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Cortisol and Testosterone Induce BAX and BCL-2 Gene Expression and Caspases-8 and 9 Activity in Colon Cancer Cells (HT29) and Reduced the Cell Viability

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Research Article

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ABSTRACT

Cortisol and testosterone can inhibit the proliferation of colorectal cancer cells. Cortisol may augment the anti-cancer activity of testosterone in colorectal cancer cells. This research aimed to assess the impact of cortisol and testosterone on the viability of colon cancer cells (HTCs). The cytotoxic effects of cortisol and testosterone were evaluated using the MTT assay. BAX and Bcl-2 expression levels were determined using real-time PCR. The colorimetric method was used to assess the activity of caspase-8 and 9 enzymes. The expression levels of BAX and BCL-2 genes significantly increased ($p<0.001$), as well as the activity levels of caspase-8 and 9, were elevated ($p<0.001$). Testosterone may exert cytotoxic activity in colon cancer cells in the presence of cortisol, and cortisol and testosterone cotreatment may contribute to the elevated BAX and BCL-2 genes expression and caspase 8 and 9 activity enhancement in colorectal cancer cells.

INTRODUCTION

Colorectal cancer, also known as colon cancer, is primarily defined by the uncontrolled growth of epithelial cells in the colon or rectum (a continuation of the sigmoid colon, which connects to the anus) [1]. Cancer is pathologically defined by the irregular growth of cells that can assault or spread to other regions of the body. Weight loss, abnormalities in bowel motions, blood in the stool, and persistent tiredness are some of the most common clinical signs of colon cancer. Most types of colorectal cancer are caused by environmental factors and lifestyle, and in some cases, genetic predisposition. Among environmental factors, a high-protein diet, obesity, smoking, sedentary work, and consumption of alcohol is recognized as colon cancer risk factors [2]. Cortisol is a steroid hormone belonging to the glucocorticoid family. Cortisol is prescribed as hydrocortisone, and it is naturally produced by adrenal glands present in the adrenal cortex of kidneys. This hormone is secreted in response to stress and low blood glucose levels which increases blood glucose through gluconeogenesis, leading to a weakened immune system, increased lipid, protein, and carbohydrate metabolism, as well as facilitating bone resorption [3]. A few of studies indicated that cortisol has an important role in the progress of colon cancer [4]. In this respect, colorectal cancers have been found to be a significant source of immunoregulatory glucocorticoids, and Tumor-derived glucocorticoids, according to researchers, may aid immune evasion by suppressing immune cell activation and stimulating apoptosis. Glucocorticoids are steroid hormones that are anti-inflammatory and pro-apoptotic. Even though previous descriptions of steroid production have occurred in a tumor derived from primary steroid organisms such as testis, ovaries, and adrenals, for the first time, this investigation demonstrates that a tumor derived from nonendocrine tissues has synthesized glucocorticoids. The function of nuclear receptor and transcription factor liver receptor homolog-1(LRH-1, NR5a2) is particularly intriguing in this regardLRH-1 is a transcription factor that is becoming more well-known for its function in steroid production, cell cycle control, and metabolism. The production of immune-cellulose glucocorticoids in the intestine of LRH-1 is significantly controlled by steroidogenic activation, which is more likely to lead to progress of experimental colitis by LRH-1-deficient mice. In colorectal tumor cells, as in primordial intestinal crypt cells, LRH-1 is a major regulator of glucocorticoid synthesis. Not unexpectedly, LRH-1 is substantially overexpressed in colorectal tumor cells while initial epithelial cells expressing the LRH-1 only in proliferatory crypt cells. The formation of colorectal cancers is based on the dual function of LRH-1. Although the stimulation of the production of glucocorticoids may support the removal of tumor-infiltration immune cells and the ability to evade destruction *via* cytotoxic effector mechanisms, LRH-1 also stimulates proliferation of the tumor cells directly through cyclin D1 and E1. Glucocorticoid synthetization of tumor cells such as LRH-1 is most likely to be an epithelial immune evasion strategy [5,6].

Testosterone, which is the primary sex hormone in males and categorized as anabolic steroids, plays a crucial role in carbohydrate, lipid, and protein metabolism Testosterone is recognized as a vital molecule in the production of muscle mass and body fat in males. Increased fat mass (especially central obesity), increased triglycerides and cholesterol, weakened glucose tolerance, reduced insulin sensitivity are all linked to testosterone deficiency [7] and also by effected on caspase pathway express p53 gene and leading to the degradation of some protein activation of other caspases. The BCL2 associated X gene, called Bax, belongs to a member of the BCL-2 gene family and serves as a regulator for apoptosis. Bax and BCL-2 proteins are able to form a heterodimer that can trigger apoptosis [8].

Studies indicated that Bax protein facilitates the voltage-dependent opening of mitochondrial anion channels, as a result, the mitochondrial membrane potential is lost, and cytochrome C is released. Tumor suppressor P53 controls the expression of the Bax gene, and consequently, promotes programmed cell death in a p53-dependent manner.

Furthermore, Caspases are a family of enzymes that play a role in apoptosis by decomposing their specific substrates in a particular site, leading to the degradation of some protein activation of other caspases. In terms of priority and delay in participation in the process of cell death, caspases are classified into two categories of initiators and executioners. Initiator caspases, such as caspase-8, are activated at the initiation of the cell death process, while executioner caspases, such as caspase-3 and 9, are activated in later stages in response to initiator caspases [9]. The activation of executioner caspases ensures the beginning of caspase cascades and, subsequently, results in apoptosis. Studies show an association between steroid hormones and the development of some types of cancer [10]. Moreover, a variety of research has confirmed the correlation of steroid hormones with the development of gastrointestinal cancer [11].

Some findings suggest that steroid hormones influence the pathogenesis of colorectal cancer [12]. Some investigations have also revealed the relation of cortisol and various cancer types [13], especially gastrointestinal cancer [14]. Some clinical analyses assigned a strong correlation between cortisol and the development of colorectal cancer. It is now known that sex hormones are potential biomarkers for the assessment of colorectal cancer risk in individuals [15]. It is shown testosterone can induce apoptosis in colon cancer cells, which is controlled by the PI3K/Rac1 signaling pathway [16,17]. Several lines of evidence showed a direct association between steroid/glucocorticoid hormones and the expression of some genes responsible for the modulation of apoptosis [18-22]. Evidence also implies that steroid hormones and cortisol have a close relationship with the activation of caspases-8 and 9 [23,24]. However, in contrast, some studies have shown the inhibitory effects of cortisol on cancer progression [25]. Also, the effect of stress reduction on salivary cortisol and interleukin-6 secretion was evaluated in patients with advanced cancer using the Mindfulness-based stress reduction (MBSR) program [26,27]. In contrast to the above statements, cortisol has been shown to inhibit apoptosis [28].

Based on evidence mentioned earlier, as well as the presence of contradictory results of steroid effects on cancer progression. The study's objective was to determine the influence on the viabilities of colorectal cancer cells, the HC29 cell line, of cortisol and testosterone. Besides, the BAX and BCL-2 gene expression levels were evaluated together with the Caspase-8 and 9 activity levels

MATERIAL AND METHODS

Cell culture and treatments

Colon Cancer (HT29) cell line was obtained from the National Cell Bank of Iran (NCBI), which is affiliated with the Pasteur Institute of Iran (Pasteur Institute, Tehran, Iran). The cell culture process was conducted at the Javid Biotechnology Research Center One month before the preparation of cortisol and testosterone. The stock solution of cortisol and testosterone was serially diluted to prepare different concentration. Cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 g/mL) at 37°C in a humidified water-jacked incubator with 5% CO_2 (all purchased from Sigma, Germany). Cortisol and testosterone were purchased from the Pharma Shimi Pharmaceutical Company (Tehran, Iran). The MTT assay was

used to determine cortisol's cytotoxicity, which is based on the ability of viable cells to convert yellow tetrazolium salt MTT to purple formazan crystals *via* mitochondrial dehydrogenases. Briefly, cells were seeded at a density of 15000 per well in 96-well plates; subsequently, after 24 h incubation, they were treated with various concentrations of cortisol, testosterone (1000, 500, 250, 125, 62/5 µg/mL) and testosterone+cortisol for 24 h. The untreated wells were considered negative controls. Afterward, the supernatants were discarded and 20 µl of 5 mg/mL MTT solution was added to each well and further incubated for 4 h at 37°C. Subsequently, the resulting supernatants were removed from the wells before, and then 200 µL DMSO and 50 µL Sorenson buffer were added to each well. The plate was incubated for 30 min with gentle shaking for 5 min. By measuring each well's absorbance at the wavelength 570 nm, the cytotoxic effects of cortisol were monitored.

RNA extraction and real-time PCR

Total RNA was isolated from the cells by means of the RNA extraction kit (RNeasy, Qiagen, Inc., Chatsworth, CA) as said by the manufacturer's protocols. The isolated RNA was qualitatively and quantitatively evaluated by running the samples on the agarose gel and reading the optical absorbance of specimens by a Nanodrop instrument. Then, the extracted RNA was reverse-transcribed into complementary DNA (cDNA) using the First-Aid Reverse Transcription Kit (Fermentase). Real-time PCR reactions were performed using the SYBR Green master mix on the LightCycler 480 (LC480) instrument. The sequences of primer were designed by the NCBI primer design tool and synthesized by Primer Design (Ltd., Southampton, UK). The primer sequences of BAX, BCL-2, and GAPDH genes are depicted in Table 1. The relative expression (RQ) of target genes was assayed in comparison with the reference gene using the $2^{-\Delta\Delta CT}$ formula.

Table 1. List of primers.

Gene	Primer	
BAX	Forward	5'CGGCAACTTCAACTGGGG-3'
	Reverse	5'TCCAGCCCAACAGCCG-3'
BCL-2	Forward	5'GGTGCCGGTTCAGGTACTCA-3'
	Reverse	5'TTGTGGCCCTTCTTTGAGTTCG-3'
GAPDH	Forward	5'CCCACTCCTCCACCTTTGAC-3'
	Reverse	5'CATACCAGGAAATGAGCTTGACAA-3'

Caspase assay

First, the laminar airflow was turned on, and the equipment under the airflow instrument, along with non-glass surfaces, was disinfected with 15% ethanol. Then, the cell culture flasks were opened, and the culture medium was removed using a pipette. Next, the PBS solution was added to cells cultured in special flasks. Then, the flasks were agitated for a few minutes, and the resulting solution was extracted. The trypsin enzyme was then removed with a pipette and released into the wall of the flask to avoid injury to the cells. Then, the lid of the flask was closed, and it was placed in an incubator for 3–4 min to separate the cells. Afterward, the wall of the flask was touched with the palm to separate the rest of the cells, and the activity and morphology of cells under a light microscope were seen. To eliminate the trypsin enzyme, 2 ml of the fresh cell culture medium was poured into the flasks under the airflow apparatus and repeatedly pipetted. The contents of the flask were then separated by a pipette and poured into a

15 ml Falcon and centrifuged at 1000 rpm for 10 min. The supernatants of Falcons containing the trypsin enzyme and dead cells were then discarded by a pipette (the pipette did not hit the bottom of falcons where viable cells were accumulated). Next, 4 ml of the fresh cell culture medium was poured into falcons and pipetted several times to isolate the cell aggregates. Then, 30 µl was then removed from the cell culture media and added to 30 µl Trypan Blue dye in a 96-well plate. Then, 10 µl of the mixture was placed on the Neubauer chamber for counting the cells. Then, the contents of falcons were poured into a 6-well plate until 50% of the wells were filled. Approximately 900 µl of the contents of falcons was poured into each well. Next, 100µl of the FBS solution was added to each well. About 5×10^5 cells were seeded onto each well. For the culture of the cells, the 6-well plate was positioned at an angle of 31° in an incubator for 24 h to fill 50% of the surface of the wells.

The plate was then sealed and stored at 37°C for 2–3 min to allow the cells to be fully trypsinized. For the control and treatment groups, two 1.5-ml microtubes were chosen individually. The contents of the three control groups were poured into a 1.5-ml microtube. After this period, microtubes were centrifuged at 1120,000 g for 1 min. Finally, 50 µl of the Caspase Assay Solution was added to each microtube under the laminar airflow (the amounts of Caspase Assay and Lysis buffer were the same). Microtubes were subsequently stored at 37°C for 2 h. The Eliza Reader device was then set at a wavelength of 400 nm, and the optical absorbance of each group was recorded.

Statistical analysis

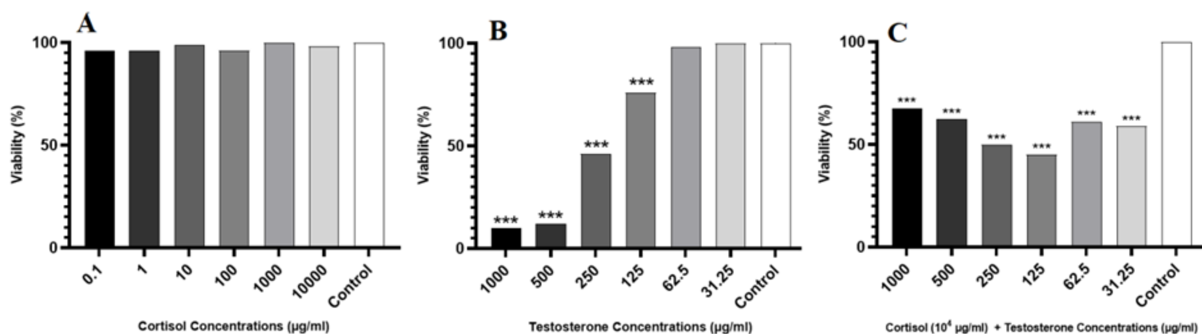
The data analysis was carried out using the SPSS program version 16. The difference between the experimental groups was analyzed using one-way analysis of variance (one-way ANOVA) followed by Tukey's post hoc test. The Kolmogorov-Smirnov test was also utilized to examine whether the data were normally distributed. In the case of normality, the Independent T-test was applied to compare the difference in the effects of cortisol and testosterone on the activity levels of Caspase-8 and 9 between the treated and control groups.

RESULTS

Cell cytotoxicity assay

Cortisol showed no significant toxicity on the viability of colon cancer cells when they were treated with various concentrations of cortisol (Figure 1A). With regards to Figure 1B, the viability of colon cancer cells at an IC50 of 250 µg/ml of testosterone is shown. 1000, 500, 250 and 125 µg/ml concentrations of testosterone significantly reduced viability percent in comparison to control. Cotreatment cortisol (10^4 µg/ml) + testosterone significantly reduced viability percent in 1000, 500, 250, 125, 62.5 and 31.25 µg/ml concentrations in comparison to control. Figure 1C also displays the viability of colon cancer cells at an IC50 of 106 µg/ml of testosterone+cortisol.

Figure 1. Viability of HCT cells exposed to various concentrations of testosterone and cortisol.



(A) Various concentrations of cortisol (0.1-104 μg/ml) had no significant toxicity on the viability of colon cancer cells. (B) treatment with several concentrations of testosterone showed significant reduced viability percent (C) cortisol (104 μg/ml)+testosterone several concentrations cotreatment significantly reduced viability percent

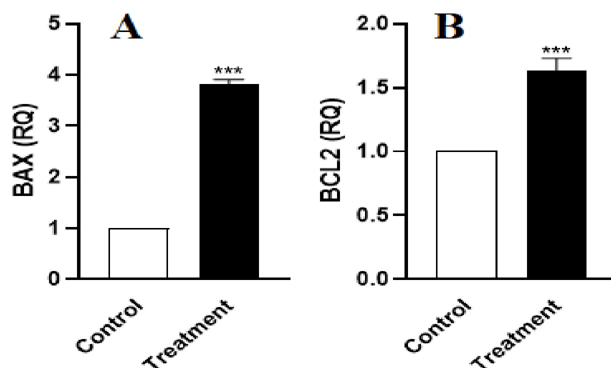
represents significant difference compared to control group ($p<0.001$).

Bax and BCL-2 expressions

In comparison to the control group, cortisol and testosterone co-treatment (IC50 concentration) resulted in a significant increase in BAX gene expression. ($p<0.01$) (Figure 2A). In comparison to the control group, cortisol and testosterone co-treatment significantly increased BCL-2 gene expression ($p<0.01$). (Figure 2B) demonstrates the impact of testosterone + cortisol (IC50 concentration) on the expression of the BCL-2 gene.

Figure 2. Partial expression level (RQ) of BAX and BCL-2 in HCT cells exposed to effective concentration of Cortisol (10^4 μg/m) and Testosterone (500 μg/m). (B)

represents significant difference compared to control group ($p<0.001$).

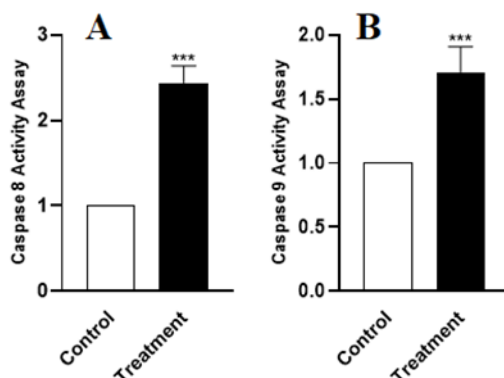


Caspase activity

Figure 3A exhibits the effect of testosterone+cortisol (IC50) on the activity rate of caspase-8, which has

increased remarkably compared to the control group (p<0.01). Figure 3B shows the effect of testosterone+cortisol (IC50) on the activity rate of caspase-9, which has increased remarkably compared to the control group (p<0.01).

Figure 3. Activity of caspase 8 and caspase 9 (B) in HCT cells exposed to effective concentration Cortisol (104 µg/m) and Testosterone (500 µg/m). ***represents significant difference compared to control group (***p<0.001).



DISCUSSION

In-vitro cytotoxicity of cortisol and testosterone was determined in the HC29 cell line using the MTT assay. Our findings showed that cortisol has considerable cytotoxicity on the HC29 cell line at a dose of 250 µg/ml. In accordance with this finding, several studies showed the cytotoxic effects of cortisol and testosterone on colon cancer cells *in-vivo* and *in-vitro*. One *in-vitro* investigation indicated the anti-proliferative properties of cortisol and testosterone on colon cancer cells [29]. The inhibitory effect of testosterone on colon cancer cells has also been reported in the literature [30,31]. Recent studies indicated that cortisol inhibits colon cancer cell invasion and migration [32,33]; whereas some reports indicated that cortisol may promote colon cancer cell proliferation. Cortisol is capable of enhancing tumor cell proliferation and migration in sporadic colon cancer by a decrease in the secretion of interleukin-6. However, the testosterone-induced proliferation of colon cancer cells might be accompanied by using lower doses of testosterone during the research.

Our findings revealed that the treatment of HC29 cells with 0/1, 1, 10, 100, 1000, and 10000 µg/ml of cortisol did not significantly change the cell viability of HC29 cells *in-vitro*. Previous studies have shown that cortisol has a significant effect on colon cancer proliferation. In this respect, a group of studies confirmed that colon cancer cells produce glucocorticoids with immunomodulatory and anti-depressant properties. It was first thought that glucocorticoids are exclusively produced by suprarenal glands; however, evidence indicates the presence of additional resources for the synthesis of these compounds. It has been shown that cortisol secretion has a profound effect on the LRH-1 expression. In colorectal tumor cells, LRH-1 is also an important regulator for glucocorticoid synthesis. Not unexpectedly, LRH-1 is substantially overexpressed in colorectal tumor cells while primary epithelial cells expressing the LRH-1 only in proliferatory crypt cells. In developing colorectal cancers, LRH-1 acting a double role. Although the stimulation of the production of glucocorticoids may support the removal of tumor-infiltration immune cells and the ability to avoid being destroyed by cytotoxic effector mechanisms, LRH-1 also stimulates proliferation of the tumor cells directly through cyclin D1 and E1 [34].

Sex steroid hormones were also found to have a significant impact on osteosarcoma cell proliferation, colony formation, apoptosis, migration, and invasion [35]. Sexual steroids, especially cortisol and testosterone, have proved to be crucial in the control of human cell proliferation [36]. Cortisol increases blood glucose through a process called gluconeogenesis, leading to a weakened immune system, stimulation of lipid, protein, and carbohydrate metabolism, and increased bone resorption [37]. Of note, cortisol had no significant cytotoxicity on colon cancer cells. While colorectal cancer is generally not considered a hormonal malignancy, accumulating evidence suggests that the effectiveness of sex hormones helps to develop and/or prevent certain types of malignancies [38]. The real-time PCR technique was employed to analyze the expression levels of BAX and BCL-2 genes in HC29 cells treated with an effective concentration of cortisol (10000 µg/ml) and testosterone (500 µg/ml). Our findings revealed an increase in the anti-apoptotic activity of BCL-2, as well as the pro-apoptotic activity of the BAX, in cancer cells. These results indicated that the cytotoxicity of cortisol and testosterone on HC29 cells is mediated through BAX-dependent apoptosis. The roles of cortisol and testosterone in the induction of BAX-dependent apoptosis have been addressed in previous studies. In addition, progesterone has been shown to induce apoptosis in cervical, ovarian, and colon cancer cells through the BAX-dependent pathway [29, 38-40].

The protective effects of androgens on colorectal cancer have been reported in experimental and clinical studies. Adrenocortical steroids, Dehydroepiandrosterone (DHEA), are increasingly shown to be critical mammalian hormones, as the use of DHEA by mice and rats inhibits skin, colon, lungs, breast and lymphatic tissues from developing induced tumors [41,42].

In order to distinguish the extrinsic and intrinsic apoptotic pathways, the activity of the initiator caspase-8 and 9 was calorimetrically measured in HC29 cells treated with an effective concentration of cortisol (10000 µg/ml) and testosterone (500 µg/ml). Our findings indicated a significant increase in the activity levels of caspases-8, 9 in HC29 cells. Regarding the involvement of caspase-9 in the intrinsic pathway and caspase-8 in the extrinsic apoptotic pathway [43], it is conceived that cortisol and testosterone induce both pathways in HC29 cells. Apoptosis in cortisol and testosterone-treated HC29 cells was also confirmed by the Caspase kit and Real-time PCR methods. Consistent with our findings, there are some reports indicating that an effective concentration of cortisol (10000 µg/ml) and testosterone (500 µg/ml) induce apoptosis in cancer cells by triggering the caspase cascade. A number of studies indicated that that the activation of Caspas-8 pathway induces apoptosis in colonic cancer cells [44]. Caspase-8 has also been shown to be associated with the development of colon cancer, and such a correlation may be influenced by the activation of cortisol and testosterone receptors [24,45]. Based on our knowledge, the effects of cortisol and testosterone on the caspase cascade in osteosarcoma cells have not been reported in previous studies.

CONCLUSION

In conclusion, our findings show that cotreatment of cortisol and testosterone may cause apoptosis in colon cancer cells. In this regard, the combination of cortisol and testosterone increases the relative expression of BAX and Bcl-2 genes. Moreover, the IC50 value of cortisol+testosterone increased the activity levels of caspases-8 and 9. Consequently, the IC50 concentration of cortisol+testosterone may induce BAX-dependent apoptosis *via* triggering the intrinsic and extrinsic apoptosis pathways in colon cancer cells.

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DECLARATIONS

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Conflict of intrest

All the authors have declared that no conflict interest exists.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate

Not applicable.

Consent for publication

All authors consent for publication of the work

Availability of data and material

All related data is deposited in the manuscript.

Code availability

Not applicable.

REFERENCES

1. Arisan ED, et al. Diclofenac induced apoptosis *via* altering PI3K/Akt/MAPK signaling axis in HCT 116 more efficiently compared to SW480 colon cancer cells. Mol Biol Rep. 2018; 45:2175-84. [Crossref] [Google Scholar] [PubMed]
2. Todaro M, et al. Colon cancer stem cells: Promise of targeted therapy. Gastroenterology. 2010; 138:2151-62. [Crossref] [Google Scholar] [PubMed]
3. Gerards J, et al. Impaired glucose metabolism in primary aldosteronism is associated with cortisol cosecretion. J Clin Endocrinol Metab. 2019; 104:3192-202. [Crossref] [Google Scholar] [PubMed]
4. Geiger AM Pitts KP, et al. Cortisol-dependent stress effects on cell distribution in healthy individuals and individuals suffering from chronic adrenal insufficiency. BBI. 2015; 50:241-8. [Crossref] [Google Scholar] [PubMed]
5. Sidler D, et al. Colon cancer cells produce immunoregulatory glucocorticoids. Oncogene. 2011; 30:2411-9. [Crossref] [Google Scholar] [PubMed]

6. Maingi JW, et al. Targeting estrogen receptors in colorectal cancer. Mol Biol Rep. 2020:1-5. [Crossref] [Google Scholar] [PubMed]
7. Kelly DM, et al. Testosterone: a metabolic hormone in health and disease. J Endocrinol. 2013; 217:R25-45. [Crossref] [Google Scholar] [PubMed]
8. Park WH, et al. Arsenic trioxide induces human pulmonary fibroblast cell death *via* the regulation of Bcl-2 family and caspase-8. Mol Biol Rep. 2012; 39:4311-8. [Crossref] [Google Scholar] [PubMed]
9. Mitupatum T, et al. Hep88 mAb-mediated paraptosis-like apoptosis in HepG2 cells *via* downstream upregulation and activation of caspase-3, caspase-8 and caspase-9. Asian Pac J Cancer Prev. 2015; 16:1771-9. [Crossref] [Google Scholar] [PubMed]
10. Capper CP, et al. The metabolism, analysis, and targeting of steroid hormones in breast and prostate cancer. Horm Cancer. 2016; 7:149-64. [Crossref] [Google Scholar] [PubMed]
11. Murakami H, et al. Octreotide acetate–steroid combination therapy for malignant gastrointestinal obstruction. Anticancer Res. 2013; 33:5557-60. [Google Scholar] [PubMed]
12. Proto MC, et al. Interaction of endocannabinoid system and steroid hormones in the control of colon cancer cell growth. J Cell Physiol. 2012; 227:250-8. [Crossref] [Google Scholar] [PubMed]
13. Drebert Z, et al. Glucocorticoids and the non-steroidal selective glucocorticoid receptor modulator, compound A, differentially affect colon cancer-derived myofibroblasts. J Steroid Biochem Mol Bio. 2015; 149:92-105. [Crossref] [Google Scholar] [PubMed]
14. Ramanathan ML, et al. Is perioperative systemic inflammation the result of insufficient cortisol production in patients with colorectal cancer?. Ann Surg Oncol. 2013; 20:2172-9. [Crossref] [Google Scholar] [PubMed]
15. Roshan MH, et al. The role of testosterone in colorectal carcinoma: Pathomechanisms and open questions. Epma Journal. 2016; 7:22. [Crossref] [Google Scholar] [PubMed]
16. Alkahtani S. Testosterone induced apoptosis in colon cancer cells is regulated by PI3K/Rac1 signaling. Asian J Androl. 2013; 15:831. [Crossref] [Google Scholar] [PubMed]
17. Heng MP, et al. Mitochondria-Dependent Apoptosis inducer: Testosterone-N4-ethylthiosemicarbazonate and its metal complexes with selective cytotoxicity towards human colorectal carcinoma cell line (HCT 116). Inorganica Chim Acta. 2020:119581. [Crossref] [Google Scholar]
18. Huang G-Y, et al. Effects of steroid hormones on reproduction-and detoxification-related gene expression in adult male mosquitofish, Gambusia affinis. Comp Biochem Physiol Part-C: Toxicol Pharmacol. 2013; 158:36-43. [Crossref] [Google Scholar] [PubMed]
19. Bas D, et al. Altered expression of Bcl-2 and Bax in follicles within dehydroepiandrosterone-induced polycystic ovaries in rats. Cell Biol Int. 2011; 35:423-9. [Crossref] [Google Scholar] [PubMed]
20. Wilmot Roussel H, et al. Identification of gene expression profiles associated with cortisol secretion in adrenocortical adenomas. J Clin Endocrinol Metab. 2013; 98:E1109-E21. [Crossref] [Google Scholar] [PubMed]
21. Wan X, et al. Elucidating a molecular mechanism that the deterioration of porcine meat quality responds to increased cortisol based on transcriptome sequencing. Sci Rep. 2016; 6:36589. [Crossref] [Google Scholar] [PubMed]

22. Wang D, et al. The steroid hormone 20-hydroxyecdysone promotes the cytoplasmic localization of yorkie to suppress cell proliferation and induce apoptosis. J Biol Chem. 2016; 291:21761-70. [Crossref] [Google Scholar] [PubMed]
23. Marchetti MC, et al. Dexamethasone-induced apoptosis of thymocytes: Role of glucocorticoid receptor–associated Src kinase and caspase-8 activation. BLOOAW. 2003; 101:585-93. [Crossref] [Google Scholar] [PubMed]
24. Pehlivanli F, et al. The value of CRP, IL-6, leptin, cortisol, and peritoneal caspase-3 monitoring in the operative strategy of secondary peritonitis. Eur J Trauma Emerg. Surg. 2011; 17:390-5. [Crossref] [Google Scholar] [PubMed]
25. Xing K, et al. Dexamethasone enhances Programmed Cell Death 1 (PD-1) expression during T cell activation: an insight into the optimum application of glucocorticoids in anti-cancer therapy. BMC Immunol. 2015; 16:1-9. [Crossref] [Google Scholar] [PubMed]
26. Lengacher CA, et al. A pilot study evaluating the effect of mindfulness-based stress reduction on psychological status, physical status, salivary cortisol, and interleukin-6 among advanced-stage cancer patients and their caregivers. Holist Nurs Pract. 2012; 30:170-85. [Crossref] [Google Scholar] [PubMed]
27. DiNicolantonio JJ, et al. Fructose-induced inflammation and increased cortisol: A new mechanism for how sugar induces visceral adiposity. Prog Cardiovasc Dis. 2018; 61:3-9. [Crossref] [Google Scholar] [PubMed]
28. Saeij JP, et al. Daily handling stress reduces resistance of carp to *Trypanoplasma borreli: in vitro* modulatory effects of cortisol on leukocyte function and apoptosis. Dev Comp Immunol. 2003; 27:233-45. [Crossref] [Google Scholar] [PubMed]
29. Hu Z, et al. The effect of progesterone on proliferation and apoptosis in ovarian cancer cell. Zhonghua Fu Chan Ke Za Zhi. 2000; 35(7):423-6. [Google Scholar] [PubMed]
30. Im JY, et al. Molecular Mechanism of progesterone-induced apoptosis in human breast cancer T47D Cells. Cancer Prev Res. 2008; 13:177-83. [Google Scholar]
31. Chávez-Riveros A, et al. Synthesis and cytotoxic effect on cancer cell lines and macrophages of novel progesterone derivatives having an ester or a carbamate function at C-3 and C-17. Eur J Med Chem. 2014; 82:498-505. [Crossref] [Google Scholar] [PubMed]
32. Godbole M, et al. Progesterone suppresses the invasion and migration of breast cancer cells irrespective of their progesterone receptor status: A short report. Cell Oncol. 2017; 40:411-7. [Crossref] [Google Scholar] [PubMed]
33. Buoso E, et al. Cortisol-induced SRSF3 expression promotes GR splicing, RACK1 expression and breast cancer cells migration. Pharmacol Res. 2019; 143:17-26. [Crossref] [Google Scholar] [PubMed]
34. MacNamara P, et al. Progesterone receptors are expressed in human osteoblast-like cell lines and in primary human osteoblast cultures. Calcif Tissue Int. 1995; 57:436-41. [Crossref] [Google Scholar] [PubMed]
35. Fang D, et al. 17beta-estradiol regulates cell proliferation, colony formation, migration, invasion and promotes apoptosis by upregulating miR-9 and thus degrades MALAT-1 in osteosarcoma cell MG-63 in an estrogen receptor-independent manner. Biochem Biophys Res Commun. 2015; 457:500-6. [Crossref] [Google Scholar] [PubMed]

36. Dohi O, et al. Sex steroid receptors expression and hormone-induced cell proliferation in human osteosarcoma. Cancer Sci. 2008; 99:518-23. [Crossref] [Google Scholar] [PubMed]
37. Liang M, et al. Effects of progesterone and 18-methyl levonorgestrel on osteoblastic cells. Endocr Res. 2003; 29:483-501. [Crossref] [Google Scholar] [PubMed]
38. Rickard DJ, et al. Bone growth and turnover in progesterone receptor knockout mice. Endocrinology. 2008; 149:2383-90. [Crossref] [Google Scholar] [PubMed]
39. Pompei L, et al. Effects of estradiol, progestogens, and of tibolone on breast proliferation and apoptosis. Climacteric. 2015; 18:518-22. [Crossref] [Google Scholar] [PubMed]
40. Fonseca-Moutinho J, et al. Estrogen receptor, progesterone receptor, and bcl-2 are markers with prognostic significance in CIN III. IJGC. 2004; 14:911-20. [Crossref] [Google Scholar] [PubMed]
41. Wang Q-P, et al. Effect of progesterone on apoptosis of murine MC3T3-E1 osteoblastic cells. Amino acids. 2009; 36:57-63. [Crossref] [Google Scholar] [PubMed]
42. Pietri E, et al. Phase II study of dehydroepiandrosterone in androgen receptor-positive metastatic breast cancer. The oncologist. 2019; 24:743. [Crossref] [Google Scholar] [PubMed]
43. Aral K, et al. The role of caspase-8, caspase-9, and apoptosis inducing factor in periodontal disease. J Periodontol. 2019; 90:288-94. [Crossref] [Google Scholar] [PubMed]
44. Syed V, et al. Progesterone-induced apoptosis in immortalized normal and malignant human ovarian surface epithelial cells involves enhanced expression of FasL. Oncogene. 2003; 22:6883. [Crossref] [Google Scholar] [PubMed]
45. Boege Y, et al. A dual role of caspase-8 in triggering and sensing proliferation-associated DNA damage, a key determinant of liver cancer development. Cancer cell. 2017; 32:342-59. e10. [Crossref] [Google Scholar] [PubMed]