Endocrine-Disrupting Chemicals and Vitamin D Deficiency in the Pathogenesis of Uterine Fibroids

Hoda Elkafas, Osama Badary, Engy Elmorsy, Rehab Kamel, Qiwei Yang, Ayman Al-Hendy

1Department of Pharmacology and Toxicology, Egyptian Drug Authority (EDA) formally, (NODCAR), Cairo 35521, Egypt. 2Department of Anesthesiology, University of Illinois at Chicago, Chicago, IL 60612, USA. 3Department of Clinical Pharmacy, Faculty of Pharmacy, British University in Egypt, Cairo 11837, Egypt. 4Department of Pharmacology and Toxicology, Faculty of Pharmacy, Helwan University, Cairo 11795, Egypt. 5Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL, 60637, USA.

*Corresponding author: Ayman Al-Hendy, Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL, 60637, USA. Tel. (+1)773-834-0742 Email address: Aalhendy@bsd.uchicago.edu

Submitted on: 08-03-2020; Revised on: 24-03-2021; Accepted on: 26-03-2021


ABSTRACT

Uterine fibroids (UFs) are the most prevalent gynecologic neoplasm, affecting 70–80% of women over their lifespan. Although UFs are benign they can become life-threatening and require invasive surgeries such as myomectomy and hysterectomy. Notwithstanding the significant negative influence UFs have on female reproductive health, very little is known about early events that initiate tumor development. Several risk factors for UFs have been identified including vitamin D deficiency, inflammation, DNA repair deficiency, and environmental exposures to endocrine-disrupting chemicals (EDCs). EDCs have come under scrutiny recently due to their role in UF development. Epidemiologic studies have found an association between increased risk for early UF diagnosis and in utero EDC exposure. Environmental exposure to EDCs during uterine development increases UF incidence in a UF animal model. Notably, several studies demonstrated that abnormal myometrial stem cells (MMSCs) are the cell origin for UFs development. Our recent studies demonstrated that early-life EDC exposure reprogrammed the MMSCs toward a pro-fibroid landscape and altered the DNA repair and inflammation pathways. Notably, Vitamin D3 (VITD3) as a natural compound shrunk the UF growth concomitantly with the reversion of several abnormal biological pathways and ameliorated the developmental exposure-induced DNA damage and pro-inflammation pathway in primed MMSCs. This review highlights and emphasizes the importance of multiple pathway interactions in the context of hypovitaminosis D at the MMSCs level and provides proof-of-concept information that can help develop a safe, long-term, durable, and non-surgical therapeutic option for UFs.

Keywords: Uterine fibroids; EDCs; Vitamin D3, Inflammation, DNA damage.

INTRODUCTION

Uterine fibroids (UFs, AKA: leiomyoma) are the most widespread pelvic tumors in women of reproductive age. They affect 70–80% of women throughout their lives and lead to hysterectomies in premenopausal women. About 200,000 surgeries are performed annually in the US with a cost of up to $34 billion. There has also been an increase in women who want to save their uteri for different purposes.
Therefore, treatment should be selected with these purposes in mind. Surgery is still the best cure rate method; however, there is a massive shift to minimally-invasive techniques in recent years. Given the recent safety concerns regarding some drugs currently available, there is a need for safe and effective alternative therapies to prevent and treat UFs. Although UFs are benign tumors, they can cause various symptoms such as severe abdominal and pelvic pain, severe uterine bleeding, bladder dysfunction, and more obstetric complications leading to sub-infertility, miscarriage, and other reproductive dysfunctions.

**Burden and prevalence of UFs**

The prevalence of UFs increases with age, especially during the premenopausal stage and is remarkably higher in the fourth and fifth decades of life. In contrast, after age 50, UFs appeared even in 80% of women. Forty percent of Caucasian women younger than 35 are affected, while the percentage was almost 70% in women aged 50 and older. UFs are not seen in preteen girls and are rare in teenagers. Nevertheless, the factors which may contribute to their development at such an early age are unknown. The cause of UFs is still mostly unknown. However, there are proven risk factors such as age at menopause, genetics, African American (AA) ethnicity, vitamin D3 (VITD3) deficiency, and early life exposure to EDCs. While other risk factors for this disease are hormone-related, the underlying mechanism for AA women's increase in risk compared to Caucasian women remains to be identified.

**Origin of UFs**

UFs are monoclonal tumors that originate from uterine smooth muscle (myometrium), and a characteristic hallmark is their dependence on the ovarian steroids estrogen (E2) and progesterone (P4). The exact mechanisms of pathophysiology remain unclear. However, various studies have identified the pathophysiological conversion of myometrium stem cells to tumor-initiating cells in UFs and several studies have been performed to identify tumor-initiating cells in UFs. The main difference between myometrial stem cells (MSCs) and fibroid stem cells at the DNA level is genomic instability, leading to an increase in the proliferation rate. MED12 mutations were detected in fibroid stem cells but not in MMSCs.

**The influence of steroid hormones in UFs**

UFs are hormonally dependent upon estrogen (E2), progesterone (P4), and other steroid hormones and growth decreases after menopause. UF development also depends on the ovarian steroid hormone, and typically relapse after menopause. E2 exerts its genomic and nongenomic effects by the E2 receptors (ERα and ERβ). Genomic pathways involve the direct junction of ER complexes to specific sequences in gene promoters whereas nongenomic signaling involves activation of signaling cascades that result in indirect alterations in gene expression. Furthermore, progesterone binds progesterone receptors (PR-A and PR-B) and transduces its effects primarily via the genomic signaling pathways and works through non-classical signaling pathways.

The Eker rat has been marked as an excellent in-vivo animal model for determining the gene by environment interactions and the role of hormones in UFs. Eker rats bearing a germline mutation in tuberous sclerosis 2 (Tsc2) tumor suppressor gene develop UFs in with an incidence of 65% between age 12 and 16 months. These UFs mimic the anatomic, histologic, and biologic traits of human UFs. Therefore, Eker rats serve as an excellent preclinical model to assess the potency and therapeutic efficacy of compounds for nonsurgical UF therapy. In 2002, the Walker group reported the hormonal factors required in developing UFs in the Eker rat. Thus, the dependence of these tumors on ovarian hormones has been demonstrated clearly. Ovariectomy on these rats at four months of age almost erodes tumor occurrence, although animals undergoing sham surgeries have a tumor incidence of about 65%.

**Genetic factors and mutations in UFs**

Besides hormonal influences, UFs are affected by genetic abnormalities like growth factor signaling pathways. Translocations in the high mobility group genes HMGA1 and HMGA2 were recorded, probably influencing fibroblast growth factor (FGF) pathway activity and increased the mass of lesions. The main mutations were identified within the gene coding the mediator complex subunit 12 (MED12) in women with UFs. This mutation is found in approximately 70% of UFs patients. All mutations of the MED12 gene were located in exon 2, and they may be responsible for the tumorigenesis. Fumarate hydratase (FH) is also a genetic predisposition to UFs. The FH gene can be mutated and has been seen in patients with hereditary leiomyomatosis and renal cancer (HLRCC). FH is an enzyme in the TCA cycle and acts as a standard tumor suppressor and mutations in FH significantly increased the risk of UFs and tumors in other tissues in patients with HLRCC.

**Endocrine-Disrupting Chemicals and UFs**

Endocrine-disrupting chemicals and their adverse health effects

The National Institute of Environmental Health Sciences defines endocrine-disrupting chemicals (EDCs) as "chemicals that intervene with the body's endocrine..."
system function and generate adverse developmental, neurological, reproductive, and immune responses." The endocrine system consists of glands distributed throughout the body and releases hormones into the circulatory system to control physiological and homeostatic functions. These glands are not restricted to the hypothalamus, pituitary, thyroid, and reproductive organs. EDCs can be natural or artificial and include dioxins, plasticizers, polychlorinated biphenyls (PCBs), organochlorines, pesticides, and polyfluoroalkyls (PFOAs), Bisphenol-A (BPA) and Diethylstilbestrol (DES), and phthalates. The common routes of exposure to EDCs in humans are ingestion, inhalation, and skin absorption, with a method of exposure dependent on each EDC's nature. In 1971, DES was the first pharmaceutical estrogen to be orally administered to decrease the risk of preterm labor or spontaneous abortion and related complications of pregnancy. Researchers associated fetal DES exposure with a variety of carcinoma of the cervix and vagina called adenocarcinoma. Shortly after its approval, The Food and Drug Administration (FDA) recommended discontinued the use of DES for pregnant women. EDCs can show non-monotonic dose-response curves, and low doses of EDCs can cause a pathophysiological effect. EDCs can show non-monotonic dose-response curves, and low doses of EDCs can cause a pathophysiological effect. EDC binding to nuclear receptors can change hormonal functions by imitating naturally occurring hormones in the body and preventing the endogenous hormone from binding or instead by interfering with the production of control hormones and/or receptors. Each EDC member may interact with more than one receptor, and many EDCs can combine with the same receptor, pointing to the complexity of animal and human responses to environmental EDC exposures. For example, the xenoestrogen BPA has been shown to bind and stimulate (ER), estrogen-related receptor gamma (ERRγ) and pregnant X receptor (PXR). Furthermore, other EDCs (i.e., DES, PCBs, ******

Table 1. Summary of environmental EDC exposure in animal and human experimental models in hormone-dependent diseases.

<table>
<thead>
<tr>
<th>EDCs Compound</th>
<th>Model exposure</th>
<th>stage of exposure</th>
<th>Main DNA damage and epigenetics modification</th>
<th>Outcomes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisphenol-A (BPA)</td>
<td>In mice utero</td>
<td></td>
<td>Increase EZH2 expression in mammary glands</td>
<td>Breast Cancer</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>In mice utero</td>
<td></td>
<td>Increase the mRNA level and expression protein of Hoxa10</td>
<td>Endometrial cancer</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>In Rat neonatal</td>
<td></td>
<td>Hypermethylation in ER in testis, and epigenetic modification</td>
<td>Affect the spermatogenesis and fertility</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>In Rat postnatal</td>
<td></td>
<td>Silencing of HOXA 10 gene in uterine stromal cells</td>
<td>Affect Embryo implantation</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>In Vitro Human fibroid cell line</td>
<td></td>
<td>Activation MAPK/ERK/c-fos Pathway</td>
<td>Uterine fibroids</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>In Vitro Human fibroid cell line</td>
<td></td>
<td>Activation of non-genomic signaling Src, EGFR, RAS, ERK</td>
<td>Uterine fibroids</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>In rat Postnatal</td>
<td></td>
<td>Increased ER expression in hypothalamus and increased AR expression in prostate</td>
<td>Prostate cancer</td>
<td>64,65</td>
</tr>
<tr>
<td>Vinlozolin (Pesticide)</td>
<td>Fetal rat</td>
<td></td>
<td>Epigenetic modification by altered DNA methylation in germ cell lines</td>
<td>Undescended testes, delayed puberty, and prostate disease</td>
<td>66,67</td>
</tr>
<tr>
<td>Diethylstilbestrol (DES)</td>
<td>In Rat Postnatal</td>
<td>Prescribed drug at 1970</td>
<td>Increase DNA damage γ-H2AX and repressing DNA repair sensor MRN complex</td>
<td>Uterine fibroids</td>
<td>36,38</td>
</tr>
<tr>
<td></td>
<td>In Rat Postnatal</td>
<td></td>
<td>Increase in H3K27 by upregulation of EZH2 expression</td>
<td>Uterine fibroids and adenocarcinoma</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>In mice utero</td>
<td></td>
<td>Increase the EZH2 expression</td>
<td>Breast cancer</td>
<td>58</td>
</tr>
<tr>
<td>Permethrin (Insecticide)</td>
<td>In Rat postnatal</td>
<td></td>
<td>Increase TNFα, IL-1β, IL-2, IFN-γ and RANTES</td>
<td>Suppression of the immune system and Induce leukocytosis, and suppress T- and NK-cell functions</td>
<td>69</td>
</tr>
</tbody>
</table>


http://aprh.journals.ekb.eg/
PFOA, and phthalates) can also bind to ERs. EDCs can also induce both genomic and non-genomic signaling. Both BPA and DES, for example, have been shown to activate nongenomic signaling pathways by ER. Notwithstanding the mechanism of action, EDCs have been connected to several adverse health effects including cardiovascular disease, diabetes, obesity, reproductive tract disorders, and neurodevelopmental dysfunctions. Hormone-related cancers are connected to exposure to EDCs (Table 1).

Endocrine-disrupting chemicals and immune system response

EDCs exposure directly impacts inflammatory processes may lead to the immune response’s weakness against bacteria, fungi, viruses, and cancer cells. Based on the literature that describes the link between the endocrine and immune systems, many investigators prove the theory that the immune system and natural hormones are targets of EDCs. EDCs compounds can also generate an immune response in macrophages throughout their specific receptors, i.e., ERs. ERs are parts of the nuclear receptor family of ligand-dependent transcription factors. Hence, macrophages’ exposure to estrogenic compounds may stimulate or repress the expression of nuclear factor kappa B (NFkB), which leads to remodeling of inflammatory mediators and cytokine secretion. Activated macrophages induce inflammatory responses by causing the secretion of various inflammatory mediators and pro-inflammatory cytokines such as nitric oxide (NO) and interleukin 6 (IL-6).

The impact of developmental exposure to endocrine-disrupting chemicals on UF development

Two extensive prospective studies found a positive relationship between developmental EDC exposure and UF risk. In the Nurses’ Health Study II, 11,831 cases of UFs were diagnosed in 1.3 million person-years of follow-up (over 20 years), making this the most comprehensive prospective study to assess the impact of prenatal DES exposure on UFs. In this study, prenatal exposure to DES raised the risk for UFs by 13% in women above 35 years of age. Moreover, exposure through the first trimester of gestation was the most dangerous with an increased risk of 21% compared to unexposed women. Another study, the NIEHS Uterine Fibroid Study, defined a positive correlation between DES exposure and UFs, in which 1364 DES-exposed or unexposed women aged 35–49 in the Washington DC area were selected for UFs diagnosis. In this study, huge UF masses were more common in those exposed to prenatal DES. The odds ratio for white women exposed to DES was 2.4, which was even higher for large UFs. However, there were not enough data about exposed AA women for significant evaluations. A further study used a subset of the NIEHS Sister Study cohort to estimate the risk for UFs after prenatal exposures in 3,534 AA women aged 35–59. In this study, DES exposure, maternal or gestational diabetes, and monozygotic twins with a relative risk (RR) of 2.02, 1.54, and 1.94, respectively, were the most critical factors associated with increased risk of UFs. Nevertheless, not all data were consistent. When a larger portion of NIEHS Sister Study data was assessed, the correlation between DES and UFs was not clear. This study included 19,972 Caucasian women in a prospective analysis and observed five early life factors to be associated with a greater than 20% higher risk for UFs:

(i) Prenatal DES exposure
(ii) Gestational diabetes
(iii) Pregnancy diabetes (mothers who had diabetes before pregnancy)
(iv) Soy formula
(v) Gestational age at birth

The most influential relationships were women whose mothers had pregnancy diabetes and those born more than a month early. DES exposure appeared in an adjusted RR of 1.42, and gestational diabetes and soy formula had a similar RR of 1.28 and 1.25, sequentially. Although there is an inconsistency in this study: when dividing women who reported to have ’definitely’ or ‘probably’ been exposed to DES, women exposed showed no increased risk, while those who classified as being exposed had a RR of 2.07, this study also described that experiencing menarche under the age of 10 or 11 resulted in RR of 1.54 and 1.32, respectively, and being poor had a RR of 1.24. Another study from 85 UF cases reported an association between prenatal DES and UF risk. Experimental animal studies have shown a clear link between DES exposure and UFs; the Eker rat animal was used to illustrate the increasing risk of genomic instability due to early-life DES exposure. Developmental exposure to DES can alter the rat’s myometrium stem cells (MMSCs) ability to repair and reverse DNA damage. It has been shown that DES-MMSCs cause DNA damage accumulation than vehicle-exposed (VEH)-MMSCs, and showed less DNA machinery to repair it.

Endocrine-disrupting chemicals and DNA damage in UFs

DNA damage and repair mechanisms

Accumulated studies showed that several risky human-made agents were added to the environment’s naturally existing agents. Furthermore, shifting in the individual’s lifestyle and food intake and hormonal imbalances, medicines, illness, travel, radiation or chemical exposures, stress, insomnia, and many factors can influence the body’s intracellular environment, which affects the most vital biomolecule i.e., DNA. Exposure to these agents exhibits direct and indirect
effects on the cell, causing an abundance of changes resulting in inflammatory responses, mutation, genomic instability, and tumorigenesis. Varying by exposure, different types of DNA damage were found in the uterus such as single-strand breaks (SSBs), primary sites (AP sites), base damages, mismatched bases, or double-strand breaks (DSBs) which can occur as separated lesions or appear in batches (two or more separate lesions). If it remains unrepaird, SSBs can be turned into DSBs on the sister chromatids through DNA replication, which may exacerbate damaging consequences. Various studies reported that recurrent incidents of damage to the myometrial cells might affect the repair capacity, thus shifting the uterine environment to chronic inflammation. This inflammation milieu creates more DNA damage as a closed loop cycle which generates a typical UF predisposition environment. Accumulation of unrepaired DNA damages has been incorporated with many health and disease risk outcomes, although various DNA repair pathways can avoid these damages naturally in the human body. Some DNA repair pathways either go precisely through the lesion or in coordination to secure regular repair. Nucleotide excision repair (NER) is the mammalian pathway to eliminate massive DNA injuries like those developed by UV light, environmental hazards, and chemotherapeutic agents. Xerodermia pigmentosum (XP) group of proteins such as XPC, XPF, XPD, XPG, and XPB help repair polymerase structural proteins (PCNA, CSA, RPA, RAD23, RFC, CSB, ERCC1) and ligases. Deviation in the XPC gene diminishes the NER repair function, leading to increased UV light sensitivity and more susceptibility to skin tumors. Homologous recombination (HR) and/or Non-homologous end joining (NHEJ) is the primary mechanism implicated in the DSB repair depending on the cell cycle status and the tumor protein 53 (TP53) level in the cell which, need a high level of accuracy to preserve genome integrity. Homologous recombination (HR) and Non-homologous end joining (NHEJ) is the primary mechanism implicated in the DSB repair depending on the cell cycle status and the tumor protein 53 (TP53) level in the cell which, need a high level of accuracy to preserve genome integrity. Homologous recombination (HR) and Non-homologous end joining (NHEJ) is the primary mechanism implicated in the DSB repair depending on the cell cycle status and the tumor protein 53 (TP53) level in the cell which, need a high level of accuracy to preserve genome integrity. Homologous recombination (HR) and Non-homologous end joining (NHEJ) is the primary mechanism implicated in the DSB repair depending on the cell cycle status and the tumor protein 53 (TP53) level in the cell which, need a high level of accuracy to preserve genome integrity. Homologous recombination (HR) and Non-homologous end joining (NHEJ) is the primary mechanism implicated in the DSB repair depending on the cell cycle status and the tumor protein 53 (TP53) level in the cell which, need a high level of accuracy to preserve genome integrity. Homologous recombination (HR) and Non-homologous end joining (NHEJ) is the primary mechanism implicated in the DSB repair depending on the cell cycle status and the tumor protein 53 (TP53) level in the cell which, need a high level of accuracy to preserve genome integrity. Homologous recombination (HR) and Non-homologous end joining (NHEJ) is the primary mechanism implicated in the DSB repair depending on the cell cycle status and the tumor protein 53 (TP53) level in the cell which, need a high level of accuracy to preserve genome integrity. Homologous recombination (HR) and Non-homologous end joining (NHEJ) is the primary mechanism implicated in the DSB repair depending on the cell cycle status and the tumor protein 53 (TP53) level in the cell which, need a high level of accuracy to preserve genome integrity.

DNA damage repair sensor complex MRN (MRE11, RAD50, NBS1) and HR mediators and effectors (BRCA1, BRCA2, RAD51) and ligases. Still, HR needs a homology sequence to repair DSB, which is inherent in cycling cells with proteins such as the Breast cancer gene (BRCA1, BRCA2), Nijmegen breakage syndrome 1 (NBS1), meiotic recombination 11 (MRE11), RAD50, replication protein A (RPA), Bloom syndrome (BLM), EXO1, DNA Replication Helicase/Nuclease 2 (DNA2), and C-terminal binding protein 1 (CtIP). Reducing DNA repair genes’ expression has been characteristic of increased cancer potential in various tissues, including sex steroid hormone-regulation in breast and prostate cancer.

DNA damage and repair mechanisms in the context of UF risk factor

DNA damage and repair and various factors have been associated with developing UF risk factors. However, it is unclear which physiological pathways stimulate UF formation and whether they depend on ethnic/racial disparity or if these are entirely genetically based. Imbalances in the hormonal signaling associated with DNA damage response, consequently tumorigenesis. E2 and P4 are the main female sex steroid hormones required to develop the reproductive system (e.g., menstes regulation, fertility, pregnancy), female puberty features, and other vital biological functions. Balances are required for proper body function, and any disbalance can cause adverse effects. The elevation in E2 levels has been noted in most UF patients, while P4 is associated with UFs growth. A decrease in steroid hormones is observed in the post-menopausal period by shrinking UF size. In the uterine cells, ER and PR have a role in the DNA repair system. ER manages MDC1 (mediator of DNA damage checkpoint 1), which directly interacts with γ H2AX phosphorylated H2AX, to initiate sequences of DNA Damage Response (DDR) by recruiting MRN (MRE11, RAD50, NBS1) complex, which is essential for NHEJ and HR repair.

Furthermore, CDK2 in complex with Cyclin A and PR is needed to stimulate some progesterone target genes. ER is identified in UFs anomalies, affecting the DNA repair capacities with Cyclin A and PR is needed to stimulate some progesterone target genes. ER is identified in UFs anomalies, affecting the DNA repair capacities. In most UF patients, alterations in PRs have exhibited a vital impression on DNA repair genes and other downstream genes included in apoptosis, cell cycle progress, cellular proliferation, and tumorigenesis. Therefore, understanding the hormonal imbalances and sex hormone receptors’ alterations and their implication on DNA damage repair capacities will highlight the UF etiology. Recently the Al-Hendy group confirmed the UFs primary cells level (which are the origin propagator of UFs) and UF stem cells. Freshly isolated human UFs and adjacent normal myometrial tissues were labeled with Stro-1+/CD44+ dual-positive surface markers. The data showed a significant impairment of DNA repair capacity connected to UF genomic integrity following initiation/propagation of the UF growth significant reduction in DNA damage repair machinery by an upregulation in the expression of phosphorylation of histone H2AX at Serine 139 (γ-H2AX) as the sensor of DNA DSB marker and downregulation of DNA repair sensor complex MRN (MRE11, RAD50, NBS-1) for HR DDR initiation in fibroid stem cells. These genes linking to DSB binding (BRCA2, RAD51, RAD51AP1, and RAD52) and HR mediators and effectors (BRCA1, BRCA2)
MDC1, BARD1, CHEK1, and CHEK2) were similarly downregulated\(^{83,107}\). These data suggest that human UF stem cells have damaged DNA repair capacity compared to normal myometrium stem cells, leading to more mutagenesis followed by additional growth and propagation of UF tumors\(^{108}\). Environmental exposures to EDCs affect natural body functions. Environmental exposures and changed ER signaling followed by DNA damage accumulation reduce DNA repair genes/proteins expression followed by a shift to pro-proliferative signaling\(^{38, 109-111}\). Various epidemiological studies have shown that early life environmental-exposure to EDC leads to epigenetic modifications correlated with UF development later in life\(^{102-105}\). During specific windows of uterine development, EDC exposure induces EZH2 (Enhancer of zeste 2 protein which controls chromatin and chromosome structure) and H3K27me3 (histone 3 lysine 27 trimethylation involved in epigenetic modification to heterochromatin packaging) by receptor-based non-genomic signaling, causing a reduction in gene expression of RAD51 meaning lack of DSB repair capacity\(^{112}\).

In the Eker rat UF model, rats exposed to DES at the early stages showed accelerated UF growth at later stages compared to unexposed animals. Additional features are the significant proliferation of Stro1+/CD44+ myometrial stem cells (MMSCs), the altered role of HMGA2 in the development of smooth muscle tumors, rapid accumulation of DSB with increased mutagenesis, and decreased capacity of DNA damages repair\(^{36, 38, 113, 114}\). The Al-Hendy group reported that Eker rats exposed to EDC-DES showed significant reduction in MRN (MRE11, RAD50, and NBS-1) complex, mediators genes BRCA2, and RAD51, which elevate the level of DNA damage by a significant rise of DNA damage sensor γ-H2AX with altered baseline expression of DNA repair genes and proteins (which are essential for recognizing and repairing DSBs). A drop in functional capacity for efficiently restoring DNA DSBs has also been described in Eker rat DES-MMSCs compared to unexposed matched stem cells\(^{36, 38}\) presenting a driver for mutations that may promote tumors’ development later in adult life.

**Endocrine-disrupting chemicals and inflammation in the pathogenesis of UF**

There is a lack of information concerning the effect of EDCs on immune system response and induction of inflammation in UF pathogenesis. Recently, the Al-Hendy group generated preliminary data showing that early life exposure to DES creates a chronic inflammation milieu in Eker rat stem cells exposed to DES compared to unexposed MMSCs through upregulations of pro-inflammatory cytokines and chemokines IL-1a, IL1b, IL17, IL-18, IL-6, TNFα, INFγ, CX3CL1, CXCL5, CCL5, CCL2, and CCL7. Moreover, the data showed downregulation in anti-inflammatory cytokines IL-10 and IL1Ra\(^{115}\). More research is needed to understand the mechanism of EDCs and inflammation outcomes in UFs.

![Figure 1. VITD3 Metabolism and Pathways](http://aprh.journals.ekb.eg/)

Sunlight, diet, and supplements are the main sources of VitD in humans. VitD is synthesized in the skin from 7-dehydrocholesterol; transferred to the liver, where it converts to 25(OH)D by 25 hydroxylases; and then to the kidney to convert to the active form 1,25(OH)D. It then activates VDR Receptors in the target cells.
The Impact of VITD3 Intervention on UF Pathogenesis/Etiology

VITD3 and its metabolism

Vitamin D is a steroid derivative, which has the primary function in calcium homeostasis and bone metabolism. Vitamin D has a unique metabolism. It is synthesized in the skin when exposed to sunlight from 7-dehydrocholesterol. Subsequently, transfer to the liver turns it to 25-hydroxyvitamin D [25(OH) D] and then the kidney to the active form 1,25-dihydroxy vitamin D [1,25(OH)2D].

VITD3 deficiency and inflammation

Studies demonstrated that VITD3 deficiency raises the risk of tumor development. Inflammation is added as one of the 10 signs of cancer. Cytokines and immune cells in the inflammatory milieu work as direct growth and migratory factors for cancer cells. Studies have revealed that tissues with chronic inflammation commonly display a high tumorigenesis incidence. VITD3 can modify the innate and adaptive immune responses. Studies of tumor cells exhibited that VITD3 exerts vital regulatory effects on some pathways implicated in inflammation besides its role in the inflammatory microenvironment; however, the evidence combining immune response and VITD3 in the tumor's context is still limited.

The role of VITD3 on the proliferation and ECM deposition in UFs

Many studies have recently estimated the impact of VITD3 on UF primary cells and revealed that VITD3 represses cell proliferation through various mechanisms, including inhibition of PCNA, cyclin D1, CDK1, Bcl2, and inhibit COMT function in human fibroid cells. Raised ER-α, PR-A, and PR-B were also verified in human UFs, and treatment with VITD3 suppressed those receptors in human UF cells. Also, administering VITD3 or its analog non-hypercalcemic (paricalcitol), a potent VDR activator, efficiently represses human UF cell propagation in vitro and the Eker rat animal model.

The Al-Hendy group reported reduced levels of VDR in human UFs matched to the adjacent normal myometrium. Treatment with VITD3 significantly induced nuclear VDR expression in a concentration-dependent manner in immortalized human uterine fibroid cell line (HuLM) cells. VITD3 also significantly decreased ECM-associated collagen type I expression, proteoglycans, fibronectin, and PAI-1 in HuLM cells. Therefore, there is evidence that VITD3 and VDR agonists might be effective antitumor and anti-inflammatory agents that can be considered nonsurgical orally administered remedial choices for the efficient, safe, and long-term medical therapy and/or restriction of UFs. Nevertheless, human therapeutic and preventative clinical trials are needed still to prove the efficacy of VITD3 in patients with symptomatic UFs.

VITD3 deficiency and etiology of UFs

Hypovitaminosis D is an identified risk factor for developing UFs. Numerous studies have confirmed that (AA) women have 10 times more severe cases and a higher risk of developing UF than Caucasian women. Al-Hendy group showed that VITD3 deficiency had been connected with rising sex steroid receptors and proliferation-related gene expression increasing fibrosis and immunosuppression by Tregs (regulatory T cell implicated in preserving the homeostasis and inhibiting autoimmunity) expansion increased inflammation and DNA damage accumulation in murine myometrium, leading to increased risk of tumor development due to attenuation of the homology-dependent DSB repair.

VITD3 intervention reverses the impairment of DNA repair

The Al-Hendy group has previously determined that VITD3 efficiently represses UF growth in vitro and in UF animal models, strengthening the concept that VITD3 and its potent analogs might offer promising prevention and early treatment options for UFs. In 2018, Ali et al. reported that VITD3 treatment diminishes DNA damage and alters the DNA damage response via induction of VDR in human UF cells and upregulates the expression of DNA repair sensors genes (MRE11, RAD50, NBS1), cell cycle checkpoint (CHEK1, CHEK2, RAD17, BRCA1), and DSB binding (RAD51, BRCA2). These data indicate the antitumor effect of VITD3 in both human UFs tissues and animal model stem cells. The amelioration of pathogenic DNA damage and repair of key DDR genes’ expression diminished in VDR-deficient UF cells. In addition to the effect of VITD3 on the UFs and UF cells, recently El-Kafas et al. have shown for the first time that VITD3 treatment improves DNA repair machinery load in MMSCs early-life exposed to DES and upregulates the expression of DNA repair sensors genes (MRE11, RAD50, NBS1), mediator’s genes (CHEK1, CHEK2, and BRCA1) and DSB effector genes (RAD51, BRCA2), and downregulate the DNA damage sensor γ-H2AX. These studies provide a novel therapeutic strategy for the prevention of UF development. Collectively these data validated the relationship between VITD3 and uterine fibroid development.

[http://aprh.journals.ekb.eg/](http://aprh.journals.ekb.eg/)
Impact of chronic inflammation in UF's pathogenesis and the role of VITD3

The role of chronic inflammation in UF's

Inflammation is considered one of the main factors for tumor development; In the context of malignant tumors, the function of the immune system is not understandable. Crosstalking between cancer cells and host immune cells in the tumor microenvironment generates an immunosuppressive milieu that boosts tumor growth and helps the tumor evade immune attack\textsuperscript{133}. Several studies showed that a sustained inflammatory microenvironment could induce tumors\textsuperscript{134-137}. The tumor inflammatory microenvironment prompts the expression of numerous pro-inflammatory cytokines, enhancing angiogenesis and tumor outgrowth, invasion, and metastasis, and expedites tumor development\textsuperscript{138-140}. Chronic inflammation has been associated with increased reactive oxygen species (ROS) levels, DNA damage accumulation, and alterations in DNA repair action connected with many health problems\textsuperscript{141}. Environmental exposures or many adverse changes were occurring in the body leading to E2 and P4 signaling disfunction. Changes in sex hormonal signaling can release pro-inflammatory cytokine and chemokine in the uterine milieu, disturbing the homeostasis, which improves myometrial cells' proliferation of higher risk of producing UF\textsuperscript{142-143}. Myometrial contraction may cause ischemic destruction (or destruction from reperfusion after ischemia in the myometrial muscles), creating inflammatory responses in the uterus through menstrual bleeding. Damaged cells evading removal or apoptosis through the follicular phase are also known to work as an excellent candidate for UF progenitor cell growth\textsuperscript{144}. The uterine wall comprises healthy myometrium, which is secured against infection by natural killer (NK) cells, B lymphocytes, and T lymphocytes. Immune cells deliver cytokines (low molecular weight proteins), which interfere with and control immunity, hematopoiesis, and inflammation. For example, Interleukin 15 (IL15), known to manage the proliferation and effector function of NK cells, is also believed to be associated with menses, implantation, and decidualization\textsuperscript{143,144}. IL11 and IL13 combine with TGF-β, which is overexpressed in fibroid pathogenesis\textsuperscript{143,145,146}. P4 favors T helper 2 cells' differentiation and represses T helper 1 cells included in the menstrual cycle and has a function as an anti-inflammatory agent (targeting NK cells, monocytes, and dendritic cells) in the uterine cervix\textsuperscript{147-149}. Depending on the size of the UF tumor and a woman's age, higher levels of nuclear factor kappa B (NFkB), IL-1β, cyclooxygenase 1 (COX-2), tumor necrosis factor-alpha (TNF-α), and inducible nitric oxide synthase have been detected in UF as matched to the adjacent myometrial tissue\textsuperscript{150}. Mutation in MED12 influences NF-κB driving a rise in inflammatory responses, which induces DNA damage, making the uterus millie a perfect condition for UF growth\textsuperscript{151}. UF cells are marked by inflammatory responses\textsuperscript{152}. A recent study revealed that UF stem cells generate a chronic inflammatory microenvironment that induces tumor development\textsuperscript{115}. The Al-Hendy group

\textbf{http://aprh.journals.ekb.eg/}

267
studies confirm the release of pro-inflammatory cytokines like (TNF-α, IL-1β, NFkB, TSLP, and INFγ) in uterine tissues compared with matched normal myometrium.156

Furthermore, Serum Levels of TNF-α were elevated in symptomatic UFs patients.155,156 More studies are currently in progress to explore the role of inflammation in UF pathogenesis. Notably, chronic inflammation is correlated to DNA damage induction by generating a reactive oxygen species and oxidative stress.159-160 Recent evidence points to the functional correlation between DNA damage sensors, DNA repair mechanisms, and the innate immune responses.159 The inflammatory response and immune infiltration close cycle lead to a genomic imbalance in the uterus, encouraging more danger towards UF progress.146

**VITD3 treatment decreases inflammation in UFs**

It was well known that VITD3 alters the inflammatory microenvironment of UF tumors, including regulating the reaction between immune and tumor cells, which control the cytokines levels, inhibiting NF-kB signaling pathway, and suppressing the immune cells (macrophages, DCs, B cells, and T cells), and the prostaglandins pathway and upregulating MKP5.160,161 Blood cytokine levels are higher in colorectal cancer patients than in healthy controls.162 Another study done by Barrat et al. confirmed that VITD3 enhances IL-10 gene expression and inhibits the Th1- and Th2-specific transcription factors.163 IL-10 has a main role in controlling chronic inflammatory processes development, and its absence produces chronic inflammatory responses.164 IL-10 inhibits the production of pro-inflammatory cytokines, which, in turn, stimulates tumor growth.165 IL-6 is a pro-inflammatory cytokine with pro-tumorigenic potential and a vital key effector in prostate cancer and colorectal cancer development.166 The results demonstrated that IL-6 affects COX-2 and can increase angiogenesis and tumorigenesis.167 Moreover, VITD3 represses the activation of the p65 subunit of the NF-kB complex in colon cancer cells by blocking the binding of NF-kB to DNA.168,169 Therefore, VITD3 may work as a potent inhibitor for cancer via suppressing the NF-kB signal pathway.

To fill the gap between VITD3 and inflammation in UFs, we conducted a pioneer study using the Eker rat model with early-life EDC exposure. Our study showed that treatment of MMSCs from DES-exposed myometrium with VITD3 altered the inflammatory microenvironment by downregulation of the inflammatory cytokines and chemokines, including interleukins IL-1α, IL1β, IL17, IL-18, IL-6, tumor necrosis factor-α (TNF-α), Interferon-gamma (INFγ), CX3CL1, CXCL5, CCL5, T, and upregulating the anti-inflammatory cytokines IL-10.115

**CONCLUSIONS**

UFs significantly impact the healthcare system and women’s quality of life. The complete etiology of UF development remains largely unknown. There remains a lack of information concerning the causative agents and a lack of adequate non-invasive therapies. Although more clinical studies correlate exposure to EDCs with the development of UFs, it is now clear that both the level and the time of exposure are essential. Many epidemiological studies have confirmed that VITD3 deficiency is linked to the high frequency of UFs. Experimental data have also demonstrated that VITD3 can repress UF growth via multiple mechanisms (Figure 1). Future studies are needed to:

1. Determine the interactions between MMSCs and immune cells in response to EDCs, therefore better to understand the initiation and progression of UF pathogenesis
2. Investigate the immunomodulatory activity of VITD3 that would improve the efficacy of the immune system response,
3. Identify proper DNA repair biomarkers and anti-inflammatory agents that would help prognosticate, diagnose, and treat UFs with a safe, long-term, non-invasive therapeutic agent.

**Conflict of interest**

The authors declare that there is no conflict of interest regarding the publication of this paper.

**Funding**

This work was supported in part by the US National Institutes of Health grants: R01 HD094378, R01 ES 028615, R01 HD100367, U54 MD007602, and R01 HD094380.

**REFERENCES**

5. Trasande, L.; Zoeller, RT.; Hass, U.; Kortenkamp, A.; Grandjean, P.; Myers, JP; DiGangi, J; Bellanger,


10. Mahase, E. Uterine fibroid drug is recalled after case of liver failure requiring transplant prompts EU review. BMJ. 2020, 368, m1112.


12. Cook, JD.; Davis, BJ.; Cai, SL.; Barrett, JC.; Conti, CJ.; Walker CL. Interaction between genetic susceptibility and early-life environmental exposure determines tumor-suppressor-gene penetrance. PNAS 2005, 102 (24), 8644-8649.


18. Antoniou, A.; Pharoah PD.; Narod S.; Risch HA.; Eyfjord JE.; Hopper JL.; Loman N.; Olsson H.; Johansson, O.; Borg, Å.; Pasini B. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am. J. Human Genetics 2003, 72 (5), 1117-1130.


25. Chang, HL.; Senaratne TN.; Zhang, LH.; Szotek, PP.; Stewart, E.; Dombkowski, D.; Preffer, F.; Donahoe, PK.; Teixeira J. Uterine leiomyomas exhibit fewer stem/progenitor cell characteristics when compared with corresponding normal myometrium. Reprod. Sci. 2010, 17 (2), 158-167.


35. Howe, SR.; Gottardis MM.; Evertt JL.; Goldsworthy TL.; Wolf DC.; Walker C. Rodent model of reproductive tract leiomyomatosis. Establishment and characterization of tumor-derived cell lines. *Am. J. Pathol. 1995*, *146* (6), 1568.


43. Toro, JR.; Nickerson ML.; Wei MH.; Warren MB.; Glenn GM.; Turner ML.; Stewart L.; Duray P.; Toure O.; Sharma N.; Choyke P. Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America. *Am. J. Human Gene.* **2003**, *73* (1), 95-106.

44. Stewart, L.; Glenn, GM.; Stratton, P.; Goldstein AM.; Merino, MJ.; Tucker MA.; Linehan, WM.; Toro JR. Association of germline mutations in the fumarate hydratase gene and uterine fibroids in women with hereditary leiomyomatosis and renal cell cancer. *Arch. Dermatol.* **2008**, *144* (12), 1584-1592.


47. Gore AC.; Chappell VA.; Fenton SE.; Flaws JA.; Nadal A.; Prins GS.; Toppari J.; Zoeller RT. EDC-2: the Endocrine Society's second scientific statement on endocrine-disrupting chemicals. *Endocrine Rev.* **2015**, *36* (6), E1-50.

48. Kuiper, GG.; Lemmen JG.; Carlsson BO.; Corton JC.; Safe SH.; Van Der Saag PT.; Van Der Burg B.; Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. *Endocrinology. 1998*, *139*(10), 4252-63.


53. Henry, ND.; Fair PA. Comparison of in vitro cytotoxicity, estrogenicity and anti-estrogenicity of triclosan, perfluorooctane sulfonate and

http://aprjournals.ekb.eg/


102. Borahay, MA, Asoglu MR, Mas A, Adam S, Kilic GS, Al-Hendy A. Estrogen receptors and signaling


triggers inflammation and DNA damage profile in murine myometrium. *Int. J. Women's Health*. 2018, 10, 503.


165. Artaza, JN, Norris, KC. Vitamin D reduces the expression of collagen and key profibrotic factors by inducing an antifibrotic phenotype in mesenchymal multipotent cells. *J. Endocrinol.* 2009, 200 (2), 207.


