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# The role of estrogen receptors alpha and beta in the development of uterine leiomyomas

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**THE ROLE OF ESTROGEN RECEPTORS ALPHA AND BETA IN THE  
DEVELOPMENT OF UTERINE LEIOMYOMAS**

by

**JACQUELINE KOOMSON**

B.A., New York University, 2015

Submitted in partial fulfillment of the  
requirements for the degree of  
Master of Science

2017

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Approved by

First Reader

---

Monica Onyango, Ph.D.  
Clinical Assistant Professor, Global Health

Second Reader

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Sat Dev Batish, Ph.D.  
Chief Director, Genetics

## **DEDICATION**

I would like to dedicate this body of work to the mothers, daughters, and sisters afflicted with this condition, especially Black women.

## **ACKNOWLEDGMENTS**

I would like to thank my thesis readers, Dr. Monica Onyango and Dr. Sat Dev Batish, for providing insight and taking the time to make thoughtful and thorough edits. Thank you for your enthusiasm and guidance.

I would also like to acknowledge my family and friends who provided unconditional support throughout my education, especially during my time working towards my Master's Degree. It is your positive affirmations, motivation, and prayers that helped me get through and make it to medical school this fall. I would not have achieved this accomplishment without you.

**THE ROLE OF ESTROGEN RECEPTORS ALPHA AND BETA IN THE  
DEVELOPMENT OF UTERINE LEIOMYOMAS**

**JACQUELINE KOOMSON**

**ABSTRACT**

Uterine leiomyomas are benign tumors within the uterus, where patients present with symptoms such as abnormal bleeding, urinary retention, and pelvic pressure. The exact etiology of uterine leiomyomas is unknown, but numerous theories have been proposed, indicating a multifactorial mechanism, including lifestyle and steroid hormones. Uterine leiomyomas have become a public health concern due to the high cost of treatment as well as the high prevalence within African American communities. Currently, many treatment options exist, ranging from conservative treatments that address symptoms, to surgical intervention to remove the uterus. Research efforts thus far have determined the relationship between the role of estrogen in the growth of uterine leiomyomas (which has led to development of medications that target different approaches to estrogen synthesis) and its effects in the pathogenesis. Studies have shown that estrogen acts on estrogen receptor subtypes, ER $\alpha$  and ER $\beta$ . This study examines the role of these two receptors in estrogenic effects, and how these effects relate to the development of uterine leiomyomas. Available research has shown that each receptor has its unique functions and impacts the growth of tumors differently. There is conflicting evidence in how the number of receptors and surrounding environment modulate leiomyomas, with some studies reporting that it is the

corepressors and/or coactivators that ultimately determine the influence of estrogenic effects. However, the general consensus of such studies suggests that estrogen receptor-specific therapeutic intervention is a novel area with great potential. The primary benefit of estrogen receptor-specific treatment, such as selective estrogen receptor modulators, is the ability to regulate physiological processes that contribute to the growth of uterine leiomyomas. Future directions of research include confirming the exact roles of ER $\alpha$  and ER $\beta$  and harnessing the effects of their differing functions to manage uterine leiomyomas.

**Key Terms:** estrogen, estrogen receptor alpha, estrogen receptor beta, uterine leiomyoma, receptor-mediated therapies, uterine fibroids



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## LIST OF ABBREVIATIONS

$\alpha\beta$ ERKO	Estrogen receptors alpha and beta knock out
AF-1	Activation function-1
AF-2	Activation function-2
C-terminal	carboxyl-terminal end
CEE	Conjugated equine estrogen
DNA	Deoxyribonucleic acid
ER $\alpha$	Estrogen receptor alpha
ER $\alpha$ KO	Estrogen receptor alpha knock out
ER $\beta$	Estrogen receptor beta
ER $\beta$ KO	Estrogen receptor beta knock out
E1	Estrone
E2	Estradiol
E3	Estriol
ERE	Estrogen response element
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
GnRH $\alpha$	Gonadotropin-releasing hormone agonist
HRT	Hormone replacement therapy
LH	Luteinizing hormone
MPA	Medroxyprogesterone acetate

mRNA.....	Messenger ribonucleic acid
MTCYB.....	Mitochondrial cytochrome b
N-terminal.....	amino-terminal end
PCR.....	Polymerase chain reaction
SDF-1.....	Stromal cell-derived factor-1
SERM.....	Selective estrogen receptor modulator
VEGF.....	Vascular endothelial growth factors

## INTRODUCTION

This thesis begins with a brief overview of uterine leiomyomas, their development, and public health implications. Next, the role of an important reproductive hormone and its receptor in the development of uterine leiomyomas is explored through a review of published literature. Focusing specifically on the impact of estrogen and its mechanism towards leiomyoma growth, this work will explore clinical management options of a condition for which there is currently no non-surgical cure. Recommendations for the direction of future research will be provided, as well as the presentation of novel approaches for the management of the condition.

### ***A Brief Overview of Uterine Leiomyomas***

Uterine leiomyomas, also known as uterine fibroids, are benign tumorous growths in the smooth muscle tissue of the uterus. Leiomyomas are the most common pelvic tumor affecting nearly twenty percent of women of childbearing age (Khan, 2014). While the tumors are normally asymptomatic, the foremost symptoms that may occur are abnormal uterine bleeding, urinary retention, and pelvic pressure. The exact etiology of leiomyomas is unknown, but there are many risk factors associated with their development, with greater incidence rates among Black populations. With a significant cost of treatment, an increasing

number of research efforts have been dedicated to isolating the cause and risk factors in an attempt to decrease the burden of the disease.

Uterine leiomyomas are a public health concern in part because of the high prevalence within Black women. High cost is associated with treatment, and loss of productivity also warrants public health concern in terms of discovering more efficient, and less burdensome, treatment options. For women between the ages 18 and 44, uterine leiomyomas account for nearly 47% of hysterectomies performed. Black women, specifically, are 2.4 times more likely to have a hysterectomy compared to White women (Stewart, 2013). In terms of costs for treatment, 4 to 10 billion dollars are spent annually, and an additional 1.5 to 17.2 billion dollars are lost annually to missed work hours (Owen, 2015).

Studies by Laughlin and Stewart (2011) on women between the ages of 35 and 49 have shown that uterine fibroids in Black American women had an incidence of 60% by 35 years old, which increased to 80% by age 50. In contrast, White women had an incidence of 40% by age 35, increasing to 70% by age 50. Black women have been found to be two to three times more likely to develop fibroids, be diagnosed at a younger age, have seven or more fibroids, and have more severe symptoms. Black women are also two times more likely than White women to have a hysterectomy because of leiomyomas (Weiss, 2009). With these numbers in mind, researchers work to develop a social and historic context of uterine leiomyomas to determine the cause of this racial disparity.



Studies have been performed elucidating the relationship between socioeconomics, race, and coping strategies. In a study conducted by Timmermans and Haas (2008) Black women were observed to minimize the severity of their condition, even though Stewart et al. (2013.) explained that Black women significantly experience severe symptoms, such as heavy bleeding. Questioning the reason behind their sentiments, many reported in the Timmermans and Haas study that the inability to take time off from work was a major influencer. Some reported being socialized to be strong Black women and avoiding racial stereotypes in the workplace. Many participants were aware of the impact leiomyomas had on their quality of life, but adapted to the inconvenient symptoms by carrying emergency kits and reinforcements for bleeding. Health-seeking behavior is an important contributor to the Black women's experience with uterine fibroids. Interestingly, participants reported believing that leiomyomas developed as a result of genetics and the historical implications of being a Black woman in America. This belief is significant because the ways in which patients conceptualize their condition influence the treatments they pursue, with many concerned with preserving fertility.

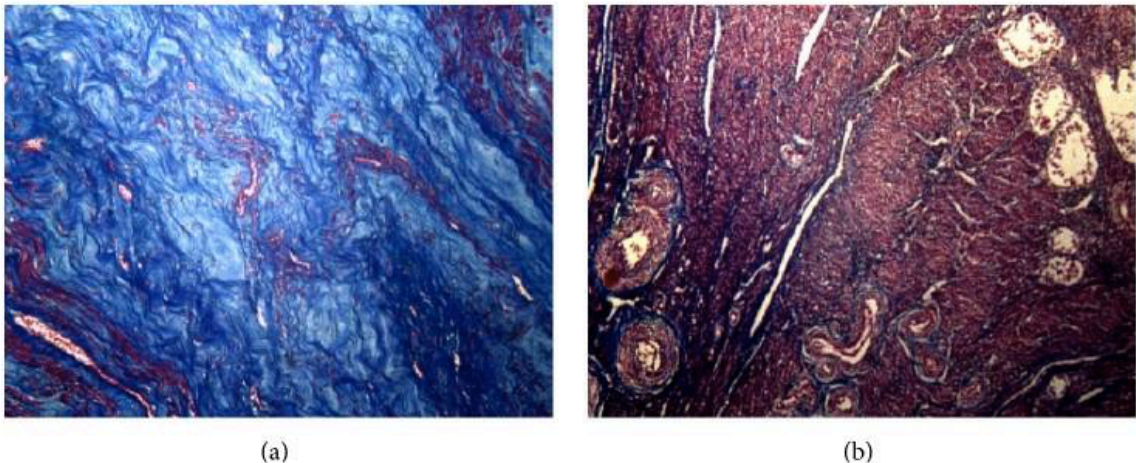
### ***Physiological and Pathological Changes that Occur with Uterine***

#### ***Leiomyomas***

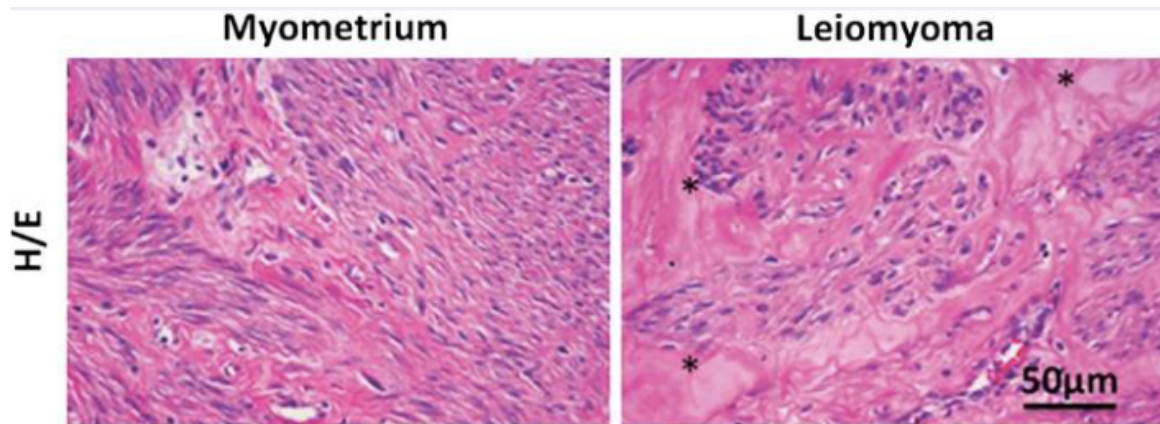
Uterine leiomyomas are composed of altered collagen fibrils, which cause rigidity in the myometrium. They are believed to be derived from the

transformation of myometrial connective tissue and smooth muscle cells into fibroblasts. They have also been found to contain altered cytokines and integrins due to tissue remodeling as well as inflammatory responses to fibroid development. Leiomyomas are typically found in a state of severe hypoxia, potentially resulting from abnormal angiogenesis as part of the inflammatory response (Owen, 2015).

A study conducted by Ligon et al. (2002) on the monoclonal origins of uterine leiomyomas found that 40 to 50% harbor cytogenetic abnormalities usually on chromosomes 6, 7, 12, and 14. Karyotyping suggests the possibility of translocations, random X inactivation, inactivation of growth suppressors, and overexpression of genes promoting self-renewal. The cytogenetic abnormalities vary among patients and are differentially expressed by race, indicating a multifactorial causative agent.



**Figure 1 Masson Trichrome of Uterine Leiomyoma** Uterine leiomyoma (a) compared to normal myometrium (b) with collagen fibers in blue and smooth muscle cells in red. Taken from Fernandes da Silva et al. (2016)



**Figure 2 Histology of Uterine Leiomyoma.** Hematoxylin and eosin stain of normal myometrium compared to a leiomyoma. Taken from Moravek et al. (2014)

Uterine leiomyomas typically appear during a woman's reproductive years, rarely occurring prior to menarche and regressing following menopause. The timing of the tumor development has piqued an interest in their development being influenced by steroid hormones (Wise, 2004). Estrogen and progesterone are the main ovarian steroid hormones synthesized during reproductive years. The smooth muscle tissues of uterine walls have receptors to these hormones, which are normally synthesized in synchrony with the ovarian and uterine cycles. Leiomyomas contain these receptors and have been found to have differential growth within different phases of the uterine cycle. Differential growth and shrinkage rates within the same woman were measured at consistent points within their cycle, and the results pointed to a multifactorial influence on growth.

While the etiology of leiomyomas is still unknown, early menarche is associated with increased fibroid size, certain fibroid types, locations, and developing multiple leiomyomas as compared to women who had menarche after age 12 (Owen, 2015) They have also been found to be increased in groups with

high rates of obesity. In a retrospective cohort study by Shikora et al. (1991) it was observed that 50% of women with uterine fibroids were obese, with 16% being morbidly obese. This suggested a possible increased risk from certain diets. Dandolu et al. (2010) was also able to conclude that body mass index was a significant contributor to uterine weight. Other hypotheses for the cause of uterine leiomyomas indicate a link to growth factors during wound healing. Roeder et al. (2012) disclosed through a histopathological study that small fibroids developed within scar formation indicating alteration during wound healing. In the process of wound healing, angiogenesis restores blood flow to the damaged area. In leiomyomas, there is an increased expression of vascular endothelial growth factors (VEGF), a key regulator of angiogenesis. VEGF is in part regulated by estrogen and progesterone, providing further evidence of the correlation between steroid hormones and leiomyoma proliferation (Chegini, 2010).

Symptoms of leiomyomas depend on their location within the uterus. The most frequent symptoms are abnormal uterine bleeding, pelvic pressure, urinary incontinence, urinary retention, pelvic pain, and spontaneous miscarriage. Abnormal uterine bleeding results from obstruction of uterine vasculature that creates endometrial venule ectasia (Lockwood, 2011). The increased uterine bleeding is associated with increased risk of developing anemia from blood loss, producing symptoms such as fatigue. The pressure and pain are the result of the

size of the myomas, which increase abdominal girth and also produce urinary symptoms as a result of ureter compression.

Leiomyomas occur subserosally, submucosally, and intramurally.

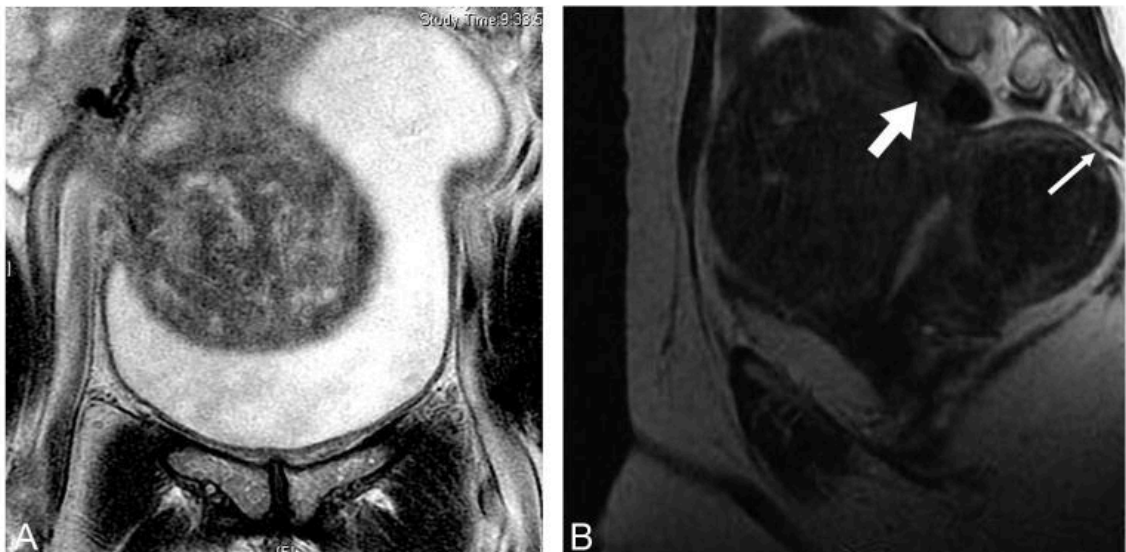
Subserosal tumors are just beneath the exterior lining of the uterine wall.

Submucosal growths are beneath the mucosal layer, and intramural myomas are beneath the interior lining of the uterine wall. Subserosal and intramural fibroids are the most common found, accounting for 95% of reported leiomyomas.

Subserosal tumors are usually asymptomatic and have no bearings on fertility, pregnancy, and birth outcomes compared to women without leiomyomas (Manta, 2016). Women with submucosal or intramural tumors have lower clinical pregnancy rates, implantation rates, and higher rates of miscarriage. In regards to submucosal fibroids, once they are surgically removed, patients become asymptomatic and have comparable pregnancy successes in comparison to women without fibroids (Pritts, 2009).

Pregnant women with uterine leiomyomas have increased risk of pregnancy-related complications. Women may experience bleeding in the first trimester, placental abruptions, and premature membrane ruptures. They also have increased risk of complications during labor and delivery, most commonly dysfunctional labor, prolonged labor, breeched positioning, pre-term delivery, and caesarean section. It was also observed that they had an increased risk for poor birth outcomes, decreased birth weight of newborns, and a decreased five minute Apgar score (Klatsky, 2008).

Since a majority of patients with uterine leiomyomas are asymptomatic, diagnosis usually results from pelvic exams during manual uterine palpations. Following the pelvic exam, ultrasounds are typically used to confirm the diagnosis. Transvaginal or abdominal ultrasounds are frequently used to detect tumors; however, they have limited ability to detect small tumors, making it difficult to identify fibroids at their onset. MRIs have better visualization, accuracy, and ability to map the exact location of small fibroids, but are used less frequently because of the cost (Lin, 2016). Cost of MRI's can range from \$1,000-\$5,000 whereas ultrasounds cost \$100-\$1,000 (Schwartz, 1994). Cost is a major deterrent in health-seeking behaviors and possibly contributes to the prevalence of uterine leiomyomas in Black women as compared to White women, who have stronger socioeconomic foundations.



**Figure 3 MRI of Uterine Leiomyoma.** Shown compressing the bladder (A) and the spine (thin arrow) and colon (thick arrow) (B). Taken from Laughlin and Stewart (2011).

There are numerous options for the clinical management of uterine leiomyomas, ranging from conservative approaches that manage symptoms and preserve fertility, to approaches that eliminate symptoms but also eliminate fertility options. Pharmaceutical options for symptoms include NSAIDs to address pain from cramping and bleeding. Newer approaches include aromatase inhibitors, selective progesterone receptor modulators, selective estrogen receptor modulators and GnRH agonists. These methods aim to inhibit the influence of sex steroid hormones on the growth and proliferation of fibroids (Khan, 2014). Surgical options include myomectomies and hysterectomies. Hysterectomies are the only method to cure uterine leiomyomas, but simultaneously render the patient infertile. Myomectomies remove fibroids, and depending on the size and location can be done laparotomically, laparoscopically, or hysteroscopically. They allow for the uterus to be left in place and maintain fertility.

Medical management approaches are gauged based on growth rate of existing myomas. In non-pregnant young women, or post-menopausal women with rapid growth, there is suspicion of malignancy and suggests surgical removal. Though it is rare that leiomyomas become cancerous, proactive removal is commonly performed as a cautionary measure. Once patients are found to have fibroids, they have serial follow-ups if they are small and not growing rapidly. Initially, these follow-ups occur every three months to establish growth intervals, and afterwards, follow-ups occur every four to six months. It is

important to have check-ins at a uniform time in a cycle in order to prevent erroneous conclusions on the growth and/or shrink rate of the fibroids. Initially, many physicians require follow-ups at three-month intervals in order to identify the tumors growth patterns. If the growth pattern is stationary, the intervals can be moved to four to six months (Wallach, 2004).

### ***Common Medical Management of Uterine Leiomyomas***

There are various medical options available in managing uterine leiomyoma, including both surgical and hormonal interventions. A myomectomy is the removal of a uterine fibroid while preserving the primary structure and function of the uterus. The surgery can be performed in three different ways: hysteroscopy, laparotomy, or laparoscopically. A myomectomy is the preferred surgical option for women who are considering having children. With advancing reproductive therapies available, women now have options and greater possibilities to conceive, and the preservation of the uterus is important in their decision to have a myomectomy (Wallach and Vlahos, 2004). Determination of the efficacy of myomectomy is based on two important criteria: the position and size of the leiomyoma. A large fibroid (>3cm) may result in a uterus that is not functional after a myomectomy, unable to perform its reproductive responses such as maintaining the ovum and establishing a placenta. The position of the fibroid may also prove to be too risky to attempt to remove, due to apprehension of causing damage to nearby structures, such as the bowel and urinary tract.



Hysterectomy, surgical removal of the uterus, or a large part of it, is the second most frequent major surgery performed on women, following cesarean sections. This surgical option eliminates any possibility of recurrence, unlike myomectomy, which is not a solution to the underlying cause of leiomyoma. When symptomatic women are close to menopause and/or have fulfilled their desire to bear children, having a hysterectomy becomes an easier decision. A hysterectomy offers the immediate relief of symptoms, including pain, bleeding, urinary tract symptoms, and pelvic pain (Rowe, 1999). Women are shown to be satisfied with their decision to have a hysterectomy because of symptom relief and believed to have better quality of life (Carlson et al., 1994).

Hysterectomy is the only method of clinical management that completely cures women of uterine fibroids. However, surgical risk and the loss of a functioning uterus have expanded the search to find effective alternative treatments of uterine leiomyomas. Hormonal treatments have been developed in hopes of alleviating symptoms, reducing the volume of fibroids, and targeting the underlying cause of leiomyomas. Gonadotropin releasing hormone agonists are one venture into non-surgical treatment options.

GnRH agonists work by increasing the release of gonadotropins. The drastic increase in gonadotropins causes the receptors to become desensitized. The ensuing downregulation creates a hypogonadotropic state that is similar to hormonal state during menopause. GnRH agonists have been clinically approved as a medical treatment that reduces the size of uterine leiomyomas and relieves

some of the symptoms in as early as the 4<sup>th</sup> week (Mizutani et al., 2005). However, the drawback to GnRH agonists is the drastic hypoestrogenic state that causes adverse effects, including osteoporosis (Stewart, 2001). It was also observed that once administration of GnRHa was discontinued, volume of the uterus began to increase, as it reverted back to developing its pathology. The need for safe and effective methods of treating uterine leiomyomas has elicited novel hormonal treatments that focus on one proven contributor to the development of uterine fibroids, for example, estrogen.

**Table 1 Common Medical Management of Uterine Leiomyomas** As adapted from (<http://www.fibroiddoc.com/treatment-options/>)

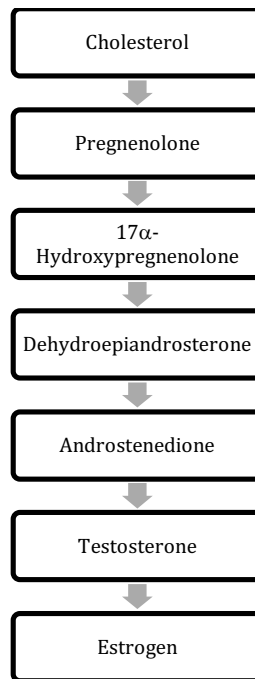
<b>Treatment</b>	<b>Type</b>	<b>Target</b>	<b>Advantages</b>	<b>Disadvantages</b>
Myomectomy	Surgical	Uterine fibroid	Symptom relief  Removes problematic fibroids  Preserves uterus for future pregnancies	Recurrence  Surgical risks
Hysterectomy	Surgical	Uterus	No recurrence  Symptom Relief  Only known cure	Highly invasive  Loss of fertility  Cost  Recovery time
GnRH	Hormonal	Gonadotropin releasing hormone receptors.	Fibroid volume reduction  Symptom relief  No procedure  Preserves uterus	Risk of osteoporosis  Recurrence  Menopause symptoms

### ***Importance of Estrogen in the Female Reproductive System***

Estrogen plays a vital role in the function of specific cells, tissues, and organs. It is primarily a growth hormone for the reproductive system, a trigger for ovulation, and also functions to maintain the development of oocytes. Estrogen is produced in the ovaries, adrenal glands, and placenta during pregnancy. As one of the most important reproductive hormones, it is required for maintenance of positive sexual and reproductive health (Simpson, 2003). Outside the reproductive system, estrogen also contributes to the synthesis of binding proteins, lipoproteins, and blood clotting proteins. There are three major and naturally occurring estrogens in women: estrone (E1), estradiol (E2), and estriol (E3). Estradiol is the most active and profound estrogen during reproductive years (Nelson and Bulun, 2001). For the purposes of this thesis, estrogen will be referring to E2, estradiol only.

The effects of estrogen are mediated through nuclear receptors. As a nuclear hormone, estrogen's mode of action in cells is regulation of transcription in the nucleus. The interaction of estrogen with its receptor causes the induction of mRNA, which results in distinct proteins to be synthesized by ribosomes. The proteins then produce the desired effects of estrogen on the cell (DeMayo et al. 2002). Estrogen synthesis begins in the ovary's theca cells where androgen precursors are synthesized from the steroid, cholesterol. The precursors are

converted to the final product, estrogen, in granulosa cells by the enzyme aromatase (Hojo et al 2011).

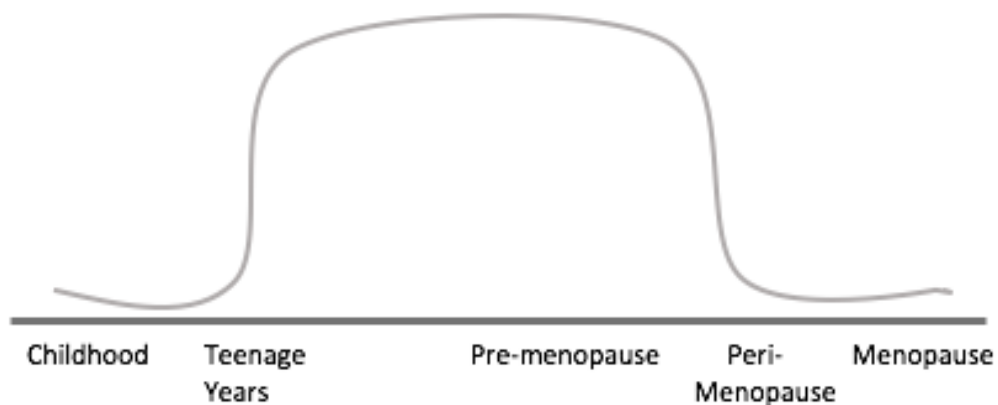


**Figure 4 Synthesis of Estrogen.** Adapted from Hojo et al. (2011)

Estrogen levels peak during a woman's reproductive years. Understanding the hypothalamic-pituitary-gonadal axis is important in assessing estrogen levels at any given time. Regulation of the axis impacts steroid-sensitive organs such as the uterus and works to maintain the homeostasis of body processes, including reproduction. The axis begins with the hypothalamus secreting GnRH, which binds to receptors on the anterior pituitary. The anterior pituitary releases LH and FSH into the blood. The hormones make their way to the ovaries and stimulate the production of estrogen and inhibin. Estrogen acts as a negative stimulus to the hypothalamus, and causes a decrease in the production of GnRH,

and thus, further production of estrogen (Plant, 2015). This complex interaction is the foundation of menarche and future menstrual cycles.

During the menstrual cycle, specifically at the follicular phase, estrogen induces ovulation by causing a surge in luteinizing hormone. During the luteal phase, estrogen works to proliferate the uterus lining for implantation with the influence of progesterone (Gleicher, 2000). The menstrual cycle provides the resources necessary to restore and replenish the reproductive tract, in order to maintain the wellness of sexual and reproductive health. Estrogen levels experience a drastic decrease and production slows at perimenopause, which is considered to be months before a woman's last period. After the onset of menopause, women will no longer menstruate, however there is still a small synthesis of estrogen in the ovaries. Low levels of estrogen are responsible for the hot flashes, sleep disturbances, and mood swings that encompass a woman's experience postmenopause (Prior 2006).



**Figure 5 Estrogen Levels during a Women's Life Cycle** Adapted from Prior (2006).

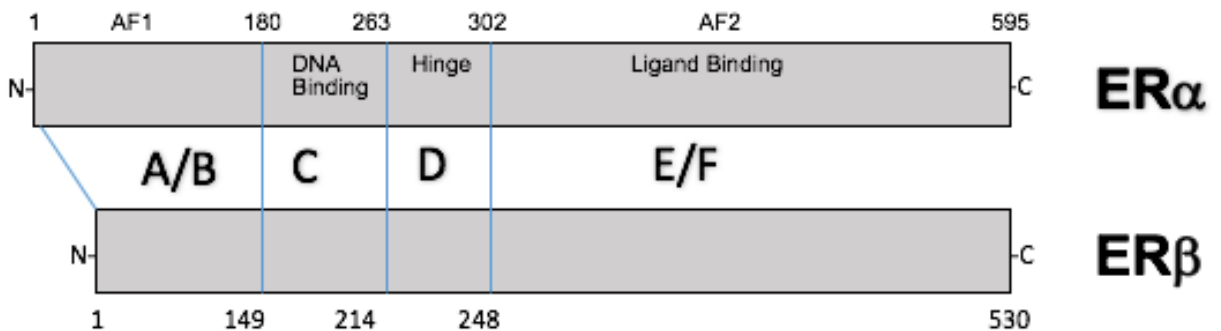
### ***ER $\alpha$ and ER $\beta$***

The estrogen receptors are nuclear receptors that contain structurally and functionally distinct domains. The two estrogen receptors, ER $\alpha$  and ER $\beta$ , have similar affinities for estrogen, bind the same DNA response elements, and have conserved sequence homology except in their N-terminal. The DNA-binding domain is responsible for DNA recognition and binding, and it is the most conserved area. The ligand-binding domain is multifunctional and occurs at the C-terminal. The N-terminal represents the most variable domain in both sequence and in length. Two activation functions, AF-1 and AF-2, assist transcriptional activation. AF-1 is continuously active and located at the N-terminal while AF-2 is located at the C terminal and is ligand- dependent. Ligand-dependent estrogen signaling is cell-specific and depends on the composition of coregulatory proteins in a given cell and signaling is initiated with the binding of estrogen to the receptors (Katzenellenbogen, 2000).

It was initially believed that ER $\alpha$  was the only estrogen receptor that mediated the extensive effects of estrogen. PCR primers were used to clone ER $\beta$  from humans and it was discovered that the ER $\alpha$  and ER $\beta$  were encoded by and located on different genes, chromosome 6 and chromosome 14, respectively, indicating that they are both distinct receptors. The identification of ER $\beta$  revealed the complexity of estrogen signaling. This discovery helped explain the estrogen action in tissues that do not express ER $\alpha$ . Due to the structure of the two receptors, they are observed to form heterodimers that have both similar and

distinct roles in estrogen-dependent action. Their different distributions contribute to differing actions in specific tissues (Dechering, 2000).

When both ER $\alpha$  and ER $\beta$  are expressed in cells, ER $\beta$  can antagonize the transcription processes of ER $\alpha$  and inhibit the action of ER $\alpha$ . The mechanisms regulating the estrogen receptors, and interaction of ER $\alpha$  and ER $\beta$  with each other present a means to understand estrogen-mediated processes in normal and pathological tissues. The role of estrogen in various cells is dictated by the structure of the ligand, the distribution and type of estrogen receptor, and the surrounding environment of coactivators and corepressors. Novel synthesis of estrogen receptor-specific agonists and antagonists will prove to be an important field of therapy (Katzenellenbogen, 2000).



**Figure 6 Schematic of structure and homology of ER $\alpha$  and ER $\beta$ .** The domains A-F and activation functions, AF-1 and AF-2, are shown. Adapted from Katzenellenbogen et al (2000).

## **SPECIFIC AIMS**

The specific aims of this thesis are:

1. Indicate the importance of estrogen dependency on the growth of uterine fibroids.
2. Examine the role of ER $\alpha$  and ER $\beta$ .
3. Examine the effects of receptor-mediated therapies.
4. Recommend other areas of interest to address possible pathways for the disease.



## **PUBLISHED STUDIES**

### ***Biological and Clinical Evidence Indicating Estrogen Dependency of Uterine Leiomyomas***

This section will focus on various biological and clinical trials involving treatment options that indicate a strong estrogen dependency on the growth and size of uterine leiomyomas. Proposed treatment options, such as aromatase inhibitors and gonadotropin releasing hormone agonists, are a few therapies used to decrease the amount of estrogen in the uterus that subsequently decreases the size of the fibroids. Many of these therapies are directed towards premenopausal women, who have high levels of estrogen due to the natural menstrual cycle, as compared to post-menopausal women, who have a significantly lower amount of estrogen.

Marshall et al. (1998) determined through a prospective study that the incidence of uterine fibroids increased with early onset of menarche ( $\leq 10$ ) and those with late onset ( $\geq 16$ ) had a lower risk. Early onset menarche had a relative risk of 1.24 with a 95% confidence interval 1.08-1.4, whereas late onset menarche had a relative risk of 0.68 with a 95% confidence interval of 0.53-0.88. Wise et al. (2004) explained that early onset of menarche increased the risk of uterine fibroids because the myomas respond to estrogen, and their incidence parallels the development and changes in reproductive hormones. Early onset menarche leaves women with an increased number of ovulatory cycles. The

luteal phase is a stage of the menstrual cycle where the lining of the uterus thickens. More encounters with the luteal phase increases the risk of developing a fibroid.

Uterine leiomyomas predominantly occur during premenopausal years, which indicates that estrogen plays an important role in its development because after the onset of menopause, estrogen levels dramatically decrease.

Templeman et al. (2009) assessed the relative risk factors for pre-, peri-, and post-menopausal women. They determined that the relative risk was dramatically less for post-menopausal women (1.00) compared to pre (5.31, with a 95% confidence interval of 3.61-7.82). It was also found that perimenopause women had decreased risk than premenopausal women. During perimenopause, the body is approaching menopause and estrogen production decelerates, indicating the important role of estrogen.

Templeman et al. (2009) also found that women who used hormone replacement therapy post-menopause had an increased relative risk compared to those who did not use HRT. Whether they used only estrogen, or a combined or mixed hormone therapy, the risk was greater (RR 2.03, 2.38, 3.25, respectively) than the relative risk of no HRT (1.00). The hormone therapy replenishes some of the estrogen that is lost with the onset of menopause. This could hinder the reduction of pre- and perimenopausal fibroids, which would otherwise shrink on their own post-menopause because of the lack of high levels of estrogen (Ciarmela et al. 2014).

Ishikawa et al. observed that aromatase mRNA levels in leiomyoma tissues were strikingly higher compared to non-leiomyoma myometrium. This paralleled the increase in levels of estrogen within myomas, which indicates that myomas produce estrogen de novo as well as receiving the hormone from the ovaries. Furthermore, low levels of  $17\beta$ -hydroxysteroid dehydrogenase have been found in myomas.  $17\beta$ -hydroxysteroid dehydrogenase converts estradiol to estrone. Estradiol is the most active form of estrogen, whereas estrone has lesser effect (Reed et al. 1985). Without  $17\beta$ -hydroxysteroid dehydrogenase, estradiol accumulates in the cells, and leads to up-regulation and hyper-responsiveness to estrogen, resulting in myoma growth (Parker, 2007).

Aromatization of androgens to estrogens is catalyzed by the cytochrome P450 enzyme, aromatase, specifically converting testosterone to estradiol and androstenedione to estrone. Aromatase's structure contains a steroid-binding site and an iron-containing site. This sets up the potential for the enzyme to be inhibited by two methods: blocking the steroid-binding site or interfering with the iron-binding site (Fishman, 1982). Aromatase inhibitors are the ideal means to target estrogen because the aromatase enzyme catalyzes the final step in estrogen synthesis. This decreases the chance of affecting other cholesterol derived products, including cortisol and aldosterone (Murphy, 1998).

Shozu et al. (2003) reported the first case of a premenopausal patient who used an aromatase inhibitor to manage a large uterine fibroid. The middle-aged woman began therapy with 2 mg of fadrozole, an aromatase inhibitor. Eight

weeks post onset of therapy, the volume of the large fibroid had decreased by 71%. E<sub>2</sub> levels had lowered to <10 pg/mL from the initial 80 pg/mL found in her serum upon first examination. At the conclusion of the treatment, there was no regrowth of the fibroid and her initial symptoms had ceased. Aromatase inhibitors suppress estrogen synthesis directly. The case report suggests that rapid decrease in serum levels of E<sub>2</sub> decreased the patient's symptoms and volume of the fibroid. It was reasonable to conclude the shrinkage of the fibroid was due to the decrease in estrogen.

Maggiore et al. (2014) conducted a non-randomized comparative study where they secondarily evaluated the effect of an aromatase inhibitor, letrozole, on the size of uterine myomas prior to a myomectomy. This 80-patient study was divided into two groups: group A received the three-month treatment and group B did not. Total myoma volume was decreased ( $-41.2 \pm 7.7\%$ ), which provided a significant decrease in the amount of time it took to complete the operation to remove the myomas. The study confirmed that letrozole is a safe therapy in reducing the size of uterine fibroids and showed that the blocking of estrogen can cut off the supply that allows fibroids to grow.

Hilario et al. (2009) designed a clinical study to assess the role of aromatase inhibitors in the management of uterine leiomyomas size, symptoms, and serum levels of estradiol and FSH. Statistical analysis showed significant reduction in the volume of the uterine leiomyoma from T1 to T2 with aromatase inhibitor treatment. Although there was reduction in size, serum levels of estradiol

did not have a significant decrease, unlike what was presented in the studies evaluated by Shozu et al. (2003) and Maggiore (2014). There was still a decrease in the size of the fibroid even though hypoestrogenism was not present. They hypothesized that it was due to the low dosage of treatment that was unable to sufficiently block the synthesis of estradiol in the ovary. With an increased dosage, it is possible that the levels of estradiol would significantly decrease and have a greater impact on alleviating symptoms.

Aromatase inhibitors are one important estrogen-suppressing treatment for uterine leiomyomas. While they directly inhibit the synthesis of estrogen, gonadotropin releasing hormone agonists work indirectly to create a state of hypoestrogenism in order to decrease the volume of uterine fibroids. Parsanezhad et al. (2010) compared the efficacy of aromatase inhibitors and GnRHa. They designed a randomized and controlled clinical trial where 60 subjects that had a uterine leiomyoma greater than 5cm were randomized into group A and group B and completed the study. Group A received the aromatase inhibitor, letrozole, while group B received the GnRHa, triptorelin. Both groups had significant reduction in the size of the myoma. Group A decreased by 45.6% and group B decreased by 33.2% at the end of the 12 weeks of treatment.

Chegini et al. (1996) described the role of GnRH in the development of leiomyomas and how GnRHa inhibits its mechanisms. GnRHa suppressed the rate of [<sup>3</sup>H] thymidine incorporated into myometrial smooth muscle cells, which is significantly stimulated in the presence of E<sub>2</sub>. Increased levels of [<sup>3</sup>H] thymidine

contributes to the proliferation of the smooth muscle cells and its response to GnRHa indicated that there are receptors present in myometrial smooth muscle cells that respond to GnRH. Chegini et al. (1996) provided the first data to indicate the direct action of GnRH in myometrial smooth muscle cells. This is a different perspective to the mode of action than those who focus on the indirect nature of GnRHa. GnRHa is more commonly known to downregulate the pituitary-ovarian-gonadal axis, which leads to the inhibition of estrogen production to levels similar to those measured during menopause (Beshay and Carr 2013). Cessation of GnRHa has resulted in an increase in leiomyoma volume as explained by Friedman (1993) and Broekman (1996). This again suggests that estrogen has a strong impact on the development of uterine leiomyomas, and indicates that there are possible novel avenues to treat estrogen levels.

Mizutani et al. (2005) confirmed that GnRHa reduce the size of uterine fibroids by assessing the effects of three add-back therapies: CEE, MPA, and CEE plus MPA. The hypoestrogenism that GnRHa causes has been clinically shown to produce disorders, such as osteoporosis (Friedman, 1993). Fibroids were obtained from patients treated with GnRHa, GnRHa and an add-back therapy, and those who were not given any treatment. Mizutani et al. (2005) quantified the size of the leiomyoma by the number of Ki-67-positive leiomyoma cells/cm<sup>2</sup>, a marker for cell proliferation activity. GnRHa alone caused the biggest decrease in Ki-67-positive cells (30% at the end of the 4<sup>th</sup> week, and 14% by the

12<sup>th</sup> week). The add-back therapies were considered to have a possible benefit in preventing the development of adverse disorders, however, the reduction of Ki-67-positive cells, and in relation, the size of the uterine leiomyomas, were not as significant had GnRHa been used alone.

It is biologically and clinically confirmed that the development of uterine fibroids is estrogen dependent. Had it not been, therapies, such as aromatase inhibitors and gonadotropin releasing hormone agonists, would not be effective. However, these treatments have been able to reduce the volume of uterine myomas, alleviate symptoms, and also reduce operative time of removing the fibroids. The reduction of estrogen levels and subsequent shrinkage of the benign tumors confirm the role of estrogen in developing and maintaining the size of the fibroids. Continued exploration in endocrine therapy is an important effective way to treat conditions because it decreases the need to perform surgery.

### ***ER $\alpha$ and ER $\beta$ role in Uterine Leiomyomas***

Despite research demonstrating a discernable role of ER $\alpha$ , ER $\beta$ 's influences on estrogen-dependent processes, the functions and growths have not been clarified. Several studies have indicated that in benign tumors, the ratio of ER $\alpha$  and ER $\beta$  is higher compared to normal tissues. While ER $\alpha$  is shown to have a proliferative action in target tissues, ER $\beta$  is hypothesized to be inhibitory to excess proliferation. The balance and influence of the two receptors on

estrogen maintenance and proliferation provide a point of research for receptor-mediated therapies.

Fuqua et al. (2003) created a monoclonal antibody to detect ER $\beta$  at the protein level in breast cancer tissues in a study of 242 patients. Their results indicated that ER $\beta$  was less significant in determining tumor grade and proliferation in comparison to ER $\alpha$ . ER $\beta$  was only observed to have a correlation with aneuploidy. The rate of aneuploidy was greater in ER $\beta$  -positive tumors than tumors that lack both ER $\alpha$  and ER $\beta$ . This result indicated that ER $\beta$  -positive tumors might be more assertive than others. In contrast, Park et al. (2003) used in situ hybridization of mRNA to compare ER $\beta$  mRNA levels in varying breast tissue. It was discovered that ER $\beta$  expression in metastatic lymph node tissues and breast cancer biopsies was decreased compared to those in normal mammary tissue.

Similarly, Rutherford et al. (2000) assessed the expression of ER $\alpha$  and ER $\beta$  mRNA and protein levels in normal ovarian and metastatic tumor biopsies. Western blot analysis was used to determine protein levels with the use of antibodies. Of the 25 participants, nine had normal biopsies, eight were of a primary tumor, and eight had a metastatic tumor. A primer was used to amplify the expression of ER $\alpha$  and ER $\beta$  through a reverse transcriptase polymerase chain reaction and it was quantified. They found that amounts of ER $\alpha$  and ER $\beta$  varied in the different types of biopsies. Normal ovaries had both ER $\alpha$  and ER $\beta$ ,



with ER $\beta$  being greatly transcribed at a 1:2 ratio, which was determined by the mRNA and proteins present. Primary tumor biopsies were shown to have ER $\alpha$  in greater abundance, with drastically lower levels of ER $\beta$ . In metastatic tumor biopsies, there was only ER $\alpha$  present. This suggested that ER $\alpha$  was responsible for the proliferation of undesired growth, and ER $\beta$  provided inhibition to this process.

Pujol et al. (1998) also determined that the ER $\alpha$ /ER $\beta$  ratio increase was evident in ovarian cancers compared to normal ovaries and cysts. 60% of ovarian cancers were shown to have an ER $\alpha$ /ER $\beta$  ratio of greater than one, and they showed that ER $\beta$  was predominant in normal ovarian tissues. These findings resonate with those found by Rutherford et al. (2000), that ER $\alpha$  overexpression and/or loss of ER $\beta$  contribute to tumorigenesis. ER $\alpha$  again is shown to have proliferative actions, while ER $\beta$  is present to put a halt on excess growth. The balance between the two subtypes of the estrogen receptor is indicated to be essential in maintaining cellular homeostasis.

There are few studies on knockout ER $\alpha$  or ER $\beta$  receptors for uterine leiomyomas, but there are ample studies on breast cancer. Boccinfuso and Korach (1997) investigated the role of the estrogen/ER signaling pathway on the mammary development and tumorigenesis. The presence of ER $\alpha$  in both ductal epithelial cells and stromal cells revealed that there is a strong estrogen link in estrogen and mammary gland development, which was shown to secrete growth

factors in the presence of estrogen. They utilized a knockout study to understand the direct link between the estrogen receptor alpha and mammary development. The mammary oncogene Wnt-1 was included to assess how the tissue would respond when it lacked estrogen receptors. ERKO/Wnt-1 mice still developed mammary tumors, but had delayed onset. This suggests that while estrogen receptor knockouts cannot remove the carcinogenic effects of Wnt-1, they present an important role in the stimulation of tumorigenesis.

Couse et al (1995) created a knockout study to investigate estrogen insensitivity in mice that lacked the estrogen receptor alpha gene. Reverse transcriptase PCR illustrated that no wild type ER $\alpha$  mRNA was observed after the gene knockout. Their study demonstrated that estrogen insensitivity was present in ER $\alpha$ KO mice, which was confirmed by increased estrogen levels in the serum, which increased ten-fold. The lack of response to estrogen treatment resulted in significantly decreased DNA synthesis, and transcription of downstream post-estrogen action genes. This indicates that the presence of the estrogen receptors is important for estrogen-mediated action.

Couse et al. (1999) continued to explore the roles of ER $\alpha$  and ER $\beta$  to understand how they affect target tissues. They found that mice homozygous for both receptor knockout, coined  $\alpha\beta$ ERKO, created mice that had normal reproductive development, but were infertile, indicating the importance of the receptors for the ability to conceive. The  $\alpha\beta$ ERKO phenotype suggests that both estrogen receptors are essential for ovarian function. Although the mechanism is

still unclear, Couse et al. attribute the loss of function to be influenced by elevated expression of the MIS gene, which has been observed in oocyte and granulosa cell death.

Dupont et al. (2000) performed a knockout study on ER $\alpha$  and ER $\beta$  on the reproductive phenotypes of mice. In order to create the ER $\alpha$  knockout, they altered exon 3, which encodes the first zinc finger of the DNA-binding domain. For ER $\beta$ , they inserted the *neo* gene into the *SpeI* site of exon 3 through homologous recombination. They controlled these disruptions with immunohistochemistry, using staining to detect the signals of the different ER $\alpha$ , ER $\beta$ , ER $\alpha$ KO, and ER $\beta$ KO. Through this study Dupont et al. determined that with ER $\beta$ KO the incomplete penetrance of weakened follicular growth indicated the existence of a partial functional compensation by ER $\alpha$ . They also showed that although ER $\alpha$  is the predominant estrogen receptor in the uterus, with an ER $\alpha$  knockout, ER $\beta$  could possibly compensate for the loss of ER $\alpha$ .

Krege et al. (1998) also used the *neo* gene in exon 3 by homologous recombination to create phenotypic mice lacking the estrogen beta receptor. Their RNA analysis revealed that the tissues did not have functional ER $\beta$  mRNA and proteins in mice that were ER $\beta$   $-/-$ . Development and functionality of the breasts were not affected by the absence of ER $\beta$ , whereas mice lacking ER $\alpha$  were unable to develop breast tissue further than what had occurred prior to menarche. The urogenital and reproductive organs of the mice were functional

and normal in the ER $\beta$  -/- mice. Krege et al. determined that ER $\alpha$ , rather than ER $\beta$ , was the estrogen receptor subtype that was essential for normal growth and function.

Jakimiuk et al. (2004) designed a prospective study to assess the differences of ER $\alpha$  and ER $\beta$  expression in uterine leiomyomas and healthy myometrium. Sensitivity of leiomyomas to estrogen was shown to have a greater significance than increased expression. They observed that there was no significant difference between the expression of ER $\alpha$  and ER $\beta$  in uterine leiomyomas compared to normal myometrium. This led to the conclusion that it was due to the sensitivity of the ER receptors, not the increased or decreased expression of the receptors, that influenced the development of uterine leiomyomas.

However, Hall and McDonnell (1999) stated that although the sensitivity of the estrogen receptors plays a significant role in the development of uterine leiomyomas, the expression of the receptors is also a great influencer. They suggested that ER $\beta$  is capable of regulating the transcriptional activity of ER $\alpha$ . They proposed the explanation that ER $\beta$  can suppress ER $\alpha$ , because ER $\beta$  can bind its response element in a constitutive way and challenge ER $\alpha$  for the access of the DNA target. The testing of Hall and McDonnell's hypothesis revealed that ER $\beta$  functions as a trans-dominant inhibitor of ER $\alpha$  transcriptional activity by decreasing the cells sensitivity to estrogen. Therefore, the expression of the

estrogen receptors sways the cellular response to estrogen, estrogen agonists, and estrogen antagonists.

Bakas et al. (2008) utilized samples from biopsies of normal myometrial tissue and uterine fibroids to determine the DNA-binding status of ER $\alpha$  and ER $\beta$ . They assessed whether increased level of estrogen receptors in leiomyoma was caused by increased gene expression of ER $\alpha$  and/or ER $\beta$  and how it affected their binding to ERE. Bakas et al. accomplished this by using samples from 35 women and revealed that the level of expression of ER $\alpha$  and ER $\beta$  mRNA in leiomyomas were greater than levels found in normal myometrium, which resulted in higher levels of estrogen receptors. This increase in ER $\alpha$  in the leiomyoma allowed the receptor to bind to ERE as a homodimer and ER $\beta$  as a heterodimer, a feat that is not seen in normal myometrium. Down-regulation of ER $\alpha$  in uterine leiomyomas is a potential therapy of their growth

ER $\alpha$  was once thought to be the only type of estrogen receptor, however, presence of ER $\beta$  indicates its physiologic role in uterine leiomyomas. Valladares et al. (2006) explored the cellular localization of the estrogen receptor subtypes in uterine leiomyomas with a retrospective study. ER $\alpha$  was observed to be present in only smooth muscle cells of the leiomyoma. ER $\beta$ , on the other hand, was present in smooth muscle cells, connective tissue cells, and endothelial cells. Smooth muscle cells, connective tissue cells, and endothelial cells produce various factors, including VEGF. Valladares et al. demonstrated that the

presence of factors regulated the estrogenic effects of ER $\alpha$  and ER $\beta$ . ER $\beta$ 's location in endothelial cells and connective tissue cells indicate that the factors produced in these cells specifically modulate ER $\beta$ , since ER $\alpha$  is not present in these cells. They concluded that it is possible that ER $\beta$ , and not ER $\alpha$ , is the major contributor to the development of uterine leiomyomas.

Brandon et al. (1995) attempted to characterize the molecular mechanisms that are the basis for developing uterine leiomyomas. They suggested that overexpression of estrogen receptors results in increased sensitivity to estrogen and also caused increased expression of progesterone receptors in leiomyomas. Through Northern analysis, they showed that ER mRNA levels were increased from 1.4 to 12.6-fold in leiomyomas compared to healthy myometrium. Scatchard analysis determined that in leiomyomas, ER binding capacity to estrogen increased, but ER binding affinity was not significantly different from normal myometrium. This finding revealed that it is possibly the estrogen signal transduction pathway that may be more enhanced in uterine leiomyomas, which is a reasonable mechanism to account for the increased expression of progesterone receptors in leiomyomas. It is possible that leiomyomas autonomously increase expression of both estrogen and progesterone receptors.

Glance et al. (2009) characterized the pharmacological regulation of stromal cell-derived factor-1 to determine its use as a biomarker for estrogenic effects. They explained that some pharmacological models used to determine

estrogenic activity are limited by the low throughput and lengthiness of treatment. They used microarray analysis to distinguish differences in gene expression in their rat analog. Glace et al. revealed that a rat leiomyoma cell line that lacked ER $\alpha$  did not have estrogen-dependent SDF-1 regulation, suggesting the roles of estrogen in regulated SDF-1 expression. After treatment with estrogen on functional ER $\alpha$ , the expression of SDF-1 increased. They were unable to confirm that SDF-1 played a significant role in proliferation, but they determined that SDF-1 gene regulation is conserved and could potentially be used as a biomarker.

Shaik et al. (2011) explored the expression of ER $\alpha$  and mitochondrial cytochrome b (MTCYB) transcripts in premenopausal uterine leiomyomas. Estrogen was observed to stimulate the expression of mitochondrial genes. This study proposes that mitochondrial genes are important in leiomyomas. Overexpression in ER $\alpha$  and MTCYB transcripts were observed at  $9.18 \pm 0.79$  fold and  $5.24 \pm 0.48$  fold, respectively, in uterine leiomyomas compared to normal myometrium. This increase was also correlated with the fact that leiomyomas with CC genotype had increased levels of ER $\alpha$  at  $11.9 \pm 1.02$  fold. Increased transcript resulted in elevated ER $\alpha$  levels, which influence estrogenic activity. Their results suggested that the elevation of ER $\alpha$  and MTCYB transcript levels and its relationship with ER $\alpha$ , -397 CC genotype suggests the mitochondrial-mediated role of estrogen as an influencer of the progression of leiomyoma development.

Premenopausal leiomyomas have been observed to express higher levels of ER $\alpha$  and ER $\beta$  than healthy myometrium. Strissel et al. (2007) assessed the expression of ER $\alpha$  and ER $\beta$  in postmenopausal women. They evaluated a group of 14 postmenopausal patients using reverse transcriptase PCR and realtime PCR. Mean average results demonstrated that ER $\beta$  was 2.5-fold overexpressed in postmenopausal women (p=0.038). ER $\alpha$  was not significantly different in postmenopausal leiomyomas compared to normal myometrium. This suggested that upregulation of ER $\beta$  was evident at the transcriptional level and the actions of estrogen functioned predominantly in the ER $\beta$  pathway compared to the ERA. Strissel et al. accounted for the increased expression of ER $\beta$  as being responsible for growth suppression and maintenance.

Sakaguchi et al. (2003) assessed the expression of ER $\alpha$  and ER $\beta$  in premenopausal and postmenopausal myometrium. Competitive reverse transcriptase polymerase chain reaction-Southern blot analysis was used to quantify the levels of ER $\alpha$  and ER $\beta$ . In premenopausal women, the ratio of ER $\beta$  to ER $\alpha$  was evaluated to be 0.6-1.5, signifying the presence of more ER $\alpha$  than ER $\beta$ . In contrast, for postmenopause women, the ratio was determined to be 2.5-7.6. This suggested that between premenopause to postmenopause, there was a change in the ratio of ER $\beta$  to ER $\alpha$ . Administration of estrogen was shown to change the ER $\beta$  to ER $\alpha$  ratio in postmenopausal women to that of premenopausal women.



Sakaguchi et al. also showed that in premenopausal women, GnRHa decreased expression of ER $\alpha$  to levels similar to postmenopausal women, but increased ER $\beta$  expression greater than that found in postmenopausal women. Relative scores of Western blotting for premenopausal women were (183  $\pm$  49) in ER $\alpha$  pre-treatment with GnRHa. After treatment, the relative scores were (122  $\pm$  36). WS values for ER $\beta$  were 14  $\pm$  13 and increased to (82  $\pm$  27). For postmenopausal women, ER $\alpha$  WS values were (110  $\pm$  26) to (197  $\pm$  34) post-treatment and ER $\beta$  was (86  $\pm$  22) to (37  $\pm$  17). Overall, GnRHa increased the ratio of ER $\beta$  expression to ER $\alpha$  expression.

Shao et al. (2015) assess the expression of ER $\alpha$  and ER $\beta$  in solitary and multiple uterine leiomyomas, indicating a possible correlation between estrogen receptor subtypes and the subtypes of leiomyomas. They used ELISA assay to quantify estrogen concentration, realtime PCR was used for mRNA expression of both receptors, and Western blot was used for detection of ER $\alpha$  and ER $\beta$  protein expression. They observed that ER $\beta$  mRNA and protein levels in multiple leiomyomas were significantly greater than those in solitary growths. However, ER $\alpha$  mRNA and protein levels in multiple leiomyomas were lower than those in singular leiomyomas. High ER $\beta$  and lower ER $\alpha$  was characteristic of multiple leiomyomas compared to solitary tumors. In both solitary and multiple leiomyomas, ER $\alpha$  expression was greater than ER $\beta$  and the concentration of estrogen was dependent on ER $\alpha$  expression.

### ***Studies Concerning Therapies to Remediate the Effects of ER $\alpha$ and ER $\beta$***

Selective estrogen receptor modulators (SERMs) are non-steroidal compounds that act on estrogen receptors as either agonists or antagonists. The most commonly used SERMs are tamoxifen and raloxifene. In different tissues of the body, they can have a stimulatory or inhibitory effect, with varying sensitivity. They are used for estrogen-responsive cancers, such as breast cancer. The ability for SERMs to have an antiestrogenic effect on estrogen receptors led to the development of a SERMs to manage uterine fibroids (Lethaby, 2008).

Due to the fact that SERMs can have different actions in target tissues, the complete molecular network it is of great importance. The number of coactivators and corepressors influence the effect of SERMs through the estrogen receptor complex. Some coactivators are ubiquitous and can amplify the stimulatory action of the estrogen receptor complex by altering the genes involved in the ligand-activated initiation. A tissue site that contains more coactivators or less corepressors will more likely be an estrogenic site, and the opposite is true for anti-estrogenic sites (Lonard, 2006).

SERMs act on the two estrogen receptors, ER $\alpha$  and ER $\beta$  after diffusing into cells. The receptors experience conformational and structural changes that allows them to interact with estrogen response elements, which results in specific estrogen effects. The ratio of ER $\alpha$  and ER $\beta$  plays an important role in the anti- and estrogenic effects of SERMs. Roger et al. (2001) observed that a low ER $\alpha$ /ER $\beta$  had protective effects on cell proliferation. Higher levels of ER $\beta$

correlated with low levels of Ki67 ( $r$  0.333). In contrast, a high ratio ER $\alpha$ /ER $\beta$  proved to have a proliferative response. This observation provides insight in assigning SERM antiestrogen preventative therapy for individualized treatment.

One class of SERMs, tamoxifen, is an antiestrogenic drug used to treat breast cancer. Powles et al. (2007) demonstrated that tamoxifen was successful in reducing the risk of developing an ER-positive breast cancer through their 8-year study of assigning the drug versus a placebo in almost 2500 women. They divided the participants in half, those who received tamoxifen, and those who received the placebo, and discovered that posttreatment, those with tamoxifen had less of a risk with a hazard ratio of .048 at a 95% confidence interval. Cuzick et al. (2007) also noted that while there was a significant decrease in breast cancer risk with tamoxifen, this reduction was only seen in ER-positive cancers. There was no significant reduction in ER-negative cancers. Tamoxifen was able to have success in creating an antiestrogenic effect on breast cancer cells, however, it is not used for the treatment of uterine leiomyomas because it has a risk of causing endometrial carcinoma. Therefore, there were no randomized control trials on uterine leiomyomas because of the adverse effects.

Raloxifene is another member of approved SERM that has been shown to have antiestrogenic actions on uterine leiomyomas. Unlike tamoxifen, it does not have the adverse side effects of osteoporosis, thrombolytic events, and endometrial carcinoma. Vogel et al (2006) conducted a study to compare the effectiveness of both tamoxifen and raloxifene in their ability to reduce ER-

positive breast cancer risk. Their results concluded that raloxifene was just as effective as tamoxifen on the risk reduction of invasive breast cancer. They also discovered that those who received tamoxifen treatment developed endometrial hyperplasia, a precursor to endometrial cancer, more commonly than those receiving raloxifene. There was also a downward trend of uterine cancer incidence in the raloxifene group. Raloxifene was observed to be a less toxic and therefore a safer option in comparison to tamoxifen for treating invasive breast cancer.

Palomba et al. (2001) designed a randomized, double blind, placebo-controlled study to assess the effectiveness of raloxifene on the size of uterine leiomyomas. 70 women were split into those who received 60 mg/day of the treatment and those who received the placebo. 28 cycles of treatment were given, and during the early cycles, no significant variations were observed with the two groups. Six cycles into the study, fibroid size had decreased with raloxifene. They determined that in postmenopausal women, raloxifene was successful in reducing the size of uterine leiomyomas. However, mean E<sub>2</sub> levels of the subjects were greater in those who were receiving the treatment compared to those who were receiving the placebo (11.4 ± 4.8 vs. 10.9 ± 4.6). Palomba et al. attributed this to the fact that there are ER $\alpha$  variants that lack E<sub>2</sub> binding sites or the incorrect translation of ER $\alpha$  mRNA.

Raloxifene has been approved to successfully reduce uterine leiomyomas in postmenopausal women. Jirecek et al. (2004) assessed raloxifene's success

in middle aged premenopausal women after three months of treatment. Of the 25 patients in the study, 13 received raloxifene treatment and 12 were the control group. Raloxifene was shown to induce a 22.2% decrease in the volume of leiomyomas after 3 months. Those on the raloxifene treatment had a baseline value of  $59.0 \pm 48.1$  and their end point values were  $54.4 \pm 47.9$ . This reduction was in stark contrast with the control group, who had a baseline of  $68.1 \pm 48.0$  and end values of  $78.4 \pm 62.3$ , an increase in size. They determined that high-dose raloxifene was effective in inhibiting the development of uterine leiomyomas. Importantly, raloxifene was also clinically tolerated with limited adverse side effects.

Palomba et al. (2004) provided an evaluation of the effectiveness of combining raloxifene with a GnRHa as an add-back treatment. They discovered that after six months, there was a significant decrease in leiomyoma compared to the baseline. Clinically, the treatment was tolerated by the premenopausal patients. There was evidence of decreased bleeding observed with patients as treatment cycles progressed. Their data also indicated that raloxifene was able to protect patients from risk of osteoporosis and hypoestrogenic side effects of GnRHa. Raloxifene as an add-back therapy to GnRHa was determined to be a safe and effective method to manage uterine leiomyomas and continuation of the treatment continued to suppress the size of the leiomyoma.

## DISCUSSION

Estrogen has been confirmed to influence the development of uterine leiomyomas. This dependency is observed naturally between premenopausal and postmenopausal women (Templeman et al., 2009). Postmenopausal women are reported to have less incidence of uterine fibroids because estrogen levels drop drastically at menopause. Early onset of menarche led to increased susceptibility to uterine fibroids because of prolonged elevated estrogen exposure and the proliferation stage of the menstrual cycle (Marshall et al., 1998).

Pharmacological manipulation of estrogen levels also demonstrated the importance of the hormone on uterine fibroid growth. For example, hormone replacement therapy after menopause was shown to increase risk of uterine fibroids because it raised the levels of estrogen in women (Templeman et al., 2009). While HRT was administered to increase estrogen levels, aromatase inhibitors and GnRH agonists were provided to premenopausal women to reduce estrogen levels. They were both shown to be effective in reducing estrogenic effects and the volume of uterine leiomyomas (Shozu et al., 2003, Maggiore et al., 2014, Chegini et al., 1996, Mizutani et al., 2005). However, with aromatase inhibitors and GnRH agonists, there is the risk of creating a hypoestrogenic state that have adverse side effects, including osteoporosis and symptoms associated with menopause.

The mechanism of how estrogen relays its effects on uterine growth is still unclear, however it has been validated that estrogenic effects are mediated by the estrogen receptors alpha and beta. The activation of estrogen receptors results in the subsequent activation of other genes through estrogen response elements and the production of growth factors. The estrogen receptors were shown to elevate the SDF-1 gene and MTCYB transcripts (Glance et al., 2009, Shaik et al., 2011). While the estrogen receptors are important in the development of aberrant growths, it was observed that its inhibition did not completely block the initiation of growths, but rather delays the promotion of growth (Boccanfuso and Korach, 1997).

Of the two known subtypes of estrogen receptor, ER $\alpha$  and ER $\beta$ , ER $\alpha$  has a more clarified role. ER $\alpha$  has been confirmed to be essential for regulated growth. Knockout studies have shown that ER $\alpha$  has a proliferative action in target tissues (Krege et al., 1998) with tumors having a greater transcription of ER $\alpha$  (Rutherford et al., 2000). ER $\beta$ 's role is less clear, but it is hypothesized to impede excess proliferation. ER $\beta$  was observed to be greater in normal tissue, indicating that it plays a role in maintaining the level of growth in a tissue and preventing abnormal growth (Pujol et al., 1998). However, ER $\beta$  was also seen to compensate for ER $\alpha$  after it had been knocked out (Dupont et al., 2000), offering a unique, yet vague understanding of multifunctional role of ER $\beta$ .

With the opposing effects of ER $\alpha$  and ER $\beta$ , some researchers have attempted to elucidate which receptor plays the greatest role in the development

of uterine leiomyomas. ER $\alpha$  is present in smooth muscles cells, however, ER $\beta$  is present in smooth muscle cells, connective tissue cells, and endothelial cells (Valladares et al., 2006) indicating a vaster influence. There is an alteration of ER $\alpha$ /ER $\beta$  ratio in uterine leiomyomas compared to normal myometrium. ER $\alpha$  in premenopausal women have more significant elevation in uterine leiomyomas than the increase in ER $\beta$  transcript (Strissel et al., 2007). In postmenopausal women, ER $\beta$  has a more significant flux of transcription. ER $\beta$  elevation is observed in leiomyomas, compared to premenopausal women who have less ER $\beta$  in uterine leiomyomas. This increase is caused by upregulation in order to maintain the development of the growths (Sakaguchi et al., 2002). Therefore, depending on the status of the woman's reproductive age, there are different possibilities to modulate the receptors to manage uterine leiomyomas. A ER $\beta$  agonist could be used to increase ER $\beta$  transcript to inhibit the growth of uterine leiomyomas, or an ER $\alpha$  antagonist could be administered to block the role of ER $\alpha$  and its promotion of proliferation.

Selective estrogen receptor modulators have immense potential for future research. They have been confirmed to decrease the volume of uterine leiomyomas. The ratio of ER $\alpha$  to ER $\beta$  needs to be assessed in order to determine which SERM is best to use, antagonist or agonist (Roger et al., 2001). Characterization of the estrogen receptor ratio provides insight on the coregulatory molecules present, which will modulate the ER-SERM complex (Lonard, 2006). Tamoxifen, for example, recruits corepressors and if they are low



in a particular fibroid, tamoxifen will not be able to produce its targeted effects. Tamoxifen has also been reported to have severe adverse effects, including hypoestrogenic-induced osteoporosis and thrombotic events. Building on the development of raloxifene, synthesis of novel SERMs is an important approach for future research of uterine leiomyomas.

The developing molecular and physiological understanding of estrogen and its receptors alpha and beta can be further exploited to design effective treatment of uterine leiomyomas. Using a combination of pharmacological treatments could potentially be effective in managing uterine leiomyomas. Using aromatase inhibitors to block the synthesis of estrogen and using SERMs to target the estrogen receptors would be very effective in ceasing the estrogenic effects on uterine leiomyomas. However, this will probably cause a hypoestrogenic state that is observed with GnRHa treatment because estrogen is a vast and ubiquitous hormone. The knock out studies performed by researchers highlighted another area of interest, gene therapy. Gene therapy, such as CRISPR, could be used to alter the transcript of ER $\alpha$  and ER $\beta$  and modulate the estrogenic effects of estrogen-dependent uterine leiomyomas.

## CONCLUSION

Endocrine manipulations are one of the least toxic therapeutic interventions for hormone-responsive conditions and present a growing field of medical research. It is clear that ER $\alpha$  and ER $\beta$  play an important role in the development of uterine leiomyomas although their complexities have yet to be fully understood. Future research on estrogen and uterine leiomyomas should focus on characterizing and enhancing the value of ER $\alpha$  and ER $\beta$ 's unique function in order to synthesize individualized selective estrogen receptor modulators. With ER $\alpha$  possibly contributing to proliferation and ER $\beta$  inhibiting excess growth, there is opportunity to design a treatment plan catered to a patient's tumor environment. Estrogen receptor agonists could be therapeutic for patients who have a low ER $\alpha$ /ER $\beta$  ratio and abundance of corepressors. Antagonists would be beneficial for those who have high ER $\alpha$ /ER $\beta$  ratio with an analysis of the complete molecular network of the leiomyoma.

With current research, physicians can mediate symptoms in premenopausal and postmenopausal women. Using a combination of clinical management approaches can be beneficial, including using SERMs in conjunction with surgical treatments. SERMs could reduce the volume of the uterine leiomyoma, which would decrease risk and surgical-time frame associated myomectomies. Patients could also opt for a combination of two non-surgical interventions, such as GnRH agonists with SERMs. While monitoring the

adverse of GnRHa, including hypoestrogenism, SERMs could contribute to volume reduction of uterine leiomyomas.

Estrogen's role in physiology has resulted in the development of relatively safe and effective treatments that target uterine leiomyomas. The benefits of non-surgical treatments are vast. These treatment options offer a less expensive and less invasive approach to manage uterine leiomyomas. However, further research must occur in order to access the full potential of endocrine modulation of the tumors. Targeting  $ER\alpha$  and  $ER\beta$  as a means to treat uterine leiomyomas can bring medicine a step closer to developing a non-surgical cure and can be applied to other endocrine-sensitive conditions, such as breast cancer.

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## CURRICULUM VITAE

