to reveal other side effects of p53. To overcome overexpression in normal cells, it is necessary to discover stable vectors and improve cancer cell specificity to control p53 expression in normal cells and cancer cells to an appropriate level. Currently, it is recommended that the function recovery method of p53 that changes the expression level of p53 is used as a supplementary method and that the specific expression characteristics of p53 are used as in the presented method. And combination with drug will promote the synergy of anticancer drugs and enable appropriate treatment without significantly increasing p53 expression.

The goal of cancer treatment is to fundamentally suppress the occurrence of cancer. Therefore, treatment using p53 should also ultimately move in the direction of identifying the reason for p53 abnormalities, that is, the cause of cancer, and should not be limited to suppressing cancer cells that have already occurred.

While extensive research has focused on p53 in humans, veterinary medicine has lagged behind. However, studying p53 in animals, especially species with natural cancer resistance like elephants, can offer valuable insights for both animal health and human medicine. Investigating p53 in dogs has revealed mutations in lymphoma cases, potentially impacting chemotherapy response and survival. Furthermore, the study of p53 in apocrine sweat gland carcinomas in dogs unveiled unique mutations, suggesting a role for UV radiation in their development. These findings highlight the importance of bridging the gap in veterinary research to unlock the full potential of p53 in safeguarding the health and well-being of both humans and the diverse animal kingdom, ultimately fostering a more comprehensive understanding of this critical protein's role in biology.

References

1. Amanda M Goh, Cynthia R Coffill and David P Lane (2010) p53 Laboratory, A.Star, Singapore, Translational Research, Institute of Molecular and Cell Biology, Singapore. The role of mutant p53 in human cancer. 223: 116~117, 120

2. Andrei V. Gudkov and Elena A. Komarova (2007) Department of Molecular Genetics, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA. Dangerous habits of a security guard: the two faces of p53 as a drug target. R67

3. Cooper, Geofferey M. (2000) Oncogene. World Science. 145~146

4. Guhung Jung. (2011) Department of Lice Science, Seoul University. Cancer metastasis increasing effect of mutant p53.

5. Kamada R, Toguchi Y, Nomura T, Imagawa T, Sakaguchi K. (2010) Tetramer formation of tumor suppressor protein p53: structure, function, and applications. Biopolymers 2016; 106: 598–612.Joana D Amaral, Joana M Xavier, Clifford J Steer, Cecilia M Rodrigues. The Role of p53 in Apoptosis. 2~3

6. Jose G. Teodoro, Albert E. Parker, Xiaochun Zhu, Michael R. Green. (2011) p53-Mediated Inhibition of Angiogenesis Through Up-Regulation of a Collagen Prolyl Hydroxylase. 313:968

7. Jun Yeong lee. 2008. p53 Discovery of a new protein FLJ that regulates protein expression. 1

8. KEGG PATHWAY Database. p53 signaling pathway - Homo sapiens. http://www.genome.jp/kegg-bin/show_pathway?130125268214738/hsa041 15.args

9. Lauren Pecorino. (2009) Molecular biology of Cancer. Hanmi medicine 137~138, 141~145, 151~152, 153, 169, 223~224

10. Lodish, Harvey Berk, Arnold, Matsudaira Paul. (2003) Molecular cell biology. 5th ed. W. H. Freeman & Company. 959~960

11. Maureen E.Fitch, Irina V.Cross and James M. Ford.(2003) Departments of Medicine and Genetics, Division of Oncology, 1115 CCSR Bldg, 269 Campus Drive, Stanford University Medical School, Stanford, CA 94305-5151, USA. p53 responsive nucleotide excision repair gene products p48 and XPC, but not p53, localize to sites of UV-irradiation-induced DNA damage, in vivo. 843

12. Munekazu Yamakuchia, Craig D. Lotterman, Clare Bao, Ralph H. Hruban, Baktiar Karim, Joshua T. Mendell, David Husof, and Charles J. Lowenstein. (2010) P53-induced microRNA-107 inhibits HIF-1 and tumor angiogenesis. 107:6334

13. Renata Lehn Linardi1 Cláudio Corrêa Natalini1. (2006) Multi-drug resistance (MDR1) gene and P-glycoprotein influence on pharmacokinetic and pharmacodymanic of therapeutic drugs. 337~338

14. Sanjeev Shangary and Shaomeng Wang. Clin Cancer Res (2008). Targeting the MDM2-p53 Interaction for Cancer Therapy. 14:5318~5322

15. S. Kazerounian, K. O. Yee and J. Lawler. (2008) Division of Cancer Biology and Angiogenesis, Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School. Thrombospondins in cancer. 701

16. Susan Haupt, Michael Berger, Zehavit Goldberg and Ygal Haupt. (2003) Department of Pharmacy, and Lautenberg Center for General and Tumor Immunology, The Hebrew University Hadassah Medical School, Jerusalem 91120, Israel. Apoptosis – the p53 network. 4077~4082

17. Tatsuya Yamaguchi, Koichi Matsuda, Yoji Sagiya, et al. (2001) p53-regulated Cell Cycle Checkpoint p53R2-dependent Pathway for DNA Synthesis in a p53-regulated Cell Cycle Checkpoint. 8256

18. Tomasz Stokł.osaand Jakub Goł.ą.b. (2005) Department of Immunology, Center for Biostructure, Medical University of Warsaw, Warszawa, Poland. Prospects for p53-based cancer therapy. 322~325

19. V. P. Almazov, D. V. Kochetkov, and P. M. Chumakov. (2003) Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, 19991 Russia 2Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA. Use of p53 for Therapy of Human Cancer. 2, 4~10

20. Zhen Wang and Yi sun. (2009) Institute of Medicinal Biotechnology, PUMC&CAMS, Beijing, People's Republic of China, 100050. Targeting p53 for Novel Anticancer Therapy. 1~4, 6

21. Alejandro Parrales, Tomoo Iwakuma (2015) Department of Cancer Biology, University of Kansas medical Center, Kansas City, KS, USA. Targeting Oncogenic Mutant p53 for Cancer Therapy

22. Rivlin N, Brosh R, Oren M, Rotter V. Mutations in the p53 tumor suppressor gene: important milestones at the various steps of tumorigenesis. Genes Cancer (2011) 2:466

23. Vaseva AV, Moll UM. (2009) The mitochondrial p53 pathway. Biochim Biophys Acta 1787:414–20.

24. Pant V, Lozano G. (2014) Limiting the power of p53 through the ubiquitin proteasome pathway. Genes Dev 28:1739–51.

25. Brandon J Aubrey, Gemma L Kelly, Ana Janic, Marco J Herold, Andreas Strasser (2018), How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? Cell Death and Differentiation 25, 104–113

26. Fagundes R, Teixeira LK (2021) Cyclin E/CDK2: DNA Replication, Replication

Stress and Genomic Instability. Front Cell Dev Biol 9:774845.

27. Xu J, Morris GF (1999) p53-Mediated Regulation of Proliferating Cell Nuclear Antigen Expression in Cells Exposed to Ionizing Radiation. Mol Cell Biol 19:12–20.

28. Aubrey BJ, Kelly GL, Janic A, Herold MJ, Strasser A (2018) How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? Cell Death Differ 25:104–113.

29. Teodoro JG, Virologist and Cancer Biologist, Goodman Cancer Center, and Assistant Professor, Department of Biochemistry, Faculty of Medicine, McGill University (2008) The p53 Tumor-suppressor Gene—Inhibiting Tumor Angiogenesis. Oncology & Hematology Review (US)

30. Iñiguez, M. A., Rodríguez, A., Volpert, O. V., Fresno, M., & Redondo, J. M. (2003). Cyclooxygenase-2: A therapeutic target in angiogenesis. Trends in Molecular Medicine, 9(2), 73-78.

31. Levine AJ (2019) Targeting Therapies for the p53 Protein in Cancer Treatments. Annu Rev Cancer Biol 3:21–34.

32. Duffy MJ, Synnott NC, O'Grady S, Crown J (2022) Targeting p53 for the treatment of cancer. Seminars in Cancer Biology 79:58–67.

33. Chen X, Zhang T, Su W, Dou Z, Zhao D, Jin X, Lei H, Wang J, Xie X, Cheng B, Li Q, Zhang H, Di C (2022) Mutant p53 in cancer: from molecular mechanism to therapeutic modulation. Cell Death Dis 13:974.

34. Abegglen LM, Caulin AF, Chan A, Lee K, Robinson R, Campbell MS, Kiso WK, Schmitt DL, Waddell PJ, Bhaskara S, Jensen ST, Maley CC, Schiffman JD (2015) Potential Mechanisms for Cancer Resistance in Elephants and Comparative Cellular Response to DNA Damage in Humans. JAMA 314:1850–1860. https://doi.org/10.1001/jama.2015.13134

35. Li Y, Zhang M-C, Xu X-K, Zhao Y, Mahanand C, Zhu T, Deng H, Nevo E, Du J-Z, Chen X-Q (2019) Functional Diversity of p53 in Human and Wild Animals. Frontiers in Endocrinology 10:

36. Sulak M, Fong L, Mika K, Chigurupati S, Yon L, Mongan NP, Emes RD, Lynch VJ TP53 copy number expansion is associated with the evolution of increased body size and an enhanced DNA damage response in elephants. eLife 5:e11994. https://doi.org/10.7554/eLife.11994

37. Zhang J, Chen X, Kent MS, Rodriguez CO, Chen X (2009) Establishment of a dog model for the p53 family pathway and identification of a novel isoform of p21 cyclin-dependent kinase inhibitor. Mol Cancer Res 7:67–78. https://doi.org/10.1158/1541-7786.MCR-08-0347

38. Koshino A, Goto-Koshino Y, Setoguchi A, Ohno K, Tsujimoto H (2016) Mutation of p53 Gene and Its Correlation with the Clinical Outcome in Dogs with Lymphoma. Journal of Veterinary Internal Medicine 30:223–229. https://doi.org/10.1111/jvim.13807

39. Jasik A, Kycko A, Olech M, Wyrostek K, Śmiech A, Łopuszyński W, Otrocka-Domagała I, Mikiewicz M, Dolka I (2021) Mutations of p53 Gene in Canine Sweat Gland Carcinomas Probably Associated with UV Radiation. J Vet Res 65:519–526. https://doi.org/10.2478/jvetres-2021-0070

I hereby confirm that I am familiar with the content of the thesis entitled

Anti-cancer mechanism of p58 gene and its application for treatment

written by   Sihyun Han

(student name) which I deem suitable for submission and defence.

Date: Budapest, ...9.....day......11......month .....2023.....year

Bence Racz

.... Supervisor name and signature

.... ÁLLATORVOSTUDOMÁNYI EGYETEM.
Anatómiai és Szövettani Tanszék
1078 Budapest, István u. 2.
...........................1400 Budapest, Pf. 2...

.... Department

Thesis progress report for veterinary students

Name of student: Sihyun Han

Neptun code of the student: wpkpa2

Name and title of the supervisor: Dr. Rácz Bence, professor

Department: Department of Anatomy and Histology

ÁLLATORVOSTUDOMÁNYI EGYETEM
Anatómiai és Szövettani Tanszék
1078 Budapest, István u. 2.
1400 Budapest, Pf: 2.

Thesis title: Anti-cancer mechanism of p53 gene and its application for treatment

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2021	10	29	Discussion about the topic	
2.	2022	8	23	Literature review	
3.	2023	4	13	Literature review	
4.	2023	6	12	Literature review	
5.	2023	7	20	Collecting reference for additional content	

Grade achieved at the end of the first semester: ………………………………………..

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	10	10	Feedback on abstract and citations	
2.	2023	10	25	Feedback on figures and citations	
3.					
4.					
5.					

Grade achieved at the end of the second semester: ....*jó (4)*..................

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

....
signature of the supervisor

Signature of the student: ....

Signature of the secretary of the department: ....

Date of handing the thesis in 2023.11.17