REVIEW ARTICLE

GMJ.2024;13:e3525
www.gmj.ir

Received 2024-08-07
Revised 2024-09-01
Accepted 2024-10-09

Growth Factors, Signaling Pathways and other Biochemical Markers effect on Nucleotide Excision Repair (NER) Suppression in Uterine Fibroids (Leiomyomas); A Systematic Review

Fatemeh Movahed [1], Shiva Taghlidi [2], Sheida Abbasi [3], Fatemeh Asadi [4]✉, Reza Yusofvand [5]

[1] Department of Gynecology and Obstetrics, Yas Hospital, Tehran University of Medical Sciences, Tehran, Iran
[2] Università Degli Studi Di Milano-Bicocca
[3] Department of Obstetrics and Gynecology, School of Medicine, Tehran University of Medical Sciences. Tehran, Iran
[4] Department of Genetics, Izeh Branch, Islamic Azad University, Izeh, Khuzestan, Iran
[5] Department of Exceptional Talents , Faculty of Medicine Sciences , Lorestan University of Medical Sciences , Khorramabad ,Iran

Abstract

Among human malignancies, uterine fibroids (UF) rank high in frequency and severity of symptoms. Vitamins, mutant genes, growth factors, microRNAs, steroid signaling pathways, and a host of other substances can influence UF. This study set out to compile the effects of various agents, such as growth factors and signaling pathways, on nucleotide excision repair (NER) in uterine fibroids. For this review, the PRISMA guidelines were followed. The following search phrases were utilized in the PubMed, Ovid, and Cochrane Library databases: "fibroid, leiomyomata, leiomyoma, TGF-*, vitamin D, RACK 1, WNT, miR, mutant gene." Every study, in vitro, in vivo, and clinical, was also taken into account. From January 1, 2000, to December 31, 2023, we looked for any proceedings published by the Pacific Coast Fertility Society, SGI,American Society for Reproductive Medicine, Association of Gynecologic Laparoscopists, ASRM, or ASRM. Relevance was determined by looking at titles first, and then abstracts. Full reports included an assessment of all citations that could be relevant. From the original pool of 990 studies, 24 were included in the data analysis. Methods, steroid signaling, growth factors, microRNAs, vitamins, and mutant genes were the main categories into which studies were subdivided. Proliferation of cells and an excess of disordered extracellular matrix (ECM) were identified as hallmarks of uterine leiomyomas. This pair of activities is carefully controlled by a network of signaling pathways. Furthermore, future research should employ current procedures consistently in order to conduct meta-analysis on the results. This will greatly assist in developing treatment protocols.
[GMJ.2024;13:e3525] DOI:10.31661/gmj.v13i.3525

Keywords: Uterus Fibroids; TGF-ß, Vitamins, RACK1

Introduction

The most common tumors found in the female genital tract are uterine leiomyomas, which were first described by a British pathologist from St. George's Hospital, Matthew Baillie [1, 2]. Several gynecological issues, as well as a heavy financial and health care cost, have been associated with them since then. The truth is that leiomyomas affect any-

✉ **Correspondence to:**
Fatemeh Asadi, Department of Genetics, Izeh Branch, Islamic Azad University, Izeh, Khuzestan, Iran
Telephone Number: +987143311172
Email Address: fatemehasadi@miau.ac.ir

thing from half a million to eighty million persons [3, 4]. Their estimated yearly direct and indirect costs to the United States are $34.4 billion [5, 6]. Uterine fibroids (leiomyomas) are affected by growth hormones, signaling pathways, and other substances that decrease nucleotide excision repair (NER).

Regardless, steroid hormones influence a wide range of biological processes through binding to certain receptors, including development, reproduction, energy balance, metabolism, immunology, and behavior [7, 8]. Sex steroids (including androgens, oestrogens, and progesterones) and corticosteroids (including glucocorticoids and mineralocorticoids) are two ways to classify them. Nanomolar concentrations of steroid hormones in bodily fluids allow the endocrine system to maintain constant communication with the neurological and immunological systems. The body's natural and synthetic components, known as endocrine-disrupting chemicals (EDCs), are increasingly implicated in a host of harmful health effects, including reproductive dysfunction [9, 10], cognitive impairment [11, 12], cognitive deficits [13], metabolic diseases and disorders [14] and cancers [15, 16]. Due to their hormonal properties, which include oestrogen and androgen activity, EDCs have been associated with an elevated risk of tumors when present.

Although EDCs are still largely unknown, they play a significant role in regulating the tumor microenvironment. Therefore, RACK1 is an immunological EDC target that has been extensively studie [17] and this molecular actor mediates the progression of cancer.

Several investigations have found that uterine fibroids are associated with obesity, a positive family history, low vitamin D levels, and high TGF-Î²3 blood concentrations. Two other studies also found a correlation between vitamin D and uterine fibroids. The growth of smooth muscles and UFs in the uterus are controlled by a TGF-β receptor. Laboratory investigations have shown that the uterine smooth muscle directly touching the fibroid expresses a large amount of TGF-β [18, 19]. Expression of TGF- β is nearly double in UF tissue compared to normal smooth muscle, the control group [20, 21]. In contrast to healthy myometrium tissue, UF tissue contains roughly five times the amount of TGF- β 3 [22, 23]. The process of TGF- β signaling is highly involved in creating UFs. Myometrium and UF cancers express different types of TGF- β and the receptors that bind to them. By suppressing proliferation and inducing apoptosis, TGF- β effectively suppresses tumors in smooth muscle cells. In addition, UFs show an overexpression of TGF- β, which seems to be crucial for their development and advancemen [24-26].

Although uterine fibroids have a huge influence, the specific pathobiology of them is still a mystery. As a result, there is currently no viable medical therapy option. Leiomyoma formation is thought to begin with the proliferation of a smooth muscle cell [27, 28]. More changes to the signaling pathways are supposedly required [29].

Identifying the role of innate immunity in cancer development and comprehending endocrine-mediated microenvironment modulation may depend on how EDCs regulate RACK1 in both settings. Hence, our review covered the fact that RACK1 is involved in reacting both immune and cancer cells to EDCs. The alterations to these signaling pathways and their linkages in order to identify possible treatment targets.Our research focuses on uterine fibroids (leiomyomas) and the mechanisms by which growth hormones, signaling pathways, and other substances inhibit NER.

Materials and Methods

Search Strategy

Protocol for the Reporting of Systematic Reviews and Meta-Analyses, or PRISMA, was followed throughout the course of this review (Figure-1). We utilized search phrases such "fibroid, leiomyomata, leiomyoma, TGF-*, vitamin D, RACK 1, WNT, miR, Mutant gene" to extensively explore the Ovid, PubMed, and Cochrane Library databases. Phrases related to the outcome and the population were used in all database searches in the same way. From 2000-01-30 through 2023-12-30, all databases were searched. Only English dates and publications were supplied.

Clinical, in vitro, and in vivo investigations were all part of this study. From 2000 to 2023, the proceedings of the following societies

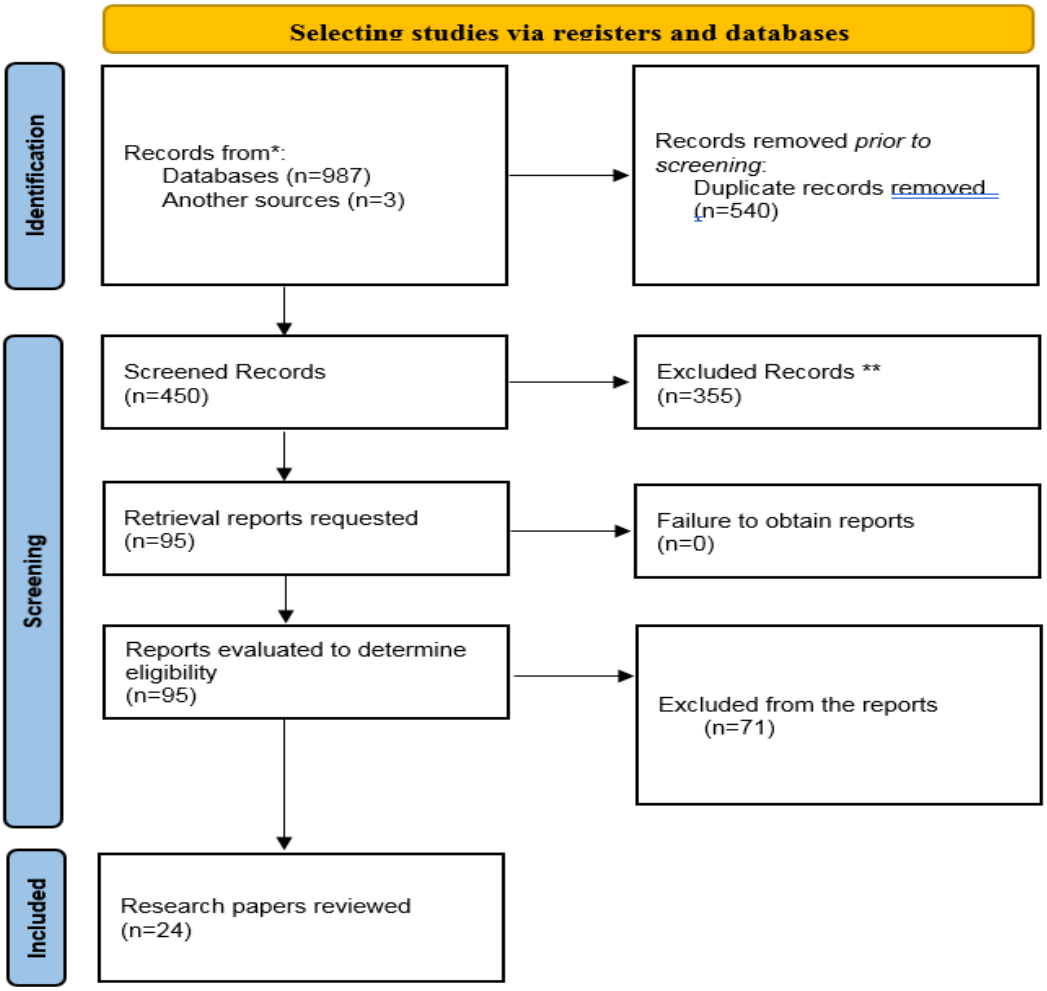

Figure 1. A PRISMA flow diagram including 24 out of 990 records.

were hand-searched: Pacific Coast Ripeness Society, American Society for Regenerative Pharmaceutical, American Association of Gynecologic Laparoscopists, and Society for Gynecologic Examination. After perusing the titles, we moved on to the abstracts to find any relevant information. All potentially relevant citations were reviewed in full reports.

Selection Criteria

This review included studies about steroid signaling, signaling pathways, growth factors, miRNAs, vitamins, and mutant genes. A paper that lacked molecular investigation on UFs, a study focusing on another disease or a specific population of patients, a randomized controlled trial, a case study, a review, an editorial, or a letter was not included.

Main Outcome Variables

Suppression of nucleotide excision repair (NER) in uterine fibroids (leiomyomas) was the primary outcome variable. Research on the suppression of nucleotide excision repair (NER) in uterine fibroids (leiomyomas) was included in all studies that assessed growth hormones, signaling pathways, or other agents. Following the guidelines laid out by the PICO (Population, Intervention/Exposure, Comparison, and Result) framework for effective surveys, we drafted the following audit address to guide the investigation: People with leiomyomas, or uterine fibroids, were the intended recipients. It failed to identify a particular intervention or comparison due to the extremely varied exposures for leiomyomas. The following factors were considered:

Table 1. Distribution of the Studies according to the Authers, orgine and Year

Reference	Main Auther	orgine	Year
[18]	Arici	New Haven, Conn	2003
[32]	Halder	Nashville, Tennessee	2013
[33]	Arici	USA	2000
[34]	Levens	USA	2005
[35]	MichałCiebiera	Poland	2016
[36]	Yangyu Zhao	USA	2007
[37]	Halder	Tennessee	2011
[38]	Xi-Xi Cao	China	2011
[39]	Xi-Xi Cao	China	2010
[40]	Buoso	Italy	2019
[41]	Asada	Japan	2008
[42]	Panagiotis	Greece	2008
[43]	Govindan, Sujatha	Russia	2009
[44]	Zhu	China	2021
[45]	Gallagher	USA	2018
[46]	Makwe	NGA	2021
[47]	Vinita	India	2019
[48]	Li	China	2020
[49]	Ciebiera	Poland	2018
[50]	Ciebiera	Poland	2018
[51]	Markowski	Germany	2012
[52]	Wang	Newark, NJ	2007
[53]	Markowski	Bremen	2014
[54]	Jokinen	Finland	2023

Signaling based on steroids, TGF-ß, 25-hydroxyvitamin D, RACK1, Wnt/β-catenin, vitamin effects, mutant genes, and so on.

Data Extraction

All citations of included studies were exported to Mendeley. After deleting duplicates, all references were manually screened and categorized. To choose studies that could be included in the review, one of the writers looked at the titles and abstracts to see if they were eligible. Then, they read the entire texts to see if they were. Final decisions regarding manuscript screening and data extraction were made by one person for consistency. Finally, the required data of the full-text screening were exported into extraction table. In this study, general information (title, author, journal, year), cell source type, type of investigation, method of testing and effect on UF was collected from each of the studies (Table-2). Also, in Table-3,: patients characteristics, method of measurement of variables, animals (If the study is carried out on animals), cell line, steroid signaling, signaling pathway, grows factors, miRNA, vitamin, mutant gene and finally results were extracted from the included studies in this systematic review.

Risk of Bias Assessment

In order to evaluate the possibility of bias, we employed the revised Cochrane risk-of-inclination device (RoB-2), which consists of five distinct parts: randomization handle, deviations from planned treatments, lost result information, result estimation, and detailed result determination. The findings of the analysis for potential bias are presented in Table-2.

Results

About 24 papers out of 990 were evaluated

Table 2. Summary of Studies

Cell source type	Type of investigation	Method of testing	Effect on UF	Risk of bias	Reference
Human	TGF-β1	Northern blot analysis and ELIZA	At low doses, factor-β1 primarily induces cell proliferation in leiomyoma cells.	Risk of bias attributable to the randomization process: low Concerns with the risk of bias stemming from departures from the targeted interventions. Insufficient outcome data: little Risk of bias in outcome measurement: low Risk of bias in the selection of the reported outcome: low	[18]
Human	1,25-Dihydroxyvitamin D3	Analyzed by Western blotting using antibodies against VDR and β-actin	VDR expression is lower in human uterine fibroids in comparison to the surrounding normal myometrium.	The randomization approach poses a low risk of bias. Potential for bias as a result of interventions that deviate from the intended course: some worries Insufficient data on outcomes: low Potential for bias in outcome measurement: little Very little chance of prejudice in choosing this result to report	[32]
Human	TGF-β3	Northern analysis was used to assess the levels of TGF-β3 and fibronectin messenger RNA.	In comparison to the myometrial samples, the leiomyoma samples had a 3.5-fold higher quantity of TGF-β3 mRNA.	The randomization approach poses a low risk of bias. Potential for bias as a result of interventions that deviate from the intended course: some worries Insufficient data on outcomes: low Potential for bias in outcome measurement: little Very little chance of prejudice in choosing this result to report	[33]
Human	TGF-β3	A vaginal ultrasound scan, blood and tissue samples, and the measurement of TGF-β3 concentrations in serum and tissues	One significant aspect of ulipristal acetate's (UPA) impact on UF biology may be the decrease in serum and tissue TGF-β3 concentrations in UFs.	Potential for bias as a result of randomization: little Concerns about the possibility of bias arising from treatments that deviate from their intended goals Results data missing: minimal There is little chance of bias in the results. Selective bias in reporting results: little	[34]

continus on next page

Continue of **Table 2.** Summary of Studies

Human	TGF-β and Smad3 FMOD gene	By using immunohistochemistry and western blotting	The various ways in which TGF-β and GnRHa are regulated in vitro indicate that FMOD, an important regulator of tissue organization, is crucial in the fibrotic features of leiomyomas.	Very little chance of bias as a result of randomization Disruptions to the planned interventions pose a potential risk of bias, which raises some problems Unavailable results: poor The possibility of bias in the results assessment is minimal. Possible bias in choosing this result to report: little	[35]
Human	vitamin D and TGF-β3	Performing an ultrasound scan of the vagina or the abdomen, drawing blood samples, and measuring the quantities of vitamin D and TGF-β3 in the blood.	Risk factors for uterine fibroids were shown by this investigation to include lower vitamin D levels and greater TGF-β3 serum concentrations.	The randomization approach poses a low risk of bias. Some worries about the possibility of bias resulting from treatments that deviate from their intended approaches Lack of outcome data: little Mitigation of measurement bias in the outcome: minimal Potential for bias in the selection of the stated result: little	[36]
Human And smooth muscle cells (LSMC)	TGF-ß fibrillin-1 (FBN-1)	The following methods: real-time PCR, immunofluorescence labeling, and immunohistochemistry employing an avidin-biotin alkaline phosphatase technique	The expression of FBN-1 did not differ between the groups.	The randomization approach poses a low risk of bias. Potential for bias as a result of interventions that deviate from the intended course: some worries Insufficient data on outcomes: low Potential for bias in outcome assessment: some worries Possible bias in the results that were reported: some worries	[37]

continus on next page

Continue of **Table 2.** Summary of Studies

HuLM cells	Smad2, TGF-β3,Vitamin D3	Vitamin D3's impact on TGF-β3-induced Smad activation was confirmed through the use of Western blots and immunofluorescence studies.	Smad2 phosphorylation was enhanced by TGF-β3, but all of these effects mediated by TGF-β3 were substantially diminished by vitamin D.	The randomization approach poses a low risk of bias. Potential for bias as a result of interventions that deviate from the intended course: some worries Insufficient data on outcomes: low Potential for bias in outcome measurement: little Very little chance of prejudice in choosing this result to report	[38]
Human	RACK1 Gene	Immunohistochemistry , Immunofluorescence analysis, Migration analysis	By interacting with RhoA and activating the RhoA/Rho kinase pathway, RACK1 promotes migration.	Potential for bias as a result of randomization: little Concerns about the possibility of bias arising from treatments that deviate from their intended goals Results data missing: minimal There is little chance of bias in the results. Selective bias in reporting results: little	[39]
The TNBC cell line MDA-MB-231(ATCC®)	RACK1	Plasmid DNA preparation,cloning Pcr,Realtime PCR	elucidate RACK1 transcriptional regulation and demonstrate that SRSF3 involvement in cells migration implies its role in controlling different pathways	Very little chance of bias as a result of randomization Disruptions to the planned interventions pose a potential risk of bias, which raises some problems Unavailable results: poor The possibility of bias in the results assessment is minimal. Possible bias in choosing this result to report: little	[40]
Human	ER-α	bisulfite restriction mapping, immunohistochemistry, and real-time RT-PCR for the examination of DNA methylation status	In leiomyomas, ER-α mRNA levels were generally greater.	The randomization approach poses a low risk of bias. Some worries about the possibility of bias resulting from treatments that deviate from their intended approaches Lack of outcome data: little Mitigation of measurement bias in the outcome: minimal Potential for bias in the selection of the stated result: little	[41]

continus on next page

Continue of **Table 2.** Summary of Studies

Human	estrogen receptors α and β	Ribonucleic acid extraction,PCR,Real time PCR, mmunoprecipitation, PAGE, and immunoblotting	The estrogen receptor α is found in leiomyomas at an abnormally high level.	The randomization approach poses a low risk of bias. Potential for bias as a result of interventions that deviate from the intended course: some worries Insufficient data on outcomes: low Potential for bias in outcome measurement: little Very little chance of prejudice in choosing this result to report	[42]
Human	ER α T/C polymorphism	Following polymerase chain reaction (PCR), restriction digestion using Pvu II was performed.	a strong correlation between the C gene and endometriosis and fibroids	Potential for bias as a result of randomization: little Concerns about the possibility of bias arising from treatments that deviate from their intended goals Results data missing: minimal There is little chance of bias in the results. Selective bias in reporting results: little	[43]
HULP and MM Cells,Human	Wnt/β-catenin signaling pathway	qRT-PCR,ELIZA	Uterine fibroid tissue has more DNMT1 gene expression than myometrium.	The randomization approach poses a low risk of bias. Potential for bias as a result of interventions that deviate from the intended course: some worries Insufficient data on outcomes: low Potential for bias in outcome assessment: some worries Possible bias in the results that were reported: some worries	[44]
Human	FOXO1	PCR and Real Time	2.18-fold greater nuclear FOXO1 levels in UL patient samples was observed	The randomization approach poses a low risk of bias. Potential for bias as a result of interventions that deviate from the intended course: some worries Insufficient data on outcomes: low Potential for bias in outcome measurement: little Very little chance of prejudice in choosing this result to report	[45]
Human	D,C,Ca	vitamins were determined by utilizing vitamins that are naturally present in humans. Set of Elisa	Uterine fibroids were associated with significantly reduced vitamin C, D, and calcium blood levels in women.	Potential for bias as a result of randomization: little Concerns about the possibility of bias arising from treatments that deviate from their intended goals Results data missing: minimal There is little chance of bias in the results. Selective bias in reporting results: little	[46]

continus on next page

Continue of **Table 2.** Summary of Studies

Human	Vitamin D3	The procedure begins with a uterine ultrasound and continues with the assessment of serum FSH and vitamin D3 levels.	Uterine fibroids were associated with a markedly lower mean serum concentration of vitamin D3 in women than in controls.	Very little chance of bias as a result of randomization Disruptions to the planned interventions pose a potential risk of bias, which raises some problems Unavailable results: poor The possibility of bias in the results assessment is minimal. Possible bias in choosing this result to report: little	[47]
Human	Vitamin D	Ultrasound was used to assess the following parameters:	The serum 25OHD levels were found to be lower in women who had fibroids compared to those who did not.	The randomization approach poses a low risk of bias. Some worries about the possibility of bias resulting from treatments that deviate from their intended approaches Lack of outcome data: little Mitigation of measurement bias in the outcome: minimal Potential for bias in the selection of the stated result: little	[48]
Human	Alpha-tocopherol (AT)	high-performance liquid chromatography (HPLC) method	Major risk factors for UFs were discovered to include a higher Alpha-tocopherol (AT), a higher body mass index (BMI), a positive family history, and a poor parity.	The randomization approach poses a low risk of bias. Potential for bias as a result of interventions that deviate from the intended course: some worries Insufficient data on outcomes: low Potential for bias in outcome measurement: little Very little chance of prejudice in choosing this result to report	[49]

continus on next page

Continue of **Table 2.** Summary of Studies

Mouse	b-catenin and WNT4, Mediator subcomplex 12 (MED12)	Genetic testing, real-time polymerase chain reaction (RT-PCR), and formalin-fixed paraffin-embedded tissue samples	A mouse model has shown that β-catenin, which is known to produce leiomyoma-like lesions, is activated when estrogen and the mutant MED12 work together to stimulate the Wnt pathway.	Potential for bias as a result of randomization: little Concerns about the possibility of bias arising from treatments that deviate from their intended goals Results data missing: minimal There is little chance of bias in the results. Selective bias in reporting results: little	[50]
Human	miR-21, miR-23b, miR-29b, and miR-197	Northern Blot Analysis, Total RNA isolation was performed with kit, RT-PCR, miRNA Transfection Assay	The top five miRNAs that are dysregulated in ULMs are miR-197, miR-23b, let-7, and miR-21.	Very little chance of bias as a result of randomization Disruptions to the planned interventions pose a potential risk of bias, which raises some problems Unavailable results: poor The possibility of bias in the results assessment is minimal. Possible bias in choosing this result to report: little	[51]
Human	MED12 and HMGA2	DNA Isolation, PCR and Sequencing,	leading to the formation of new nodules through clonal processes	The randomization approach poses a low risk of bias. Potential for bias as a result of interventions that deviate from the intended course: some worries Concerns regarding missing outcome data Potential for bias in outcome measurement: little Very little chance of prejudice in choosing this result to report	[52]
Human	PCOLCE	RNA isolation,RT-PCR, Fluorescence in situ hybridization analysis		The randomization approach poses a low risk of bias. Potential for bias as a result of interventions that deviate from the intended course: some worries Insufficient data on outcomes: low Potential for bias in outcome assessment: some worries Very little chance of prejudice in choosing this result to report	[53]

that were initially reviewed (Figure-1). Table-1 shows the distribution of the research according to the authors, origin, and year. They were divided into sources of cells, methods, steroid signaling, signaling pathways, growth factors, miRNAs, vitamins, and mutant genes. 18 of these studies investigated human source cells, one studied both human and mouse, one studied both human and cell line, 3 studies examined cell line, and one study examined mouse cell line. (Table-1). Three studies investigated the influence of TGF-ß, four studied vitamins, two studied both vitamin and TGF-ß, three studied RACK1, three studied estrogen receptor, one studied miRNA, and two studies examined mutant genes.

Discussion

Our discussion indicates a convergent pathway for several signal transduction pathways. , TGF-β, vitamin D, RACK 1, WNT, miR/Wnt/β-catenin pathways are activated by growth factors, progesterone and vitamin D receptors on membranes. In cases where different pathways are treated additively, this phenomenon of convergence may have important therapeutic implications. In the following, the factors assessed in the studies included in this review are briefly mentioned.

Steroid-based Signaling

Trilogy of studies examined steroid-based signaling on human source cells. These studies examined ß and α sterogen receptors. Myometrium and leiomyomas were both examined for their DNA methylation level in one study. In these patients, the leiomyomas usually had larger levels of ER- α mRNA than their myometriums. The increased levels of ER-α mRNA expression seen in uterine leiomyomas could be attributed to an aberrant DNA methylation status at the promoter region of the ER- α gene (Figure-2) [41]. Another study compared fibroids with normal myometrial tissue in terms of estrogen receptor α and ß expression and DNA-binding status in order to better understand the molecular basis for the altered estrogen responsiveness in leiomyomas. The ratio of estrogen receptor α-to-estrogen receptor α expression effects the potential growth of myomas for the percentage of estrogen receptor α DNA-binding homodimers, not the individual expression levels [42]. Patients with endometriosis or fibroids from Asian or Indian origins were examined for ER Î± T/C polymorphism in the third study. Further research is needed to establish the ER α C allele in Asian Indian women as a potential indicator for endometriosis and fibroids. Endometriosis and fibroids risk factors include dietary variables, racial background, and ethnicity. We need to conduct more research in this area [43].

TGF-ß

Six investigations were conducted on TGF-ß. One study used pairs of myometrium and leiomyoma tissues donated by 28 women who had hysterectomy. Each tissue's total RNA was collected and examined utilizing Northern blot technique in order to detect TGF-ß 1 messenger RNA. The total and active levels of transforming growth factor-ß 1 proteins were examined using an enzyme-linked immunosorbent assay. TGF-ß 1 (0.01-1 ng/mL), anti-transforming growth factor-ß antibody (0.01-10 ng/mL), or platelet-derived growth factor (10 ng/mL) were employed to measure the proliferation of pure human myometrial and leiomyoma cells using [3H] thymidine incorporation Also, transforming growth factor-ß 1 only promotes cell proliferation in leiomyoma cells at low doses, just like its bimodal and dose-dependent activities in smooth muscle cells elsewhere (Figure-3) [18]. In their study, Arici *et al*. assessed the amounts of TGF-β3 mRNA and protein in the myometrium and leiomyoma. In the subsequent steps, they injected TGF-β3 into the developed myometrial and leiomyoma cells. Leiomyoma samples contained 3.5 times more TGF-*3 mRNA than myometrial samples. The amount of TGF-β3 mRNA was five times higher in leiomyoma samples collected during the midsecretory phase compared to samples taken during the proliferative phase [30-33]. During the menstrual cycle, Eric Levens *et al*. found that FMOD expression was approved in leiomyoma and myometrium (N=20). Seven women were given gonadotropin-releasing hormone analogue (GnRHa) as treatment in the trial, whereas twenty women were given transforming growth factor (TGF)- β in addi-

Table 3. Studies included in the Systematic Review

Auther	Patients	Method	Animals	Cell line	Steroid signaling	Signaling pathway	Grows factors	miRNA	Vitamin	Mutant gene	Results
Arici [18]2003	Pairs of myometrium and leiomyoma tissues were collected from 28 hysterectomy patients.	Tissue pairs of myometrium and leiomyoma were obtained from 28 patients who had hysterectomy. Northern blot analysis was employed to identify TGF-β1 messenger RNA following the extraction of total RNA from each tissue sample. The quantity of active and total transforming growth factor-β1 protein was quantified using an enzyme-linked immunosorbent assay. The proliferation of cultured human myometrial and leiomyoma cells was assessed via the [3H] thymidine incorporation method following anti-transforming growth factor-β antibody (0.01-10 ng/mL), treatment with TGF-β1 (0.01-1 ng/mL), or platelet-derived growth factor (10 ng/mL).					The assessment was conducted using TGF-β1 (0.01-1 ng/mL), anti-transforming growth factor-β antibody (0.01-10 ng/mL), or platelet-derived growth factor (10 ng/mL).				Transforming growth factor-β1 has bimodal and dose-dependent effects on smooth muscle cells in other tissues, which is consistent with the observation that, at low doses, it primarily increases cell proliferation in leiomyoma cells.

Contine of **Table 3.** Studies included in the Systematic Review

Sunil K. Halder [32]2013		studied human uterine fibroids for vitamin D receptor (VDR) protein expression levels and compared them to normal myometrium in the vicinity.	Both the immortalized human uterine fibroid cell line (HuLM) and the human normal uterine smooth muscle cell line (UTSM)		vitamin D receptor (VDR)	The therapy with 1,25(OH)2D3 can reduce the aberrant expression of essential ECM-associated proteins in HuLM cells, and human uterine fibroids have reduced levels of VDR compared to the adjacent normal myometrium. Consequently, 1,25(OH)2D3 may serve as a safe, effective, and noninvasive treatment for uterine fibroids in humans.
Arici [33]2000	Women with (n=18) leiomyoma.	Following the quantification of TGF-β3 mRNA and protein levels in the myometrium and leiomyoma, TGF-β3 was delivered to cultured cells obtained from both tissues.		TGF-β3		The leiomyoma samples exhibited a 3.5-fold greater level of TGF-β3 mRNA compared to the myometrial samples. The TGF-β3 mRNA level in leiomyoma samples during the midsecretory phase was five times higher than in those from the proliferative phase.

continus on next page

Contine of **Table 3.** Studies included in the Systematic Review

Eric Levens [34]2005	Samples of leiomyoma and corresponding myometrium were obtained from premenopausal women (N=27) scheduled for hysterectomy due to symptomatic uterine leiomyomas at the University of Florida associated Shands Hospital.	Investigated the expression of FMOD in paired leiomyoma and myometrium (**N**=20) during menstruation in women undergoing GnRHa therapy (**N**=7), as well as in leiomyoma and myometrial smooth muscle cells (SMC) following TGF-β and GnRHa treatment.	Smad3	**TGF-β**	Western blotting and immunohistochemistry showed that FMOD was present in many different tissues, including LSMC, MSMC, arterial walls, connective tissue fibroblasts, myometrial and leiomyoma extracts, and fibroblasts in connective tissue. The expression of FMOD in MSMC was elevated in a time- and cell-dependent manner by TGF-β1 (2.5 ng/ml), whereas GnRHa (0.1 μM) inhibited this effect in both MSMC and LSMC (**P**<0.05). Prior to pretreatment with Smad3 SiRNA for LSMC and U0126 (a MEK1/2 inhibitor) for MSMC, the influence of TGF-β on FMOD expression was mitigated.
MichałCiebiera [35]2016	A total of 188 women participated in the trial, comprising 105 patients admitted for uterine fibroid surgery as the study group and 83 healthy women of comparable age as controls.	To evaluate the influence of variables such as weight, familial predisposition, serum levels of 25-hydroxyvitamin D and transforming growth factor β3 (TGF-β3), on the risk of developing uterine fibroids.		vitamin D and TGF-β3	Our study indicates that uterine fibroids may be exacerbated by an elevated body mass index (BMI), a favorable familial history, diminished vitamin D levels, and increased TGF-β3 blood concentrations.

continus on next page

Contine of **Table 3.** Studies included in the Systematic Review

Yangyu Zhao [36]2007	During hysterectomy, matching samples of unaffected myometrium and biopsies of intramural leiomyomata were obtained from premenopausal women.	Compared the expression of latent TGF-ß binding protein-1 (LTBP-1) and fibrillin-1 (FBN-1) in leiomyomata and myometrium, correlating with the size of leiomyomata.			TGF-ß binding fibrillin-1 (FBN-1) and protein-1 (LTBP-1)	FBN-1 expression remained consistent across the groups during both stages. 17β-estradiol (E2) significantly augmented the gene and protein expression of LTBP-1 and FBN-1 in cultured leiomyoma smooth muscle cells (LSMC) ($P<0.05$). Following treatment of cells with E2 and progesterone, no significant alteration in the expression of FBN-1 and LTBP-1 was observed. Estrogen may influence the expression of LTBP-1 and FBN-1 in leiomyomata. Metabolic alterations in the extracellular matrix may differ in medium-sized leiomyomas.
Halder [37]2011		To investigate the impact of 1,25-dihydroxyvitamin D3 (vitamin D3) on the expression of fibrosis-related proteins produced by TGF-β3 in immortalized human uterine leiomyoma (HuLM) cells.	HuLM cells	Smad2	1,25-dihydroxyvitamin D3 (vitamin D3) on TGF-β3-induced fibrosis-associated proteins	In HuLM cells, TGF-β3 induced the phosphorylation of Smad2 and facilitated the translocation of Smad2 and Smad3 to the nucleus; however, vitamin D significantly attenuated all these actions mediated by TGF-β3. Our data indicate that Vitamin D3 consistently reduces the effects of TGF-β3, which are associated with fibrosis in human leiomyoma cells.

continus on next page

Contine of **Table 3.** Studies included in the Systematic Review

Xi-Xi Cao [38]2010	conducted immunohistochemistry in 160 breast carcinoma samples.	sought to learn how RACK1 contributes to the migration and metastases of breast cancer. Breast cancer cell lines were used to perform migration tests. Additional tools utilized were the Rho kinase inhibitor and siRNA directed against RACK1. The connection between RACK1 and RhoA was investigated using immunofluorescence and immunoprecipitation.			RACK1	Based on in vitro experiments, RACK1 activates the RhoA/Rho kinase pathway and interacts with RhoA to induce migration. A substantial correlation between RACK1 and approved tumor spread indices and RhoA was found by immunohistochemistry in 160 cases.
Xi-Xi Cao[39] 2009	seek out the role(s) played by RACK1 in the processes of breast cancer cell proliferation, invasion, and metastasis.	nude mouse models	breast carcinoma cell lines		RACK1	Proliferation and invasion/metastasis of breast cancer are enhanced in vitro and in vivo by RACK1, an independent prognosis-related factor. A sneak peek at the content available to subscribers,
Buoso[40] 2019	Investigated its role in regulating RACK1, a scaffolding protein associated with breast cancer cells, which possesses a Glucocorticoid Response Element (GRE) site on its promoter and is involved in cellular migration and invasion.		The TNBC cell line MDA-MB-231(ATCC®)		RACK1	Illuminate the regulation of RACK1 transcription and demonstrate that SRSF3 participates in cell migration, indicating its involvement in regulating several pathways; this emphasizes the necessity to investigate other contributors in GR-positive TNBC.

continus on next page

Contine of **Table 3.** Studies included in the Systematic Review

Hiromi Asada(2008) [41]	18	Nine patients' leiomyomas had hypomethylated CpG sites in the distal area of the ER-α promoter, according to an examination of eleven matched myometrium and leiomyoma samples that used bisulfite restriction mapping to determine DNA methylation status.	-	-	ER-α -	-	ER-α mRNA levels were often elevated in the leiomyoma.
PanagiotisBakasM.D.(2007) [42]	35	- Biopsy specimens from uterine fibroids and adjacent normal myometrial tissue during the follicular phase of the menstrual cycle			estrogen receptors α and β		Estrogen receptor α is significantly elevated in leiomyomas.
Govindan, Sujatha(2009) [43]	367	PCR was performed to amplify the ER α gene, subsequently followed by restriction digestion using Pvu II.			ER α T/C polymorphism		Notable correlation of the C allele with endometriosis and fibroids

continus on next page

Contine of **Table 3.** Studies included in the Systematic Review

María Cristina Carbajo-García(2021) [44]	16	Gene expression was assessed via qRT-PCR and DNMT activity through ELISA.		HULP and MM cells		Wnt/β-catenin signaling pathway			DNMT1 gene expression was elevated in uterine fibroids compared to the myometrium.
C. Scott Gallagher(2018) [45]	5	PCR and Real Time						FOXO1	2.18-fold greater nuclear FOXO1 levels in UL patient samples was observed
Christian C(2021) [46]	88	Blood samples were collected and examined for serum concentrations of specific micronutrients (vitamins A, C, D, and E) and trace elements.					D,C,Ca		Uterine fibroids were associated with significantly reduced vitamin C, D, and calcium blood levels in women.
Vinita Singh(2019) [47]	144	-Uterine fibroids were associated with a markedly reduced mean serum concentration of vitamin D3 in women when compared to controls.	-	-	-	-	-	- D	Prior to assessing serum FSH levels (on the third day of menstruation) and serum vitamin D3, all women received a uterine ultrasound.

continus on next page

Contine of **Table 3.** Studies included in the Systematic Review

Saisai Li(2020) [48]	546	Serum 25OHD levels were seen to be reduced in women with fibroids relative to those without.			Vitamin D		the following parameters were evaluated by ultrasound
Michał Ciebiera(2018) [49]	162	Elevated levels of Alpha-tocopherol (AT), body mass index (BMI), a positive family history, and poor parity were identified as significant risk factors for uterine fibroids (UFs).		Alpha-tocopherol (AT)			the following parameters were evaluated by ultrasound
MichałCiebieraM.D(2018) [50]	141	One significant aspect of ulipristal acetate's (UPA) impact on UF biology may be the decrease in serum and tissue TGF-β3 concentrations in UFs.		TGF-β3	.		a scan of the vaginal area, blood and tissue samples, and the determination of the amounts of TGF-β3 in the blood and tissues
Dominique Nadine Markowski(2011) [51]	50	Genomic DNA sequencing was conducted on specimens derived from 15 lipomas and 21 endometrial polyps maintained in formalin-fixed paraffin-embedded tissue (FFPE samples).	b-catenin and WNT4		.	Mediator subcomplex 12 (MED12)	A mouse model has shown that β-catenin, which is known to produce leiomyoma-like lesions, is activated when estrogen and the mutant MED12 work together to stimulate the Wnt pathway.

continus on next page

Contine of **Table 3.** Studies included in the Systematic Review

Tongsheng Wang(2007) [52]	55	The mirVana™ miRNA Isolation Kit (Ambion) was utilized for total RNA isolation and small RNA enrichment.	mouse model	miR-21, miR-23b, miR-29b, and miR-197		The top five dysregulated miRNAs in ULMs are the let-7 family, miR-21, miR-23b, miR-29b, and miR-197
Markowski(2014) [53]	120	Isolating DNA from frozen tissue samples and running PCR were the steps involved in DNA-sequencing, which was done either during or immediately following surgery. The next step was to use agarose gel electrophoresis to separate the PCR products. genes were examined, along with their expression.		.	MED12 and HMGA2	resulting in an independent clonal outgrowth of nodules
Azra HLigon(2002) [54]	18 women	This trial does not establish a definitive function for PCOLCE in fibroid growth at this time.			PCOLCE	The expression and deletion status of PCOLCE were assessed using fluorescent in situ hybridization and reverse-transcriptase polymerase chain reaction (RT-PCR).

continus on next page

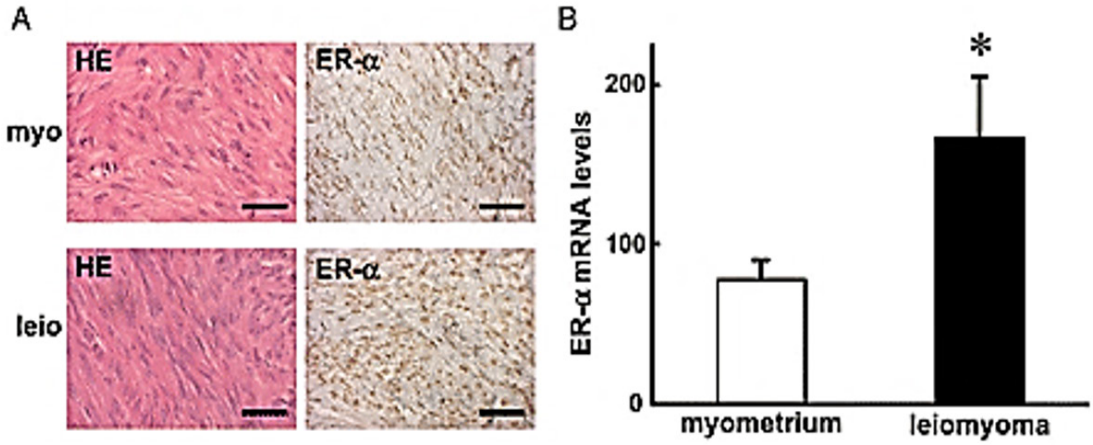

Figure 2. ER-α expression in uterine leiomyoma and normal myometrium[41].

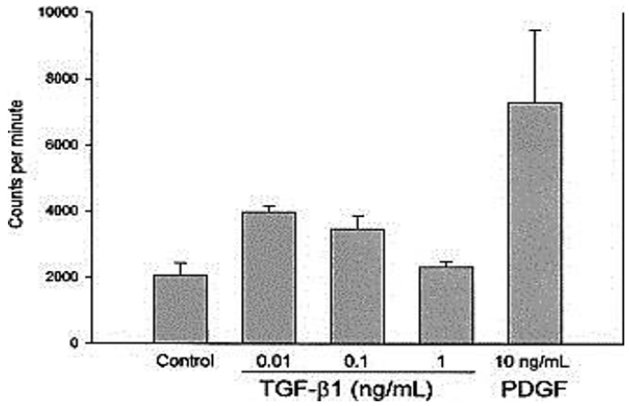

Figure 3. The dosage response for TGF-β1 and PDGF therapy over one day in cultured leiomyoma cells: tritiated thymidine consolidation. Data are reported as averages ± SEM for 8 replicates [18]

tion to GnRHa. Immunohistochemistry and western blotting confirmed the presence of immunoreactive FMOD in LSMC, MSMC, vascular walls, and connective tissue fibroblasts. The treatment of MSMC with TGF- β 1 (2.5 ng/ml) led to an increase in the expression of FMOD, which was time- and cell-dependent. Nevertheless, upon treatment with GnRHa (0.1 m), FMOD expression was shown to be downregulated (P 0.05) in LSMC. The pretreatment of LSMC and MSMC with Smad3 SiRNA and U0126, an inhibitor of MEK1/2, reduced the impact of TGF- β and GnRHa on FMOD production [34]. The levels of TGF- β 3 in the myometrium and leiomyoma were assessed in a study carried out by Arici *et al*. The cells were subsequently treated with TGF-β 3. The level of TGF- β 3 mRNA was 3.5 times higher in leiomyoma samples compared to myometrial samples. Compared to leiomyoma samples taken during the proliferative phase, those taken during the midsecretory phase had TGF- β 3 mRNA levels that were five times greater [33]. The study conducted by Micha*Ciebiera *et al*. [50] found that ulipristal acetate (UPA) can decrease blood and tissue TGF- β 3 concentrations, which could significantly impact UF biology.

25-hydroxyvitamin D

Chebiera, Micha investigated the impact of variables such as body mass index (BMI), serum levels of 25-hydroxyvitamin D and altering growth factor β 3 (TGF- β 3), and personal and family medical history on uterine fibroids formation. Factors that could increase the

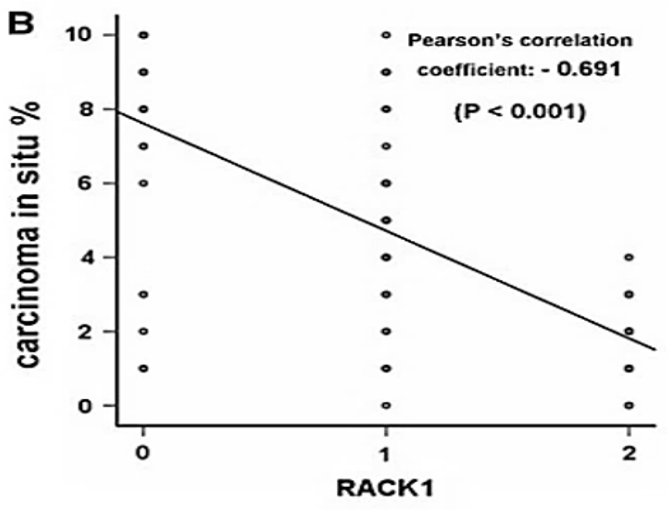

Figure 4. Percentage of carcinoma in situ[38]

likelihood of uterine fibroids include a high body mass index (BMI), a positive genetic background, low vitamin D levels, and high concentrations of TGF- β 3 in the blood. None of the groups showed any difference in the expression of FBN-1. The expression of LTBP-1 and FBN-1 mRNA and protein was enhanced in cultured leiomyoma smooth muscle cells (LSMC) by 17 β -eestradiol (E2) (P<0.05). E2 did not influence FBN-1 or LTBP-1 levels when given alongside progesterone. A response to estrogen triggers the secretion of LTBP-1 and FBN-1 by leiomyomata. It is possible that the extracellular matrix metabolism varies in medium-sized leiomyomas [35]. Halder We hope to learn more about the effects of vitamin D3 on TGF-Î²3-induced protein fibrosis in immortalized human uterine leiomyoma (HuLM) cells in this study. Vitamin D3's effects on fibrosis-related protein production in immortalized human uterine leiomyoma (HuLM) cells were studied in relation to TGF- β 3. By lowering the nuclear translocation and phosphorylation of Smad2 and Smad3 in HuLM cells, vitamin D3 considerably diminished the effects of TGF- β 3 on fibrosis in human leiomyoma cells [37].

RACK1

The function of RACK1 has been the subject of research. Xi-Xi Cao set out to discover the molecular mechanisms by which RACK1 aids in the migration and metastasis of breast cancer. To perform migration tests, cell lines derived from breast carcinomas were utilized. Additionally, the Rho kinase inhibitor and siRNA targeting RACK1 were employed. RACK1/RhoA interactions were studied using immunoprecipitation and immunofluorescence. The RhoA/Rho kinase pathway is activated by RACK1 through interaction with RhoA in vitro. The RACK1 protein is highly correlated with RhoA and accepted tumor spread indicators in 160 samples studied by immunohistochemistry [38] (Figure-4). To learn how RACK1 contributes to breast cancer progression, invasion, and metastasis, Xi-Xi Cao *et al*. performed their own investigation. In vivo and in vitro investigations have shown that RACK1 promotes breast cancer cell growth and metastasis [39, 40].

Wnt/β-catenin

One study looked at the Wnt/Î²-catenin signaling pathway. Gene expression and DNMT activity were examined in Marla *et al*. E. using qRT-PCR and ELISA. There was an increase in DNMT1 gene expression in uterine fibroid tissue compared to myometrium. A potential therapeutic target was identified in HCC cells—a new positive feedback loop involving DNMT1/miR-378a-3p/TRAF1/NF- κ [44]. Twenty-one endometrial polyps and fifteen lipomas were subjected to DNA sequencing analysis using FFPE-samples obtained by Dominique *et al*. The combination of estrogen

and mutant MED12 promotes the Wnt pathway, which in turn activates Î³ -catenin, leading to the development of lesions similar to leiomyomas in mice [51].

Effects of Vitamins

A vitamin's impact was the subject of one research. The results of every study corroborated its effectiveness. Uterine fibroids and surrounding normal myometrium were compared in terms of vitamin D receptor (VDR) protein expression by Sunil K. Hald *et al*. By lowering VDR levels, 1,25(OH)2D3 treatment for human uterine fibroids diminishes aberrant expression of key ECM-related to proteins in HuLM cells. One potential noninvasive, effective, and safe method for treating uterine fibroids in humans is the use of 1,25(OH)2D3 [32]. Christian *et al*. analyzed trace element and micronutrient levels in serum (vitamins A, C, D, and E). Uterine fibroids were associated with decreased serum calcium, vitamin D, and vitamin C levels in women [46]. Vinita Singh *et al*. discovered that compared to healthy controls, women who had uterine fibroids had far lower quantities of vitamin D3 in their blood. All women had their blood FSH and vitamin D levels checked after uterine ultrasounds [47]. This was done on the third day of menstruation. One study looked into beta-tocopherol (into). UFs are linked to elevated levels of alpha-tocopherol (AT), body mass index (BMI), a favorable family history, and low parity, as reported by Michal Ciebiera *et al*. [49], The impacts of miR-21, miR-29b, miR-23b, and miR-197 were examined in a study. The top five dysregulated microRNAs (miRNAs) in ULMs were determined by Wang *et al*. to be mutations in miR-21, miR-29b, miR-23b, and miR-32 [52].

Mutant Genes

Mutant genes were the subject of two investigations. Markowski looked at MED12, which is a quality code for a go-between subcomplex 12, and HMGA2, which is a quality code for a high-mobility bunch protein 2, in one of these. Based on these results, it seems that the pathophysiology of fibroids can vary. Separate clonal outgrowths may occur if an unidentified "mutator" mutated MED12 in the majority of these populations. There is a modest but recognized risk of MED12-mutated fibroids turning into cancers following a leiomyoma, hence the latter mutation cannot be utilized as a signal for benign development.-Leomyosarcoma sequence -STUMP (small tumors of smooth muscle with unclear cancer risk) [53, 55, 56]. According to the results of an RT-PCR and FISH analysis, PCOLCE is a potential chromosomal 7 locus whose product, PCPE, cleaves sort I procollagen C-propeptide. The following reasons make PCOLCE an intriguing gene for the study of uterine fibroids: its high levels of expression in the uterus, the fact that rodent fibroblasts undergo anchorage-independent development when subjected to retroviral-mediated insertional mutagenesis, and, lastly, its position on the human chromosomee (7q21.3*q22) [54, 55].

Conclusion

Uterine leiomyomas are characterized by cell proliferation and an excess of disorganized extracellular matrix (ECM). This pair of activities is carefully controlled by a network of signaling pathways. For instance, the ECM contains dormant transforming growth factors. Once tissue proteases have activated cell surface receptors, they are ready to be used. Two key features of leiomyoma signaling pathways are interconnectedness and convergence. They need to communicate with one other and work together in a number of contexts. Leiomyoma cells' intricate connection to the ECM is an intriguing illustration of this. Cellular secretion of transforming growth factors increases collagen synthesis and, by extension, ECM stiffness. A number of research compounds, such as liarozole, use this relationship as an advantage. Our discussion indicates a convergent pathway for several signal transduction pathways. , TGF-β, vitamin D, RACK 1, WNT, miR/Wnt/β-catenin pathways are activated by growth factors, progesterone and vitamin D receptors on membranes. Important therapeutic implications may arise from this phenomenon of convergence in situations where various pathways are addressed additively. Targeting numerous signaling pathways at once is the foundation of dual targeting and multitargeting. As a result, additive or synergistic effects may occur. Some can-

cers, like prostate cancer, are currently being treated using this multifocal signal modulation approach.

Future research on multifocal targeting in leiomyoma could benefit from a better understanding of signaling pathways in the tumor. It is suggested that future studies on the issue related to the factors of the present study be done with a larger sample size than the existing studies and in the form of cohort designs. In addition, the use of modern techniques in a homogeneous manner in future studies to perform meta-analysis on the obtained results can help significantly in providing treatment protocols. Furthermore, it should be emphasized that these results should be taken with caution, even though the risk of predisposition assessment is minimal for most of the components discussed in this research.

Conflict of Interest

None declared.

References

1. Okolo S. Incidence, aetiology and epidemiology of uterine fibroids. Best practice & research Clinical obstetrics & gynaecology. 2008; 22(4): 571-588.
2. Żak K, et al. Preoperative differentiation of uterine leiomyomas and leiomyosarcomas: current possibilities and future directions. Cancers. 2022; 14(8): 1966.
3. Baird D.D, et al. High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence. American journal of obstetrics and gynecology. 2003; 188(1): 100-107.
4. Berta D.G, et al. Deficient H2A Z deposition is associated with genesis of uterine leiomyoma. Nature. 2021; 596(7872): 398-403.
5. Cardozo E.R, et al. The estimated annual cost of uterine leiomyomata in the United States. American journal of obstetrics and gynecology. 2012; 206(3): 1-211.
6. Lin K.Y.-H, et al. Uterine leiomyoma is associated with the risk of developing endometriosis: A nationwide cohort study involving 156,195 women. Plos one. 2021; 16(8): e0256772.
7. Carbajo-García M.C, et al. Integrative analysis of the DNA methylome and transcriptome in uterine leiomyoma shows altered regulation of genes involved in metabolism, proliferation, extracellular matrix and vesicles. The Journal of Pathology. 2022;257(5):663-73.
8. Gargiulo A.R. Yen & Jaffe's reproductive endocrinology. Physiology, pathophysiology, and clinical management: 2017; Elsevier Health Sciences.
9. La Merrill M.A, et al. Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. Nature Reviews Endocrinology. 2020; 16(1): 45-57.
10. Afrin S, et al. Simvastatin modulates estrogen signaling in uterine leiomyoma via regulating receptor palmitoylation, trafficking and degradation. Pharmacological Research. 2021; 172: 105856.
11. Axelstad M, et al. EDC IMPACT: Reduced sperm counts in rats exposed to human relevant mixtures of endocrine disrupters. Endocrine connections. 2018; 7(1): 139-148.
12. Johansson H.K.L, et al. Environmental influences on ovarian dysgenesis—developmental windows sensitive to chemical exposures. Nature Reviews Endocrinology. 2017; 13(7): 400-414.
13. Amano I, et al. Effects of mild perinatal hypothyroidism on cognitive function of adult male offspring. Endocrinology. 2018; 159(4): 1910-1921.
14. Alonso-Magdalena P, et al. Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environmental health perspectives. 2010; 118(9): 1243-1250.
15. Heindel J.J, et al. Review of developmental origins of health and disease publications in environmental epidemiology. Reproductive toxicology. 2017; 68: 34-48.
16. Ponomarenko I, et al. Candidate genes for age at menarche are associated with uterine leiomyoma. Frontiers in genetics. 2021; 11: 512940.
17. Buoso E, et al. Transcriptional regulation of RACK1 and modulation of its expression: role of steroid hormones and significance in health and aging. Cellular Signalling. 2017; 35: 264-271.

18. Arici A, Sozen I. Expression, menstrual cycle-dependent activation, and bimodal mitogenic effect of transforming growth factor-β1 in human myometrium and leiomyoma. American journal of obstetrics and gynecology. 2003; 188(1): 76-83.
19. Sahly N.N, et al. Molecular differential analysis of uterine leiomyomas and leiomyosarcomas through weighted gene network and pathway tracing approaches. Systems biology in reproductive medicine. 2021; 67(3): 209-220.
20. Zeyneloglu H.B, et al. Immunohistochemical characteristics of intramural leiomyomata that enlarge during controlled ovarian hyperstimulation for in vitro fertilization. Gynecologic and obstetric investigation. 2008; 65(4): 252-257.
21. Kim M, et al. MicroRNAs as potential indicators of the development and progression of uterine leiomyoma. PloS one, 2022. 17(5): p. e0268793.
22. Joseph D.S, et al. Myometrial cells undergo fibrotic transformation under the influence of transforming growth factor β-3. Fertility and sterility. 2010; 93(5): 1500-1508.
23. Mathew R.P, et al. Uterine leiomyomas revisited with review of literature. Abdominal Radiology. 2021; 46(10): 4908-4926.
24. Ciebiera M, et al. Role of transforming growth factor β in uterine fibroid biology. International Journal of Molecular Sciences. 2017; 18(11): 2435.
25. Shved N, et al. Development of primary monolayer cell model and organotypic model of uterine leiomyoma. Methods and Protocols. 2022; 5(1): 16.
26. Ming X, et al. A prognostic index model for predicting long-term recurrence of uterine leiomyoma after myomectomy. PloS one. 2021; 16(7): e0254142.
27. Townsend D.E, et al. Unicellular histogenesis of uterine leiomyomas as determined by electrophoresis of glucose-6-phosphate dehydrogenase. American journal of obstetrics and gynecology. 1970; 107(8): 1168-1173.
28. Pandis N, et al. Chromosome analysis of 96 uterine leiomyomas. Cancer genetics and cytogenetics. 1991; 55(1): 11-18.
29. Rein M.S. Advances in uterine leiomyoma research: the progesterone hypothesis. Environmental health perspectives. 2000;: 791-793.
30. Buoso E, et al. Ribosomes as a nexus between translation and cancer progression: Focus on ribosomal Receptor for Activated C Kinase 1 (RACK1) in breast cancer. British journal of pharmacology. 2022; 179(12): 2813-2828.
31. Watanabe K, et al. Development of Uterine Leiomyosarcoma During Follow-up After Caesarean Section in a Woman With Uterine Leiomyoma. Anticancer Research. 2021; 41(6): 3001-3010.
32. Halder S.K, Osteen K.G, Al-Hendy A. 1, 25-dihydroxyvitamin d3 reduces extracellular matrix-associated protein expression in human uterine fibroid cells. Biology of reproduction. 2013; 89(6):1-13.
33. Arici A, Sozen I. Transforming growth factor-β3 is expressed at high levels in leiomyoma where it stimulates fibronectin expression and cell proliferation. Fertility and sterility. 2000; 73(5): 1006-1011.
34. Levens E, et al. Fibromodulin is expressed in leiomyoma and myometrium and regulated by gonadotropin-releasing hormone analogue therapy and TGF-β through Smad and MAPK-mediated signalling. Molecular human reproduction. 2005; 11(7): 489-494.
35. Ciebiera M, et al. Influence of vitamin D and transforming growth factor β3 serum concentrations, obesity, and family history on the risk for uterine fibroids. Fertility and Sterility. 2016;106(7):1787-1792.
36. Zhao Y, et al. Increased expression of latent TGF-ß binding protein-1 and fibrillin-1 in human uterine leiomyomata. Molecular human reproduction. 2007; 13(5): 343-349.
37. Halder SK, Goodwin J.S, Al-Hendy A. 1, 25-Dihydroxyvitamin D3 reduces TGF-β3-induced fibrosis-related gene expression in human uterine leiomyoma cells. The Journal of Clinical Endocrinology & Metabolism. 2011; 96(4): E754-E762.
38. Cao X.-X, et al. RACK1 promotes breast carcinoma migration/metastasis via activation of the RhoA/Rho kinase pathway. Breast cancer research and treatment. 2011; 126(3): 555-563.
39. Cao X.-X, et al. RACK1 promotes breast carcinoma proliferation and invasion/metastasis in vitro and in vivo. Breast cancer research and treatment. 2010; 123(2): 375-386.
40. Buoso E, et al. Cortisol-induced SRSF3 expression promotes GR splicing, RACK1 expression and breast cancer cells migration. Pharmacological research. 2019; 143: 17-26.
41. Asada H, et al. Potential link between estrogen receptor-α gene hypomethylation

and uterine fibroid formation. Molecular human reproduction. 2008; 14(9): 539-545.
42. Bakas P, et al. Estrogen receptor α and β in uterine fibroids: a basis for altered estrogen responsiveness. Fertility and sterility. 2008;90(5): 1878-1885.
43. Govindan S, et al. Estrogen receptor-α gene (T/C) Pvu II polymorphism in endometriosis and uterine fibroids. Disease markers. 2009; 26(4): 149-154.
44. Zhu B, et al. DNMT1-induced miR-378a-3p silencing promotes angiogenesis via the NF-κB signaling pathway by targeting TRAF1 in hepatocellular carcinoma. Journal of Experimental & Clinical Cancer Research. 2021; 40(1): 1-19.
45. Gallagher CS. Genetic, Functional, and Epidemiologic Analyses of Genomic Variants in Uterine Leiomyomata. Harvard University. 2018;:28221125.
46. Makwe C.C, et al. Micronutrient and Trace Element Levels in Serum of Women With Uterine Fibroids in Lagos. Cureus. 2021; 13(10): e18638.
47. Singh V, Barik A, Imam N. Vitamin D3 level in women with uterine fibroid: an observational study in eastern Indian population. The Journal of Obstetrics and Gynecology of India. 2019; 69(2): 161-165.
48. Li S, et al. The associations between serum vitamin D, calcium and uterine fibroids in Chinese women: A case-controlled study. Journal of International Medical Research. 2020; 48(5): 0300060520923492.
49. Ciebiera M, et al. Alpha-tocopherol serum levels are increased in Caucasian women with uterine fibroids: a pilot study. BioMed Research International. 2018; 2018(1):6793726.
50. Ciebiera M, et al. Ulipristal acetate decreases transforming growth factor β3 serum and tumor tissue concentrations in patients with uterine fibroids. Fertility and sterility. 2018; 109(3): 501-507.
51. Markowski D.N, et al. MED12 mutations in uterine fibroids—their relationship to cytogenetic subgroups. International journal of cancer. 2012; 131(7): 1528-1536.
52. Wang T, et al. A micro-RNA signature associated with race, tumor size, and target gene activity in human uterine leiomyomas. Genes, Chromosomes and Cancer. 2007; 46(4): 336-347.
53. Markowski D.N, et al. Uterine fibroids: do we deal with more than one disease? International journal of gynecological pathology. 2014; 33(6): 568-572.
54. Jokinen V, Mehine M, Reinikka S, Khamaiseh S, Ahvenainen T, Äyräväinen A, Härkki P, Bützow R, Pasanen A, Vahteristo P. 3′ RNA and whole-genome sequencing of archival uterine leiomyomas reveal a tumor subtype with chromosomal rearrangements affecting either HMGA2, HMGA1, or PLAG1. Genes, Chromosomes and Cancer. 2023 Jan;62(1):27-38.
55. Movahed F, Ehymayed HM, Kalavi S, Shahrtash SA, Al-Hijazi AY, Daemi A, Mahmoud HM, Kashanizadeh MG, Alsalamy AA. Development of an electrochemical sensor for detection of lupron as a drug for fibroids treatment and uterine myoma in pharmaceutical waste and water sources. Journal of Food Measurement and Characterization. 2024 Jun 7:1-1.
56. Mohammadi M, Seifi A, Mokhtaryan M, Ghahan A, Ghaderkhani S, Mohammadnejad E, et al. Device-associated Infections (DAIs) in ICU: Using new CDC/NHSN definitions. Indian Journal of Forensic Medicine & Toxicology. 2020 Apr 1;14(2):3095.