ORIGINAL ARTICLE
DOI: 10.4274/tjps.galenos.2020.58827

Investigation of dehydroepiandrosterone induced in vitro physiological effects in human endothelial and ovarian cancer cells

İnsan ovaryum kanseri ve kanserli olmayan insan endotel hücrelerinde dehidroepiandrosteronun neden olduğu in vitro fizyolojik etkilerin araştırılması

Gül Ipek Gundogan[1], Cenk Kig[2], Meric Karacan[3], HÜSNIYE DOGRUMAN[1]
[1]Department Of Histology And Embryology, Istanbul Yeni Yuzyil University, Istanbul Turkey
[2]Department Of Medical Biology And Genetics, Istanbul Yeni Yuzyil University, Istanbul Turkey
[3]Department Of Gynecology And Obstetrics, Istanbul Yeni Yuzyil University, Istanbul Turkey

Corresponding Author
Gül Ipek Gundogan
gulipekgundogan@gmail.com
orcid.org/0000-0002-9438-6113
05418581094
11.03.2020
14.04.2020

ÖZ

GİRİŞ ve AMAÇ: Dehidroepiandrosteron (DHEA), bir dizi hücresel reseptör için ligand görevi görebilen endojen bir hormondur. Dolaşımdaki DHEA seviyelerinde yaşa bağlı gerçekleşen düşüş, fizyolojik işlevlerdeki değişikliklerle bağlantılıdır. Jinekolojik klinik uygulamada DHEA genellikle ovulasyon indüksiyonu için tercih edilmektedir. Bazı klinik çalışmalar, yüksek DHEA konsantrasyonları ile ovaryum kanseri gelişme riskinde artış arasında pozitif bir ilişki olduğunu bildirmiştir. Bununla birlikte, DHEA'nın ovaryum kanseri hücreleri üzerindeki in vitro fizyolojik etkileri şimdiye kadar araştırılmamıştır. Bu çalışmada, DHEA inkübasyonunun (0-200 uM, 24-72 saat) MDAH-2774 insan ovaryum kanseri ve kanserli olmayan HuVeC insan endotel hücre hatları üzerindeki fizyolojik etkileri araştırılmıştır.

YÖNTEM ve GEREÇLER: DHEA inkübasyonunun (0-200 uM, 24-72 saat) MDAH-2774 insan ovaryum kanseri ve kanserli olmayan HuVeC insan endotel hücre hatları üzerindeki fizyolojik etkileri; MTT testi, AO/EtBr boyaması ve yara kapanma testi ile araştırılmıştır.
BULGULAR: DHEA inkübasyonu, doza bağlı bir şekilde MDAH-2774 kanser hücre hattının proliferasyonunu indüklemiştir ($r = 0,6906$, 24 saat boyunca $P<0,0001$) ($r = 0,6802$, 48 saat boyunca $P<0,0001$) ($r = 0,7969$ $P <0,0001$, 72 saat). Aksine, DHEA kanserli olmayan HuVeC hücrelerinin proliferasyonunu ise inhibe etmiştir ($r = 0,9490$, 24 saat için $P<0,0001$) ($r = 0,9533$, $P<0,0001$, 48 saat için) ($r = 0,9584$ $P<0,0001$, 72 saat için). Bununla birlikte, DHEA

inkübasyonu sonucu nekrotik veya apoptotik hücre sayısı MDAH-2774 hücre hattında önemli ölçüde değişmemiştir. Ayrıca DHEA inkübasyonunun kanserli olmayan HuVeC hücrelerinin migrasyon oranını doza bağlı bir şekilde azaltırken (r = 0,9868, P<0,0001), MDAH-2774 ovaryum kanseri hücre hattında (r = 0,8938, P<0,05) arttırdığı tespit edilmişir.

TARTIŞMA ve SONUÇ: Bulgularımız DHEA'nın in vitro olarak ovaryum kanseri hücrelerinin doza bağımlı bir şekilde çoğalmasını teşvik ettiğini göstermektedir. Ayrıca DHEA'nın nekrozu indüklediği ve kanserli olmayan endotelyal hücre hattında proliferasyonu inhibe ettiği tespit edilmiştir. Mekanistik olarak doğrulanması gerekse de ön bulgularımız, yüksek dozlardaki DHEA maruziyetinin ovaryum kanseri gelişme riskiyle ilişkili olabileceğini göstermektedir.
Anahtar Kelimeler: Dehidroepiandrosteron, MDAH-2774, HuVeC, ovaryum kanseri
ABSTRACT

INTRODUCTION: Dehydroepiandrosterone (DHEA) is an endogenous hormone that acts as a ligand for several cellular receptors. An age-dependent decline in circulating levels of DHEA is linked to changes in physiological functions. In gynecological clinical practice, DHEA is commonly prescribed for the induction of ovulation. Some clinical studies report a positive association between high concentrations of DHEA and increased risk in developing ovarian cancer. However, in vitro physiological effects of DHEA on ovarian cancerous cells have not been explored so far. In this study, we aimed to investigate the physiological effects of DHEA treatment (10-200uM, 24-72h) on MDAH-2774 human ovarian cancer and non-cancerous HUVEC human endothelial cell lines.

METHODS: Physiological effects of DHEA treatment (10-200uM, 24-72 hours) on MDAH-2774 human ovarian cancer and non-cancerous HUVEC human endothelial cell lines investigated with MTT test, AO/EtBr staining and scratch assay.

RESULTS: DHEA treatment was able to promote proliferation of MDAH-2774 cancer cell line in a dose-dependent manner (r=0.6906, P<0.0001 for 24 hours) (r=0.6802, P<0.0001, for 48 hours) (r=0,7969 P<0.0001, for 72 hours). In contrast, DHEA inhibited proliferation of the non-cancerous HuVeC cells (r=0.9490, P<0.0001 for 24 hours) (r=0.9533, P<0.0001, for 48 hours) (r=0,9584 P<0.0001, for 72 hours). In agreement with these observations, DHEA treatment resulted in a dose-dependent increase in the number of necrotic cells in the non-cancerous HuVeC cell line (r=0.97, P<0.0001). However, the number of necrotic or apoptotic cells did not significantly change in the MDAH-2774 cell line when exposed to DHEA. Moreover, we found that DHEA treatment reduced the migration rate of non-cancerous HuVeC cells in a dose-dependent manner (r=0.9868, P<0.0001) whereas, only a slight increase was observed in MDAH-2774 ovarian cancer cell line (r=0.8938, P<0.05).

DISCUSSION AND CONCLUSION: Our findings suggest that DHEA promotes the proliferation of ovarian cancer cells in a dose-dependent manner in vitro. Moreover, we found that DHEA induces necrosis and inhibits proliferation in the non-cancerous endothelial cell line. Although mechanistic evidence is required, our preliminary findings imply that exposure to high doses of DHEA can be associated with an increased risk for the development of ovarian cancer.
Keywords: Dehydroepiandrosterone, MDAH-2774, HuVeC, ovarian cancer

1. Introduction

DHEA is one of the most abundant circulating steroid hormones produced by adrenal glands[1] and it can also serve as a precursor for other steroids in the brain.[2] Dehydroepiandrosterone (DHEA) is involved in the biosynthesis of sex steroids and it may act as a ligand for several nuclear receptors as well as G-protein-coupled receptors. Physiological levels of DHEA in human serum range between 1–1000 nm[3] while, prescribed pharmacological doses range between 10–100 μM.[4] An age-dependent decline in circulating levels of DHEA can induce changes in cardiovascular tissues[5], female fertility[6], metabolic and neuronal/CNS functions.[7]

Clinical findings show that DHEA administration can increase serum androgen levels, improve sexual function, improve mood and decrease fatigue.[8] DHEA is also widely used for the induction of ovulation in gynecological clinical practice. However, data also suggests that high doses or prolonged use of DHEA is associated with increased risk in developing ovarian cancer.[9,10] Cameron [8] have reported that DHEA activates estrogen receptors[11] in breast cancer and endothelial cell lines. [4,12,13] In line with these observations, clinical findings also implicated DHEA as a risk factor for developing breast cancer.[14] In contrast, in vitro findings suggested an inhibitory effect of DHEA on the proliferation of breast, hepatoma, prostate, myeloma, leukemia, human colon adenocarcinoma, and cervical cancer cell lines.[15–21] Thus, our understanding of the role of DHEA as a risk factor for developing cancer is limited.

At present, in vitro effects of DHEA on normal and ovarian cancer cell lines have not been compared. Therefore, we aimed to investigate the physiological effects of DHEA on MDAH-2774 human ovarian cancer and non-cancerous HuVeC human endothelial cell lines. A better understanding of the differential effects of DHEA on normal and cancer cell lines can have important implications in clinical research.

2. Material and Methods

2.1. Cell culture and chemicals

Human ovarian carcinoma epithelial cell (MDAH-2774 (ATCC® CRLM10303 ™) and human umbilical vein endothelial (HUV-EC-C [HuVeC] (ATCC® CRL-1730™) cell lines were cultured in high glucose DMEM (Sigma, 5546) supplemented with P/S (50 U/ml penicillin and 50 μg/ml streptomycin, Biological Industries, 03-031-1B), 1% 2mM L-glutamine (Biological Industries, BI03-020-1B), %10 FBS (Biowest, S1810-500). 1,5x106 cells from each cell line were seeded into 10cm plates and split after 72 hours. Dehydroepiandrosterone tablets (about 0.6–1.2 g (2–3 pieces)) were solved in 50 ml of absolute ethanol as stock solutions. Sterilized by filtration through a 0.45 μm membrane filter and prepared fresh for every test.[4] Negative control indicates treatments of cells with ethanol (the solvent of DHEA) containing DMEM.

2.2. MTT assay

12 mM stock solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Neofrox 3580 MTT) was prepared as described by Mosmann.[22] Approximately 10^4 cells were seeded into each well of a 96-well plate at a volume of 100 μL. The MTT assay was carried out as follows: 10 μL of the 12 mM MTT stock solution was added to each well and incubated at 37°C for 4 hours. 100 μL of medium alone was included as a negative control. After 4 hours of incubation with MTT 75 μL of the medium was removed from the wells and then formazan crystals were dissolved with 50 μL of DMSO by mixing thoroughly with the pipette. After an additional incubation at 37°C for 10 minutes samples were mixed again briefly and absorbance at 540 nm was recorded.

2.3. Acridine Orange/Ethidium Bromide double staining

Acridine Orange/Ethidium Bromide (AO/EtBr) Dual staining technique was performed as described by Liu[23]. Briefly, cells were seeded in a 96-well plate at a density of approximately 10^4 cells/well. Following incubation with DHEA for 48 hours, cells were trypsinized and 10-25 µl cell suspensions were transferred onto glass slides. 1 µl of AO/EtBr staining solution (a mixture of dyes containing 100 µg/ml AO and 100 µg/ml EtBr) was added on cell suspensions and then the samples were covered with a coverslip. The morphology of cells was examined under a fluorescent microscope (Carl-Zeiss/Axio observer 3., Zen 2.3 Blue Edition software) within 20 minutes after adding Ao/EtBr stain. For statistical analysis st least 200 cells were counted and the results were expressed as mean values obtained from at least three independent experiments. Both live and dead cells are stained with AO while, ethidium bromide stains only dead cells that have lost membrane integrity. Live cells appear uniformly green whereas early apoptotic cells show green dots in their nuclei. Late apoptotic cells stain orange and show condensed and/or often fragmented nuclei. Necrotic cells stain orange, with a nuclear morphology resembling that of viable cells, but without condensed chromatin.[23]

2.4. In vitro scratch assay

In vitro scratch assay was carried out for evaluating the migration rates according to the protocol described by Liang.[24] Briefly, a scratch on the surface of the well was made with a 10 µl sterile pipette tip in 6-well plates. Following gentle washing with culture medium, photos of the scratch were taken at different time points (0-24 hours) under a microscope at a magnification of 10X (Carl-Zeiss/Axio observer 3) The gap size was analyzed using Image-J software and the rate of cell migration was calculated by comparing the cell-free areas of the scratches at 24-hour post-wounding and the area of the scratches at 0 hours. The results were expressed as the mean of triplicate experiments.

2.5. Statistical analysis

Statistical analysis was performed using GraphPad (Prism 5) software. Statistical significances of the differences between the groups were assessed by one-way ANOVA and Tukey's post-hoc test (n=3).

3. Results

Firstly, we investigated the effects of dehydroepiandrosterone on cell viability by comparing the changes in proliferation rates of MDAH-2774 (human ovarian carcinoma epithelial cell) and HuVeC (human non-cancerous umbilical vein endothelial) cell lines. For this purpose, we have used MTT assay which is a relatively simple and well-established procedure for evaluation of cell viability and proliferation.[25] We have tested the effects of four different concentrations (10µM, 50µM, 100µM, and 200µM) of dehydroepiandrosterone for 24, 48 and 72 hours. The concentration range was determined according to the literature data.[4]
Analysis of data obtained from MTT assay showed that DHEA treatment (10-200 µM) was able to promote the proliferation of MDAH-2774 cell line in a dose-dependent ($r=0.6906$, $P<0.0001$ for 24 hours) ($r=0.6802$, $P<0.0001$, for 48 hours) ($r=0,7969$ $P<0.0001$, for 72 hours). In contrast to the MDAH-2774 ovarian cancer cell line, DHEA treatment inhibited proliferation of the non-cancerous HuVeC cells. Although, 10µM (24 hours) DHEA treatment did not appear to affect the rate of cell the proliferation, higher doses of DHEA (≥50µM for 24, 48 and 72 hours) significantly inhibited proliferation of HuVeC cell in a dose-dependent fashion ($r=0.9490$, $P<0.0001$ for 24 hours) ($r=0.9533$, $P<0.0001$, for 48 hours) ($r=0,9584$ $P<0.0001$, for 72 hours) **(Figure 1b.d.f)**.
As seen in **Figure 1**, either the 48 or 72 hours of DHEA exposure exhibited the most prominent effects on cell proliferation. In this context, to avoid the possible toxic effects that

might arise due to the prolonged drug exposure we selected 48 hours as the optimum exposure time. Thus, the latter experiments were carried out by exposing the cells to 10μM, 50μM, 100μM, and 200μM of DHEA for 48 hours.

Next, we compared the changes in the ratio of apoptotic/necrotic cells using the "acridine orange/ethidium bromide" double staining protocol. Dual AO/EB staining is a reliable and inexpensive technique to detect apoptotic and necrotic cells in cell culture experiments.[23] The changes in the ratio of apoptotic and necrotic cells were evaluated upon exposure to DHEA (10-200 μM for 48 hours). Our findings demonstrated that DHEA treatment did not induce apoptosis or necrosis in MDAH-2774 cells (**Figure 2 a-b.**). On the other hand, we detected a dose-dependent increase in the number of the necrotic cells when HuVeC cells were treated with DHEA (48-hour, by 5±1% for 10uM, 14.33±6% for 50uM, 32±3% for 100uM, 54.33±5% for 200uM $P<0.05$). Suggesting that DHEA treatment induces necrotic death in the HuVeC cell line. Representative microscope images from AO/EtBr stained samples are presented in **Figure 3**.

Then, we investigated whether or not DHEA treatment could induce changes in cell migration rates. For this purpose, we have performed the scratch assay. This is a simple in vitro method which serves as a powerful tool for analyzing cell migration rates in two dimensions.[26] In this context, we tested the effect of 10- 200 μM of DHEA (48 hours) treatment on cell migration rates by in vitro scratch assay technique. Our findings indicated that DHEA treatment triggered a slight increase in the migration rate of MDAH-2774 ovarian cancer cell line (48-hour, by 7±1% for 10uM, 8±1% for 50uM, 9±1% for 100uM, 10±1% for 200uM $P<0.05$) (**Figure 4a**). On the contrary, DHEA reduced the migration rate of non-cancerous HuVeC cells in a dose-dependent manner (48-hour, by 5±3% for 10uM, 14±1% for 50uM, 31±3% for 100uM, 52±4% for 200uM $P<0.05$) (**Figure 4b**). Representative microscope images from scratch assay experiments are presented in **Figure 5**.

4. Discussion

DHEA is one of the most abundant adrenal steroids in humans. DHEA is a precursor of biologically active steroids such as testosterone and estrogen.[27] Although DHEA is shown to be able to activate estrogen receptors[11] it can also act independently of androgen and estrogen receptors in breast cancer cells and endothelial cells.[4,12,13] In gynecological clinical practice, DHEA is also prescribed for the induction of ovulation. There are conflicting findings as to the role of DHEA as a risk factor for developing cancer. Higher levels of DHEA have been correlated with increased risk of developing breast cancer.[28] On the other hand, Yoshida[29] proposed that DHEA can reduce the risk of developing breast cancer through blocking estrogen receptors. Mainly, DHEA seems to inhibit the proliferation of cancer cells according to the in vitro findings[15–21]. Several different groups demonstrated that DHEA inhibits the proliferation of breast, hepatoma, prostate, myeloma, leukemia, human colon adenocarcinoma, and cervical cancer cell lines.[15–21] On the other hand, clinical data also suggests that DHEA may be positively associated with breast cancer.[14] For example, Michels[30] have identified increased risks for endometrial cancer with high levels of adrenal androgens and estrogens.[30] A review of the literature revealed that sufficient data were not available regarding the in vitro physiological effects of DHEA on ovarian cancer cell lines. MDAH-2774 is a widely used ovarian cancer cell line[31] with a high average of tumor volume and it is accepted as a suitable model for human ovarian cancer.[32] Endothelial cells are one of the key players in maintaining physiological hemostasis[33] they are also involved in many important processes including regulation of cell migration, proliferation, and apoptosis.[34] Therefore, we have chosen a well-established endothelial cell model HUVEC cell line as a non-cancerous model. In this context, we investigated the physiological effects of DHEA on

MDAH-2774 human ovarian cancer cells in comparison with the non-cancerous HuVeC human endothelial cell line.

We have assessed the effect of DHEA on the proliferation by MTT assay which is a relatively simple and a well-established procedure for evaluation of cell viability.[25] Our findings revealed that DHEA inhibits the proliferation of the non-cancerous HuVeC cells in a dose-dependent manner (**Figure 1**). In line with our observation, Liu[35] showed that DHEA inhibited cell proliferation of non-cancerous primary rat Leydig cells. They suggested that this cell type-specific response could be due to the differences in the way DHEA was processed or metabolized inside the cell. Surprisingly we found that DHEA promoted proliferation of MDAH-2774 cancer cell line in a dose-dependent manner (**Figure 1**). This finding supports the notion that DHEA may exert its effect in a cell type-specific manner. Similarly, Joshi[36] reported cell-type specific differential effects of DHEA between mouse and human melanoma cell lines.

We have employed dual AO/EB staining technique to asses the DHEA-induced effects on apoptotic activity in cells.[23] We did not observe a significant change in the number of the necrotic or apoptotic cells in the ovarian cancer MDAH-2774 cell line (**Figure 2**). However, our findings revealed that DHEA can induce necrotic cell death in non-cancerous HuVeC cell line. Girón[19] previously reported that DHEA treatment induced both apoptotic and necrotic cell death in cervical cancer cell lines. Interestingly, supplementation of the medium with DHEA was reported to increase the number of primordial follicles by inhibiting apoptotic activity in mice.[37] In contrast, Kim [38] demonstrated that DHEA administration can induce apoptosis in the mouse ovaries. More recent evidence also suggests that DHEA supplementation can protect ovarian cells in rats rather than inducing apoptotic activity.[39] Similarly, DHEA was found to inhibit H_2O_2-induced oxidative stress damage and apoptosis in Leydig cells.[40] The presence of contradictory findings further supports the idea that DHEA can exert its' effect in a cell-type-specific manner.

Available data on the signaling mechanisms involved in DHEA-mediated physiological changes is also scarce. Girón[19] suggested that DHEA can exert its' effects through the Mitogen-Activated Protein Kinase Signaling Pathway independent of either androgen receptor or estrogen receptor. Jimenez[41] showed that DHEA may inhibit the pentose phosphate pathway and thereby alter oocyte lipid metabolism in mice. DHEA is shown to be able to directly act on plasma membrane receptors, such as the G-protein-coupled receptor in endothelial cells and aminobutyric-acid-type A in neurons. DHEA also binds androgen and estrogen receptors and can m inhibit voltage-gated T-type calcium channels.[42] Nevertheless, molecular and mechanistic approaches are necessary to dissect the mechanisms involved in this cell type-specific action of DHEA.

Previously, the migration of cervical cancer cell lines was found to be suppressed by DHEA[43]. In line with these reports, López-Marure[20] proposed that DHEA inhibited the proliferation of all breast cancer cell lines. Consistently, we found that DHEA treatment can reduce the migration rate of non-cancerous HuVeC cells in a dose-dependent manner (**Figure 4**). Curiously, however, we detected a slight increase in the migration rate of the MDAH-2774 ovarian cancer cell line (**Figure 4**). This observation suggests that DHEA may induce the migration of ovarian cancer cells. In agreement with our observation, Montt-Guevara[44] showed that DHEA stimulates cell invasion via moesin activation in the T47D breast cancer cell line.

5. Study limitations

In this study, we presented reproducible data to support our observations as to the physiological effects of DHEA on ovarian cancer and non-cancerous cells. However, we were

not able to provide an insight into the underlying molecular mechanisms and pathways due to a lack of molecular evidence. We were not able to provide mechanistic evidence to support our descriptive findings.

6. Conclusion

Although DHEA inhibited proliferation of the non-cancerous HuVeC cells, DHEA treatment was able to promote the proliferation of MDAH-2774 cancer cell line in a dose-dependent manner. We detected a dose-dependent increase in the number of necrotic cells in the non-cancerous HuVeC cell line in response to DHEA treatment. However, the number of necrotic or apoptotic cells did not significantly change in the MDAH-2774 cell line. DHEA treatment reduced the migration rate of non-cancerous HuVeC cells whereas, only a slight increase was observed in the MDAH-2774 ovarian cancer cell line. Our findings suggest that DHEA promotes the proliferation of ovarian cancer cells in a dose-dependent manner in vitro. Moreover, we found that DHEA induces necrosis and inhibits proliferation in the non-cancerous endothelial cell line. Although molecular and mechanistic evidence is required, our preliminary findings imply that exposure to high doses of DHEA can be associated with an increased risk for the development of ovarian cancer.

Ethics approval and consent to participate

Not applicable.

Availability of data and material

All the cell lines, raw experimental data, data on statistical analysis and/or detailed explanations regarding material and methods are available from the corresponding author on reasonable request.

Competing interests

No conflict of interest was declared by the authors.

Funding

Not applicable.

Authors' contributions

Gundogan G. I. carried out the experiments. Kig C. supervised the project and contributed to critical reviews. Karacan M. provided the pharmaceutical and biological materials. Gundogan

G. I. designed the model of the study and provided literature search. Kig C. and Dogruman H. contributed to processing, analysis, and collection of data. The manuscript was written by Gundogan G. I., with contributions from Kig C. and Dogruman H.

Acknowledgments
Not applicable.

References

1. Turcu A, Smith JM, Auchus R, Rainey WE. Adrenal androgens and androgen precursors-Definition, synthesis, regulation and physiologic actions. Compr Physiol [Internet] 2014 [cited 2020 Apr 6];4(4):1369–81. Available from: http://doi.wiley.com/10.1002/cphy.c140006
2. Trainor BC, Nelson RJ. Neuroendocrinology of aggression [Internet]. In: Handbook of Neuroendocrinology. Academic Press; 2012 [cited 2020 Apr 6]. page 509–20.Available from: https://www.sciencedirect.com/science/article/pii/B9780123750976100228
3. Young J, Couzinet B, Nahoul K, … SB-… J of C, 1997 U. Panhypopituitarism as a model to study the metabolism of dehydroepiandrosterone (DHEA) in humans. J Clin Endocrinol Metab [Internet] 1999 [cited 2020 Mar 2];82:2578–85. Available from: https://academic.oup.com/jcem/article-abstract/82/8/2578/2877651
4. Zapata E, Ventura JL, De La Cruz K, Rodriguez E, Damián P, Massó F, et al. Dehydroepiandrosterone inhibits the proliferation of human umbilical vein endothelial by enhancing the expression of p53 and p21, restricting the phosphorylation of retinoblastoma protein, and is androgen- and estrogen-receptor independent. FEBS J [Internet] 2005 [cited 2020 Feb 29];272(6):1343–53. Available from: http://doi.wiley.com/10.1111/j.1742-4658.2005.04563.x
5. Ohlsson C, Vandenput L, Tivesten. DHEA and mortality: What is the nature of the association? [Internet]. J. Steroid Biochem. Mol. Biol.2015 [cited 2020 Mar 5];145:248–53. Available from: https://www.sciencedirect.com/science/article/pii/S0960076014000661
6. Tartagni M, Cicinelli M V., Baldini D, Tartagni M V., Alrasheed H, DeSalvia MA, et al. Dehydroepiandrosterone decreases the age-related decline of the in vitro fertilization outcome in women younger than 40 years old. Reprod Biol Endocrinol 2015;13(1):1–6.
7. Maggio M, De Vita F, Fisichella A, Colizzi E, Provenzano S, Lauretani F, et al. DHEA and cognitive function in the elderly. J. Steroid Biochem. Mol. Biol.2015;145:281–92.
8. Cameron DR, Braunstein GD. The use of dehydroepiandrosterone therapy in clinical

practice [Internet]. Treat. Endocrinol.2005 [cited 2020 Mar 5];4(2):95–114. Available from: http://link.springer.com/10.2165/00024677-200504020-00004
9. Helzlsouer KJ, Gordon GB, Alberg AJ, Bush TL, Hoffman SC, Longcope C, et al. Serum Gonadotropins and Steroid Hormones and the Development of Ovarian Cancer. JAMA J Am Med Assoc [Internet] 1995 [cited 2020 Mar 5];274(24):1926–30. Available from: https://jamanetwork.com/journals/jama/article-abstract/393293
10. Ose J, Poole EM, Schock H, Lehtinen M, Arslan AA, Zeleniuch-Jacquotte A, et al. Androgens are differentially associated with ovarian cancer subtypes in the Ovarian Cancer Cohort Consortium. Cancer Res 2017;77(14):3951–60.
11. Chen F, Knecht K, Birzin E, Fisher J, Wilkinson H, Mojena M, et al. Direct agonist/antagonist functions of dehydroepiandrosterone. Endocrinology [Internet] 2005 [cited 2020 Mar 5];146(11):4568–76. Available from: https://academic.oup.com/endo/article-lookup/doi/10.1210/en.2005-0368
12. Gayosso V, Montano LF, López-Marure R. DHEA-induced antiproliferative effect in MCF-7 cells is androgen- and estrogen receptor-independent. Cancer J [Internet] 2006 [cited 2020 Mar 5];12(2):160–5. Available from: https://journals.lww.com/journalppo/Fulltext/2006/03000/DHEA_Induced_Antiproliferative_Effect_in_MCF_7.13.aspx
13. Liu D, Iruthayanathan M, Homan LL, Wang Y, Yang L, Wang Y, et al. Dehydroepiandrosterone stimulates endothelial proliferation and angiogenesis through extracellular signal-regulated kinase 1/2-mediated mechanisms. Endocrinology [Internet] 2008 [cited 2020 Mar 5];149(3):889–98. Available from: https://academic.oup.com/endo/article-abstract/149/3/889/2454909
14. Tworoger SS, Sluss P, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. Cancer Res 2006;66(4):2476–82.
15. Catalina F, Milewich L, Kumar V, Bennett M. Dietary Dehydroepiandrosterone Inhibits Bone Marrow and Leukemia Cell Transplants: Role of Food Restriction. Exp Biol Med [Internet] 2003 [cited 2020 Mar 5];228(11):1303–20. Available from: http://journals.sagepub.com/doi/10.1177/153537020322801109
16. Jiang Y, Miyazaki T, Honda A, Hirayama T, Yoshida S, Tanaka N, et al. Apoptosis and inhibition of the phosphatidylinositol 3-kinase/Akt signaling pathway in the anti-proliferative actions of dehydroepiandrosterone. J Gastroenterol 2005;40(5):490–7.
17. Arnold JT, Liu X, Allen JD, Le H, McFann KK, Blackman MR. Androgen receptor or estrogen receptor-β blockade alters DHEA-, DHT-, and E2-induced proliferation and PSA production in human prostate cancer cells. Prostate 2007;67(11):1152–62.
18. Ho HY, Cheng ML, Chiu HY, Weng SF, Chiu DTY. Dehydroepiandrosterone induces growth arrest of hepatoma cells via alteration of mitochondrial gene expression and function. Int J Oncol 2008;33(5):969–77.
19. Girón RA, Montaño LF, Escobar ML, López-Marure R. Dehydroepiandrosterone inhibits the proliferation and induces the death of HPV-positive and HPV-negative cervical cancer cells through an androgen- and estrogen-receptor independent mechanism. FEBS J [Internet] 2009 [cited 2020 Mar 5];276(19):5598–609. Available from: http://doi.wiley.com/10.1111/j.1742-4658.2009.07253.x
20. López-Marure R, Contreras PG, Dillon JS. Effects of dehydroepiandrosterone on proliferation, migration, and death of breast cancer cells. Eur J Pharmacol 2011;660(2–3):268–74.
21. Liu S, Ishikawa H, Li FJ, Ma Z, Otsuyama KI, Asaoku H, et al. Dehydroepiandrosterone can inhibit the proliferation of myeloma cells and the interleukin-6 production of bone marrow mononuclear cells from patients with myeloma. Cancer Res

2005;65(6):2269–76.
22. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65(1–2):55–63.
23. Liu K, Liu P, Liu R, Wu X. Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. Med Sci Monit Basic Res 2015;21:15.
24. Liang CC, Park AY, Guan JL. In vitro scratch assay: A convenient and inexpensive method for analysis of cell migration in vitro. Nat Protoc 2007;2(2):329–33.
25. Longo-Sorbello GSA, Saydam G, Banerjee D, Bertino JR. Cytotoxicity and cell growth assays [Internet]. In: Cell Biology, Four-Volume Set. 2006 [cited 2020 Apr 6]. page 315–24.Available from: https://www.sciencedirect.com/science/article/pii/B9780121647308500393
26. Nobes CD, Hall A. Rho GTPases control polarity, protrusion, and adhesion during cell movement. J Cell Biol [Internet] 1999 [cited 2020 Apr 6];144(6):1235–44. Available from: https://rupress.org/jcb/article-abstract/144/6/1235/16076
27. Okabe T, Haji M, Takayanagi R, Adachi M, Imasaki K, Kurimoto F, et al. Up-regulation of high-affinity dehydroepiandrosterone binding activity by dehydroepiandrosterone in activated human T lymphocytes. J Clin Endocrinol Metab [Internet] 1995 [cited 2020 Mar 5];80(10):2993–6. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jcem.80.10.7559886
28. Tworoger SS, Hankinson SE. Prolactin and breast cancer etiology: An epidemiologic perspective. J Mammary Gland Biol Neoplasia [Internet] 2008 [cited 2020 Apr 6];13(1):41–53. Available from: http://link.springer.com/10.1007/s10911-008-9063-y
29. Yoshida S, Honda A, Matsuzaki Y, Fukushima S, Tanaka N, Takagiwa A, et al. Anti-proliferative action of endogenous dehydroepiandrosterone metabolites on human cancer cell lines. Steroids [Internet] 2003 [cited 2020 Apr 6];68(1):73–83. Available from: https://www.sciencedirect.com/science/article/pii/S0039128X02001174
30. Michels KA, Brinton LA, Wentzensen N, Pan K, Chen C, Anderson GL, et al. Postmenopausal Androgen Metabolism and Endometrial Cancer Risk in the Women's Health Initiative Observational Study. JNCI Cancer Spectr [Internet] 2019 [cited 2020 Mar 5];3(3). Available from: https://academic.oup.com/jncics/article-abstract/3/3/pkz029/5479592
31. Freedman RS, Pihl E, Kusyk C, Gallager HS, Rutledge F. Characterization of an ovarian carcinoma cell line. Cancer [Internet] 1978 [cited 2020 Apr 6];42(5):2352–9. Available from: https://acsjournals.onlinelibrary.wiley.com/doi/abs/10.1002/1097-0142%28197811%2942%3A5%3C2352%3A%3AAID-CNCR2820420536%3E3.0.CO%3B2-%23
32. Hernandez L, Kim MK, Lyle LT, Bunch KP, House CD, Ning F, et al. Characterization of ovarian cancer cell lines as in vivo models for preclinical studies. Gynecol Oncol [Internet] 2016 [cited 2020 Apr 6];142(2):332–40. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0090825816307570
33. Galley HF, Webster NR. Physiology of the endothelium. Br J Anaesth [Internet] 2004 [cited 2020 Apr 6];93(1):105–13. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0007091217355976
34. Chien S. Mechanotransduction and endothelial cell homeostasis: The wisdom of the cell [Internet]. In: American Journal of Physiology - Heart and Circulatory Physiology. 2007 [cited 2020 Apr 6]. page H1209–24.Available from: https://www.physiology.org/doi/10.1152/ajpheart.01047.2006
35. Liu L, Wang D, Li L, Ding X, Ma H. Dehydroepiandrosterone inhibits cell proliferation and improves viability by regulating S phase and mitochondrial permeability in primary rat Leydig cells. Mol Med Rep [Internet] 2016 [cited 2020 Mar 5];14(1):705–14. Available from: https://www.spandidos-publications.com/10.3892/mmr.2016.5343

36. Joshi K, Hassan SS, Ramaraj P. Differential biological effects of dehydroepiandrosterone (DHEA) between mouse (B16F10) and human melanoma (BLM) cell lines. Dermatoendocrinol 2017;9(1):e1389360.
37. Chu N, Gui Y, Qiu X, Zhang N, Li L, Li D, et al. The effect of DHEA on apoptosis and cohesin levels in oocytes in aged mice. Biosci Trends [Internet] 2017 [cited 2020 Apr 6];11(4):427–38. Available from: https://www.jstage.jst.go.jp/article/bst/advpub/0/advpub_2017.01108/_article/-char/ja/
38. Kim HH, Shaipanich M, Hasselblatt K, Yeh J. Induction of apoptosis and ovarian cyst formation in the mouse ovary by dehydroepiandrosterone (DHEA). J Med [Internet] 2003 [cited 2020 Apr 6];34(1–6):101–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17682316
39. Sozen B, Ozekinci M, Erman M, Gunduz T, Demir N, Akouri R. Dehydroepiandrosterone supplementation attenuates ovarian ageing in a galactose-induced primary ovarian insufficiency rat model. J Assist Reprod Genet [Internet] 2019 [cited 2020 Apr 6];36(10):2181–9. Available from: http://link.springer.com/10.1007/s10815-019-01560-4
40. Ding X, Yu L, Ge C, Ma H. Protective effect of DHEA on hydrogen peroxide-induced oxidative damage and apoptosis in primary rat Leydig cells. Oncotarget [Internet] 2017 [cited 2020 Apr 6];8(10):16158–69. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28212544
41. Jimenez PT, Frolova AI, Chi MM, Grindler NM, Willcockson AR, Reynolds KA, et al. DHEA-mediated inhibition of the pentose phosphate pathway alters oocyte lipid metabolism in mice. Endocrinology [Internet] 2013 [cited 2020 Apr 6];154(12):4835–44. Available from: https://academic.oup.com/endo/article-lookup/doi/10.1210/en.2012-2140
42. Clark BJ, Prough RA, Klinge CM. Mechanisms of Action of Dehydroepiandrosterone [Internet]. In: Vitamins and Hormones. 2018 [cited 2020 Apr 6]. page 29–73.Available from: https://linkinghub.elsevier.com/retrieve/pii/S0083672918300372
43. Ortega-Calderón YN, López-Marure R. Dehydroepiandrosterone inhibits proliferation and suppresses migration of human cervical cancer cell lines. Anticancer Res [Internet] 2014 [cited 2020 Mar 5];34(8):4039–44. Available from: http://ar.iiarjournals.org/content/34/8/4039.short
44. Montt-Guevara MM, Shortrede JE, Giretti MS, Giannini A, Mannella P, Russo E, et al. Androgens regulate T47D cells motility and invasion through actin cytoskeleton remodeling. Front Endocrinol (Lausanne) [Internet] 2016 [cited 2020 Apr 6];7(SEP). Available from: http://journal.frontiersin.org/Article/10.3389/fendo.2016.00136/abstract

Figures

Figure 1. The effect of DHEA on cell proliferation. **a.c.e.** MDAH-2774 ovarian cancer, **b.d.f.** HuVeC Endothelial cell lines were treated with Dehydroepiandrosterone for 24, 48 and 72 hours in an incubator. MTT assays were performed 24, 48 and 72 hours after treatment with the indicated doses of DHEA relative % changes in proliferation rates were compared against the non-treated control group (0 µM) and Statistical significance was tested using one-way ANOVA followed by Tukey's multiple-comparisons test (*,**,*** P<0.0001, n=6). The correlation between DHEA dose and its' effect on proliferation was analysed by linear regression (GraphPAD program). MDAH: r=0.6906, P<0.0001 for 24 hours; r=0.6802, P<0.0001, for 48 hours and r=0,7969, P<0.0001 for 72 hours. HuVeC: r=0.9490, P<0.0001 for 24 hours; r=0.9533, P<0.0001, for 48 hours and r=0,9584, P<0.0001 for 72 hours.

Figure 2. DHEA induces necrosis at high concentrations. **a)** MDAH-2774, and **b)** HuVeC endothelial cell lines were treated with 10-200 µM DHEA for 48 hours in an incubator. AO/EtBr double staining was performed 48 hours after treatment with the indicated doses of DHEA. Percentage changes in the ratio of necrotic cells were compared against the non-treated control group (0 µM) and statistical significance was tested using one-way ANOVA followed by Tukey's multiple-comparisons test (**, *** $P<0.05$, n=3).

Figure 3. Representative microscope images from AO/EtBr double staining. **a)** Magnification: 10x, MDAH-2774 cells (Control: 0µM DHEA) **b)** Magnification: 40x, MDAH-2774 cells (100µM DHEA), **c)** Magnification: 10x, HuVeC cells (100µM DHEA), **d)** Magnification: 40x, HuVeC cells (100µM DHEA). Arrows point to apoptotic cells, arrow heads point to necrotic cells and double arrow heads point to live cells.

Figure 4. DHEA reduces cell migration rate at high concentrations in HuVeC while increases in MDAH-2774. **a)** MDAH-2774, **b)** HuVeC endothelial cell lines were treated with 10-200 µM DHEA for 48 hours in an incubator. Scratch assay was performed 48 hours after treatment with the indicated doses of DHEA. The rate of migration (how soon the gap has been closed) in 48 hours was calculated by measuring the gap at 0. and 48 hours after scratcing the plates. % changes in the migration rates were compared against the non-treated control group (0 µM) and statistical significance was tested using one-way ANOVA followed by Tukey's multiple-comparisons test (*, **, *** $P<0.05$, n=3).

Figure 5. Representative microscope images from in vitro scratch assay (Magnification 10X). **a)** 0.hour MDAH-2774 cells **b)** 48 hour 0µM DHEA, MDAH-2774 cells **c)** 48 hour 100µM DHEA, MDAH-2774 cells **d)** 0 hour HuVeC cells **e)** 48 hour 0µM DHEA, HuVeC cells **f)** 24 hour 100µM DHEA, HuVeC cells.