

Master Degree Project



STRESS RESPONSE IN CANCER CELL LINES

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Abstract

Globally, the cases of cancer have been on the rise. This has led to increasing research to find a lasting solution to carcinogenesis. The increase in cancer cases can be due to a change in people's lifestyles, such as diet and exercise routines which have changed for the worse. In most farms, chemicals as pesticides are added to plants to fasten their growth. These chemicals are carcinogenic, contributing to the increased number of cancer cases worldwide.

The objective of this research was to observe the cell response of brain, pancreatic, and breast cancer cells to different cortisol levels/concentrations. The three cell lines of interest in this research and present in the mentioned types of cancer are T-47D, PANC-1, and T98G. Their dynamics and roles are identified in this study. Cortisol excretion concentrations during stress and cancer growth are also monitored and compared. Additionally, the study solves the unpredictability of the impacts of stress on the three cancer types.

The three experimental setups in this study were as follows: 1. Breast cancer cells, obtained from a 54 year old woman with metastatic carcinoma. 2. Brain cancer cells, obtained from a 61 year old female with neuroblastoma. 3. Pancreatic cancer cells, obtained from a 56 year old male with metastatic carcinoma. The cells in the above setups were treated with cortisol in order to see what effect this induced stress has on cell growth.

The findings of the study concluded higher cortisol concentration increased cancer growth and spread within the body. This was that all three cancer cell types saw an increased effect of cortisol at either 24 hours or 48 hours at a specific concentration, which highlights that cortisol has an important role to play in cancer cells viability.

Popular Scientific Summary about Cancer

Typically, cells multiply and die when damaged or when they get old, and new cells take their place, which is the normal cell life cycle. However, cancer is a disease that makes cells multiply uncontrollably and spread throughout the body, resulting in tumor development, damage to the immune system, and other fatal impairments to the human immune system.

There are various noticeable differences between cancer and normal cells. First, cancer cells grow without signals telling them to grow. On the contrary, normal cells grow only when they receive the signal telling them to grow. The cancer cells also ignore signals indicating they should die due to old age or damage. Unlike most normal cells that do not move around the body, cancer cells spread everywhere without restrictions. In addition, cancer cells also lure blood vessels to grow towards the tumor, supplying the tumor with nutrients and oxygen and helping with waste management. The cancer cells also hide from the immune system tricking the immune system against eliminating the abnormal cells. Further, cancer cells also initiate changes in the chromosome, such as deletion and duplication, reducing their death rates resulting in increased tumor development. The United States recorded 15.5 million cancer-related deaths until 2016.

On average, 480,000 people die of cancer annually. Major causes or risks include heavy alcohol consumption, excess body weight, physical inactivity, and poor nutrition. However, there is an often-overlooked trending cause of cancer that many researchers find perplexing. This is the current positive correlation between cancer development and stress. Cortisol release in response to the psychological stress is claimed to produce a shift in Th1 and Th2 cytokines levels toward a Th2 response. This leads to a reduced natural dying ability of cells due to damage or old age.

The inability of cells to die increases the number of unwanted and unhealthy cells within the body, increasing the chances of developing tumors leading to cancer. Several researchers defend the correlation between cancer and stress in recent studies indicating the probability of its occurrence. Other researchers claim after one is diagnosed with cancer, one develops chronic stress. Therefore, stress increases the spread of cancer throughout the body, especially in the brain, breast, and pancreatic cancer. The major cancer treatments include chemotherapy, hormone therapy, stem cell transplantation, radiotherapy, immunotherapy, and surgery.

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List of Abbreviations

ACTH – Hormone- Adrenocorticotrophic hormone

AVP -arginine vasopressin

GCR- Glucocorticoid receptor

GCs- Glucocorticoids

CSC cancer stem cells

CRH- corticotropin-releasing hormone

FBS – Fetal Bovine Serum

GBM Glioblastoma multiforme

HER2-human epidermal growth factor receptor

HPA- Hypothalamic-pituitary-adrenal axis

HER2-human epidermal growth factor receptor

PCR – polymerase chain reaction

PVN paraventricular nucleus

SNS- sympathetic nervous system

TEM- tumor microenvironment

Introduction:

Cancer is a devastating and intricate illness that impacts millions of individuals around the globe. It is characterized by the abnormal growth of cells that damage and invade healthy tissues and organs in the body (Anand et al., 2022b). Cancer is considered a global health challenge that accounts for 16% of all deaths worldwide (Debela et al., 2021b). Cancer's agents of destruction are actually human cells that have been recruited and partly modified into pathological organisms or building blocks of tumors (Hausman, 2019). Accumulating epidemiological evidence indicates the impact of psychosocial and behavioral factors on cancer risk, progression, and mortality. (Kruk et al., 2019). This essay discusses the effect of stress on breast cancer, pancreatic cancer and brain cancer.

Breast cancer

Breast cancer is a prevalent cancer in women and is the second most frequently occurring cancer worldwide among newly-diagnosed cancers. Numerous studies have shown the impact of lifestyle and environmental factors on the development of breast cancer (Kolak et al., 2017). Breast cancer stem cells (BCSCs) play a significant role in the aggressiveness of different tumors (Barzaman et al., 2020).

T-47D cells are often used as an in vitro model for studying human breast cancer (Yu et al., 2017). Breast cancer cell lines have frequently been utilized for modeling breast cancer, which includes multiple diseases with unique phenotypical connections. Although T-47D cells offer limitless homogeneous materials for tumor research and are relatively simple to cultivate (Ostrikov et al., 2017). Among the characterized cell lines, there were 10 which had estrogen receptors positive, 12 which had human epidermal growth factor receptor 2 (HER2) amplified, and 18 triple negative breast cancer cell lines (Smith et al., 2017). One of the previous studies of human breast cancer cell lines shows that in 3D culture, each cell line takes on a colony morphology belonging to one of four main categories. Breast tumor cell lines have a high mutational frequency with many uncertainties and can not entirely capture breast cancer heterogeneity. (Ostrikov et al., 2017b)

Chronic stress promotes breast cancer via neurotransmitters released by the nervous system was covered in this article's overview of prior research on the relationship between chronic stress and the occurrence and development of breast cancer. (Liu et al., 2022)As a result, the stress granules prove to be a potential target for cancer therapy. This review aims to present a summary of the mechanism and effect of stress granule formation in cancer, using breast cancer as an example, and highlights its potential applications in cancer therapy (Hu et al., 2021). Breast cancer tumor growth and metastasis are inhibited at lower cortisol concentrations, but higher concentrations may actually promote breast cancer progression, which is not desirable (Chiriac et al., 2017).

Pancreatic Cancer

Pancreatic cancer is a type of cancer that has a high mortality rate and is associated with a poor prognosis (Cai et al., 2021) .The incidence of pancreatic cancer has doubled worldwide in the past 25 years, rendering it one of the leading causes of cancer-related deaths globally (Klein, 2021)

The PANC-1 cell line that is derived after isolation from the ductal cell's pancreatic carcinoma helps biomedical scientists to understand the behaviors of such carcinomas (Kim et al., 2019). Pancreatic ductal carcinoma, also known as pancreatic cancer, is caused by the carcinoma that develops in the cells of the duct of the pancreas (StatPearls Publishing, 2023). Pancreatic cancer, known for its aggressive biology, is among the deadliest of solid tumors (Søreide et al., 2020). One of the previous reviews assesses how pancreatic cancer's biological makeup, metabolic programming and tumor microenvironment control its progression and development (Wang et al., 2021).The cancer stem cells CSC population found in pancreatic cancers is highly resistant to chemotherapeutic drugs, making it difficult to treat and leading to tumor recurrence (Phi et al., 2018).

Recent studies have shown that this resistance to treatment may be related to the stress response in cancer cell lines (Polireddy & Chen, 2016). Previous research findings suggest that targeting abnormal signaling in cancer cells that have adapted to mechanical stress within the tumor microenvironment could be a promising way to restrict the migration of pancreatic cancer cells.

Thus, it is crucial to understand how mechanical stress regulates the potential of metastasis in pancreatic cancer (Kalli et al., 2021).

Brain cancer

Brain cancer is considered to be one of the most challenging types of cancer because it is hindered by the unique anatomy and physiology of the brain (Shah & Kochar, 2018). The identification of stem/progenitor cells within brain tumors and their role in sustaining tumor growth has highlighted the importance of understanding molecular pathways regulating neural stem cell behavior and cellular dynamics (Azzarelli et al., 2018.)

Glioblastoma is a prevalent and lethal type of brain tumor which may be caused by genetic and epigenetic alterations in normal astroglial cells (Haque et al., 2011). Glioblastoma multiforme (GBM) is among the most malignant types of tumors affecting the central nervous system (Hanif et al., 2017). In brain cancer studies, primary cell cultures have replaced established cell lines as they better represent in vivo cancer cell behaviors. Culturing glioblastoma cells is particularly challenging due to their heterogeneity and the need to preserve their original phenotype. (Ledur et al., 2017).

The microenvironment surrounding brain tumors is becoming increasingly recognized as a major factor in the progression of primary and metastatic brain cancers. Because of the unique qualities of this organ, it is necessary to have a specific approach to developing interventions that target the tumor microenvironment TME (Quail & Joyce, 2017c). According to a previous investigation, there exists a direct association between the solid pressure induced by tumors in the brain and dysfunction in the nervous system, which induces impaired neurological activity in patients (Seano et al., 2019). It has been previously noted that uncontrolled psychological distress can have a negative impact on brain tumor patients. This implies that maladaptive psychosocial and biobehavioral factors can negatively affect immune system functions in normally healthy individuals. It is still unclear how psychological conditions may impact the anti-tumor immune response of brain tumor patients (Otto-Meyer et al., 2019).

The impact of stress on Cancer:

Stress is any stimulus that elicits a biological response, and the responses to these stressors are referred to as stress responses (Sathyapalan et al., 2017). Stress, is also identified as the "health epidemic" of the current era by the World Health Organization (WHO), has a detrimental effect on the function of multiple body systems and negatively impacts the quality of life of individuals (Dogan et al., 2022b).

Chronic stress is an inevitable aspect of life and can arise due to various causes such as adversity, depression, anxiety, or loneliness. Chronic stress can lead to tumor formation and support cancer growth. This review highlights the most recent findings on molecular mechanisms underlying chronic stress-related cancer development (Dai et al., 2020). In response to various stressors, cancer cells undergo epigenetic modifications that alter their transcriptome and metabolome, ultimately suppressing their ability to proliferate or inducing apoptosis. The kind of stress and length of exposure determine whether cancer cells exhibit a protective response to stress or die. This study highlights current understanding of changes in cancer cells' epigenome and transcriptome (genetic structure) caused by exposure to various physicochemical stressful stimuli.(Mondal et al., 2021). On the other hand, there is little proof of how behavioral factors contribute to cancer initiation. Specific signaling pathways that affect cancer growth and metastasis have been identified by recent cellular and molecular studies. This article offers an overview of the connection between psychosocial factors, specifically chronic stress, and the progression of cancer (Moreno-Smith et al., 2010)

The role of cortisol as a stress hormone in tumorigenesis:

The body's response to stress involves a complex interplay between the nervous, endocrine, and immune systems. This includes the activation of the sympathetic-adreno-medullary axis, the hypothalamus-pituitary-adrenal axis, and the immune system.(Chu, 2022). The activity of the hypothalamic-pituitary-adrenal (HPA) axis is controlled by circadian rhythm and can be brought on by physical or emotional stress as shown in **Figure 1 (Timmermans et al., 2019)** .Upon activation, corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are released from the hypothalamic paraventricular nucleus (PVN), initiating the release of

adrenocorticotrophic hormone (ACTH) into the bloodstream from the pituitary gland. ACTH then triggers cortisol synthesis in the cortex of the adrenal gland. Cortisol negatively impacts HPA-axis activity by repressing the transcription of CRH and POMC through the binding of negative glucocorticoid responsive elements (nGRE) or by binding to the transcription factor Nur77 involved in the POMC expression.

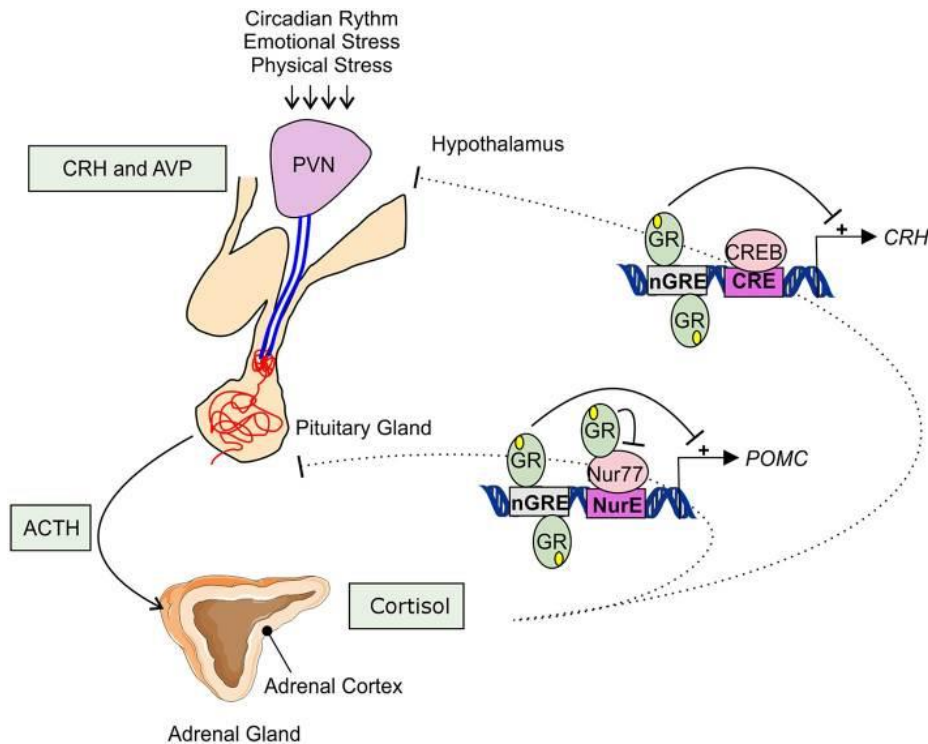


Figure 1 illustrate shows the role of cortisol as a stress hormone in tumorigenesis

Upon activation, corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) are released from the hypothalamic paraventricular nucleus (PVN), initiating the release of adrenocorticotrophic hormone (ACTH) into the bloodstream from the pituitary gland. ACTH then triggers cortisol synthesis in the cortex of the adrenal gland. Cortisol negatively impacts HPA-axis activity by repressing the transcription of CRH and POMC through the binding of negative

glucocorticoid responsive elements (nGRE) or by binding to the transcription factor Nur77 involved in the POMC expression.

Chronic stress primarily activates the classic neuroendocrine system known as the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). This can lead to a decline and dysfunction of the prefrontal cortex and the hippocampus under stress. The stress hormones produced through the activation of both the HPA axis and the SNS can promote tumorigenesis and cancer development through several mechanisms (Dai et al., 2020b). Inflammation responses have significant roles during the different stages of tumor development, which include but are not limited to initiation, promotion, malignant alteration, invasion, and metastasis (Grivennikov et al., 2010)

Using of cortisol as a treatment of cancer

One of the emerging approaches in cancer treatment is the use of cortisol, Glucocorticoids are a common form of adjuvant therapy for breast cancer patients. Although not considered an oncogene, the GC receptor differs from other steroid hormone receptors (Pufall, 2015). Recent studies have illuminated the intricate nature of GC-mediated signals, but it remains unclear whether GCs enhance or hinder tumor progression in various types of cancer (J. Pang et al., 2020).

Aim of the study:

The main aim of this study is to analyze the cellular response of breast, pancreatic, and brain cancer cells to stress. The study uses a cell viability assay to detect the responses in different cancer cells to varying cortisol concentrations. Furthermore, the study aims at exploring the relationships between cortisol excretion during stress and the growth of cancer tumors. The study's overall

purpose is to solve the inconsistencies that have existed in the past regarding the psychological impacts of stress on the progression and prognosis of the three above mentioned cancer types.

Materials and methods

Experimental design

In this experiment, the cells were harvested and seeded in both 96-well plates (for MTS assay) and in Petri-dishes for extracting mRNA. The cells were Cortisol treated at different concentrations and incubated for 24 and 48 hours. MTS assay was used to quantify the cell viability after 24 and 48 hours. The mRNA extracted from the petri dish is used for cDNA synthesis to be able to run the qPCR for gene expression.

Reagents

- mRNA kits with mRNA purification and cDNA synthesis in the same well ([RevertAid First Strand cDNA synthesis Kit K1621](#)) (1).
- Cell culture media RPMI-1640 L-glutamine + Phenol Red + 10% Fetal Bovin Serum (FBS) + 1000u/ml penicillin and 1000u/ml streptomycin.
- CellTiter 96® Aqueous One Solution Cell Proliferation Assay (MTS) (Promega-G3582).
- SYBR® Select Master Mix (Life technologies: 4472908).
- T47D – Breast cancer cell lines were derived from a 54 -years old female with metastatic carcinoma (SIGMA- Aldrich -85102201).
- T98G – Brain cancer cell lines were obtained from a bone marrow biopsy from a 5 years-old female with Neuroblastoma (SIGMA- Aldrich - 92090213).
- PANC-1 – Pancreatic cancer cell lines were derived from a 56 -years old Caucasian male with metastatic carcinoma (SIGMA- Aldrich- 87092802).

Cell culture

Three cell lines were used in this experiment (T98G, Panc-1 cells, and T47D cells) to investigate the cellular response of cancer cells to different cortisol concentration. Then , the isolated cell lines (T98G, Panc cells, and T47D cells; purchased from SIGMA- Aldrich -85102201) were cultured in DMEM (upplemented with 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 2 mM L glutamine. The medium was changed every 1 or 2 days.

Cell viability assay

Cell viability was performed using the MTS assay Promega Corporation's CellTiter 96 kit number #G3582 according to the manufacturer's protocols. The MTS assay (5-(3-carboxymethoxyphenyl)-2-(4,5-dimethyl-thiazolyl)-3-(4-sulfophenyl) tetrazolium, inner salt assay) is a colorimetric assay. This assay is based on the conversion of a tetrazolium salt into a colored formazan by the mitochondrial activity of living cells. The amount of produced formazan is dependent on the viable cell number in culture and can be measured with a spectrophotometer at the wavelength of 492 nm. The cells were treated with cortisol except for control cells at different concentrations (6, 7, and 8 μ M, denoted as "M-6, M-7, M-8" in graphs) for 24 and 48 hrs as previously described in the 96-well plate. After incubation at 37°C in a cell culture incubator, cell viability was measured at 24 and 48 hrs.

Real-time quantitative PCR

Is a technique based on the polymerase chain reaction (PCR); it is mainly used to quantify the PCR product based on fluorescent detection. PCR was performed to detect the expression of cortisol receptors. Total RNA was extracted using the QIAGEN RNeasy Plus mini kit (#74104) following the manufacturer's instructions. RNA samples were reverse transcribed to cDNA using the High Capacity cDNA Transcript Kit (#00757869) according to the manufacturer's recommendations. The cDNA was analyzed by qPCR using the SYBR Green Master Mix [4309155](#) .Real-Time PCR System. Following the manufacturer's instructions. The primers used for the cortisol receptor were GR1 (Forward Primer: ACAGCATCCCTTTCTCAACAG and Reverse Primer AGATCCTTGGCACCTATTCCAAT). The results were presented as fold change against control groups.

Statistical data analysis

Statistical analysis was performed for MTS assay to detect the viability of cells using SPSS. Statistics for the PCR results were obtained using Microsoft Excel. Differences between groups were analyzed using the independent samples t-test. The significant criterion was set as P value <0.05 . Since the study was conducted within 24 and 48 hours, the independent samples t-test was more appropriate to check the difference in the impact of cortisol concentrations as a factor of time. In this case, time will be the factor variable, while cortisol concentration will be the dependent variable.

Results

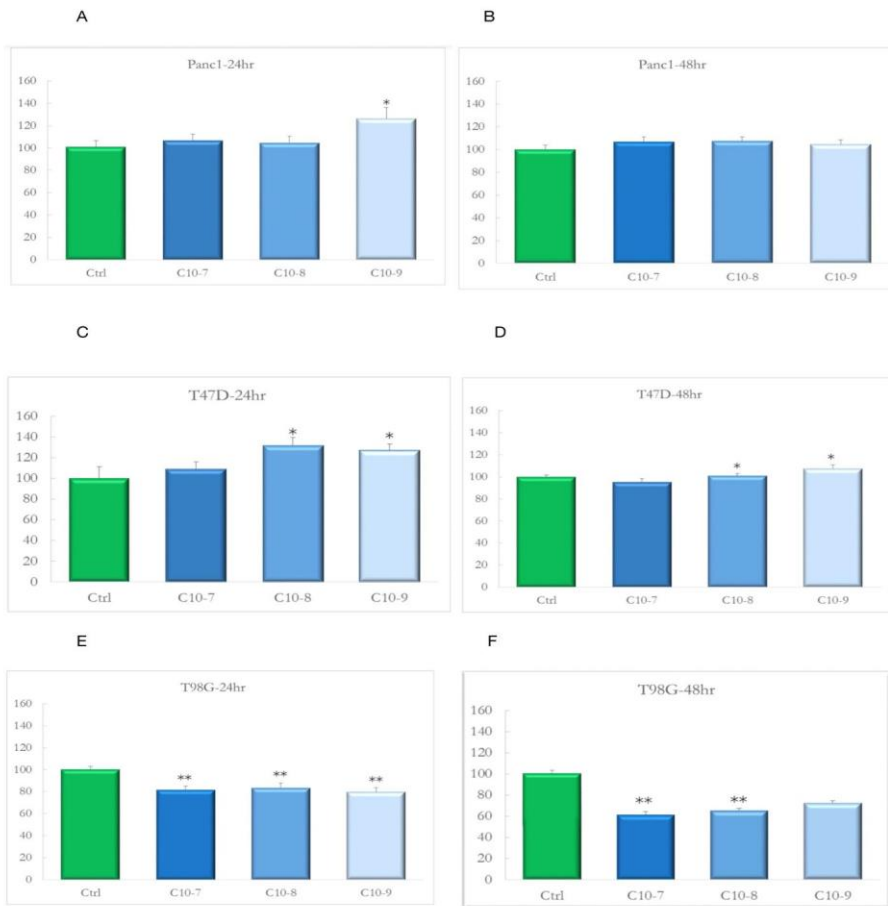


Figure 2: (A) Shows pancreatic cells at 24 h incubation and cortisol at different concentrations. Error bars are SEM values. (B) Pancreatic cancer cells at 48 h incubation with cortisol at different concentrations. (C) Shows breast cancer cells and cortisol concentration after 24 hours. (D) Breast cancer cells at different cortisol concentrations after 48 h. (E) Shows brain cancer cells and different cortisol concentrations after 24 hours duration. (F) Shows brain cancer cells and different cortisol concentrations after 48 hours duration. The stars indicate a bar with results significantly different from the expected chance performance ($p < 0.05$).

To examine the effects of cortisol as a stress agent on cell viability, breast, pancreatic, and brain cells were treated with different doses of cortisol, and cell viability was assayed by MTS assay at 24 and 48 h. The effects of cortisol varied according to concentration. Cortisol enhanced cell growth and development within short periods and inhibited the cellular proliferation of cancer cells long-term.

Stimulation of pancreatic cancer cells with cortisol, as a stress agent, induced cell viability after 24 h incubation ($p < 0.05$) (Fig.2A). We observe a significant result of cortisol on pancreatic cancer cells ($p < 0.05$). This means the stress has a good effect on the cells in a short period. These cortisol-induced effects on pancreatic cells were not significant at subsequent 48 h incubation ($p > 0.05$). There are no significant results after 48 hours ($p > 0.05$) (Fig.2B). As a stressful agent, cortisol has a positive influence on pancreatic cancer cell development within shorter periods and a negative effect for the long term.

Furthermore, no significant effect of cortisol, as a stressful agent, on breast cancer cells ($p > 0.05$) (Fig.2C) after 24 h incubation period. We observe no significant result of the cortisol as a stressful agent on breast cancer cells ($p > 0.05$). However, little effect of cortisol as a stressful agent on breast cancer cells ($p > 0.05$) (Fig.2D) after 48 h of incubation. We observe cortisol concentration has a slight long-term effect on breast cancer cells ($p > 0.05$).

A positive effect of cortisol was also detected on the brain cancer cells for the short duration of 24 h ($P < 0.05$) (Fig.2E). Here, we observe that stress has a positive effect on brain cancer cells for the short duration of 24 hours ($P < 0.05$). Likewise, there was a significant effect of cortisol on brain cancer cells at a higher concentration for the duration of 48 h ($P < 0.05$) (Fig.2F). We observe a significant effect of cortisol on brain cancer cells at a higher concentration for the duration of 48 hours ($P < 0.05$). However, the effect was insignificant at lower cortisol concentration ($P > 0.05$).

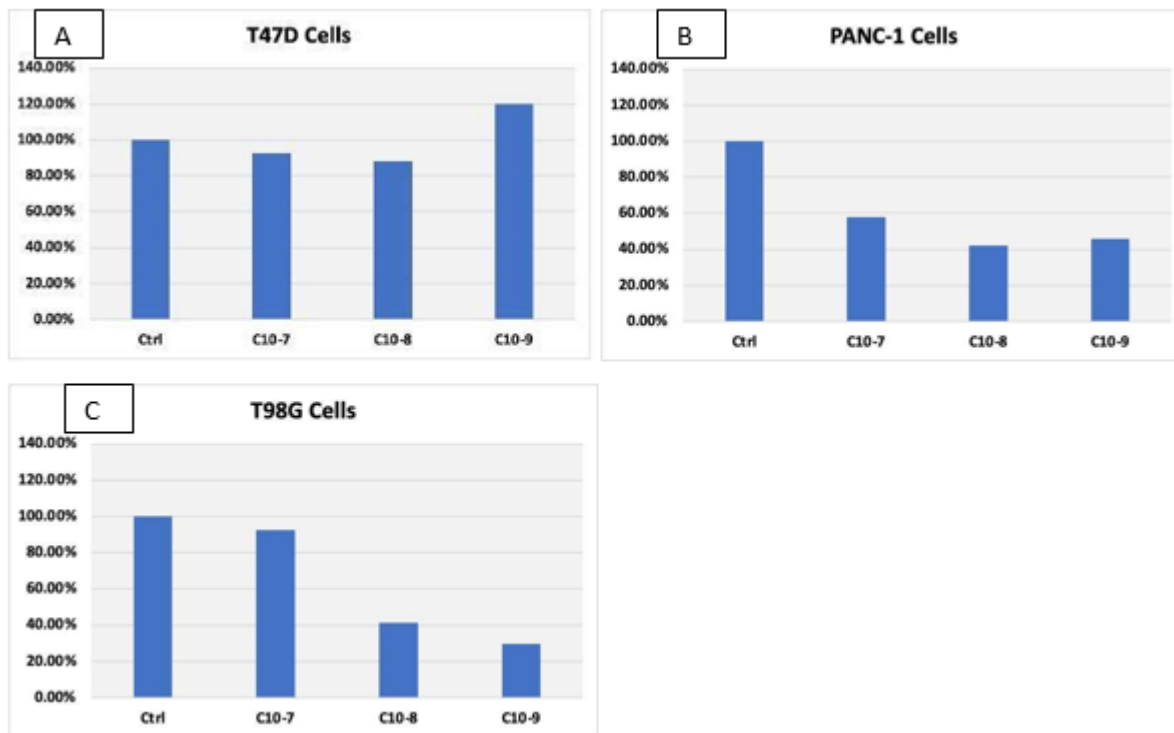


Figure 3: (A) show the Gene expression of the glucocorticoid receptor in breast cancer (T47D), (B) pancreatic cancer (PANC-1), and (C) brain cancer (T98G) cell lines after treatment with various cortisol concentrations. **There are no error bars because the genes were run only once that why the statistical analysis tools could not be applied.**

To detect the expression of glucocorticoid receptors, breast cancer (T47D), pancreatic cancer (PANC-1), and brain cancer (T98G) cell lines were analyzed after treatment with various cortisol concentrations by using qPCR. When measuring expression levels for the glucocorticoid receptor in response to varying cortisol concentrations T-47D breast, pancreatic cancer, (and brain cancer (T98G) cells exhibit a different pattern of response. (Fig.3A) showed an increase in receptor expression for the highest cortisol concentrations, while (Fig.3A,3C) showed a reduction in receptor expression. This results based more on observation and no more explanation statistical wise because the experiment run only once.

Discussion

Referring to the general outcomes of my study, these data suggest that cortisol has a potential role in cancer progression. Specific cortisol concentration levels could have an effect on cell viability for specific cancer cells, but the results are not linear and are not the same for the three cancer cell lines that were investigated in this study. Inhibitory effects of cortisol on cancer cell viability have been reported previously (Moreno-Smith et al., 2010c)

Nevertheless, the cortisol was generally tested in these studies at pharmacological concentrations (1000 nM). There are different stress effects of cortisol on carcinoma cells, depending on the hormone dose (Scatena et al., 2018). In line with our results, cortisol at different concentration levels induced varied effects on the viability of pancreatic, breast, and brain cells, and this does not necessarily mirror the impact or exposure to stress in the body.

Referring to figure 2-A our findings showed that cortisol induced cell viability of pancreatic cells after 24 h of treatment, but these results were not significant after 48h of incubation with cortisol as seen in **figure 2-B**. This suggests cortisol as a short-term stressor that does not increase cell viability in the pancreatic cancer cells; however, after 48 hours, clear increased cell viability could be seen. This would mean that stress over longer periods as a sustained result of psychological stress *in vivo* would increase cell growth (cell viability) in pancreatic cancer patients. Which is very similar to results (Zeng et al., 2019)

Referring to figure 2-C for the breast cancer cell line, the cortisol-induced stress showed no significant effect after 24 hours but increased cell viability after 48 hours. This suggests that breast cancer cells are less sensitive to short-term stress by cortisol compared to pancreatic cancer cells, although stress over time (48h) showed increased cell viability with increasing concentrations of cortisol as seen in **figure 2-D**. Studies have shown that stress is a significant risk factor that can have negative effects on the neuroendocrine and immune systems in Breast cancer (Chiriac et al., 2017b)

This mirrors the results from previous studies, which show that early exposure to a traumatic experience in animals leads to a hyperactive stress response, which in turn increases the incidence of mammary cancer (Antonova et al., 2011). These studies also highlight that there are certain windows for stress exposure that change the mechanism. For example, women diagnosed with

breast cancer were more likely to have experienced stress in childhood and adolescence in studies, which highlights that cells at certain points during development may be more susceptible to the impact of stress and cortisol on the body (Antonova et al., 2011) In this case, it may be associated with why breast cancer cell lines **Figure 2c and Figure 2D** are not responsive to stress over a short time and only at higher concentrations over a slightly longer period, as this is not related to external events.

Referring to figure 2-E in brain cancer cells, there was decreased cell viability after 24 hours; after 48 hours, as seen in **figure 2-F** a decreased cell viability for the lower cortisol concentrations could be seen for coming back to basal levels in the highest concentration. Previous studies show that brain cancer can be strongly associated with past psychological stress, although, of course, it is hard to compare the results from *in vivo* observation with *in vitro* experimentation (Otto-Meyer et al., 2019b).

According to (Abate et al., 2020) there is a conclusive correlation between psychological stress and cancer development. Researchers have supported that people who tend to experience inflammation may have a higher risk of developing brain cancer and other tumors. Though the conclusion is (Dai et al., 2020c), chronic stress creates a conducive environment where precancerous cells can flourish. However, it is not conclusive how stress increases the chances of cancer development.

Referring to figure 3: Interestingly, the treatments with varying cortisol concentrations had differential effects on different cancer cells; (Moreno-Smith et al., 2010b) confirms this claim by stating cortisol levels do not define the probability of cancer cell development as it varies among cancer types.

It is interesting that differences between specific cancer lines persist, suggesting that cortisol may show specific differences dependent on the type of cancer. For example, as reported recently, cortisol promotes both growth and migratory abilities of breast cancer cell lines, similar to our results **as shown in figure 3** (Antonova et al., 2011b). Interestingly, a recent report shows that cortisol promotes the development of metastases in the brain from breast cancer tumors, which confirms the observations by (Riebensahm et al., 2019) and provides a link to our observed positive role of cortisol in both brain cancer and breast cancer lines (Herrera et al., 2021). Both of those two studies **support the results in figure 3**. Less is known about the role of cortisol on pancreatic

cancer cells in the published literature, though it may be possible to predict stages of pancreatic cancer progression by monitoring cortisol level in the body (Larsson et al., 2021) this finding supports the out comes of my results referring to **figure 3B and figure 2A,2B.**

Conclusion

These findings suggest that cortisol as a stress agent can affect cancer cell behavior. Although this study has limitations because it is composed mainly of *in vitro* assays, the results reveal that cortisol as a stress agent can induce cell viability and could potentially affect tumor progression. Tumor progression relies on its cell viability. Cell viability defines the ease with which a cell acquires nutrients to divide further. With cancer cells, the cell does not comply with the normal cell life cycle. The cell proliferates but does not die. This leads to increased accumulation of these cells that grow old with time. The more cells produced by the cancer cells, the bigger the tumor gets. These cancer cells spread from one location to the next within the body. The study found a positive correlation between the level of cortisol and tumor progression. This implies the higher the cortisol level concentration, the higher the cell proliferation rates hence a bigger tumor. Further research is needed to assess the impact of stress responses on cancer *in vivo* and over longer durations, as psychological stress is typically experienced over weeks or months rather than 24 or 48 hours, as was the case in this study. It is hoped that this study can provide a starting point for understanding the more specific impact of cortisol on cancer cell lines *in vitro* and the mechanisms that may start proliferation in the early hours of cortisol exposure. Here we have shown that short-term stress is positive while long-term stress is negative in terms of cancer cell viability.

Ethical Aspects

(FBS) Fetal Bovine Serum is one of the major ingredients of cell culture media. FBS is obtained from slaughtered cow fetuses. The blood from the calf is harvested directly by heart puncture without anesthesia. However, the use of (FBS) Fetal Bovine Serum for culturing supposes some ethical issues. Fetuses are exposed to pain, so fetal blood harvesting is not ethically and not humane. Moreover, scientific efforts should be made to reduce FBS use (Jochems CE et al., 2002).

Currently, there is no distinctive cancer cure. Therefore, a breakthrough in studying the impact of causative agents such as cortisol concentration levels could help reduce the risk. Cancer patients will understand how stress could affect cell viability and increase growth and development, increasing tumor development. The findings of this study would help society understand how one can reduce cancer cell development risks through reduced stress levels.

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