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Examining the Role of Chronic Stress in Cancer Progression and Metastasis: p53 as a Potential Mechanism

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Examining the Role of Chronic Stress in Cancer Progression and Metastasis: p53 as a Potential Mechanism

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Abstract

The influence of stress on the development and progression of cancer has been a long-standing hypothesis. Chronic stress can have a significant impact on the immune system and inflammatory response, potentially leading to decline of the body's immune capabilities (Segerstrom & Miller, 2004). Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, which triggers the release of stress hormones, such as cortisol (Smith & Vale, 2006). When chronically activated, this can lead to corresponding changes in the immune system, including decreased activity of natural killer cells, which play a crucial role in identifying and destroying cancer cells. Chronic inflammation has also been shown to promote the growth and metastasis of cancer cells and it can also damage DNA, leading to mutations that can contribute to carcinogenesis (Singh et al., 2019).

While it's clear that chronic stress can have a negative impact on the immune system and inflammatory response and may also play an important role in cancer pathology, the mechanisms remain unclear. The tumor suppressor gene (TP53) encodes a protein called p53, which is a key modulator in the innate and adaptive immune system and is the most frequently mutated gene in cancer (Hernandez Borrero & El-Deiry, 2021). The phosphatase and tensin homolog (PTEN) gene provides instructions for producing an enzyme found in almost all tissues in the body. The enzyme acts as a tumor suppressor and is one of the p53 outputs mediating proliferation. Murine Double Minute 2 (MDM2) is a proto-oncogene that forms a negative feedback loop with p53 and acts as a negative regulator. The overexpression of MDM2 inhibits p53 expression. Here, we examine the role of chronic stress on carcinogenesis through a loss or attenuation of p53 in mouse astrocytes lacking the functional TP53 gene. To investigate this, we focus on corticosterone (main corticosteroid hormone in mice) and a select number of genes, TP53,

MDM2, and PTEN. We hypothesize that chronic stress, modeled in this study by prolonged exposure to corticosterone, promotes downregulation of proteins in the p53 regulatory pathway.

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List of Abbreviations

ACTH	Adrenocorticotropic hormone
AIP	Atrophin-1 interacting protein
APAF-1	Apoptosis protease-activating factor 1
ARF	Adenosine diphosphate-Ribosylation Factor
ATM	Ataxia-Telangiectasia Mutated
ATR	Ataxia telangiectasia and Rad3-related protein
Bax	Bcl-2 Associated X-protein
Bcl-2	B-cell lymphoma 2
Bid	BH3 Interacting Domain Death Agonist
Cdc2	Cell division control
CDK 1 or 2	Cyclin dependent kinase
CHK 1 or 2	Checkpoint kinase 1 or 2
CNS	Central nervous system
CRH	Corticotropin releasing hormone
DMP1	Dentin matrix acidic phosphoprotein 1
E2F-1	E2 promoter binding factor 1
ESCs	Embryonic stem cells
Fas	Fas Cell Surface Death Receptor
GADD45	Growth arrest and DNA damage-inducible 45
HPA	Hypothalamic-pituitary-adrenal axis
IL-1 or 6	Interleukin 1 or 6
INK4a	Family of cyclin-dependent kinase inhibitors
killer/DR5	Death receptor 5
MDMX	Murine double minute x
MDM2	Murine double minute 2
Myc	Myelocytomatosis oncogene
NF-κB	Nuclear factor kappa B
Noxa	Phorbol-12-Myristate-13-Acetate-Induced Protein 1
Nrf2	E2-transcription factor
p14ARF	Protein 14 adenosine diphosphate-ribosylation factor
P21	Protein 21
P53	Protein 53
PFT-α	Pifithrin-alpha
PTEN	Phosphatase and tensin homolog
Perp	P53 Apoptosis Effector Related to PMP22
Puma	p53 upregulated modulator of apoptosis
PIGS	Phosphatidylinositol Glycan Anchor Biosynthesis Class S
PIDD	P53-Induced Death Domain Protein
Ras	Rat sarcoma
Scotin	Protein
Ser-166/186	Serine protease
SGK1	Serine/threonine kinase 1
TME	Tumor microenvironment
TNF-α	Tumor necrosis factor

TP53	Tumor protein 53
14-3-3σ	Epithelial cell marker and tumor suppressor protein

Chapter 1: Introduction

Stress is an integral part of our lives. Every living organism experiences some form of stress during its lifetime. While stress can be psychological, psychosocial, physical, or chemical, the focus of this research will be on the physiological manifestations of chronic stress. Stress is defined as an internal process that occurs when a person is faced with a demand that is perceived to exceed the resources available to effectively respond to it, and where failure to effectively deal with the demand has undesirable consequences (Schneiderman et al., 2005). While under stress, the body responds in a way similar to how it responds to danger. Stress can elicit cellular and molecular changes in the body, and while some of these changes are manifestations of the body's defensive adaptive reactions, other responses can be related to DNA damage (Mariotti, 2015).

Stress is a key disruptor of the homeostatic balance and we are constantly in flux trying to restore homeostasis. Stress can be divided into two categories, acute and chronic stress. Acute stress typically exists during emergencies, such as fighting or fleeing, or for a short duration followed by relief, while chronic stress is considered to be long-term and pervasive (Godoy et al., 2018). While under acute stress, changes occur in both the structure and function of certain molecules in the brain and activates the stress response system allowing us to employ healthy coping mechanisms (Godoy et al., 2018). As a response, the body temporarily produces catecholamines and corticosteroids and acute stress is often considered to be beneficial to the body (Godoy et al., 2018). Chronic stress, however, threatens the ability to maintain homeostasis by exhausting the body’s capacity to compensate leading to a full-blown inflammatory response (Chovatiya & Medzhitov, 2014).

Emerging research shows that chronic stress can encourage carcinogenesis and promote cancer growth (Yan et al., 2023). Carcinogenesis is a complex process where healthy cells are

transformed into cancer cells by undergoing changes at the cellular, genetic, and epigenetic levels (Costello & Franklin, 2011). Carcinogenesis does not occur without a mutation and a single mutation is typically not enough to develop cancer, but requires several mutations in the genome over its lifetime (Costello & Franklin, 2011). These mutations can be acquired through genetic conditions or acquired during a person's lifetime called environmental factors (Costello & Franklin, 2011). While increasing bodies of evidence suggests that DNA damage is increased by exposure to stress and stress hormones, little is known about the mechanism of the molecular pathways responsible for stress-induced DNA damage (Flint & Bovbjerg, 2012).

Stress

General adaptation syndrome is how the body responds to stress (Selye, 1950). There are three stages to stress: (1) The alarm stage. During this time, the central nervous system is awakened, causing the body's defenses to assemble. This "SOS" stage results in the fight-or-flight response that is considered to be a healthy response and coping mechanism. Physiological arousal is the basis for the fight-or-flight response characterized by adaptive physiological changes like increased heart rate, increased hormonal activity, etc (Selye, 1950). (2) The resistance stage. During resistance, the body begins to repair itself and normalize heart rate, blood pressure, and return the body to its baseline prior to encountering the stressor. After the initial shock of a stressful event, the body enters the recovery phase, but remains on high alert for a period of time. The body tries to defend itself by fending off the stressor and attaining homeostasis. If the stress persists, or additional stress in any form is encountered, the third stage of exhaustion occurs, which results in the breakdown of the body's defense mechanism (Selye,

1950). (3) The exhaustion stage. Activation in the first two stages continues over time, causing a breakdown in the balance within the body. Energy sources are spent and continued exposure to stress during the stage of exhaustion can lead to the development of diseases and immunocompromisation (Selye, 1950).

Chronic stress activates the neuroendocrine system and SNS (Smith & Vale, 2006). The main component of the neuroendocrine system is the HPA axis. In addition to controlling reactions to stress, the HPA axis regulates digestion, immune function, mood, and emotions (Smith & Vale, 2006). The hypothalamus contains neuroendocrine neurons that synthesize and secrete vasopressin and corticotropin-releasing hormone (CRH), which regulate the pituitary gland and stimulate the production of adrenocorticotropic hormone (ACTH) (Smith & Vale, 2006). In turn, ACTH stimulates the adrenal cortices to produce glucocorticoids, mainly cortisol. Glucocorticoids act on the hypothalamus and pituitary to suppress the production of CRH and ACTH, completing this negative feedback loop to extinguish the stress response (Smith & Vale, 2006) (figure 1).

Real or interpreted threats to homeostasis induce the release of adrenaline and noradrenaline along with glucocorticoids initiating the fight-or-flight response during periods of acute stress (Goldstein, 2010). When exposed to chronic stress, the HPA axis becomes continuously activated for prolonged periods, leading to hyperactivity. This excessive activity causes other biological systems to overcompensate, leading to eventual collapse (Juster et al., 2010). The resulting HPA axis dysfunction may manifest as hypercortisolism, hypocortisolism, or diurnal dysrhythmia. Chronic stress elevates ACTH levels, which in turn, increases cortisol while depleting other glucocorticoids (Juster et al., 2010).

The HPA axis therefore shifts from a hyper-responsive state to a hypo-responsive state, and eventually to an unresponsive state known as “adrenal fatigue” (Dhabhar, 2009). This attenuation of the stress response might be a defensive mechanism to prevent long term suppression of immune function (Dhabhar, 2009). Stress hormones can affect human tumor biology in various ways. The activation of the HPA axis can alter immune defense mechanisms and anti-tumor immune capabilities, promoting tumor progression in cancer patients (Hong et al., 2021). Cancer-related systemic inflammation is associated with poor outcomes, irrespective of tumor stage (Roxburgh & McMillan, 2014). Additionally, clinical studies suggest that stress and depression can lead to decreased cellular immunity and immune alterations in cancer patients (Liu et al., 2022). SNS hormones, which signal through β-adrenergic receptors, have been shown to regulate disease progression and metastasis. Stimulation of these receptors can impact cancer cell biology, especially metastasis (Cole et al., 2015).

Cortisol

Cortisol is a glucocorticoid synthesized by the adrenal cortex and has important immunosuppressive and anti-inflammatory effects making it an ideal starting point for researching cancer (Coutinho & Chapman, 2011). Cortisol is involved in metabolism, immune response, inflammation, and triggers the fight or flight response making it an ideal starting point for better understanding the role of stress in cancer progression (Coutinho & Chapman, 2011). The amount of cortisol in the blood undergoes diurnal variation—it peaks in the early morning and reaches its lowest level approximately three to five hours after the onset of sleep (Mohd Amzi et al, 2021). Studies conducted on healthy adults suggest that chronic stressors that threaten physical integrity, are uncontrollable, or involve trauma, tend to result in a high daily release of cortisol with lower than normal levels in the morning and higher than normal levels in

the evening (Michaud et al., 2008). At the cellular level, there are changes in the structural integrity of mitochondria, fragmentation of the Golgi complex, aggregation of cytoskeleton structures, and collapse of vimentin containing intermediate filaments around the nucleus (Machamer, 2015).

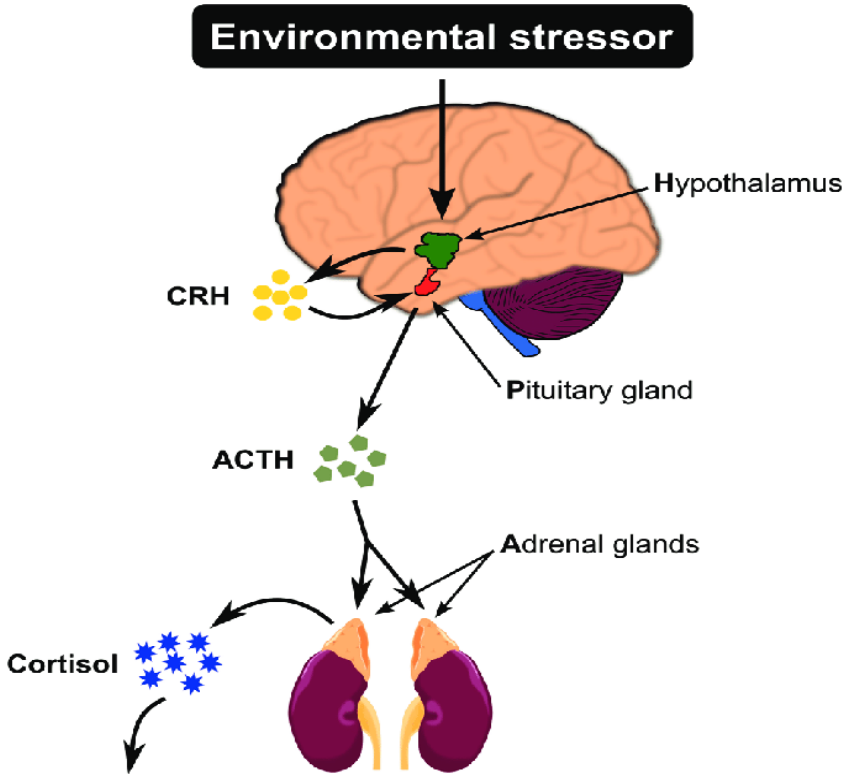

Figure 1. When the brain detects an environmental stressor, the Hypothalamic-pituitary-adrenal (HPA) axis is activated. This results in the release of corticotrophin-releasing hormone (CRH) by the hypothalamus. CRH then stimulates the secretion of adrenocorticotropic hormone (ACTH) by the anterior lobe of the pituitary gland. In response to ACTH, the adrenal glands produce glucocorticoids (cortisol), which generate a stress response (Lanoix & Plusquellec, 2013)

Cancer

The term ‘cancer’ does not refer to one single disease, rather a collection of different diseases that are related by certain qualities and characteristics (Hanahan & Weinberg, 2011). All cancer cells are derived from normal human cells, and therefore retain many features of the original cells. This means that almost any type of cell in the body has the potential to become cancerous (Rossi et al., 2020). There are over 100 types of cancer each exhibiting unique behaviors. These dissimilarities require tailored treatment approaches and research efforts that concentrate on specific cancer types rather than the disease in its entirety (Hanahan & Weinberg, 2011).

Given that cancer is a general term that encompasses various types of cancer, the fundamental question arises - what exactly *is* cancer? To answer this question, we can rely on Hanahan and Weinberg's eight hallmarks of cancer. All cancers share the following characteristics: (1) Sustaining proliferative signaling; (2) Evading growth suppressors; (3) Resisting cell death; (4) Enabling replicative immortality; (5) Inducing angiogenesis; (6) Activating invasion and metastasis; (7) Deregulating cellular energetics; (8) Evading immune destruction (Hanahan, 2022). Without most of these eight hallmarks, cancer would no longer be cancer. The focus of this research will relate to hallmarks one, two, and three.

Sustaining proliferative signaling

The first and arguably most significant hallmark is that cancer cells continue to grow infinitely, while normal healthy cells do not. The body has a highly controlled and regulated process that ensures cell growth is tightly regulated and monitored (Feitelson et al., 2015). This is a delicate balance throughout a person's lifetime that is lost when any one of the checks and

balances are not functioning properly (Lee & Muller, 2010). Normal cell growth is tightly regulated by specialized proteins and other biological molecules, which are controlled by genes (Feitelson et al., 2015). With cancer, there are two types of genes: proto-oncogenes and tumor suppressor genes (Lee & Muller, 2010). Proto-oncogenes increase growth, while tumor suppressor genes decrease it and normally these genes operate synergistically (Lee & Muller, 2010). Even with a genetic mutation that excessively activates or over-expresses oncogenes, cancer must sustain its proliferative capacity and undergo a complex process that leads to metastasis (Feitelson et al., 2015). This process requires a large amount of energy, coordination, and timing, and a single point mutation is usually not enough for all of these things to occur (Feitelson et al., 2015).

Evading Growth Suppressors

Proto-oncogenes actively suppress cell growth. Some of the most commonly affected genes in cancer are tumor suppressor genes such as TP53 and PTEN (Cooper, 2000). If DNA damage is not recognized and repaired by these genes, mutated cells can proliferate (Cooper, 2000). Tumor suppressor genes are activated under these circumstances that normally inhibit proliferation of damaged or mutated cells by arresting cell cycle progression and inducing apoptosis (Cooper, 2000). These genes play a key role in determining whether cells undergo apoptosis or proliferate (Cooper, 2000).

Resisting Cell Death

When normal cells age or become damaged they undergo senescence or apoptosis. Apoptosis is the highly-organized destruction of a cell and is governed by a set of tightly regulated signals (Elmore, 2007). Apoptosis is a crucial element in several biological processes,

such as regular cell renewal, embryonic growth, immune function, and proper development (Elmore, 2007). It is a controlled and natural aspect of an organism's life cycle. Additionally, apoptosis acts as a protective mechanism, such as during immune responses or when cells are harmed by diseases (Elmore, 2007). This method of programmed cell death is defined by specific morphological features and energy-dependent biochemical mechanisms (Elmore, 2007).

This is an active process that requires energy and is so important it has been evolutionarily conserved amongst all multicellular organisms (Renehan, 2001). To avoid excessive growth, the number of old cells removed must be carefully balanced by the number of newer cells (Wong, 2011). Cancer cells resist undergoing apoptosis, offsetting the balance of cell division and cell death allowing for excessive growth (Wong, 2011). Cancer cells also produce telomerase, an enzyme which increases the length of telomeres at the end of chromosomes (Robinson & Schiemann, 2022). Because the telomere cap wear down at a much slower rate, cells can continue to divide, preventing apoptosis or cellular senescence (Robinson & Schiemann, 2022). Cells accumulate damage over their lifetime. Without programmed cell death, these damaged cells, which can include mutations, persist.

Nervous System Cancers

One of the most significant obstacles that oncology currently faces is nervous system cancers. Tumors in the central nervous system, which comprise both malignant and nonmalignant growths in the brain and spinal cord, pose a challenge in treatment due to the intricate nature of their biology, chemistry, and neurobiology (Park et al., 2021). Brain tumors are typically classified based on their cellular origin and may be low grade or high grade meaning less aggressive and more aggressive, respectively (Miller et al., 2021). While the cause

of primary brain tumors is largely unknown, some tumors have germ line mutations and are hereditary. The majority, however, result from somatic mutations. The most common type of brain tumor to occur at all ages is called a glioma (Miller et al., 2021).

Transformation of Glial Cells

Gliomas consist of glial cells and can be classified as an astrocytoma or ependymoma (Zong et al., 2012). Astrocytomas are the most common type of childhood glioma and typically occur in the cerebellum or brainstem. Malignant gliomas and glioblastomas, can develop anywhere in the brain and are much more aggressive than astrocytomas (Zong et al., 2012). Ependymomas are a type of glial tumor that typically arises from the cells lining the ventricles. These are often slower growing than the previously described tumors, but are most likely to recur after treatment (Zong et al., 2012). Mixed glial tumors, which typically involve a combination of astrocytes and ganglion cells, are more frequently observed in children than in adults. These tumors have the potential to arise in any part of the nervous system, although they predominantly occur in the cerebrum (Banu, 2019). During nervous system development, neuroepithelial cells differentiate into glial and nerve cells. Roughly 25% of nervous system tumors are tumors made up of poorly-differentiated neuroepithelial cells (Baba & Catoi, 2007). Choroid plexus tumors and germ cell tumors frequently arise in the region of the pineal gland and may therefore compromise HPA axis functions (Baba & Catoi, 2007).

The Cell Cycle

The eukaryotic cell cycle is broken into four parts: G1, S, G2, and M (Alberts, 2022) (figure 2). Most cells in the body undergo a cycle in which their genetic information is retained, fixed, and passed down to daughter cells through a highly-regulated process (Alberts, 2022). During interphase, some subdivisions are important to cell division and maintenance of the genetic material (G1, S, and G2) (Alberts, 2022). There are many situations where mistakes are made by the cycle or by a regulating system that causes the cell to proliferate uncontrollably (Alberts, 2022). Additionally, cells may enter into a 4th phase known as G0, when the cell no longer divides (Alberts, 2022). After mitosis, where cells divide into two cells, they enter into the G1 phase, a checkpoint for complete genetic function before the cells can start replicating DNA (Alberts, 2022). The G1 phase of the cell cycle progression also serves as a checkpoint for cells to either undergo repair mechanisms or follow the apoptotic pathway (Alberts, 2022).

When growth factors are overproduced or suppressor proteins are deficient, it can cause uncontrolled and swift cell division (Alberts, 2022). This unregulated proliferation of cells can lead to the development of cancer and result in endless mitotic activity (Alberts, 2022). During DNA replication, cancerous cells have the ability to use telomerase to append telomeric segments to the DNA ends. This allows these cells to persist for an extended period, in contrast to other somatic cells that typically have a limited lifespan, and therefore, continue to divide (Alberts, 2022).

One possible mechanism of the link between chronic stress and cancer could be telomere shortening. Each time a cell replicates, telomeres get shorter until they're so short cells can no longer divide. When cells are no longer able to divide, tissues age (Shammas, 2011). Telomerase can rebuild telomeres, but chronic stress and cortisol exposure is associated with a significant

reduction in telomerase activity (Mathur et al., 2016). This promotes a chronic inflammatory environment that supports an immunosuppressive tumor microenvironment (TME).

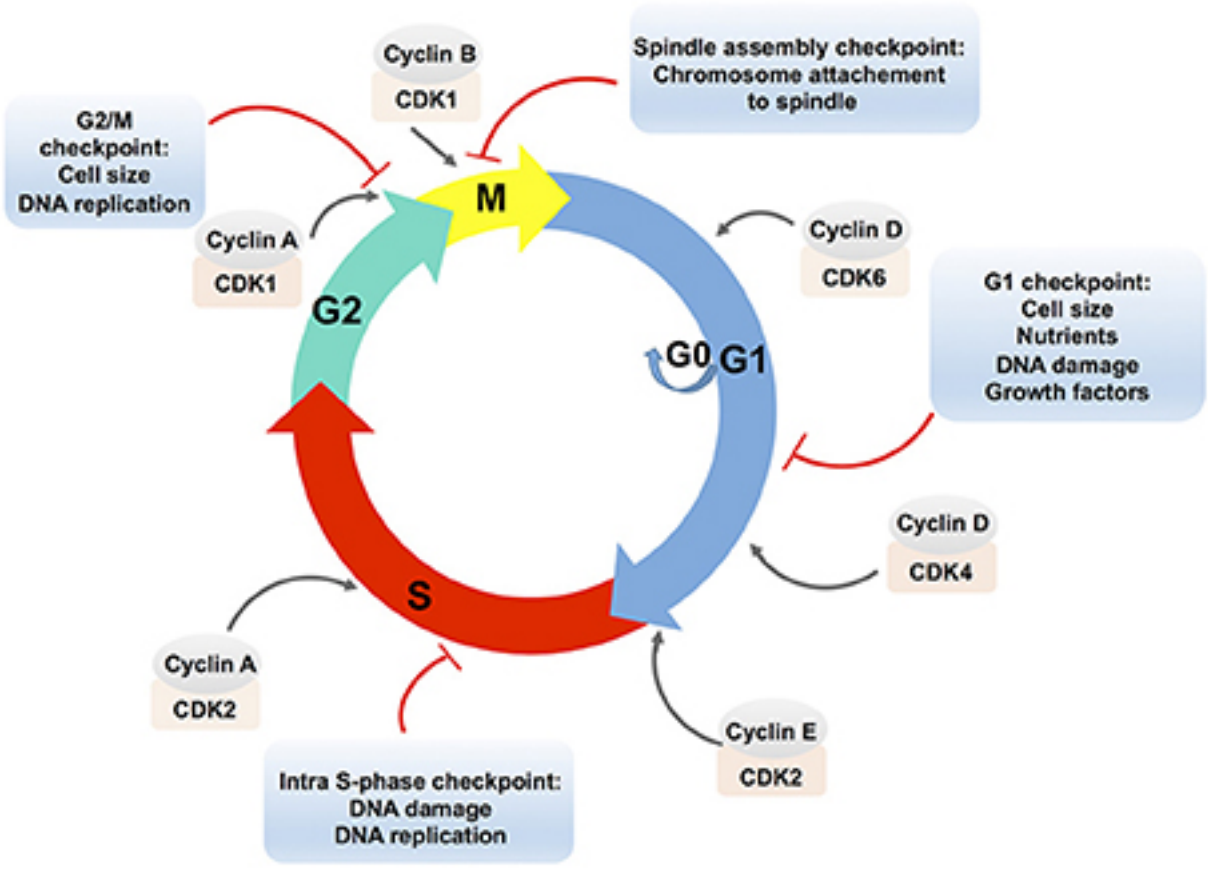

Figure 2. **The eukaryotic cell cycle** includes the G1 and G2 gap phases, the S-phase, and the M-phase of mitosis, along with a quiescent state known as G0. The colored arrows indicate the different phases of the cell cycle. Cyclin-dependent protein kinases (CDKs) bound to cyclins form complexes that regulate the cell cycle. These complexes are located at the front of the arrow corresponding to the relevant phase. Checkpoint pathways control cyclin-CDK complexes to prevent cells from advancing to the next stage if it is not permitted. A text insert outlines various stimuli that regulate checkpoint control (El-Aouar Filho et al., 2017).

The cell cycle and p53

The protein p53 plays a critical role in the cell cycle as a transcription factor that promotes growth arrest, DNA repair, apoptosis, and regulated cell death in damaging situations (Chen, 2016) (figure 3). p53 also regulates the expression of many inhibitory proteins like protein 21 (p21) and growth arrest and DNA damage inducible protein (GADD45) (E. Tamura et al., 2012). These proteins inhibit cell division cycle protein 2 homolog (Cdc2), which is crucial for the cell to progress through mitosis or meiosis (Alberts, 2022). The protein p21 allows the cell to move on from through the S phase by regulating another protein, cyclin-dependent kinase 2 (CDK2) (Alberts, 2022). Without this suppressor gene, these proteins would not be inhibited correctly, leading to cancer. If a mutation arises or an error goes uncorrected, the cell cycle's regulatory components are circumvented, allowing the mutation to propagate to all subsequent cells (Alberts 2022). Various chemotherapy drugs disrupt DNA strands to prevent cell replication once regulatory control has been subverted. Unfortunately, these medications cannot differentiate between healthy and dangerous DNA, causing the destruction of many other cells, frequently resulting in undesired off-target consequences (Alberts, 2022).

Apoptosis

The apoptotic pathways can be categorized as intrinsic and extrinsic. Extrinsic apoptosis begins by the binding of a ligand to a cell surface death receptor, such as Fas or tumor necrosis factor-α (TNF-α) (Elmore, 2007). In the extrinsic pathway, cell surface death receptor (Fas), death receptor 5 (killer/DR5), and p53-induced death domain protein (PIDD) are regulated by p53, leading to the activation of caspase 8, BH3 interacting domain death agonist (Bid), and the release of cytochrome c (Cavalcante et al., 2019). Apoptitic peptidase activating factor (APAF-1), another p53-regulated gene, then works with cytochrome c to activate caspase 9 and 3,

ultimately resulting in apoptosis (Cavalcante et al., 2019). Meanwhile, in the intrinsic pathway, various p53-regulated genes, such as Bcl-2 Associated X-protein (bax), noxa, and Bcl-2-binding component 3 (puma), may promote cytochrome c release in different cell types (Elmore, 2007). The third p53 response is cellular senescence, which may be as crucial as apoptosis in p53's tumor suppressor functions (Elmore, 2007).

Research has shown that apoptosis and the genes that control it has a profound effect on the expression of malignant phenotype (Elmore, 2007). While some oncogenic mutations disrupt apoptosis leading to tumor initiation, progression, or metastasis, other evidence indicates that different oncogenic changes promote apoptosis, thereby resulting in pressure to override apoptosis during carcinogenesis (Lowe & Lin, 2000). Most cytotoxic anticancer agents induce apoptosis, and therefore highlights the possibility that dysfunction in apoptosis contributes to cancer treatment failure (Lowe & Lin, 2000).

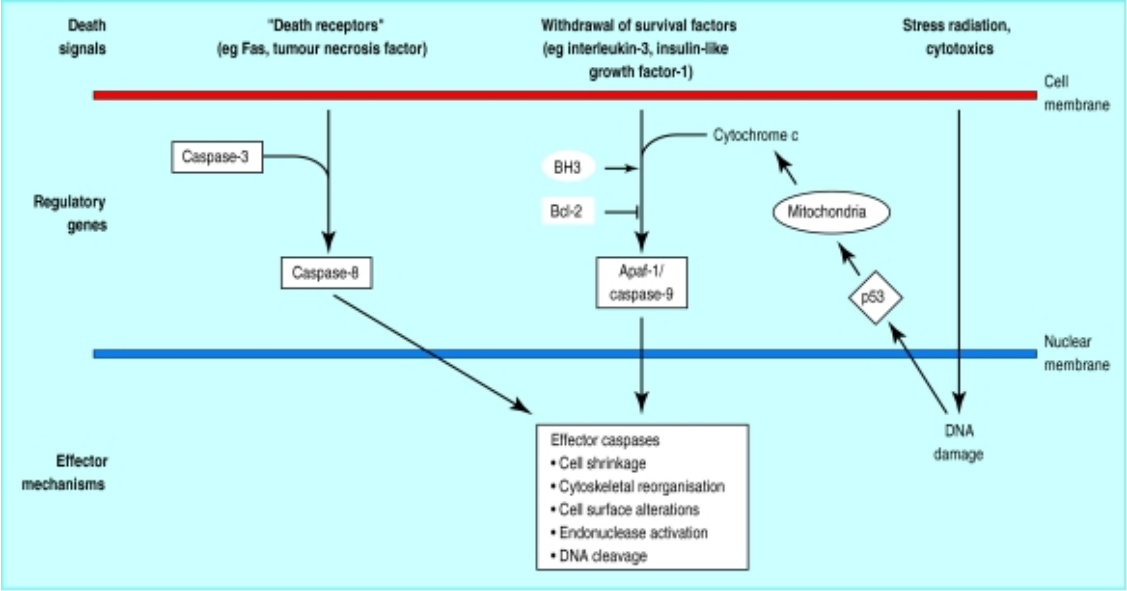

Figure 3. **Apoptotic pathways and mechanisms.** Three major pathways are shown: death signals, gene regulation, and effector mechanisms (Renehan, 2001)

P53

The p53 protein was first described in 1979, and shortly thereafter several studies were published that discussed the identification of a new cellular protein (Soussi, 2010). Unbeknownst to researchers at the time, their discovery would spur the advancement of an entirely new research field that would go on to become the most researched protein of all time. Since then, more than 150,000 papers have been published on the topic. The description of this protein and its gene has evolved from a virus-associated tumor antigen to an oncogene to a tumor suppressor gene (Soussi, 2010). This safe-guards genomic stability, a role for which p53 earned the nickname "guardian of the genome" (Mfossa et al., 2020). Researchers initially believed that p53 was an oncogene and early experiments supported this hypothesis. Over time, the complexity of its true function as a tumor suppressor and its implication in a myriad of diseases has slowly unraveled over the past forty years.

The regular function of p53 entails serving as a transcription factor that responds to damaged DNA by initiating cell cycle arrest or apoptosis, with the ultimate goal of safeguarding the DNA integrity of the cell (Chen, 2016). The outcome depends on the type and persistence of the damage and the level of p53 activation. Beyond this crucial function, TP53 also performs other roles in development, aging, and cell differentiation (Chen, 2016). Although p53 plays a significant role in various cellular activities, it is an unstable protein, and most of the mutations that impact its function occur on the DNA binding core domain, accounting for 95% of such mutations (Ozaki & Nakagawara, 2011). Through p53 regulated genes, the protein produces stress signal-carrying proteins that communicate with neighboring cells, facilitate the prevention and repair of damaged DNA, and create feedback loops that either amplify or reduce p53 activity, while interacting with other signal transduction pathways (Ozaki & Nakagawara, 2011).

p53 pathway

The p53 pathways comprises numerous genes and their corresponding products that respond to a diverse range of stress signals (figure 3) (Levine et al., 2006). The pathway can be divided into five parts; (1) Input signals that trigger or induce the network into a functional state. (2) Upstream mediators that detect and interpret those signals that initiate the functional pathway and relay the inputs to p53. (3) The core set of proteins, including p53 itself, which regulates p53 activity and function. (4) The downstream events which are composed of a set of genes and their proteins that are regulated by p53. (5) The cellular outputs of these downstream events which include cell cycle arrest, cellular senescence or apoptosis (Levine et al., 2006).

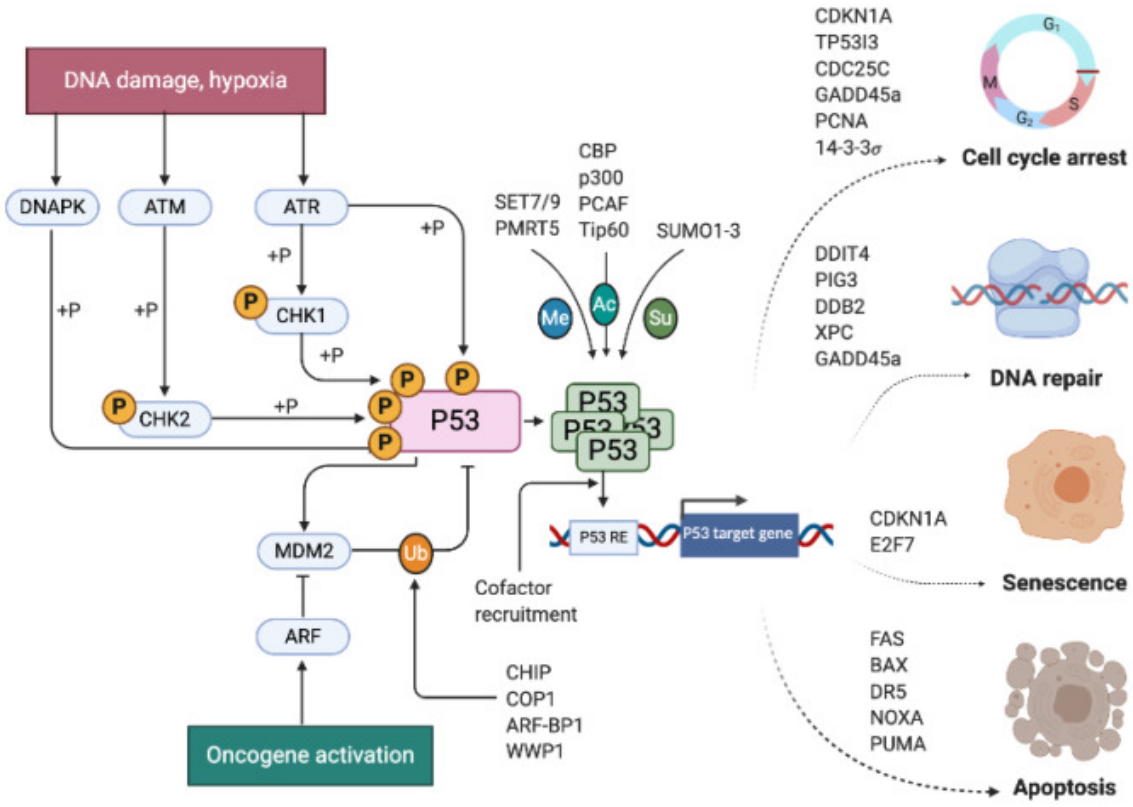

Figure 4. **The input signals, mediators, and core functions of the p53 pathway.** Stress signals are detected by the cell and communicated to p53. The regulation of P53 stability primarily relies on MDM2,

which, as a p53-target, creates a negative feedback loop. Additionally, protein modifiers and cofactors that bind to the p53 protein modulate the transcriptional activity of its target genes. Through this multi-step process, the activation of p53 ultimately governs the stress response, determining the appropriate outcome (Hernandez Borrero & El-Diery, 2021)

Input Signals

The signaling pathway of the p53 protein comprises a group of genes and their corresponding proteins that react to diverse intrinsic and extrinsic stress signals, regulating the output of many biological processes (Hernandez Borrero & El-Diery, 2021). This pathway serves to oversee and regulate DNA damage and cell division to maintain cellular homeostasis. Various stressors can cause diverse types of DNA damage through different mechanisms, and each kind of damage is detected by specific proteins and repaired by distinct enzymes (Chatterjee & Walker, 2017). Although there exist multiple DNA damage detection and repair systems in the cell, the p53 pathway is responsible for responding to the majority of them, serving as a checkpoint for these processes and typically eliminating cells with errors (Ozaki & Nakagawara, 2011). As cells divide and telomeres shrink, the p53 protein is notified of the presence of these shorter telomeres, resulting in a p53-dependent response (Dang et al., 2008). Therefore, the question arises as to what other stress signals can trigger a p53 response, and whether chronic psychological stress or nervous system activity can activate p53.

Upstream Mediators

In response to these stresses, p53 undergoes post-translational modifications, promoting the transcription of genes involved in specific cellular responses according to the type of stressor (Hernandez Borrero & El-Deiry, 2021). The fundamental regulatory system comprises p53,

MDM2, p14ARF, and E2F-1, which are arranged in two feedback loops (Jin & Levine, 2001). One loop consists of p53 and MDM2, where p53 activates MDM2 transcription to positively regulate it, while MDM2 promotes the degradation of p53 by ubiquitination to negatively regulate it. The other loop is formed by E2F-1 and ARF, where E2F-1 induces ARF transcription, and ARF facilitates the degradation of E2F-1. The two feedback loops are interconnected in two ways: ARF blocks MDM2 mediated p53 degradation, thus stabilizing p53, and p53 inhibits ARF gene transcription (Jin & Levine, 2001). This intricate circuitry is crucial for controlling p53 intracellular levels and functions. However, many cancers exhibit defects in this system due to missense mutations of p53, MDM2 amplification, ARF silencing or deletion, and loss of E2F-1 regulation through RB mutation, among other mechanisms (Jin & Levine, 2001).

Cellular stresses signal p53 by disturbing the p53 regulatory circuit either directly or indirectly (Jin & Levine, 2001). DNA damage activates p53 through p53 upstream mediators, which include protein kinases, transcriptional coactivator complexes, transcriptional co-repressor complexes, and other p53 activity modulators (Jin & Levine, 2001). Most of these upstream mediators target p53 for post-translational modification (Jin & Levine, 2001). For example, DNA damage activates the protein kinases serine/threonine kinase (ATM), ataxia telangiectasia and Rad3 (ATR), checkpoint kinase (CHK1) and (CHK2), which in turn phosphorylate p53 and or MDM2 (Jin & Levine, 2001). Phosphorylation of p53 and or MDM2 activates p53 through three mechanisms: (1) stabilizing p53 by disrupting p53-MDM2 interaction; (2) regulating p53 transactivation activity; (3) promoting p53 nuclear localization. The oncogenes E2F-1, Ras, DMP1, and Myc enhance ARF transcription, whereas others downregulate it. (Jin & Levine, 2001)

Research has shown that the p53 and MDM2 proteins are extensively modified after a stress signal (Ashcroft et al., 2000). Activation is the increase in the concentration of p53 and increased activity transcription of a set of genes that are transcriptionally regulated by p53 (Chen, 2016). The levels of p53 are regulated by its proteolytic turnover and the protein has a short half-life of 6–20 minutes conferred by the MDM2 protein (Ashcroft et al., 2000). p53 is also extensively modified by different protein kinases, histone acetyl-transferases, methylases, and other enzymes. These modifications include phosphorylation, acetylation, methylation, ubiquitination, and some of these modifications are involved in the response to DNA damage (Reed & Quelle, 2014). The p53 transcriptional output after each stressor is different, and these protein modifications dictate which p53 responsive genes are transcribed by the cell based upon the specific protein modifications of the p53 protein (Reed & Quelle, 2014).

Downstream Mediators

Upon activation, p53 activates or represses the transcription of its downstream effector genes (figure 4) (Chen, 2016). p53 may induce G1-S arrest by activating p21, a CDK2 inhibitor, or it may trigger G2-M by inducing 14-3-3σ and GADD45 (Chen, 2016). The p53 protein induces apoptosis by activating pro-apoptosis genes, which ultimately activate Apaf-1 and caspase 9 through different pathways. p53 can also activate genes involved in DNA repair (Chen, 2016).

Once p53 becomes activated in response to a stress signal, it gains the ability to bind to p53 responsive DNA sequence elements in the genome (Kearns et al., 2016). It has become clear that different types of stress signals as inputs result in different genes being transcribed under p53 control, but the mechanism is not fully understood (Chen, 2016). There are some p53

regulated genes that are transcribed in response to different types of stress signals and in all tissues responding to the stress, such as, p21, MDM2, GADD45, and others that are either stress or tissue specific, such as PTEN (Vousden & Prives, 2009). Still, what regulates these differences remains unclear.

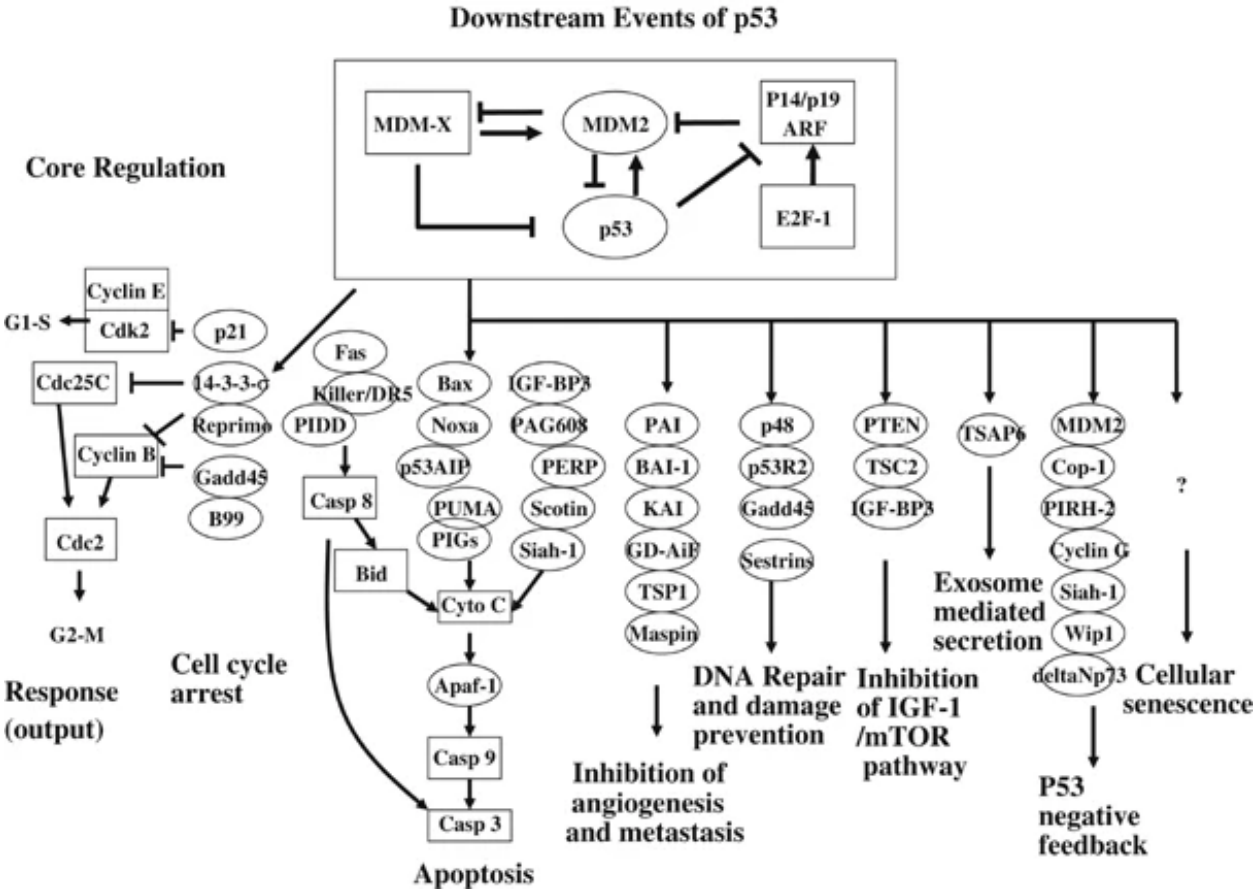

Figure 5. **The functions of p53**, the genes controlled by p53 downstream, and the results of the p53 pathway. The p53 protein functions as an activator of transcription for genes regulated by p53, leading to three primary outcomes: stopping the cell cycle, inducing cellular senescence, or triggering apoptosis. Additionally, other p53-controlled genes help to interact with neighboring cells, repair DNA damage, or

establish feedback loops that boost or weaken p53 protein functions while integrating these stress responses with other signal transduction pathways (Levine et al., 2006)

Loss of p53 function

When p53 loses its function, and becomes destabilized, it can lead to the development of cancer. When this loss of function occurs, cells proliferate uncontrollably, the first hallmark of cancer growth. Mutations or functional inactivation of p53 is indicated in many cancer types and roughly half of these cases display mutant p53 that has lost its tumor suppressor function, while the other half exhibit decreased p53 levels due to overexpression of the E3 ubiquitin ligases murine double minute 2 (MDM2) and MDMX (Brooks & Gu, 2006). MDM2 decreases p53 levels by acting as a transcriptional inhibitor and as an E3 ligase facilitating degradation of p53 via the proteasome (Brooks & Gu, 2006). Overexpression of p53 and MDM2 is seen in many malignant cancers and is associated with poor prognosis due to the upregulation of genes involved with cellular growth (Brooks & Gu, 2006). There is overwhelming evidence that the major role of MDM2 and its related complexes is the targeting of p53 for proteasomal degradation (Brooks & Gu, 2006).

Typically, loss of p53 function happens in one of two ways: (1) point mutations in the p53 gene or (2) dysfunction or destabilization of protein(s) function that is/are involved in the p53 signaling pathways (Pflaum et al., 2014). In response to DNA damage, cellular stress and certain oncogenic proteins like p53 transactivates genes involved in one of three outcomes: (1) temporary cell cycle arrest, (2) permanent cell cycle arrest in the form of senescence, and (3) programmed cell death (Pflaum et al., 2014). These processes are essential for removing defective cells from the actively dividing cell population. Cells are motivated to repair mutations by halting the cell cycle or eliminate themselves through senescence or apoptosis, and p53

guarantees that these mechanisms take place. When p53 is inactive and incapable of carrying out this duty, the defective cell will persist through the cell cycle, multiply rapidly, and introduce faulty cells to the population (Chen, 2016).

The fact that p53 is absent or nonfunctional in a large quantity of human cancers implies its significance. Given this importance and the thousands of complex processes and safeguards our bodies undergo, it begs the question, why isn't there an alternative pathway should the first one experience dysfunction or damage? Having one central protein receive all the information is more efficient than several proteins receiving partial information, leading to the most effective response. However, this creates challenges when pathways destabilize as all roads seemingly lead back to p53. What specifically causes p53 to choose cell cycle arrest or senescence versus apoptosis and adopt one p53 dependent response over another is not fully understood.

Chronic stress and Cancer

Link between stress and cancer

Numerous clinical, epidemiological, and animal-based studies have explored the link between stress-related behaviors and cancer (Moreno-Smith et al., 2010). Evidence suggests that stress may have adverse effects on tumor progression and survival (Dai et al., 2020). This effect is mediated by neuroendocrine pathways and inflammation, which play a critical role in cancer progression. In addition, chronic stress may promote metastasis by interacting with the tumor microenvironment and enhancing processes that favor tumor growth (Dai et al., 2020).

Studies have also shown that increased cortisol levels have been associated with worse prognosis in cancer patients (Figueira et al., 2022). Chronic stress can result in neurohormonal

dysregulation affecting the immune system and therefore have an effect on cancer progression. Since the neuroendocrine and immune systems share common mediators and receptor signals, this suggests that the brain plays an immunoregulatory role (Haykin & Rolls, 2021). Cortisol may also promote DNA damage and interfere with DNA repair, mediated by glucocorticoid receptors (Antonova et al., 2011). In a preclinical model of chronic stress, researchers observed increased tumorigenesis and attenuation of p53 function which were hypothesized to be mediated by elevated glucocorticoids (Feng et al., 2012). These results suggest that cortisol downregulates p53 through glucocorticoid signaling. Furthermore, cancer patients who have abnormal plasma cortisol levels have been linked to experiencing shorter periods of being disease-free (Figueira et al., 2022). It's no surprise that cancer patients experience abnormally high levels of stress, anxiety, and depression during different phases of diagnosis and treatment. Newly diagnosed lung cancer patients shared both higher levels of depression and higher salivary cortisol levels compared to healthy adults (Hong et al., 2021). Stress exposure has also been proposed to contribute to the etiology of breast cancer, ovarian cancer, thyroid cancer, and possibly brain cancer, but there's a lack of knowledge regarding the intracellular pathways involved (Antonova et al., 2011).

Potential Mechanisms of Action

A meta-analysis of 165 studies on chronic stress demonstrated that psychosocial factors and chronic stressful life experiences are associated with higher cancer incidence, poorer cancer survival and outcomes, and higher mortality rates (Chida et al., 2008). Glucocorticoids are also believed to play an important role in malignant transformation, especially in solid tumors (Azher et al., 2016). Yan et al., conducted a study on the link between emotional stresses and the origin

and development of different types of tumor cells. The results reveal that excessive activation of the HPA axis and cortisol binding to glucocorticoid receptors reduces the activity of both NF-κB and Nrf2 inside cells and inhibits cellular immune responses (Yan et al., 2023). They therefore concluded that defects in these mechanisms can promote cancer cell growth via their escape from the typical immune surveillance safeguards (Yan et al., 2023).

The effects of catecholamines are mediated by nine α-adrenergic and β-adrenergic G-protein coupled receptors, which are present on a wide range of cell types, including cancer cells Flint & Bovbjerg, 2012). Evidence suggests that the accumulation of DNA damage following chronic adrenergic stimulation as a result of chronic stress may be the result of β-adrenergic stimulation on two molecular pathways (Archer et al., 2021). The first one directly leading to DNA damage and the second leading to a reduction in p53 activity (Archer et al., 2021). Both pathways are activated as a result of stimulation of β2-adrenoreceptors, with the ARBB1 facilitating AKT-mediated activation of MDMX, which in turn promotes MDM2 to bind and degrade p53 through phosphorylation of MDM2 Ser-166/186 (Hu et al., 2012). This effect is mediated by glucocorticoid induced protein kinase (SGK1), which is regulated by glucocorticoids (Hu et al., 2012). A retrospective analysis documented how cancer patients who were exposed to β-blockers for treatment of hypertension were less likely to experience tumor metastasis and survive longer (Na et al., 2018).

Stressors activate the SNS and the HPA-axis, resulting in the release of catecholamines, glucocorticoids, and pro-inflammatory cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6), and TNF-α (Liu et al., 2017). The release of these sympathetic, neuroendocrine and immune mediators has been shown to have effects on immune function, physiology, and tumor biology. If neurohormones that are elevated during chronic stress activate MDM2 and subsequently

down-regulate p53, this could be a potential mechanism by which chronic stress promotes carcinogenesis. Chronic exposure to stress has also been linked to increased expression of inflammatory biomarkers in various cancer types. Inflammatory mediators, including IL-6, IL-12, IFN-γ, and TNF-α, have been shown to play a critical role in tumor growth and progression (Singh et al., 2019). It can therefore be hypothesized that CNS cancers are particularly vulnerable to the effects of stress related pathways suggesting that chronic emotions and chronic stress could contribute to the pathology of cancers. Collectively, this research suggests that stress is a modulator of cancer progression and metastasis through multiple intracellular pathways.

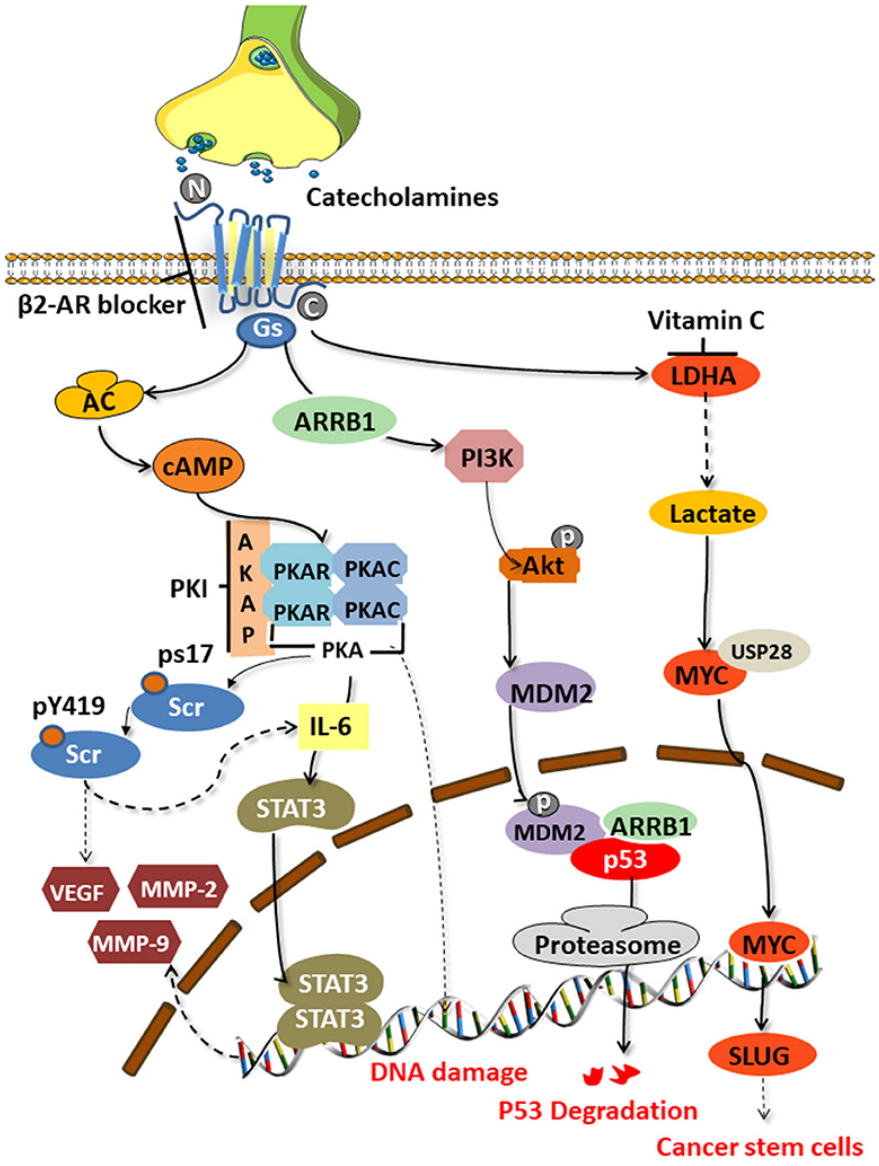

Figure 6. **Schematic view of the effects of catecholamine's on DNA damage.** Stress induced catecholamines activate the cAMP-PKA signaling pathway via β2-andrenergic receptors. This downstream reaction results in a variety of outputs such as DNA damage and p53 degradation. There are two pathways by which catecholamines can have an effect: one is through Gs-protein kinase A, and the other is through ARRB. Both pathways are triggered when β2-adrenoreceptors are stimulated. ARBB1 helps activate MDMX via AKT, which then encourages MDM2 to attach to and break down p53 (Dai et al., 2020).

Chapter 2: Materials and Methods

Cell Culture and Treatment

C8-S murine astrocytes (CRL-2535) and NE-4C p53 deficient murine astrocytes (CRL-2925) were obtained from the American Type Culture Collection (ATCC) and cultured in Dulbecco's modified Eagle's medium supplemented (DMEM) with 10% fetal bovine serum (FBS) (Gibco, USA) at 37℃, 5% CO_2. Supplemented media was changed every 2-3 days. Cells were split 1:10 and plated onto 6-well and 96-well plates (BD Falcon) and incubated with supplemented media for 24 h followed by pretreatment with corticosterone, (0μM-50μM) for 24 h and 48 h.

Cell Viability and Proliferation Assay

To examine viability, cells were dissociated and diluted with equal volumes of trypan blue dye (4%). Cell count averages were taken from hemocytometer to determine cell number and viability. The CyQUANT XTT Cell Viability Assay (Invitrogen) was used to assess the proliferation of NE-4C cells and C8-S cells exposed to corticosterone. Cultured cells were transferred to a 96-well plate in DMEM supplemented with 10% FBS. Cell proliferation was monitored by measuring the absorbance at 450 nm and 650 nm 24 h and 48 h using a microplate reader.

RNA Extraction and cDNA synthesis

C8-S cells and NE-4C cells were homogenized with RNA Lysis Buffer (Zymogen). Following the manufacturers' protocols, spin-column purification and extraction of total RNA was performed on homogenized cells (Quick-RNA Miniprep Kit, Zymogen). RNA preparations were

treated with DNase I. Isolated total RNA then underwent first-strand cDNA synthesis using Superscript II Reverse Transcriptase with oligo(dT) primers (Invitrogen).

Gene Expression Analysis using RTqPCR

Expression of three genes (p53, PTEN, and MDM2) were quantified (Table 1). Primers were designed using Primer-BLAST and validated in literature and obtained from ThermoFisher Scientific. Real-time PCR (qPCR) was run on undiluted cDNA samples using the designed primers, and iQTM SYBR® Green Supermix (Bio-Rad). Samples were run in duplicate with the following parameters: 2 minutes at 94°C, 40 cycles at 94°C for 15 seconds, then 1 minute at 60°C.

Table 1.

Gene Symbol	**Identification**	**Sequence**	
P53	Tumor protein	Forward Reverse	GACTCAGCCTCTTGATGACT GCAACAGATCGTCCATGCAG
PTEN	Phosphatase and tensin homolog	Forward Reverse	ATTTCTGCGGCTGTTCCTCT ACGAGCTCGCTAATCCAGTG
MDM2	Murine Double Minute 2	Forward Reverse	CTCACCCTGGCTAAAGTTCTGT AATGGTTTTGGTCTAACCTGG
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Forward Reverse	AGAGGGATGCTGCCCTTACC TGTCTACGGGACGAGGAAAC

Data Analysis

Gene expression graphs show Cq value against concentration of corticosterone. Specific absorbance values from viability testing are also displayed graphed against concentration of cortisol. All RT-qPCR and absorbance data was compiled and analyzed in Microsoft Excel and

one-way ANOVA using Prism. Amplification and melt curve graphs were produced in CFX Maestro qPCR analysis software.

Chapter 3: Results

Proliferation Assay and Cell Viability

Specific absorbance 24 hrs post-corticosterone treatment was highest in the control and 50uM for NE-4C cells (figure 1). There was a significant decrease in proliferation at 1.5uM-6.25uM, ($P < 0.01$), followed by an increase in proliferation from 6.25uM-12.5uM that was also statistically significant ($P < 0.01$). We observed a statistically significant increase in proliferation in the control from 24 hrs to 48 hrs ($P<0.001$), and a statistically significant increase in proliferation between 24 hrs and 48 hrs in 12.5uM-50uM ($P<0.001$). In 1.5uM-6.25uM there was also a significant increase in proliferation from 24 h to 48 h ($P<0.01$). At 48 hrs there was a significant decrease in proliferation from the control to 1.5uM-6.25uM ($P < 0.001$), followed by a significant increase in proliferation from 6.25uM-12.5uM ($P < 0.01$). At 48 hrs there was also a significant increase in proliferation from 12.5uM and 25uM to 50uM ($P < 0.01$).

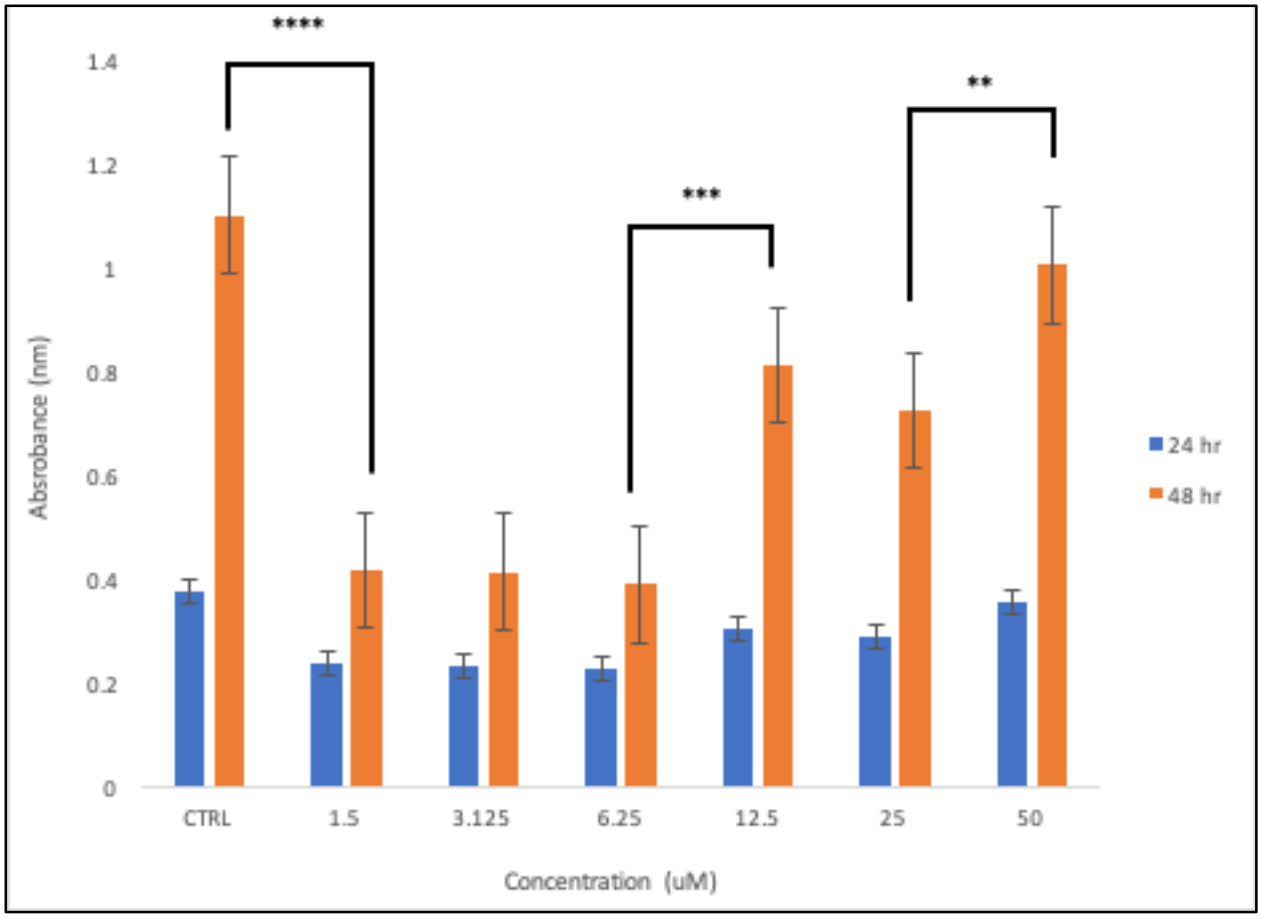

Figure 1. **Proliferation and Viability Assay of NE-4C Cells.** Cells treated with 0uM-50uM cortisone and cell proliferation quantified using the cyquant xtt assay. Absorbance measure and 24 and 48 hrs post treatment at 450 nm and 650 nm.

Proliferation Assay and Cell Viability

Specific absorbance values were negligible at 24 hrs in C8-S cells (figure 2). At 48 hrs there was a significant increase in proliferation in the control, 1.5uM and 3.125uM sample ($P < 0.001$). There was a trending increase in proliferation from 3.125uM-50uM, however this was not statistically significant. The increase in proliferation observed at 24 hrs in the control was statistically significant compared to 48 hrs in 50uM ($P < 0.001$). There was also a statistically significant increase in proliferation at 24 hrs and 48 hrs in the 50uM concentration. ($P < 0.001$).

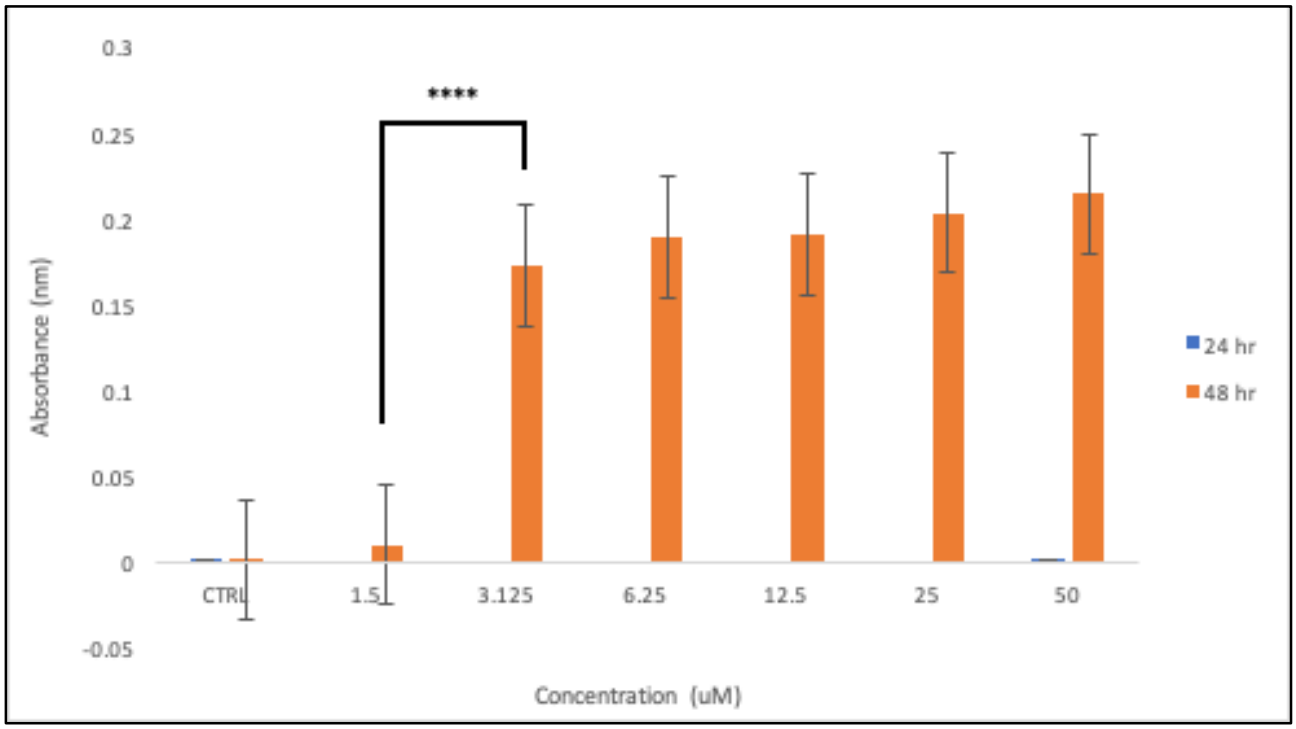

Figure 2. **Proliferation and Viability Assay of C8-S cells.** Cells treated with 0uM-50uM cortisone and cell proliferation quantified using the cyquant xtt assay. Absorbance measure and 24 and 48 hrs post treatment at 450 nm and 650 nm.

Mean Gene Expression

There were no statistically significant differences in expression of the housekeeping gene, GAPDH, across varying corticosterone concentrations for either cell type (figure 3).

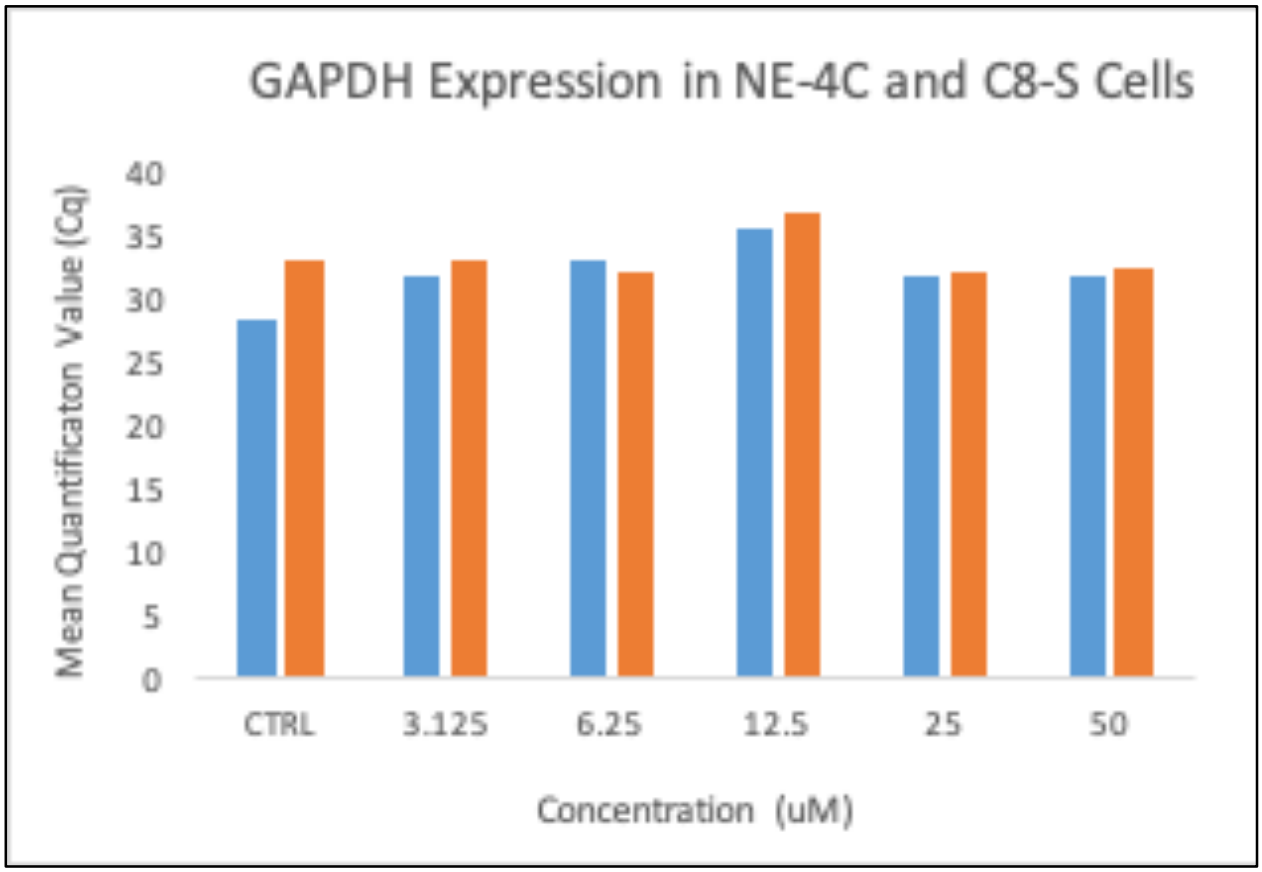

Figure 3. **Gene expression analysis in treated NE-4C and C8-S cells at 48hr.** NE-4C cells indicated in blue and C8-S cells indicated in orange.

Mean Gene Expression

NE-4C cells (indicated in blue) showed a statistically significant decrease in p53 expression from the control to 3.125uM ($P < 0.001$) (figure 4). The decrease in expression seen at 3.125uM to 6.25uM was also statistically significant ($P<0.001$). There were no significant differences across 6.25uM-25uM. The decrease in expression from 6.25uM to 50uM was statistically significant ($P < 0.01$). The decrease in expression from the control to 50uM was statistically significant ($P<0.001$). There were no significant differences in expression across C8-S cells (indicated in orange). There was a decrease in expression from the control and 3.125uM to 6.25uM followed by a slight increase at 12.5uM and another decrease at 25uM. None of these changes were significant ($P > 0.05$). There was a statistically significant increase in expression seen in NE-4C cells compared to that of C8-S cells across concentrations ($P < 0.001$).

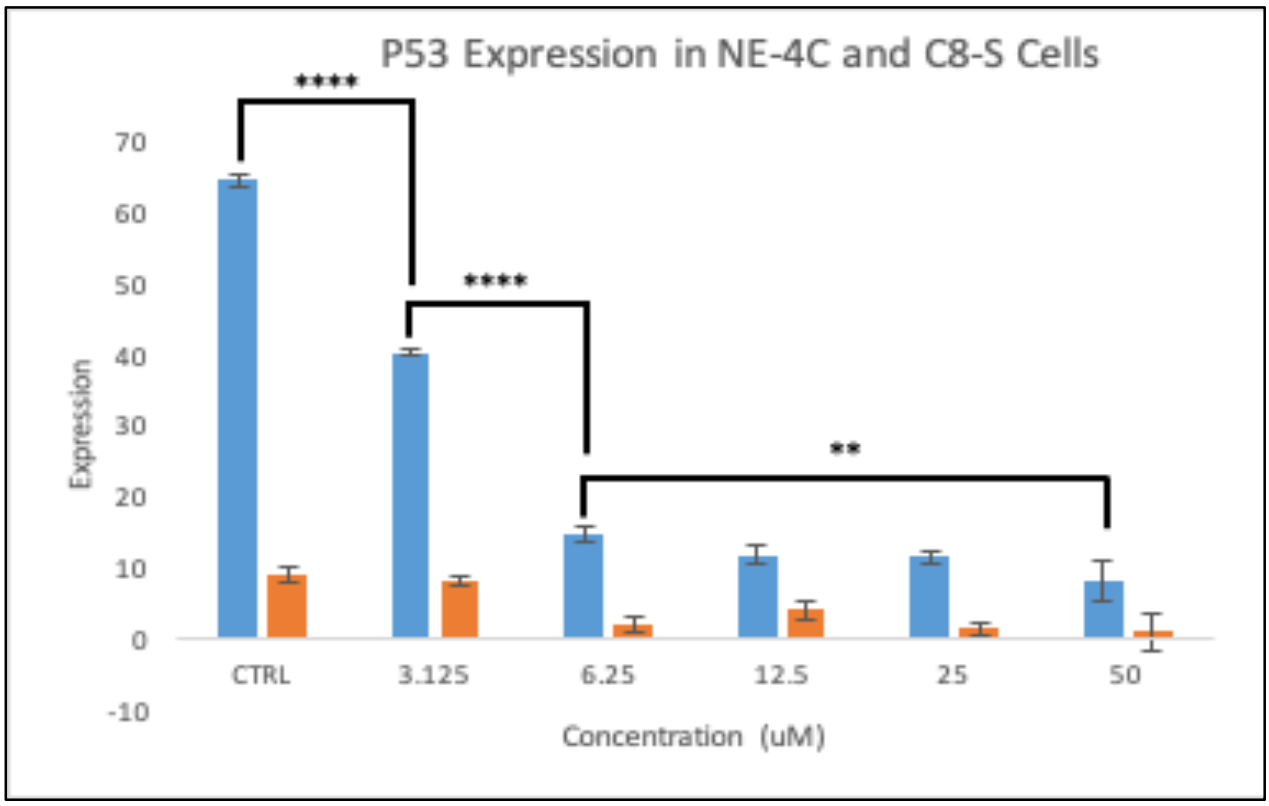

Figure 4. **Gene expression analysis in treated NE-4C and C8-S cells at 48hr.** NE-4C cells indicated in blue and C8-S cells indicated in orange.

Mean Gene Expression

NE-4C cells (indicated in blue) showed several significant differences in MDM2 expression (figure 5). There was no significant decrease in expression between the control and 3.125uM ($P > 0.05$), however there was a significant increase in expression from 3.125uM to 6.25uM ($P < 0.05$). There was no significant change in expression from 6.25uM to 12.5uM, however the increase in MDM2 expression from 12.5uM to 25uM is significant ($P < 0.01$). The increase in expression from 25uM to 50uM was not significant ($P > 0.05$). There were no significant changes in MDM2 expression in C8-S cells (indicated in orange). There was however a significant difference in expression between C8-S and NE-4C cells across concentrations ($P < 0.001$).

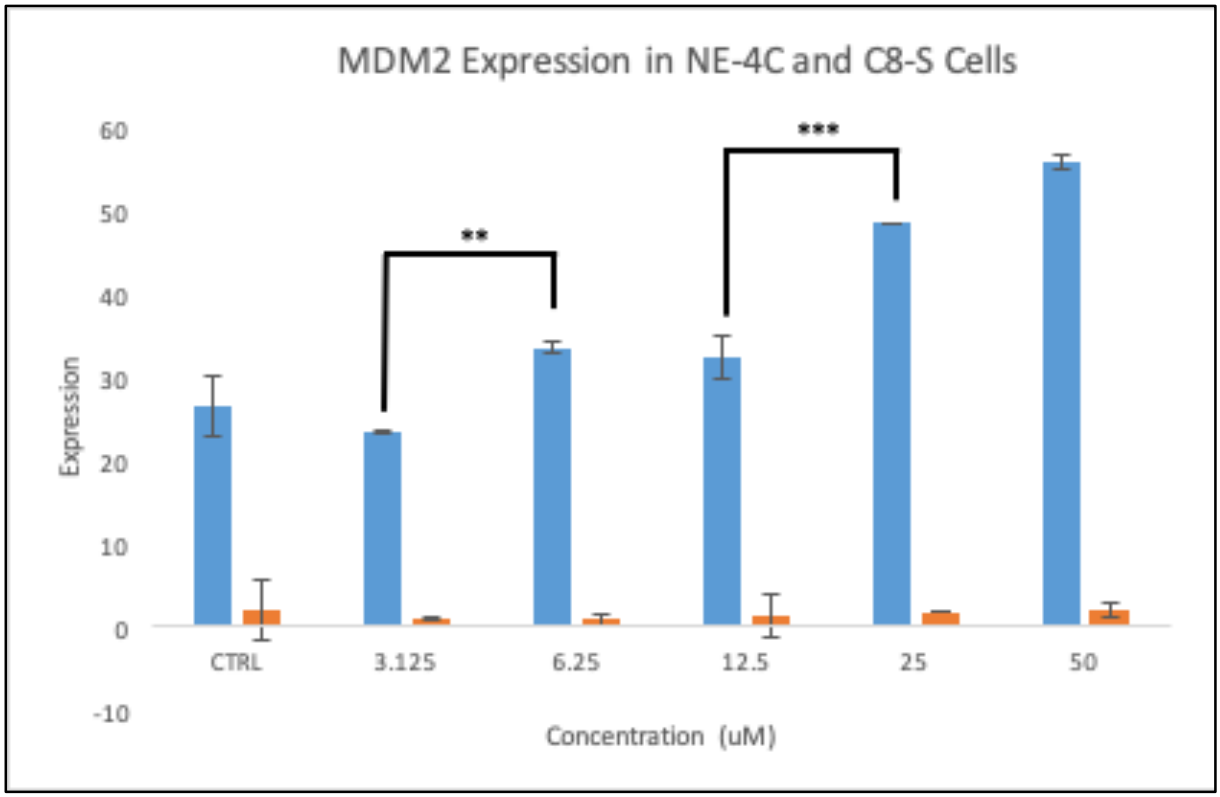

Figure 5. **Gene expression analysis in treated NE-4C and C8-S cells at 48hr.** NE-4C cells indicated in blue and C8-S cells indicated in orange.

Mean Gene Expression

Changes in PTEN expression across concentrations in C8-S cells (indicated in orange) were not statistically significant (figure 6). NE-4C cells (indicated in blue) showed several significant changes in expression. There was no significant decrease in expression from the control to 3.125uM. There was a significant decrease in expression from 3.125uM to 6.25uM ($P < 0.05$). The decrease in expression from 6.25uM to 12.5uM was statistically significant ($P < 0.01$). The increase in expression from 12.5uM to 25uM was not significant and neither was the decrease in expression from 25uM to 50uM ($P > 0.05$). The difference in expression between C8-S cells and NE-4C cells was statistically significant ($P < 0.001$).

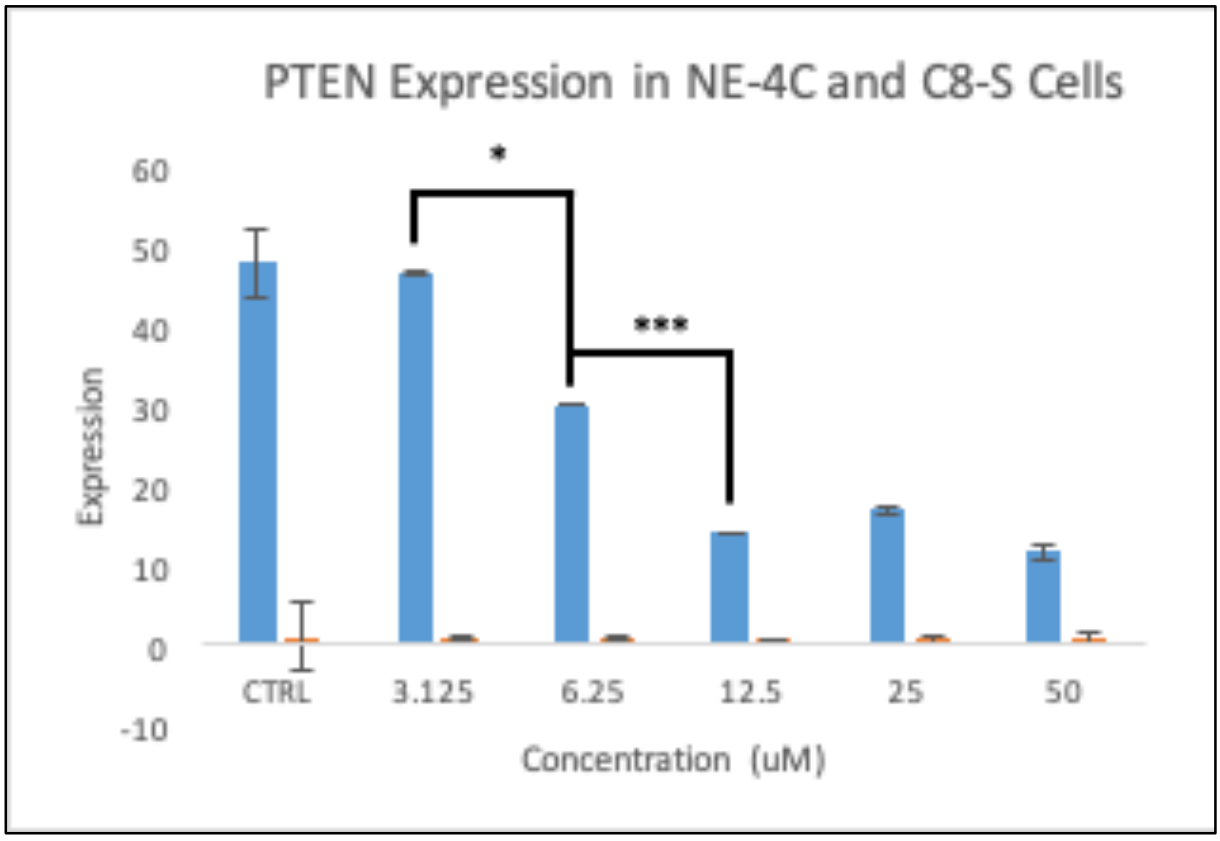

Figure 6. **Gene expression analysis in treated NE-4C and C8-S cells at 48hr.** NE-4C cells indicated in blue and C8-S cells indicated in orange.

Chapter 4: Discussion

Proliferation Assay and Cell Viability

NE-4C and C8-S cells were treated with corticosterone concentrations ranging from 0uM-50uM. At 24 hrs and 48 hrs absorbance was read and mean absorbance was calculated by subtracting 450 nm from 650 nm and the averages for each concentration was calculated. The difference in proliferation between cell types was statistically significant ($P < 0.001$) with the highest absorbance value for NE-4C cells at 1.1 nm and the highest absorbance value for C8-S cells was around 0.2 nm (figures 1 & 2).

It was expected that C8-S cells would not demonstrate high levels of proliferation as they are unmodified and healthy. Proliferation is highly regulated in healthy cells and is a necessary process for normal development and maintenance over the lifespan. This level of absorbance is indicative of normal cell division in healthy unmodified cells. This was also evident by the fact that the absorbance levels at 24 hrs were very low and negligible in the presence of corticosterone. This indicates that there was no rapid and uncontrolled proliferation occurring in the cells as a response to high levels of acute stress.

Although there was an increase in absorbance from 24 hrs to 48 hrs that was significant ($P < 0.001$), the absorbance levels were still low compared to that of NE-4C cells. While the change in absorbance at 48 hrs was significant, as was the control compared to 50uM, there were no significant differences in absorbance between 3.125uM and 50uM. This indicates that corticosterone has an insignificant effect on healthy cells and the cells were likely undergoing normal cell division. The effect of corticosterone on healthy cells alone doesn't indicate that it's enough to mutate healthy cells into cancerous cells without another mutation already present.

NE-4C cells that were p53 deficient showed significantly higher levels of absorbance and proliferation. At 24 hrs the absorbance of NE-4C cells surpassed the highest levels of absorbance seen at 48 hrs in C8-S cells. The absorbance seen in the control to 1.5uM-6.25uM of cortisone significantly decreased ($P < 0.01$), suggesting that low to moderate levels of cortisol or acute stressors could potentially be beneficial for regulating uncontrolled proliferation. The significant increase in absorbance seen at higher concentrations of cortisol ($P < 0.01$), suggests that exposure to high levels of cortisol or chronic stress might be contributing to or worsening proliferation in already mutated cells. This increase in proliferation was consistent with the decrease in p53 expression in higher concentrations of cortisone in the gene expression analysis.

Gene Expression Analysis

Expression in the housekeeping gene, GAPDH showed no significant changes or variation across cell type or concentration. In figure 4, we see the highest levels of p53 expression across NE-4C cells. There was a significant decrease in expression from the control to 3.125uM-50uM ($P < 0.001$). Corticosterone is causing a significant decrease in p53 expression in mutated cells, suggesting that corticosterone is somehow modifying p53 expression possibly through the MDM2 feedback loop through the increased expression seen in MDM2. In C8-S cells, corticosterone does not have a significant effect on p53 expression. p53 is expressed at very low levels in healthy cells, so we wouldn't expect C8-S cells to undergo significant changes in p53 expression as the result of exposure to corticosterone. This further indicates that exposure to cortisol or acute stressors does not have a significant impact on proto-oncogenes in healthy cells. This exemplifies the significant difference between NE-4C cells and C8-S cells as p53

mutated is frequently mutated in cancer and plays a vital role in cell cycle regulation, apoptosis, and uncontrolled proliferation.

Figure 5 shows MDM2 expression completely opposite of p53 expression in NE-4C cells. MDM2 expression significantly increased as corticosterone concentrations increased ($P < 0.001$). There was no significant change across expression in C8-S cells, which was expected because MDM2 expression is normally kept low by MDMX in healthy cells. The opposite expression patterns in MDM2 and p53 indicate that the overexpression of MDM2 as a result of exposure to corticosterone is negatively regulating p53 function causing a decrease in expression. This is also consistent with the proliferation assay in NE-4C cells. Because MDM2 negatively inhibits p53, its increase in response to increasing corticosterone concentrations in NE-4C cells is important and may point to a potential target mechanism.

PTEN expression in C8-S cells (figure 6) was generally low and showed no significant changes across concentration. Since PTEN is downstream of p53 it would be expected that PTEN expression would be somewhat similar to p53. In NE-4C cells, PTEN followed a similar pattern to p53, where increasing corticosterone concentrations generally caused a decrease in PTEN expression. In NE-4C cells there was a significant decrease in expression in the control compared to 50uM ($P < 0.001$). This suggests that exposure to high levels of corticosterone is modifying the p53-MDM2 feedback loop and pathway. PTEN is a critical regulator in the proliferation pathway, making this result significant given the role of proliferation as a hallmark of cancer.

Conclusion

As previously discussed, increased glucocorticoid activity is associated with up-regulation of MDM2, which the results confirm. It was hypothesized that increasing corticosterone concentrations as a result of chronic stress would mediate p53 and PTEN through the induction of the SGK1 pathway. This would cause increased MDM2 activity and decreased p53 and PTEN function through the MDM2-p53 feedback loop. MDM2 is the primary negative regulator of p53 and overexpression of MDM2 causes decreased p53 levels and attenuates p53 function. The findings of this study demonstrate that where MDM2 is up-regulated with increasing corticosterone concentrations, it causes corresponding decreases in p53 and PTEN function. This was also confirmed by the increase in proliferation seen in NE-4C cells.

In NE-4C cells, MDM2 expression generally increased with increasing concentrations of corticosterone, whereas p53 expression decreased as cortisol concentrations increased. Similarly, PTEN expression decreased as corticosterone concentrations increased. This suggests that corticosterone or chronic stress affects MDM2 and subsequently p53 as part of its feedback loop, which then affects PTEN as a downstream mediator. Since PTEN is part of the IGF-1/mTOR pathway, which regulates proliferation, it was expected to see an increase in proliferation as expression of p53 and PTEN decreased. The proliferation assay confirms this, as proliferation increased with increasing corticosterone concentrations. In untransformed C8-S cells, MDM2, p53, and PTEN expression were much lower. Exposure to cortisol may or may not be affecting healthy cells. It's likely that the effect is not significant enough to cause cancer, but might cause cells to behave differently in the presence of a mutation.

Future directions for this project include exposing C8-S cells to corticosterone for a

longer period of time and measuring proliferation. Additionally, looking at protein expression using western blot would be necessary to see how the pathways are being affected on a protein level. It's also important to consider the regulation that occurs between mRNA and active protein, and further explore post-transcriptional regulation. Finally, expanding the gene and protein analysis to a different or larger portion of the pathway would be necessary to see if these changes are also occurring in other parts of the stress and p53 pathway. The results of this experiment indicate that corticosterone is acting directly on MDM2, not p53. Therefore, MDM2 could serve as a good target to modulate p53 when developing cancer therapeutics and performing cancer research.

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