Cortisol and Breast Cancer: A review of clinical and molecular evidence

Mohammad Al Sorkhy¹, Zina Fahl¹ and Jenna Ritchie²

¹) Faculty of pharmacy, Al Ain University of Science and Technology, Al Ain, UAE.
²) Humber College Institute of Technology and Advanced Learning, Toronto, Canada.

Abstract

Breast cancer (BC) is a commonly diagnosed cancer amongst women and the second leading cause of cancer deaths in the world. BC has created huge challenges to healthcare providers regarding the identification of main risk factors and how they contribute to the development of the disease. Several studies suggest that biological risk factors such as duration of breast feeding, age at menarche, menopausal status and the use of contraceptive pills have contributed to the increase of BC diagnoses. Moreover, psychological factors such as depression, stress and negative lifestyles are gaining more attention as a major contributor to this type of cancer. The role of psychological stress regarding BC has been widely demonstrated in the literature across several fields including but not exclusive to epidemiology, physiology, and molecular biology which all show a clear relationship between intracellular stress signaling and protumorigenic pathways within breast cells. Cortisol is primary stress hormone of the human body and a growing body of research both clinically and molecularly are revealing a positive correlation of high cortisol levels and the progression of BC. This review attempts to establish and highlight how cortisol levels impact breast cancer development and progression.

Key words: Breast Cancer, Cortisol, Stress.

(Introduced: March 1, 2018; Accepted: March 8, 2018)

Introduction

According to the National Cancer Institute (NIH), 125 per 100,000 women will be diagnosed with breast cancer with 21.5 per 100,000 will succumb to this disease every year. Among the numerous factors affecting breast tumor development and progression, stress has been linked to cancer progression and patient survival. Both acute and chronic stress have been studied with respect to relapse of breast cancer with most studies showing that chronic stress is related to breast cancer relapse compared to acute stress³. One of the most dependable methods to measure an individual’s physiological stress is the measurement of cortisol levels¹. It has been found that more than 60% of women with breast cancer had abnormal levels of cortisol during their day, in addition, the mortality rate in these women was significantly higher than women with normal cortisol levels³.

Despite these numbers, studies fail to offer a unified conclusion on how stress contributes to the initiation and progression of BC due to various design methodologies, stress type, and timing of stress⁴. This review summarizes the physiological role cortisol plays within the body, breast development and highlights the importance of cortisol levels in breast cancer initiation and progression and identifies areas that require further exploration.

Physiological Role of Cortisol in a Healthy Individual

Cortisol is regulated via the Hypothalamic -Pituitary-Adrenal (HPA) axis and is the primary hormone responsible for stress response and restoration of homeostasis after exposure to stress. Cortisol’s effects are widely distributed throughout the body and is produced in large quantities when a person is in “fight of flight” to help an individual respond and manage physical and psychological stress. Stress is generally defined as any factor that allows the body to deviate from homeostasis. Exercise, emotional disturbances, trauma, hemorrhage and fever are a few key examples of stress inducing events⁵. Cortisol promotes carbohydrates and protein disassembly and exerts multiple and sophisticated actions on fat distribution and breakdown. Cortisol is imperatively included with immune and inflammatory processes; which are pivotal for many actions involved with the body immune defense mechanisms. It’s observed that cortisol protects the host from stress by halting the pathophysiological response that the body undergoes in the case of tissue
damage and inflammation\(^6\). In normal cells, cortisol acts as an anti-inflammatory by causing apoptosis through the mitochondrial pathway requiring Apaf-1 and caspase-9\(^6\). Furthermore, cortisol works indirectly by causing an increase in blood pressure through affecting the tissue-sensitivity to catecholamines. Cortisol synthesis affects bone formation, and exhibits both positive and negative effects on cellular proliferation and cell death. Other effects of cortisol include mood and behavioral changes; increase or decrease in food intake, body temperature changes, sensitivity towards pain as well as neuroendocrine functioning\(^6\).

Although only 5% of cortisol is considered to be active, producing effects on the body it is possible for bound cortisol to become free. These are known as 11\(\beta\)-hydroxysteroid dehydrogenase (HSD). To date, only 2 unique isoforms of 11\(\beta\)-hydroxysteroid dehydrogenase (HSD) have been discovered both with different actions. HSD type I reduces cortisone to hormone cortisol [active form] and works on the glucocorticoid receptors (GR) and is found mainly in the liver and adipose tissues. HSD type I is sensitive to numerous factors including cortisol, stress and cytokines. In vitro, 11\(\beta\)-HSD1 showed a bidirectional function as it acts as a dehydrogenase and reductase\(^6\). 11\(\beta\)-HSD1 is usually found where GR is plentiful, but shows a low affinity for cortisol. This reflects that the main role of 11\(\beta\)-HSD1 is to generate and to increase the local amount of ‘free cortisol’ in which cortisol is critical in endocrine functioning\(^6\). In normal and cancerous breast tissue, the number of GR present is extremely correlated with the enzyme 11\(\beta\)-HSD1.

Alternatively, HSD type II oxidizes cortisol to cortisone rendering any active cortisol inactive. HSD type II is co-expressed in the mineral corticoid receptor (MR), the parotid and sweat glands, kidneys as well as smooth muscle tissue. HSD type II is also present in the brain and in placenta and acts as a protective agent against the possible harmful effects of excess cortisol\(^6\).

**GR Signaling**

Due to its lipophilicity, cortisol links to the GR via passive diffusion through the cellular membrane. However, it has been reported that a membrane transporter might be used by the glucocorticoids to shift into the cytoplasm of the cell\(^7\). GR depends on 3 factors that are fundamental for its activation: the amount of cortisol available for binding, the receptor, and the induction of other transcription factors and proteins. Acting as a monomer, the GR can bind and inhibit proinflammatory transcription factors (TFs) such as NF-kB and AP-1, non-genomically\(^8\). As a homodimer, the GR complex shuttles into the nucleus and binds to unique DNA elements, within the promoter regions (GREs), these elements specific to proinflammatory gene targets thereby blocking gene expression.

The genomic activity of the GR requires a “conformational change” upon binding cortisol whereby the hsps are separated\(^9\). In addition to the direct GR binding, the active GR complex also aids and interacts with other DNA bound transcription factors to regulate transcriptional effects\(^10\). A suitable GR-DNA complex with other proteins adjust the communication of other co-activator complexes on specific GR target promoters. The GR can’t induct all cofactors that are essential around the target promoters, but with the help of steroid coactivators that are recruited by GR, this enables the recruitment of other coactivators as well as chromatin remodeling complexes to form a transcription initiation complex permitting for local chromatin remodeling\(^11\).

GR-GRE recruitment causes either an increase or decrease of GRE-dependent gene transcription\(^6\). Moreover, the binding of corticosteroid to the GR transcriptionally activates the expression of ribonucleases as well as mRNA destabilizing proteins that target anti-inflammatory genes, thereby indirectly setting gene expression. However, according to a therapeutic standpoint, the most widely studied action of glucocorticoids is to curb the transcription of cytokines and chemokines genes implemented in inflammation\(^7\). GR activation also affects in the activation of many genes implicated in metabolic homeostasis such as raising blood glucose levels, glucogenesis, and the recruitment of amino and fatty acids\(^10\).

**Normal Breast Development: Links to Carcinogenesis**

The mammary gland is a unique organ that undergoes the majority of development post- embryonically. At adulthood (approximately day 21 in the mouse), the adult mammary stem cells are subjected to a burst of proliferation, as well as degradation of the extra cellular matrix (ECM) to create the primitive ductal tree. Once pregnancy occurs, a huge rise in hormones induces another wave of proliferation to create the alveolar epithelium necessary to produce milk proteins during lactation. In mid-pregnancy (usually at day 10 of the pregnancy), functional differentiation of the gland begins by modifying the morphology of the gland and initiating gene expression important to synthesize milk proteins. Upon the birth of the pups, the gland halt to proliferate and the alveoli starts to produce milk. Genes regulating proliferation shows potential targets that may be aberrantly regulated in breast cancer to aid uncontrolled proliferation. Similarly, genes responsible for regulating differentiation might act as tumor suppressor genes that either mutate or down-regulate in carcinogenesis. It’s important to understand normal breast development because it has revealed critical regulators of specific forms of breast cancer such as c-Myc\(^12\), ras\(^13\) and ErbB2/Neu\(^14\). In metastatic breast cancer, abnormal morphology of ECM is highly preva-
Little data regarding cortisol and normal breast development has been studied with literature proposing that glucocorticoids promote expression of lactating proteins, casein and lactalbumin. Interestingly, 11β-HSD is the enzyme responsible for inactivating glucocorticoids, which was high in both virgin and pregnant rat mammary cells, but the ability of 11β-HSD to inactivate glucocorticoids was decreased by 75% during lactation. In normal mammary epithelial cells (MECs), cortisol promotes morphology changes such as ‘alveolar and multilobular branching’. The fundamental GR- responsive genes responding to cortisol during normal development has yet to be isolated.

**Cortisol and Cancer: Clinical Evidence**

The correlation between stress as a negative impact on human health has been growing over the past 2 decades where studies have shown increased rates of stress can increase the chances of developing ulcers, migraines, and heart attacks. This trend has also been observed with cancer with more than 70% of breast cancer patients showing high levels of cortisol. This correlation between cortisol levels and cancer severity has been demonstrated in several clinical studies in addition to an increase in mortality rate and the recurrence of breast cancer. In 2000, Sephton et al. linked flatter slopes of cortisol levels to fatigue between breast cancer patients and suggested shorter life span which was independent of other prognostic factors. Women with metastatic breast cancer have been studied by Ambercrombie et al. and also revealed flatter cortisol slopes compared to a healthy control population. It has been assumed that such abnormalities could be due to many causes such as a Hypothalamic-Pituitary-Adrenal (HPA) feedback system malfunction, hypersensitivity towards stress, inability to inactivate cortisol and even sleep irregularities. Nilsen et al., concluded after a 19 year follow up survey of 18,932 women that those working in highly fast paced jobs are more prone to breast cancer than women working at a slower pace. Highly fast paced jobs are considered to be more demanding and therefore more psychologically stressful than moderate or slower paced jobs. Furthermore, a 24 year follow up study by Gross et al. showed a statistical difference in depression and an increased level of anxiety for breast cancer women. Eskelinen and Ollonen, designed a case-control study that exhibit that the outcome of negative life events and the level of personal pressure among breast cancer women is significantly different from healthy women or women with benign disease. More recently several comparative case control studies have examined the relation between lifestyle and psychological stress on the onset of breast cancer. These studies have clearly demonstrated that frequent depression and negative emotional experiences are linked to the early development of breast cancer with a risk ratio of 1.32 (95% CI: 1.00 – 1.75) and 1.15 (95% CI: 1.03 – 1.29) respectively.

Spiegel et al. suggested that flatter cortisol levels can be associated with the failure to suppress cortisol which was assessed through the dexamethasone (DEX) suppression test, hyperactivity of Adrenocorticotropic Hormone (ACTH), as well as a higher sensitivity to stress. During the study, metastatic breast cancer patients were subjected to cortisol measurement through saliva samples. During two successive days, saliva samples have been collected at 5 specific time points throughout the day according to the following sequence: at the waking time, 30 minutes after waking, at noon, 5 and 9 pm. The results showed an increasing daytime cortisol slope with increased cortisol concentrations 30 minutes post waking, and this trend continued and was strongly associated with flatter cortisol slopes throughout the day. In addition, patients with rhythmically higher levels of cortisol during the day didn’t reveal any changes during the administered stress measures. Additionally, flatter day time cortisol slopes were linked with HPA failure to suppress cortisol levels via the DEX suppression test suggesting dysfunction of the negative feedback system within the HPA.

Turner-Cobb et al. examined the possible relationship of cancer patient support networks with endocrine levels of cortisol. The researchers hypothesized that if a patient had a strong social network and better quality of support the lower the cortisol levels would be. The participants were randomly divided to either the psychosocial treatment test group or the educational control group. Participants were asked to complete baseline questionnaires that estimated their perceived social support, the quality of social support, in addition to measures assessing the quality of support before the treatment. Saliva samples were collected for 3 successive days and during different time intervals starting from 8 am, 12pm, 5pm, and 9am. Turner-Cobb et al. found a significantly negative correlation with positive appraisal, belonging and supports with respect to cortisol levels. No advocacy was found for their hypothesis for slope of cortisol with respect to quantity, however they did find statistical significance for quality of support received. The previous results are also comparable to other studies that measures other endocrine and immune responses where social support was strongly correlated with proper immune functioning.

Despite many studies reporting similar findings, there are studies that can’t replicate them or find different results together. Palesh et al. suggested that women who tolerate stressful or traumatic events, or even grasp these events to be particularly stressful or traumatic have a decreased ability to combat tumor formation. In the same
study, Palesh et al. didn’t find any significant differences between the participants who experienced or perceived stressful events and the participants who experienced or perceived traumatic ones despite that there was some significant difference in the cortisol levels between the test group and the control group with respect to perceived psychological-social support. As a result, these findings do not clearly link breast cancer to cortisol levels\(^{25}\). Kagaya, and colleagues studied psychological problems and cortisol levels in breast cancer patients during their treatment. The patients were evaluated for a set of disorders related to depression – dejection and tension – anxiety. They reported that 35% of the patients had elevated cortisol levels throughout their treatment course which was not dependent upon the psychiatric disorders found within the group, and as a result of this finding Kagaya et al. hypothesized that high levels of cortisol were related to the breast cancer itself. Another study also demonstrates that the perception of stress or a hypersensitivity to stress might not aid BC from initiating or progressing\(^{26}\). A cross sectional study using 69,886 US women aged from 46 to 71 years from the Nurses’ Health Study participated where blood samples and hormones levels were collected to examine the association between self-reported levels of stress and informal caregiving with the incidence of breast cancer. Results showed that there is no relationship between high number of caregiving hours and high self-reported stress with the increase of incidence of breast cancer. However, the authors did find that stress is involved with the development of breast cancer by down regulating the immune system\(^{27}\).

Vedhara, Tuinstra, Miles, Sanderman, & Ranchor used 4 different indicators that are widely used amongst the scientific community to compare between breast cancer patients with healthy controls. The indicators used are: AUCg, AUCl, EPQR-S and HADS respectively. The first 2 indicators are highly used to measure cortisol levels, whereas the latter 2 indicators are used to measure distress. For 2 successive days, saliva samples were taken from the participants at specific time intervals to measure the cortisol levels using a variety of methods. It was detected that the reliability between the measurements was very low, thus producing low validity. No statistical difference was found when both groups were compared\(^{28}\). Interestingly, Nunes, Rodriguez, da Silva Hoffmann, Luz, Filho, Miller, & Bauer compared breast cancer patients who are receiving both ‘radiotherapy’ and treatment for stress relief to patient who are receiving solely ‘radiotherapy’ treatment. During a 24 days’ period, the researchers found that relaxation techniques facilitated in lowering psychological problems such as stress, anxiety and depression but found that cortisol levels are independent on such therapeutic aids\(^{29}\).

Cortisol and Cancer: Clinical data limitations

Although progression in clinical research has occurred, clear conclusions are still debatable thus producing low reliability between studies. The main cause of the problems in regards to clinical data in relation to support systems and cortisol are due to the methodology. Many contributing factors are affecting the vague data such as how the cortisol samples were collected, the time of day cortisol levels were assessed at, the type of support given, differences between the participants and the integrating compounding variables such as the use of different medications. Additional caveats to the available in vivo clinical studies conducted on this topic includes time conflict allotted for testing. It’s difficult to generalize long term information because most of the studies collected data for a period less than or equal to 1 year. Also, many confounding matters are deep-rooted regarding how to deal with human populations; in support studies, even if congruent support is provided, the perception of this support is mainly dependable on the patients’ level of sense. According to many studies, researchers count on the patients to measure their own cortisol levels, thus allowing the researchers to be highly dependable on the patients’ willingness, accuracy and ability to precisely record data. To compound the issue further cortisol is extremely tissue-specific and each location in the body has diverse concentrations of cortisol\(^{30}\) making any specific conclusions to the role of cortisol in breast cancer non-specific. Due to these reasons, it is crucial to define the cellular effects of cortisol and to clearly identify the underlying molecular mechanisms of cortisol in both normal breast growth and development as well as in breast tumorigenesis.

Cortisol and Breast Cancer: Molecular Evidence

The GR has been found in a considerable number of primary human breast cancers. Aberrant GR expression has been observed in breast cancer stroma, especially at advanced stages of tumor development\(^{31}\). Lien et al., examined GR expression levels using immunohistochemistry in 400 human breast tissue samples extending from normal tissues to invasive lesion\(^{32}\). Studies indicate approximately 20% of breast cancer profiles are caused due to BRCA1 and 2, TP53, PTEN and ATM mutations\(^{33}\), with an additional number of genes participating to the overall survival of the patient\(^{34}\). Both psychosocial and psychological stress has been correlated as causative factors\(^{35}\). Previously, Holden, Pakula, & Mooney detected that prolonged stress increased the amount of TNF in various organ carcinomas\(^{36}\). Another hypothesis suggests that stress increases both the chance of breast cancer and the chance of overcoming the disease by re-
Reducing the ability of the cell to respond to DNA damage or even apoptosis. Kroenke et al found that the immune system is adversely suppressed by high cortisol levels which are released in response to stress\textsuperscript{27}. The impact of high levels of cortisol revealed the immune systems reduced capability to eliminate mutated cells leading to rapid development of cancer. Kroenke and colleagues proposed that stress resulting in higher cortisol levels may be involved in enhancing cancer through DNA damage and suppression of apoptosis which acts as a precursor to many hormonal and lymphatic cancers\textsuperscript{37}. More recently, Flaherty et al. examined if acute stress reduces a cell’s ability to repair DNA damage. Several GR positive cancer cell lines, MCF-7 and MDA-MB-231 were incubated with cortisol and norepinephrine for 30 minutes where controls included incubation with hydrogen peroxide. A comet assay assessed DNA damage and repair and Flaherty et al. demonstrated that acute exposure to cortisol increased DNA damage and repair was reduced\textsuperscript{39}.

Numerous studies in mice as well as humans reveal significant differences in the cell's ability to repair DNA damage or to undergo apoptosis while under stress compared to cells with no stressful conditions \cite{Glaser, Tarr, Kelly, Cohen, Marshall, Cheng, Agarwal, Wei, Amsterdam and Sasson}. To date, no model put forth explains these findings. It is likely to consider a model where cortisol may change the ability of proteins which are responsible and fundamental in DNA repair and apoptosis to be hindered. Antonova and Mueller revealed that BRCA1 within healthy mammary gland cell line in the mouse, EPH4, was down-regulated by cortisone, the active form of cortisol (270nM). The authors suggest that these observations shed light on a molecular pathway for experimental manipulation to study possible modifications in DNA repair mechanisms and cell survival during stressful periods. Through enabling the tumor suppressor role of BRCA1, cortisone affects the ability of cells to maintain genomic stability and may aid the cells’ alterations to reveal carcinogenic characteristics\textsuperscript{40}. Former reports showed that BRCA1 expression levels increases during cellular proliferation in mammary gland\textsuperscript{40}. Antonova and Mueller were curious if cell proliferation hinders the normal repressive effects of cortisone on BRCA1 promoter activity and found that cells treated completely with cortisone repressed BRCA1 promoter\textsuperscript{40}. It is well known that estrogen enhances the expression of BRCA1 in proliferating cells \cite{Romagnolo, et al.}. To establish whether estrogen-induced BRCA1 expression prevents the repressive capabilities of cortisone, Antonova and Mueller treated EPH4-L6 cells with 10nM estrogen with and without cortisone at a concentration of 1 µg/mL. Predictably, estrogen increased the expression of BRCA1 promoter without cortisone, but when cortisone was added, BRCA1 promoter was repressed regardless of estrogen treatment. It was concluded that cortisone can eliminate the promoter activity of BRCA1 by estrogen\textsuperscript{40}.

Cortisol, a glucocorticoid [GC] mediates its effects primarily through the GR. However, as described above cortisol can affect the PR. As the GR, ER, PR and MR are under the steroid receptor family is it possible that cortisol could mediate undesirable affects through cross-talk with other steroid receptors? Using the cell line MDA-MB-231, which is negative for both PR and ER but positive for the GR, Anderson, Ma, Raj, Cidlowski, Helle, Knutson, Krutilina, Seagroves and Lange \cite{2016} allowed the cells to be in a hypoxic and nutrient lacking state to mimic a stressful cellular environment. Cells were also treated with increasing concentrations of demethasone, a corticosteroid, which is similar to cortisol. Anderson et al. found there to be extensive cross-talk between the GR and the hypoxia-inducible factors (HIF) where the HIF created upregulation of Brk, a breast tumor kinase. The authors suggest that identifying ways to target and reduce Brk expression could be helpful in treating patients whose tumors are both PR and ER negative\textsuperscript{43}.

**Conclusion**

While all clinical data does not point in one direction there are similar findings amongst these published works that cannot be ignored. These findings can be groups into 2 categories: that increased levels of cortisol are correlated with increased risk of mortality and that perceived stress or negative events do not necessarily reflect cortisol levels. Studies that do not show correlations between perceived stress and levels of cortisol do acknowledge that the levels of cortisol may be caused by underlying physiological, not psychological factors. It is this acknowledgement that makes molecular research on this topic necessary to help determine if (i) cortisol alone is creating undesired effects; (ii) if it is a combination of increased cortisol levels and interacting with increased GR in BC tumors and (iii) is cortisol working with other members of the steroid family and if so what are the effects from each receptor. As discussed above, it has been shown that cortisol primarily mediates its effects through the GR but it has also shown to not only have cross-talk capabilities but can affect other steroid receptors such as the PR. This promiscuity may be a factor to help explain irregularities discussed in the clinical data. Due to this information and the need to clarify the role of cortisol as outlined above future research should focus on determining the effects of cortisol through each receptor and how cross-talk could have an impact.

Another area warranted for further research includes the interactions of 11β-HSD type I and II. If type I is responsible for generating more free cortisol to act within...
the body and it is correlated with more GR is it the cortisol or type I creating an issue? An interesting note on this topic is several studies have shown that when BC do not have ER or PR, the expression of the GR has shown to be as high as 40% more than normal [Buxant, Engohan-Aloghe and Noel, 2010; Belova, Delgado, Kocherginsky, Melhem, Olopade and Conzen, 2009; Pan, Kocherginsky, and Conzen, 2011]. 11β-HSD type II inactivates active cortisol within the body but it has not been investigated as a potential target. Is it possible that 11β-HSD type II is being rendered inactive in BC thus allowing for more active cortisol to remain in the body?

Our ongoing research on this topic had revealed very interesting data, we have examined the effect of cortisol on different breast cancer cell lines, effects on proliferation migration and adhesion were assessed (Unpublished Data). These experiments showed remarkable results on the abovementioned parameters; to explain these effects we performed cortisol-mediated gene expression assay in invasive human breast cancer cells. This microarray revealed numerous abrogated gens, 19 genes were down regulated and 12 gens we found to be upregulated (un published data); analysis of theses hits are underway along with signaling pathway analysis of some famous genes.

This review has highlighted the findings of both clinical and molecular studies that have spanned more than 20 years. Based on these findings it is clear that there is a correlation between cortisol and BC but in what capacity is cortisol related and potentially affecting BC patients remains elusive. As more and more cancers become a patient by patient treatment in comparison to a 1 treatment fits all approach the same could be hypothesized for cortisol and BC. These and future studies could help add to a more personalized treatment plan that can help increase the rate of remission.

Conflict of interest
The authors declare no conflict of interests.

References


