Chapter 3

Emerging Biomarkers and Clinical Implications in Endometrial Carcinoma

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Abstract

Endometrial cancer (EmCa) is the most common type of gynecological cancer. EmCa is the fourth most common cancer in the United States, which has been linked to increased incidence of obesity. EmCa can be classified into two main types: Type I and Type II, which include the major histological subtypes. Type I EmCa is hormonally driven, less aggressive, and has a more favorable prognosis. In contrast, Type II EmCa grows independently of hormonal signals, is more aggressive, and generally has an unfavorable prognosis. Various tumor biomarkers [*i.e.*, tumor suppressor p53, hypoxia-inducible factor 1-alpha (HIF1-α), human epidermal growth factor receptor 2 (HER2/neu), and vascular endothelial growth factor (VEGF)] have been identified in EmCa. Biomarkers of treatment effectiveness involve immunosuppressive factors targeted by microRNA (miRNA)-based therapy. However, there are no reliable biomarker tests for early detection of EmCa and treatment effectiveness. A potential new biomarker is Notch, Interleukin-1, leptin crosstalk outcome (NILCO) that could affect the progression of Type II EmCa. NILCO expression in EmCa might be dependent on patient's obesity status. This chapter presents updated information on these, and other potential emerging biomarkers for EmCa, and discusses current challenges and clinical implications on this area of research.

Keywords: Endometrial cancer, Biomarkers, NILCO, Leptin, Obesity

1. Introduction

1.1. Endometrium

The uterus is a pear-shaped hollow organ, with a virtual cavity, composed of the cervix and corpus (body of uterus). The corpus has three tissue layers: the endometrium, myometrium, and the perimetrium. The endometrium is the innermost layer, is comprised of endometrial glands, stroma, and blood vessel, and is the most active layer in responding to cyclic hormonal cues. The endometrium is essential for reproductive function (**Figure 1**) [1]. The myometrium or the muscle layer comprises interwoven spirals of smooth muscle fibers more compactly arranged adjacent to mucosa as visualized by magnetic resonance imaging (MRI) studies [2]. It

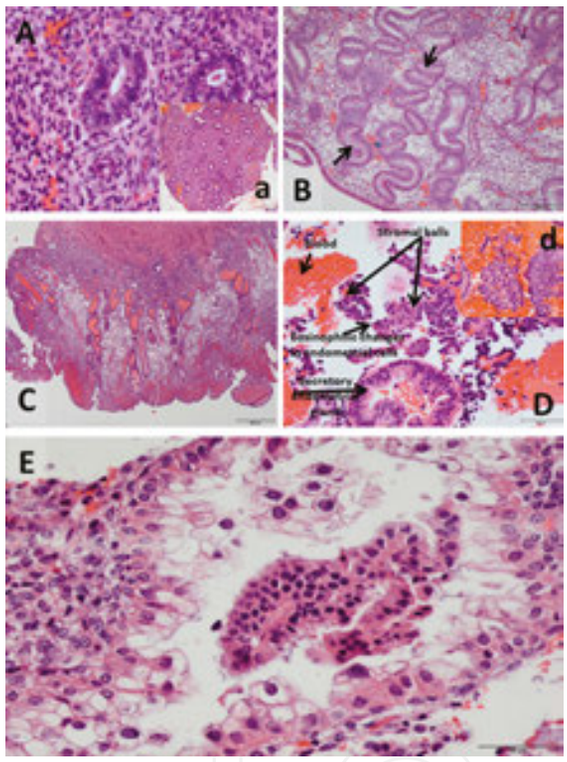

Figure 1. Representative pictures from hematoxylin and eosin staining of endometrial tissue from different menstrual phases and pregnancy. (**A**) Proliferative-phase endometrium shows round to oval endometrial glands lined by columnar cells with basally located nuclei. Multiple mitoses are present during the proliferative phase (40×). (**a**) The thumb image is a lower magnification of proliferative-phase endometrium revealing the evenly spaced round-to-oval glands within the endometrial stroma (10×). (**B**) Early secretory endometrium that shows the saw-tooth appearance of endometrial glands during secretory phase; some of the subnuclear secretions of the early phase are marked with short arrows (10×). (**C**) Secretory-phase endometrium that shows the basalis and functional layers. Upper arrows: basalis. Lower arrow: functionalis, composed of compact layer, situated ad-luminal, formed by the necks of endometrial glands and spongy layer underlies the compact layer, and is formed by the tortuous endometrial glands (10×). (**D**) Menstrual endometrium. The functionalis layer sheds during menstruation. The endometrial glands shed and may show eosinophilic change. The stromal cells condensate and form "stromal balls" a characteristic finding in shedding endometrium. A residual secretory gland is also visible. The background is blood (10×). (**d**) Stromal balls are depicted in the thumb image. (**E**) Pregnancy endometrium: Arias-Stella reaction showing enlarged endometrial glands with abundant clear or eosinophilic cytoplasm and marked nuclear changes. The nuclei are large, hyperchromatic, pleomorphic, and smudged). Rare mitotic figures may be found. The stroma is decidualized (40×).

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is responsible for uterine contractions that occur during the entire menstrual cycle, varying in frequency and intensity during the follicular and luteal phase and at the time of menstruation and delivery [2, 3]. The outer most layer, the perimetrium, oftentimes referred to as the (tunica) serosa lines the entire uterus and consists of a thin layer or epithelial cells [1]. The uterus functions in receiving the embryo, housing the fetus throughout pregnancy and labor and delivery of the infant [3]. Implantation occurs in the endometrium layer and its function, and morphology is dependent on the release of sexual hormones. The morphology of the endometrium in the absence of hormonal influence (*i.e.*, pre-pubescent females and postmenopausal women) is constant and maintains a certain thickness. After the onset of menarche, the uterus prepares to receive a fertilized oocyte during the menstrual cycle. If implantation fails to occur, the functional layer of the endometrium sheds which leads to menstruation [4].

1.2. Menstrual cycle

At puberty, females undergo monthly cyclic changes controlled by the hypothalamus. The hypothalamus produces and releases gonadotropin-releasing hormone (GnRH), which acts on the anterior pituitary gland to stimulate the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) to initiate and control these cyclic changes [4]. Throughout the menstrual cycle, estrogen and progesterone are responsible for the morphological and biological changes that occur in the endometrium, cervix, and vagina. Additionally, estrogen and progesterone are responsible for the feedback of FSH and LH secretion [5].

The phases of the menstrual cycle are as follows: menstrual phase, proliferative or follicular phase, ovulation, and luteal or secretory phase [4]. The first day of the menstrual cycle begins with menstrual bleeding due to the regression and shedding of the outer layer of the endometrium, which is the functional layer. The menstrual period or menses typically lasts 3–4 days. The proliferative phase or follicular phase lasts on average 8–10 days. During the follicular phase, ovarian follicles begin to develop and secrete 17β-estradiol. In addition, FSH and LH receptors are upregulated in ovarian theca and granulosa cells. FSH stimulates rapid growth of ovarian follicles. An increase in 17β-estradiol induces cell proliferation of the endometrium and reconstructing the outer layer lost during menstruation [6]. Ovulation occurs on day 14 and is followed by an increase in estradiol secretion at the end of the proliferative phase. A surge in FSH and LH causes ovulation of the ovum. Estradiol levels decrease shortly after ovulation and increase during the luteal phase. The luteal phase or secretory phase begins after ovulation where the formation of the corpus luteum is evident [6]. The corpus luteum synthesizes and secretes estradiol and progesterone. Progesterone increases the vascularity of the endometrium and prepares the endometrium to receive the fertilized ovum with the endometrium reaching its maximum thickness. If fertilization does not occur, the corpus luteum regresses, thus decreasing the levels of estradiol and progesterone in circulation [5]. Menses follows for the beginning of the next menstrual cycle (see **Figure 1**).

1.3. Menopause

The menstrual cycle occurs in women of reproductive age and continues until the onset of menopause. Menopause usually occurs between the ages of 45–55, but can begin as early as

40. The age of onset could be determined by various factors such as genetics, diet, hysterectomy, or damage to ovary due to the chemotherapy or radiation. Common symptoms associated with menopause include as follows: irregular vaginal bleeding, hot flashes, changes in mood, and urinary and vaginal symptoms [7, 8].

Menopause is defined as the permanent cessation of menstruation, which results from the loss of ovarian function [7]. In other words, the ovaries become less sensitive to gonadotropin stimulation, which is associated with follicular attrition. Throughout a woman's life, oocytes undergo atresia, which results in the decline of the quality and quantity of ovarian follicles. Normally, follicles mature and release their ova for the purpose of ovulation and secretion hormones; and the failure to ovulate alters the menstrual pattern immensely. During menopause, estrogen levels decline dramatically, leading to a decrease in the number and size of ovarian follicles. As a consequence of declining estrogen levels, FSH and LH levels are elevated during menopause due to the follicular changes in sensitivity to gonadotropins and negative endocrine feedback [5]. Then, menopause is characterized by the loss of progesterone synthesis, and the increase in body weight and androgen levels [9].

The surge of androgens augments aromatization and production of estrogen by adipose tissue that further increases EmCa risk. In addition, estrogen can be produced by the aromatization of androgens in the ovarian stroma as well as in other tissues and organs such as bone, muscle, bone marrow, liver, fibroblasts, and hair roots [10]. Consequently, estrogen production that is accompanied by sharp decrease of progesterone leads to an unopposed estrogen status. This can result in endometrial hyperplasia that could possibly develop into EmCa [10, 11]. Also, postmenopausal women having increased levels of estrone are also under EmCa risk. Furthermore, there is evidence that chronic hyperinsulinemia is an EmCa risk factor [9]. The unopposed estrogen hypothesis proposes that EmCa is a result of the mitogenic effects of unbalanced estrogens. Then, situations showing chronic anovulation and progesterone deficiency lead to hyperandrogenism, which together with nutritional lifestyle factors increase EmCa risk. Indeed, pre- and postmenopausal women having elevated plasma androstenedione and testosterone also have increased EmCa risk. Approximately 75% of women with EmCa are postmenopausal; the most common symptom is postmenopausal bleeding [9].

2. Endometrial cancer

EmCa is a malignancy of the endometrial glands of the uterus and is the most frequent malignancy of the female pelvic reproductive tract [12]. EmCa comprises a series of malignant diseases of the endometrium with diverse phenotypes. Although it is not categorical, EmCa can be subdivided into two main different types based on the histologic examination: endometrioid and non-endometrioid with their variants [12]. EmCa may also be classified based on epidemiological, histologic, and behavioral information into two types: Type I EmCa and Type II EmCa (**Figure 2**) [12]. Type I EmCa comprising the endometrioid carcinomas is the most common type of adenocarcinoma. Then, Type I accounts for 85% of all EmCa cases and is more common than Type II EmCa (non-endometrioid carcinoma) [12]. Type I EmCa is dependent on estrogen hormonal stimulation, less aggressive, and shows a favorable prog-

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nosis [12, 13]. Endometrioid carcinoma is well differentiated, closely resembles the endometrial glands and can be developed from atypical hyperplasia [14]. A common variant of Type I EmCa displays squamous cells adjacent to glandular elements, representing a tumor with squamous differentiation. Rare variants of endometrioid carcinomas are ciliated carcinoma, secretory carcinoma, and villoglandular adenocarcinoma [13]. Type II EmCas are the non-endometrioid type. Type II EmCa includes serous adenocarcinoma, clear cell carcinoma, uterine carcinosarcoma, mucinous adenocarcinoma, squamous cell carcinoma, mixed type of carcinoma, and undifferentiated carcinoma [14]. Type II EmCa is high grade and stage, independent of estrogen stimulation, poorly differentiated, and more aggressive with a poor prognosis. Most Type II EmCas have metastasized outside the uterus at the time of diagnosis [13, 14].

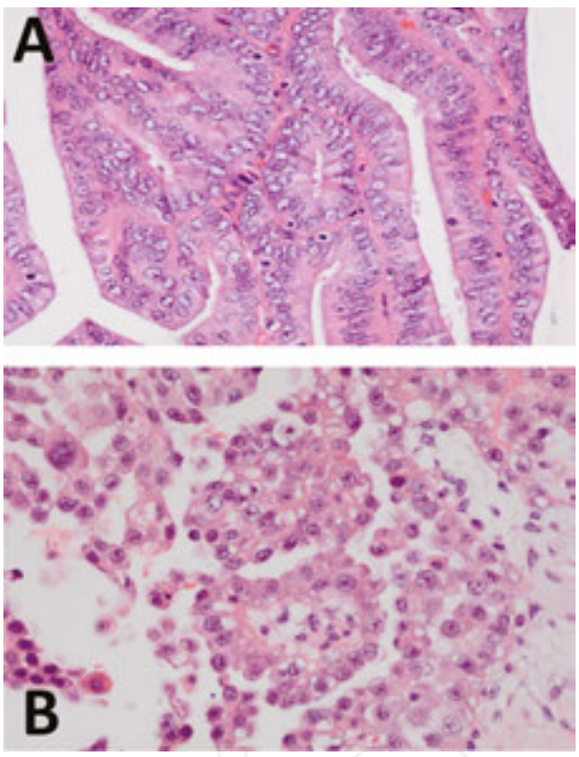

Figure 2. Histopathological features of endometrial cancer. (**A**) Type I (endometrioid) endometrial carcinoma: This is a 40× magnification of an H&E stain revealing columnar cells with basally located nuclei. This tumor shows slender villous architecture (better seen at the right of the picture) as well as glandular architecture (central). (**B**) Type II (serous) endometrial carcinoma. This 40× magnification of an H&E stain shows a high-grade tumor with micropapillary architecture. The nuclei are enlarged with irregular nuclear membrane, often protruding, giving a "hob nail" appearance. They show hyperchromasia or most often nuclear clearing with prominent, sometimes multiple nucleoli.

According to the system of the International Federation of Gynecology and Obstetrics (FIGO), grading of EmCa is determined by how closely similar the cancer forming glands appear when compared to benign endometrium [13]. Low-grade tumors form more glands and are well differentiated whereas high-grade tumors do not form glands, and are poorly differentiated [13]. Grade 1 tumors have well-formed glands with roughly 95% of the cancer forming glands and no more than 5% of solid non-squamous areas [13]. Grade 2 tumors have 50–94% of cancerous forming glands, while Grade 3 tumors have less than 50% of cancerous forming

glands. Grade 3 tumors are considered high-grade and are more aggressive than lower-grade tumors [14]. Similarly, the FIGO system is also used for staging [15]. According to the National Cancer Institute (NCI), Stage I cancer is limited to the uterus; Stage II extends into the cervix; Stage III cancer has spread outside of the uterus, but is limited to the pelvic region; Stage IV cancer invades the bladder, bowel, and distant locations [16]. Staging is further stratified (*i.e.*, IA, IIB, IIIC) based on myometrial invasiveness [17].

In the United States, EmCa is the fourth most common cancer among women after breast, lung and bronchus, and colorectal cancer. In 2015, there were approximately 54,870 new EmCa cases diagnosed in the United States with roughly 10,170 estimated deaths. The overall 5-year survival rate is 96% when diagnosed at the local site, and 67% when diagnosed at the regional area [18]. The survival rate drastically decreases to 16% when diagnosed at a distant site.

The incidence of EmCa has been steady since 2004 for most ethnic groups, but is increasing by 1.9% in African-American women. The incidence rates in Caucasian women are the highest when compared to all ethnic groups. In Caucasian women, the incidence rate is 24.8/100,000 when compared to African-American women at 21.8/100,000 [19]. Even though the incidence of EmCa is higher in Caucasian women, the mortality rates are more than two times higher in African-American women (3.9/100,000 and 7.3/100,000, respectively). When comparing the survival rates between both ethnic groups, Caucasian women exceed that for African-American women roughly by 7% at each stage of diagnosis. Possible multifactorial reasons for EmCa health disparities usually include socioeconomic status, limited access to healthcare, comorbidities, etc., but the exact causes for this disparity are unknown [20].

2.1. Risks factors

A major role in endometrial carcinogenesis is represented by estrogen actions, both endogenous and exogenous. Increased exposure to estrogen augments the risk of EmCa. Postmenopausal women on estrogen replacement therapy have an increased risk of developing EmCa, and the risk further increases with the duration of replacement therapy use [21]. It has been reported that the relative risk of developing EmCa rises to 9.5:1 when the use of exogenous unopposed estrogen last for 10 years or longer [21]. Moreover, EmCa risk in these women persists for several years after estrogen discontinuation [22].

Tamoxifen is widely used as an adjuvant therapy in patients with estrogen receptor positive breast cancer. However, tamoxifen use also increases EmCa risk due to its agonistic effects on the endometrium [23]. However, the majority of tamoxifen-related carcinomas present mainly at early stages and show low grade [23, 24].

Ovarian tumors and conditions, such as granulosa cell tumor, thecoma, polycystic ovary disease, and hyperthecosis, causing prolonged unopposed estrogen production may lead to endometrial hyperplasia, and usually low-grade endometrioid carcinoma. Granulosa cell tumor is a relatively uncommon sex cord–stromal tumor, which affects mainly perimenopausal women. These tumors are associated with increased estrogen production. EmCa occur in 9–13% of women with granulosa cell tumors [25]. Thecomas are benign ovarian neoplasms developed by ovarian theca cells, which affect women of any age, but predominantly in women

older than 40 years of age. EmCa have been reported in up to 21% of women with thecomas [26]. Polycystic ovarian disease (PCOD) occurs in young, usually infertile women with menstrual irregularities. Multiple cysts, stromal hyperplasia, and hyperthecosis enlarge the ovaries. These conditions show elevated estrogen and androgen serum levels. However, endometrioid carcinoma may occur in less than 5% of these women [27]. Hyperthecosis may occur independently for PCOD and may be associated with increased androgen production and virilization, or it may produce estrogen. Data from a small series of patients showed that a third of them have developed EmCa [28].

Major endogenous risk factors associated with EmCa are as follows: age, obesity, hypertension, and reproductive characteristics (late menopause, low parity and infertility). These conditions are associated with increased levels of estrogen.

Most women diagnosed with EmCa are postmenopausal or 50 years and older [20]. Approximately 15% of women diagnosed with EmCa are younger than 50 years of age, while 5% are diagnosed before the age of 40 [29]. Metabolic syndrome including obesity, hypertension, insulin resistance, diabetes, and dyslipidemia increase the risk of developing multiple malignancies, particularly EmCa [30]. Younger women diagnosed with EmCa are usually obese, and their carcinomas show a well-differentiated histology [20]. Obesity is a major risk factor for EmCa [12]. EmCa incidence is higher in well-developed countries where obesity is on the rise [18]. Hypertension has been linked to an increase in the incidence of EmCa, but it is unclear whether it is an independent risk factor or could be related to comorbidities of conditions and diseases (*i.e.,* obesity and diabetes) [31].

Lastly, as it was mentioned, infertility, late age onset of menopause, early age of menarche, and nulliparity increase EmCa risk. However, smoking decreases the risk of developing EmCa as well as oral contraceptive use lowers the risk [20, 32]. In regards to smoking, the anti-EmCa effect is probably related to its actions on estrogen metabolism. This anti-EmCa effect is primarily found in postmenopausal women, with current smokers showing the greatest risk reduction, in contrast to former smokers [32]. The greatest extent of risk reduction for EmCa is reported in postmenopausal, multiparous, obese, women who had no exogenous hormones [33]. Additionally, about 50% of women that used combined oral contraceptives (COCs, which is related to use of progestins and estrogens) show decrease EmCa risk. In most of these studies, this protective effect persisted for more than 15–20 years after cessation of the COC [34]. The adverse effects of oral contraceptive have been investigated extensively, whereas their non-contraceptive benefits have been underestimated. COC therapy could also reduce the risk of developing EmCa after menopause [35].

3. Endometrial cancer biomarkers

A biomarker is a characteristic or substance that can be quantified or measured objectively, and predicts the incidence and outcome of disease or normal biological function/process [36]. Cancer and non-malignant cells produce tumor markers. Biomarkers are molecules produced by cancer or non-cancer cells in response to malignant or benign conditions. Tumor markers

are expressed higher under cancerous conditions. They can be present in urine, tumor tissue, blood, and bodily fluids. Most biomarkers are of protein origin (*i.e.*, growth and angiogenic factors, oncogenes, tumor suppressor, cytokines, and serum proteins, etc.). Recent studies have shown that alterations in DNA and gene expression can also be used as tumor markers (*i.e.*, mRNA, miRNA) (**Figure 3**) [12, 36].

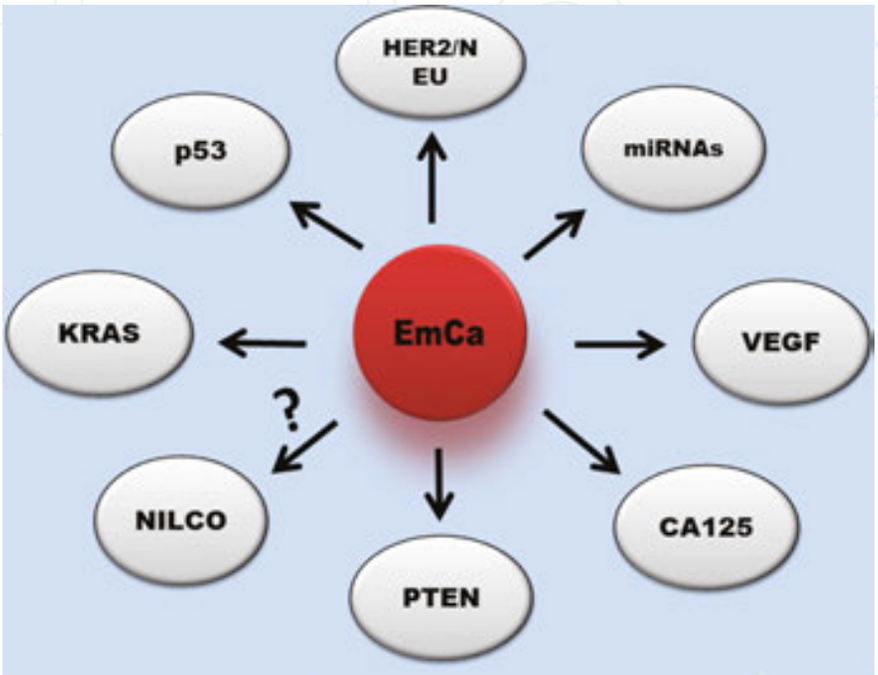

Figure 3. Main biomarkers of endometrial cancer (EmCa).

Tumor biomarkers have been instrumental in designing treatments of certain types of cancer. Tumor markers can be used for early detection, screening, diagnosis and prognosis, recurrence of cancer, and response to therapy. Studies on serum and plasma biomarkers are emerging and promising areas of research for early screening, treatment effectiveness, and recurrence in EmCa. Although these molecules have potential for the early detection of this disease, the impact of risk factors on EmCa and biomarkers is an area of promising research.

3.1. Tumor suppressors

Normally, tumor suppressor genes act to inhibit or arrest cell proliferation and tumor development [37]. However; when mutated, tumor suppressors become inactive, thus permitting tumor growth. For example, mutations in p53 have been determined in various cancers such as breast, colon, lung, endometrium, leukemias, and carcinomas of many tissues. These p53 mutations are found in approximately 50% of all cancers [38]. Roughly 10–20% of endometrial carcinomas exhibit p53 mutations [37]. Additionally, overexpression of mutated tumor suppressor p53 has been associated with Type II EmCa (poor histologic grade, non-endometrioid histology, advanced stage, and poor survival). African-American women present with stage I EmCa are three times more likely to have overexpression of mutant p53 and also have

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higher recurrence with poor survival rates when compared to Caucasian women [20]. Similarly, the tumor suppressor phosphatase and tensin homolog (PTEN) is the natural inhibitor of PI3K/AKT, which is involved in the progression of many cancers. PTEN can affect the regulation of cell cycle; enabling apoptosis and inhibiting the AKT survival pathway. Therefore, mutated PTEN causes an increase in cell proliferation, survival, and angiogenesis of cancer cells. PTEN mutations occur in 83% of all EmCa and are typically associated with Type I EmCa, which shows a more favorable prognosis and less aggressiveness [12]. Caucasian women have higher PTEN mutations, which may be related to a better overall survival rate when compared to African-American women [20].

3.2. Oncogenes

Oncogenes have the capacity to accelerate cell-cycle progression and induce the expression of several factors that induce tumor growth. These proteins are highly mutated and are overexpressed in many cancers. Oncogenes come from proto-oncogenes, which are involved in cell growth and differentiation [39]. For example, the overexpression of the oncogene HER2/neu (human epidermal growth factor receptor 2) has been associated with poor prognosis and resistance to treatment in breast, ovary, and EmCa [20]. Indeed, HER2/neu is involved in 20% of endometrioid (Type I EmCa) and serous carcinomas (Type II EmCa) [12]. In a study, African-American women with uterine papillary serous carcinoma showed three times higher HER2/neu overexpression when compared to Caucasian women showing this disease [20].

RAS (Rat Sarcoma Viral Oncogene Homolog) gene encodes GTPases involved in signal transduction [40]. Mutations in Kirsten mutated RAS (KRAS) have been associated with the progression of many malignancies [12]. An estimated 10–30% of EmCa cases exhibit RAS mutations that are predominantly observed in Type I EmCa, and also in non-malignant conditions such as endometrial hyperplasia. [40].

3.3. Vascular endothelial growth factor

Angiogenesis is important for tumor growth and the development of metastases [41]. Angiogenesis is controlled by pro-angiogenic and anti-angiogenic factors. An important angiogenic factor is vascular endothelial growth factor (VEGF), which was firstly identified by Senger et al. [42]. The overexpression of VEGF by cancer cells enhances tumor growth and metastasis of colorectal, head and neck, ovarian, and EmCa [41]. Elevated levels of VEGF and other angiogenic markers are associated with poor survival rates in EmCa [43]. Therapeutic targets for VEGF such as bevacizumab (anti-VEGF antibody) could be promising in inhibiting tumor growth in EmCa [44].

3.4. Hypoxia-inducible factor-*1α*

Hypoxia-inducible factor 1 (HIF-1α) is a major regulator of cellular processes that constitutes a biological response to hypoxic conditions. During hypoxia, HIF-1α is produced and accumulated within cells. HIF-1α is translocated to the nucleus, where it binds to hypoxia response elements (HREs), in the promoter region of several genes (*i.e.*, VEGF), thus activating angio-

genesis and other processes that facilitate adaptation and survival of cells and the whole organism from normoxia. To date, there are more than one hundred HIF-1α downstream genes identified with varying functions, including erythropoiesis/iron metabolism, angiogenesis, vascular tone, matrix and glucose metabolism, cell proliferation/survival, and apoptosis [45]. In a study, HIF-1α expression was detected in approximately 49% of EmCa. Additionally, a strong correlation between HIF-1α and well-differentiated EmCa was found.

Since hypoxia enhances tumor progression and is a major obstacle for chemotherapy and radiation, HIF1-α could be used as a useful tool to predict patient outcome after surgery and radiation [46].

3.5. Serum markers

Several serum tumor markers have been identified as potential useful tools for detecting early relapse and monitoring response to therapy. Increased levels of Cancer Antigen 125 (CA 125) have been detected in many malignancies and are associated with endometrial proliferation and EmCa [47]. Approximately 11–33.9% of EmCa patients have increased CA 125 levels (>35 U/ml) [47]. Moreover, CA 125 is positively correlated with tumor size and stage in EmCa and is significantly associated with poorer survival rates in EmCa patients [48].

Other tumor-associated serum markers for EmCa include CA 15.3 and CA 19.9 that are detected in 24–32.1 and 22.3% of EmCa cases, respectively [48]. Surprisingly, 47% of patients with occult stage III EmCa exhibit elevated levels of CA 15.3 levels (>30 U/ml) when compared to stage I–II in which 18% of this tumor marker was observed [5]. CA 125 levels in combination with CA 19.9 levels could be used as a predictor of recurrence [49].

3.6. Epigenetic markers

The epigenetic change associated with gene regulation is an emerging area of research. MiRNAs or microRNAs regulates gene expression by binding to target mRNAs, resulting in the degradation of RNA or the repression of mRNA expression. MiRNAs can influence signaling pathways by functioning as a promoter or repressor in tumor cells and are involved in cell proliferation, migration, apoptosis, and differentiation. Targeting altered expression patterns of microRNAs could prove valuable in correcting abnormal signaling pathways observed in EmCa [50]. There are several miRNAs that might be used as biomarkers of EmCa: miR-99a, miR-199b, miR-205, miR-125b, miR-194, and miR-181b [51].

Some miRNAs have been found differentially expressed in the less aggressive endometrioid EmCa (Type I) versus the more aggressive serous papillary EmCa (Type II). MiR-99a and miR-199b expression levels were upregulated in Type I EmCa, and the combination of these two miRNAs with miR-100 could be used as diagnostic factors in the less aggressive Type I EmCa [51]. Interestingly, miR-205 levels are also increased in Type I EmCa. MiRNA-205 is a target for PTEN and is associated with poor survival [52]. In addition, miR-129-2 is involved in DNA methylation of the mismatch repair gene: human mutL homolog 1 (hMLH1) that is observed in the progression of Type I EmCa [51]. Moreover, methylated hMLH1 occurs frequently in EmCa and could induce mutations in certain cancer associated genes, which

includes type II transforming growth factor-beta (TGF-βII), PTEN, Bcl2-associated X protein (BAX) and mutS homolog 6 (hMSH6) [51, 53].

In contrast, several miRNAs positively or negatively correlate with the progression of Type II EmCa. For example, miR-125b is significantly upregulated in Type II EmCa and targets Tumor protein p53 inducible nuclear protein1 (TP53INP1) gene and V-erb-b2 erythroblast leukemia viral oncogene homolog 2 (ERBB2) gene-inducing cancer cell proliferation and invasion [54, 55]. Conversely, decreased expression levels of miR-194 were correlated with advanced stage and poor survival in Type II EmCa [51]. Studies have demonstrated the administration of miR-194 in EmCa cells targets the B cell-specific Moloney murine leukemia virus integration site 1 (BMI1) gene, which is a cell-cycle regulator, and subsequently results in the inhibition of EMT phenotype and cell invasion in EmCa [56]. Similarly, miR-181b is downregulated in cancers with RAS mutations; hence, miR-181b could be a potential prognostic marker for Type II EmCa [57].

The use of miRNAs seems to be promising as biomarkers of EmCa. However, miRNA has limitations for cancer treatment, mainly due to the lack of effective transport of miRNAs in to cells [51].

Therefore, there is an unmet need to find novel biomarkers for EmCa diagnosis, prognosis, and treatment outcome. For example, NILCO (Notch, Interleukin-1, leptin crosstalk outcome; refer to Obesity and Cancer Section, page 21) may be used as a potential biomarker. NILCO has been associated with cell proliferation, metastasis, invasion, and overall decreased survival in breast cancer patients [58, 59]. Also, NILCO overexpression has been detected in the more aggressive Type II EmCa. Thus, it may be used a biomarker of EmCa aggressive phenotype.

4. Obesity and cancer

Obesity is a global epidemic and a major risk factor for several cancers, including EmCa [60, 61]. Obesity is defined as a condition of abnormal or excessive accumulation of fat in adipose tissue and a body mass index (BMI) of 30 kg/m^2 or higher [60]. Remarkably, several studies have shown that EmCa has the strongest correlation with obesity when comparing to diverse obesity-related cancers in women [62, 63]. Roughly, half of the EmCa cases are linked to obesity. Obese women are four times more likely to develop EmCa when compared to normal weight women [61]. Noticeably, it is known that African-American women have the higher incidence of obesity in the United States. Albeit EmCa rates are slightly higher among Caucasian than African-American women, they are less likely to die from EmCa compared to African-American women. The causes of this health disparity have not yet been determined, but the gap of the mortality rates between the two ethnic groups seems to be increasing [64].

Additionally, other populations also show strong correlations between obesity and EmCa. A case–control study performed in Europe that included 305 EmCa patients and 574 matched controls showed a significant increase in the risk of EmCa in patients with elevated levels of CRP, IL-6, and IL-1Ra. However, after adjustment for BMI, the estimates were strongly reduced

and became non-significant. Nevertheless, the study provided epidemiological evidence that chronic inflammation might mediate the association between obesity and EmCa and that endometrial carcinogenesis could be promoted by an inflammatory milieu [65].

Obesity is characterized by high serum leptin levels in circulation [66]. Leptin is a 16 KD hormone (main adipokine) secreted by adipose tissue. Leptin regulates food intake, reproduction, body weight, inflammatory response, hematopoiesis, angiogenesis, bone formation, and wound healing [66]. Although leptin is mainly from adipose tissue origin, the stomach, mammary epithelium, placenta and heart, and several cancer cell types also produce this hormone. Leptin crosses the blood brain barrier and cerebrospinal fluid to bind receptors in the hypothalamus to carry out its energy-balance regulatory functions [67].

Obese individuals oftentimes exhibit resistance to leptin and show high levels of the adipokine in blood, which is known as leptin resistance [66]. The precise mechanisms involved in leptin resistance are ambiguous. One possible cause could be due to over-eating, which causes higher leptin levels in circulation. The prolonged exposure of leptin damages the hypothalamus causing it to become insensitive to the effects of leptin [68, 69]. Additionally, leptin resistance could be due to a defect in the transport system of leptin across the blood brain barrier [66].

Leptin receptor obese receptor (OB-R) has several molecular isoforms due to the posttranscriptional splicing. The long OB-R isoform (OB-RL or OB-Rb) has full signaling capabilities and is expressed in the hypothalamus and peripheral tissues [67]. The short isoform of the receptor (OB-Rb) has limited signaling capabilities and is more abundant in EmCa tissues. Evidence shows that leptin is an important pro-inflammatory, pro-angiogenic, and mitogenic factor for cancer. Leptin produced by cancer cells acts in an autocrine and paracrine manner to promote tumor cell proliferation, migration and invasion, pro-inflammation, and angiogenesis [58, 70]. High levels of leptin and OB-R are associated with metastasis and decreased survival rates in breast cancer patients [58].

Obesity is a known risk factor for several cancers, including EmCa, but there are scarce reports on the identification and detection of specific biomarkers for obesity-related EmCa. Our lab is currently investigating the relationship between an adipokine (leptin) and its crosstalk with other oncogenic factors in EmCa [12, 19].

4.1. Leptin signaling

Leptin binding to the extracellular region of OB-Rb activates Janus-activated kinase 2 (JAK2) proteins. JAK2 binding leads to the phosphorylation of tyrosine residues (Tyr985, Tyr1077, and Tyr1138) on the intracellular side of Ob-R. Phosphorylation of Tyr1138 recruits STAT3 (signal transducers and activators of transcription proteins), forming a dimer that is translocated to the nucleus to initiate transcription of target genes [71]. Additionally, JAK2 binding to OB-R causes auto-phosphorylation of JAK2 which can lead to the phosphorylation of insulin receptor proteins, recruitment of PI3K, and MAPK to activate a cascade of signaling mechanisms of downstream targets [72].

On the other hand, leptin-binding OB-R and the recruitment of JAK2 allow for the activation of tyrosine residue Tyr 985 on OB-R [71]. Src homology 2 (SH2) proteins are recruited and

activated that allows the binding of growth factor receptor-bound protein 2 (Grb-2). Grb-2 is involved in the activation of ERK in the MAPK signaling [72]. Overexpression or mutations in these signaling mechanisms can lead to malignancies [71]. Obesity and leptin significantly alter the profiles of numerous proteins linked to cellular processes in cancerous tissues such as Notch and Interleukin-1 (IL-1) [71, 77].

4.2. Notch signaling

Notch signaling is an embryonic signaling pathway also involved in various cellular processes in adult cells, some of which include: proliferation, apoptosis, cell survival, epithelial–mesenchymal transition (EMT), differentiation, and angiogenesis [74, 75]. Notch Signaling is initiated through receptor–ligand interaction expressed in adjacent cells. Currently, four Notch receptors have been identified in mammals (Notch 1–4) [76]. Each receptor consists of an extracellular domain, which is involved in ligand binding, and a cytoplasmic domain involved in signal transduction [74]. Five ligands for Notch have been identified: Jagged (JAG1 and JAG2) and Delta-like (DLL1, DLL3, and DLL4) [76]. Once the ligand binds to its receptor, the Notch receptor is proteolitically cleaved at the extracellular domain by an α-secretase (ADAM10), which is subsequently followed by the cleavage of the receptor's intracellular domain by γ-secretase, resulting in the formation of the intracellular domain of Notch (NICD or Notch-IC) [71]. The cleaved NICD then translocate to the nucleus to bind CSL transcription factor (CBF or RBP-JK) and initiate transcription of target genes such as survivin and hairy/enhancer-of-split related with YRPW motif 2 (Hey2), among others [74]. Aberrant activation of Notch signaling can lead to various pathological conditions such as cancer [74, 77]. In tumorigenesis, aberrant Notch activation can be initiated through the abnormal expression of Notch ligands, receptors, and target genes, all of which have been reported in many solid tumors, including breast, prostate, and pancreatic tumors [76]. The Notch signaling pathway exhibits oncogenic properties in some tumors and suppressive properties in others, which suggests a dual role in carcinogenesis [78]. Remarkably, we have identified leptin as an important regulator of Notch in breast cancer [58, 73, 79].

The role of Notch is poorly understood in EmCa. However, our recent research shows that leptin and Notch signaling may crosstalk in EmCa [12, 19]. Additionally, leptin upregulates IL-1 in breast and EmCa cells cultured in vitro, indicating that leptin and IL-1 could also crosstalk in these cancer types [58, 80, 81].

4.3. IL-1 system

The IL-1 system actively participates in inflammation. This system is composed of ligands (IL-1α and IL-1β), two membrane-bound receptors (IL-1RtI and IL-1RtII), and a soluble antagonist (IL-1Ra) derived from the extracellular domain of the IL-1R. IL-1β is an inflammatory and pro-angiogenic cytokine that represents the more abundant ligand, which preferably binds IL-1RtI in normal and cancer cells [80, 81]. The IL-1 system is involved in various roles in both physiological and pathological states [80]. In cancer cells, IL-1 promotes inflammation, angiogenesis, tumor growth, and metastasis [81]. IL-1 is known to be upregulated in many tumor types. Indeed, the presence of IL-1 in some human cancers is associated with aggressive

tumor biology [80]. IL-1 has been shown to upregulate leptin levels in some cancer cells. Overexpression of IL-1 is seen in breast cancer and linked to proliferation of breast cancer cells [83].

Interestingly, leptin was shown to upregulate the IL-1 system in endometrial cancer (EmCa) cells in a biunivocal manner [81] Additionally, it has been shown that IL-1 upregulates leptin and OB-R, and both cytokines upregulate β3-integrin in endometrial epithelial cells [84]. Moreover, an active leptin-IL-1 crosstalk seems to be involved in embryonic implantation [85]. Similarly, an active crosstalk between leptin, Notch, and IL-1 could lead to cancer progression [58, 59, 80, 81].

4.4. NILCO and cancer

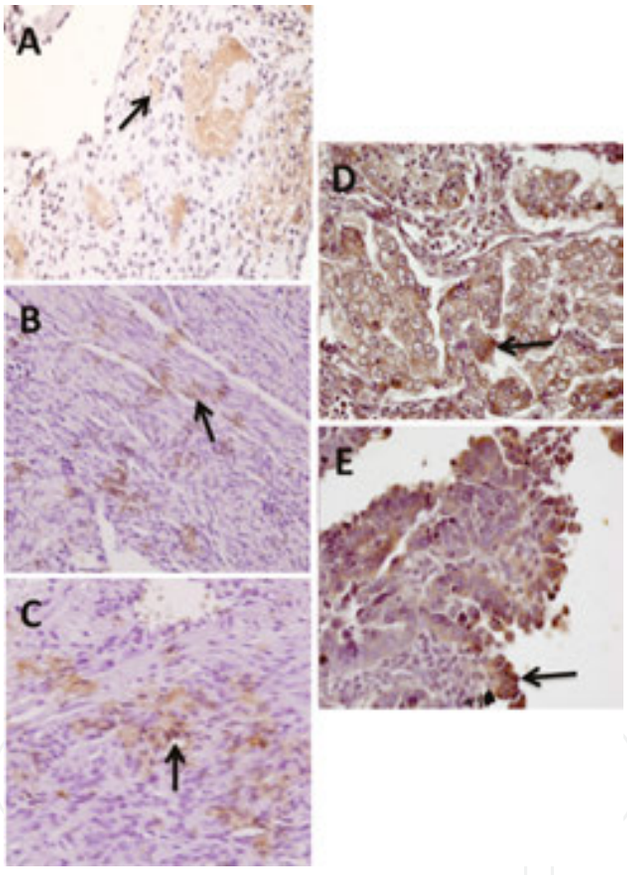

Figure 4. Immunohistochemistry (IHC) detection of NILCO in Type II endometrial cancer (Type II EmCa). Representative pictures for the IHC staining of: (**A**) Notch1, (**B**) Notch2, (**C**) Notch3, (**D**) Notch4, and (**E**) OB-R in Type II EmCa. Arrows indicate specific brown staining of NILCO antigens (40×).

A leptin-signaling crosstalk has been established in breast cancer among known pro-angiogenic factors, NILCO: Notch, IL-1, and leptin crosstalk outcome [58]. Signals triggered by these factors induce the expression of VEGF/VEGFR2 system, which is a main driver of tumor angiogenesis and tumor progression [58]. Notably, the overexpression of Notch, IL-1, and leptin has been associated with poor outcomes in breast cancer [59]. NILCO is involved in

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tumor cell proliferation and migration. Indeed, (NILCO) is correlated with decreased survival rates in breast cancer patients. Earlier studies from the Gonzalez–Perez's lab demonstrated that leptin induces Notch signaling in breast cancer [58]. Leptin was early identified as an upregulator of the IL-1 system in breast and EmCa [81, 86]. Similarly, leptin upregulates VEGF/VEGFR2 [87] and can also upregulate VEGF/VEGFR2 via IL-1 and Notch [58]. In addition, VEGF signaling could also upregulate Notch signaling in breast cancer [87]. However, these interactions have not been previously determined in EmCa.

Although obesity is a risk factor for cancer, the precise mechanisms involved in obesity-related cancer have not been explored. For the first time, our lab has shown that NILCO components are differentially expressed in EmCa, which correlated with the progression of the more aggressive Type II EmCa (**Figure 4**). NILCO components expressed in EmCa include Notch receptors (Notch1–4), ligands (DLL4 and JAG1), and targets (OB-R, IL-1RtI, Survivin, and Hey2).

Our studies have shown that in African-American (n = 20) and Chinese women (n = 75: in duplicate) suffering from EmCa, higher expression of several NILCO components was found in Type II EmCa patients compared to Type I EmCa (**Table 1**). These results suggest that the more aggressive non-hormonal responsive form of EmCa (Type II) could be more dependent on leptin signaling [12]. This would imply that Type II could be more affected by obesity than Type I EmCa.

African American Women							
	Type I (n=12)	Type II (n=17)			Type I (n=12)	Type II (n=17)	
NILCO IHC	H SCORE	H SCORE	P-value	NILCO WB	Protein Expression	Protein Expression	P-value
Notch1	1.19	1.80	<0.01	Notch1	48	58	<0.05
Notch2	1.10	1.30	=0.05	Notch2	38	36	>0.05
Notch3	1.15	1.45	>0.05	Notch3	48	44	>0.05
Notch4	1.50	1.96	<0.01	Notch4	44	98	<0.01
JAG1	1.36	2.20	<0.01	JAG1	140	172	<0.05
DLL4	1.80	2.49	<0.01	DLL4	40	115	<0.01
Survivin	1.20	1.96	<0.01	Survivin	131	230	<0.05
OB-R	1.60	1.73	<0.01	OB-R	25	70	<0.01
IL-1R tI	1.28	2.00	<0.01	IL-1R tI	59	109	<0.05
Hey2	1.14	1.45	<0.01	Hey2	46	100	>0.01
Chinese Women							
	Type I (n=97)	Type II (n=23)		NILCO qPCR	mRNA Expression	mRNA Expression	P-value

African American Women							
NILCO							
IHC	H SCORE	H SCORE	P-value	Notch1	1.00	1.30	<0.01
				Notch3	0.45	0.80	<0.05
Notch1	1.00	1.78	<0.01	Notch4	0.80	1.40	<0.01
Notch2	1.00	1.15	>0.05	JAG1	0.05	0.52	<0.01
Notch3	1.10	1.20	>0.05	DLL4	1.10	1.50	<0.01
Notch4	1.10	1.58	<0.05	Survivin	0.48	0.51	<0.05
JAG1	1.30	1.87	<0.01	OB-R	0.45	0.65	>0.05
DLL4	1.31	1.80	<0.01	IL-1R tI	0.82	1.56	<0.01
Survivin	1.17	1.60	<0.01	Hey2	0.03	0.62	<0.01
OB-R	1.10	1.50	<0.05				
IL-1R tI	1.40	1.73	<0.05				

IHC: immunohistochemistry; H SCORE[59]: semi-quantitative value calculated for each antigen and is determined by the equation HSCORE = ∑ pi (i + 1); WB: western blot; qPCR: Real-time polymerase chain reaction; Notch 1–4: transmembrane receptors; JAG1: Jagged 1; DLL4: Delta like-4 protein: Notch ligands; survivin: a cell survival factor and Notch target; OB-R: leptin receptor; IL-1R tI: interleukin 1 receptor type I; Hey2: hes-related family BHLH transcription factor with YRPW motif 2 and Notch target. Statistical significance set at $P < 0.05$.

Table 1. Expression of NILCO components in African-American and Chinese women suffering from endometrial cancer.

Our data further suggest that an active-signaling crosstalk (NILCO) triggered by obesity signals (leptin) occurs in EmCa, which might lead to the identification of novel biomarkers, particularly for Type II EmCa. NILCO investigations could lead to the identification of novel biological determinants of EmCa health disparity in African-American women [12]. However, a limitation to our preliminary data is that validation of this idea will require a larger sample size which is necessary to assess more conclusive statements.

5. Conclusions

Various biomarkers have been identified in EmCa; however, present targeted therapies have not been established in clinical practice. Clinical studies involving particular biomarkers such as VEGF and HER2 in EmCa resulted in minimal effects. Targeted therapies remain an obstacle due to the lack of specificity in EmCa cells. Therefore, more specific therapies are needed to target EmCa cells that overexpress tumor surface markers to avoid potential adverse effects on normal cells. The use of targeting epigenetic regulatory mechanisms involving miRNA biomarkers seems promising, but a more expansive approach is necessary to target the multiple signaling pathways involved in EmCa. Prognostic factors with a specific molecular biological signature may contribute to enhance tumor characterization in order to predict the

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clinical behavior of such factors. Hence, the identification of novel biomarkers could prove effective in predicting disease outcome and links to risk factors (*i.e.*, obesity). One such potential new biomarker could be NILCO, particularly for Type II EmCa. Moreover, if further proven, NILCO association with obesity-related EmCa and perhaps with race may provide new molecular evidences on the impact of chronic mild inflammation (obesity) and leptin signaling on EmCa and health disparities. Additionally, targeting NILCO could be a novel and effective way to prevent and treat EmCa, especially in obese patients.

6. Future directions

It seems that histological classifications and discoveries of reliable EmCa markers will depend heavily on molecular study findings. Establishing NILCO's role in EmCa might allow early disease detection and provide new targets for some or all components of the crosstalk. In this respect, specific and potent leptin-signaling inhibitors (*i.e.*, leptin peptide receptor antagonists: LPrA1 and LPrA2) may be used for this purpose. LPrAs for the abrogation of leptin signaling have been successfully used in several disease scenarios [84, 88]. Additionally, inhibition of IL-1 signaling via specific antibodies or the natural inhibitor, IL-1Ra, has produced satisfactory results in situations where this cytokine plays an essential role [89]. Furthermore, several inhibitors of Notch signaling have been developed and tested (*i.e.*, DAPT and other γ-secretase inhibitors) [90]. However, with the exception of LPrAs, these compounds have off target effects that could jeopardize their clinical use. LPrAs specifically block OB-R signaling, are not toxic, and have no effect on general health status, body weight, and appetite when were tested in a large number of mice. Therefore, LPrA may prove to be effective biological to disrupt NILCO and progression of EmCa.

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