# Notch and Endometrial Cancer

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**Abstract**

The human endometrium is a unique, highly dynamic tissue that undergoes cyclic changes of cell proliferation, differentiation, and death. Endometrial cancer is the most common malignancy among women in developed countries. Importantly, the incidence of endometrial cancer is rising in high-income countries. Currently histological classification is used for subtyping of endometrial cancer, while ongoing research is evaluating markers for more accurate molecular classification. Evolutionary conserved Notch signaling pathway regulates diverse cellular processes such as proliferation, differentiation, and cell invasion. Accumulating evidence links aberrant Notch signaling with diseases such as hyperplasia and endometrial cancer. This chapter summarizes the current state of Notch signaling investigations in the endometrium, endometriosis, and endometrial cancer.

**Keywords**

Notch · Endometrium · Endometrial cancer · Endometriosis · Stem cells · Leptin

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## Notch Signaling in the Normal Endometrium

The human endometrium is a unique, dynamic system that undergoes cyclic changes regulating cell proliferation, differentiation, and death during every menstrual cycle and pregnancy. Physiological changes that occur in fertile women are tightly regulated by hormones, specifically estrogen, progesterone, and chorionic gonadotropin (Banerjee and Fazleabas 2011; Maruyama and Yoshimura 2008). Therefore, it is not surprising that evolutionary conserved Notch pathway, due to its role in proliferation, differentiation, and angiogenesis, is actively involved in endometrium remodeling as well as in diseases such as hyperplasia and cancer.

Morphologically the endometrium is divided into functional and basal layers. The functional layer occupies two-thirds of the endometrial thickness and it is responsible for proliferation and secretion. During menstruations, the functional layer separates from the basal layer, while the basal layer serves as a base for endometrial regeneration and remains intact during menstruation. The endometrium is composed of different compartments: the luminal and the glandular epithelium, the stroma with stromal fibroblastic cells, and the vascular compartment (Diedrich et al. 2007; Ruiz-Alonso et al. 2012).

Notch is crucial for uterine development. In fact, it was shown that overexpression of



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NICD1 in mouse uterus leads to complete infertility, absence of uterine glands, and dysregulation of progesterone and estrogen signaling (Su et al. 2016). Mikhailik et al. have examined transcripts and demonstrated that Notch signaling is active in both the epithelial and stromal cells of the human endometrium. They have shown that all four *NOTCH* receptors are ubiquitously expressed in endometrial cells, whereas ligands *JAG1* and *DLL4* and targets *HES1* and *HEY* are predominantly expressed in endometrial epithelial cells and are scarce in stromal cells. In addition, JAG1 induces Notch signaling activity in the stromal cells resulting in regulation of 452 genes (Mikhailik et al. 2009). It is important to note that this analysis was performed following cell isolation and culturing. Other investigation has demonstrated that NOTCH1 and NOTCH3 primarily localize to the glandular epithelium, whereas NOTCH4 localize to the stroma (Cobellis et al. 2008; Mitsuhashi et al. 2012).

During menstrual cycle the human endometrium undergoes various phases including proliferative phase of endometrium regeneration, followed by a mid-secretory phase in which endometrial stromal fibroblasts undergo differentiation known as decidualization to secretory "epithelioid" cells and last late-secretory phase where the endometrium is shed in a nonpregnancy cycle. When menopause begins, the endometrium loses the ability to proliferate and differentiate and loses its functional layers (Salamonsen 2019; Mori et al. 2012; Evans et al. 2016). Notch pathway is active in the human endometrium and highly dynamic; expression patterns of Notch receptors and ligands are different according to cell type and menstrual cycle phase (Mikhailik et al. 2009; Mitsuhashi et al. 2012). Notch signaling is more active in cycling endometrium than in menopause, as the expression levels of three Notch signaling molecules, NOTCH1, NOTCH4, and JAG1, are decreased in postmenopause endometrium (Cobellis et al. 2008).

There are only a few reports regarding the role of Notch signaling in remodeling of the endometrium, and the available data is sometimes conflicting (Table 4.1). Mitsuhashi et al. have found no cyclic changes in expression of NOTCH1, JAG1, and DLL4 proteins during menstrual cycle. Only receptor NOTCH3 expression level was increased during the secretory phase in the stroma cells. The expression of these signaling molecules was investigated predominantly in glandular cells of the normal endometrium (Mitsuhashi et al. 2012). Schuring et al. have not detected cycle-dependent changes in expression of NOTCH1 and NUMB (a negative regulator of Notch) when compared to proliferative and secretory endometrium (Schuring et al. 2018). Meanwhile Cobelis et al. have performed immunohistochemical analysis of 60 samples of physiological endometrium (20 of each in proliferative and secretory phase and in menopause) and observed an increased expression level of NOTCH1 and JAG1 in the secretory phase and the opposite trend for NOTCH4. Authors proposed that these results indicate the role of NOTCH4 in cell proliferation as an attribute of proliferative phase of the cycle, whereas NOTCH1 possibly associates with cell differentiation as a characteristic of secretory phase in the human endometrium (Cobellis et al. 2008). These opposite results were obtained despite the fact that Mitsuhashi and Cobelis groups had used the same antibodies against NOTCH1 for immunodetection, however at different concentrations. Suzuki et al. have also shown the decreased level of NOTCH4 expression during secretory phase if

**Table 4.1** Changes of Notch signaling molecules in cycling endometrium secretory phase compared to proliferative phase

| | Secretory phase |
|---|---|
| NOTCH1 | ↕[a, b] ↑[c] |
| NOTCH2 | ND |
| NOTCH3 | ↓[e1]↕[e2] |
| NOTCH4 | ↓[c, d] |
| JAG1 | ↕[a] ↑[c, e1] |
| JAG2 | ND |
| DLL1 | ↑[e2] |
| DLL4 | ↕[a] ↑[f] |

[a]Mitsuhashi et al. (2012); [b]Schuring et al. (2018); [c]Cobellis et al. (2008); [d]Suzuki et al. (2000); [e]Van Sinderen et al. (2014); [f]Cuman et al. (2014)
1, luminal epithelium; 2, glandular epithelium; *ND* not determined

compared to the proliferative phase (Suzuki et al. 2000). The immunostaining of the endometrium has demonstrated that the expression of NOTCH1 receptor increased significantly in both glandular and luminal epithelium in the mid-secretory phase in comparison to the early- and the late-secretory phases. While NOTCH3 increased in the luminal epithelium during proliferative phase compared to secretory phase, no changes in expression were detected in glandular epithelium. Ligand JAG1 was detected upregulated in the luminal epithelium, and ligand DLL1 and NUMB were found in the glandular epithelium in the mid-secretory compared to the proliferative phase (Van Sinderen et al. 2014). It has been determined that DLL4 expression increases in the secretory phase of the menstrual cycle in both glandular and luminal epithelium (Cuman et al. 2014). Previously, it has been shown that NOTCH mediates uterine stromal decidualization by preventing stromal fibroblast apoptosis and regulates gene expression and cytoskeleton reorganization in mouse (Afshar et al. 2012). It has been revealed, by microarray studies, that *NOTCH2*, *NOTCH3*, and *JAG2* are expressed by the trophectoderm (polarized transporting single cell layer) of human blastocysts (Aghajanova et al. 2012).

The endometrium is involved in implantation and placental formation during establishment of pregnancy. Disorders in this process are a major reason for infertility. Critical role in implantation plays interaction of blastocyst and the endometrium. It was also demonstrated that Notch signaling is involved in implantation and placentation (Cuman et al. 2013; Cuman et al. 2014). NOTCH1, JAG1, and DLL1 are downregulated in the endometrium of women with unexplained infertility (Van Sinderen et al. 2014).

## Notch Signaling in the Endometriosis

Endometriosis is a gynecological condition characterized by the growth of ectopic endometrial cells outside the uterus. This tissue similarly to eutopic endometrium undergoes regeneration and shedding during the menstrual cycle. Endometriosis affects one in ten women of childbearing age and infertility is often associated with this disease (Hickey et al. 2014). Endometriosis shares mechanisms of estrogen stimulation and chronic inflammation with endometrial cancer; therefore it may be associated with this type of cancer (Yu et al. 2015). The deregulated expression components of the Notch signaling in endometriosis suggest an involvement of this pathway in the pathogenic process. Su et al. reported that Notch receptors NOTCH1 and NOTCH4, ligands JAG2 and DLL4, as well as target genes HEY1and HES5 were downregulated in eutopic endometrium of endometriosis patients suggesting that suppressed Notch signaling contributes to decidualization defects and is responsible for decreased fertility in woman with endometriosis (Su et al. 2015). NOTCH2 has also been shown as a regulator of decidualization (Otti et al. 2014). It was demonstrated that NOTCH1, JAG1, JAG2, and survivin significantly decrease in women with endometriosis, polycystic ovary syndrome, or repeated implantation failure, concluding that Notch signaling molecule might be associated with implantations problems and poor outcomes observed in these diseases (Amjadi et al. 2019). In addition, the expression of gene FOXO1 (NOTCH1 coactivator) was activated in decidualization. Interestingly, NOTCH1 also regulate FOXO1 expression. In the case of endometriosis, the suppression of Notch signaling results in decreased FOXO1 expression and decidualization failure (Brar et al. 2001). In contrast to the aforementioned studies, several groups have obtained opposite results. Expression of NOTCH1 in patients with deep infiltrating endometriosis and NUMB in luminal epithelium was significantly higher as compared with controls (Schuring et al. 2018). In the findings of another group, the expression levels of NOTCH1 and JAG1were upregulated in ectopic endometria than in their eutopic and normal counterparts. Moreover, estrogen regulates cell invasion in the endometriosis via activation of estrogen receptor alpha and the enhancement of Notch signaling (Li et al. 2018). After ultra-deep targeted sequencing, mutations of *NOTCH1* and

*NOTCH2* genes were observed in the ectopic endometrium and atypical endometriosis, but not in normal endometrium tissue (Er et al. 2016).

## Endometrial Cancer

Endometrial cancer (EC) is the sixth most common malignancy among women and 15th cancer in general. The rates of new diagnoses have been rising by about 1% each year. The frequency of this cancer varies among different countries – the incidence of this disease is highest in Central and Eastern Europe and North America, and it is lowest in Middle and Western Africa. Importantly, the incidence of EC is rising in high-income countries; this might be attributable to high rates of obesity, physical inactivity, late menopause, and extended life expectancy. Endometrial cancer accounts for 2% of cancer deaths in women. Fortunately, EC often causes specific symptoms at early stages, and when diagnosed at an early stage, 5-year survival rate is relatively high, reaching 69%. On the other hand, a delayed diagnosis leads to advanced stage and lower chance of survival. The majority of EC are sporadic, and 5–10% of women inherit cancer susceptibility (Sundar et al. 2017; Amant et al. 2018; Ferlay et al. 2018; World Cancer Research Fund 2019).

Endometrial carcinoma has been traditionally classified into two histological types described by Bokhman (Bokhman 1983). Type I tumors make up 80–90% of endometrial cancers and are typically characterized by a low-grade endometrioid histology (endometrioid endometrial cancer, EEC), arising on a background of atypical hyperplasia. EEC is characterized by estrogen and progesterone receptor positivity and has a favorable prognosis in most cases. Factors leading to an excess of estrogen relative to progesterone are associated with this type of cancer (Sanderson et al. 2017; Amant et al. 2018). Type II cancer is determined in 10–20% of endometrial cancers. This type of cancer is associated with typically high-grade non-endometrioid histology (serous endometrial cancer, SEC; clear cell endometrial cancer, CCEC; uterine carcinosarcoma, UCS), arising in atrophic endometria, and is usually estrogen independent. The precursor of lesion in this type endometrial carcinoma is not yet fully established. This cancer type has higher risk for metastases and less favorable prognosis (Pathiraja et al. 2013; Akhtar et al. 2019).

Aside from morphologic differences between type I and type II, the endometrial cancers are distinguished by genetic alterations. The mutations of the tumor suppressor gene PTEN (phosphatase and tensin homolog) are the most frequent genetic alteration (up to 83%) in endometrioid endometrial cancer. This mutation coexists with other mutations in PIK3CA (phosphatidylinositol 3-OH kinase), CTNNB1 (which encodes β-catenin), KRAS (proto-oncogene, GTPase), and ARID1A (AT-rich interaction domain 1A) and defects in DNA mismatch repair. Mutations in tumor protein TP53, component of ubiquitin ligase Skp1-Cullin1-F-box complex FBXW7 (F-box with 7 tandem WD40), and PPP2R1A (protein phosphatase 2 scaffold subunit alpha) are found in non-endometrioid endometrial cancer. Other frequent alterations in this type of cancer is the overexpression of oncogene HER2/neu (erb-b2 receptor tyrosine kinase 2) and p16 (cyclin-dependent kinase inhibitor 2A, CDKN2A) loss of function leading to uncontrolled cell growth and aneuploidy (McConechy et al. 2012; Matias-Guiu and Prat 2013; O'Hara and Bell 2012).

However, the accuracy of histologic classification is not sufficient in order to distribute patients into optimal treatment subgroups, since various endometrial cancer types may exhibit shared characteristics. Recently, significant progress has been made in understanding molecular events in EC, and a division of tumors into distinct prognostic groups was suggested. The Cancer Genome Atlas (TCGA) provides the most comprehensive molecular study, involving whole-genome sequencing, exome sequencing, MSI assay, and a copy number analysis. Endometrial cancers could be classified into four distinct groups: (1) POLE (polymerase ξ exonuclease) ultramutated, which have *POLE* exonuclease domain mutations; (2) MSI hypermutated tumors, which have MSI-H (microsatellite instability-

high) status and have hypermethylated *MLH1* promoter; (3) copy-number low, MSS (microsatellite stabile) tumors, characterized by a low copy number aberrations and frequent *CTNNB1* mutation; and (4) and copy-number high which are characterized by high-level copy number alterations and frequent *TP53* mutation. Women in POLE-mutated subgroup exhibit the best prognosis, whereas women from copy-number high subgroup have the worst prognosis in progression-free survival. This classification is currently not applied in routine clinical practice, but due to the evolution of the methodologies involved, it will, hopefully, be ready in the near future (Cancer Genome Atlas Research et al. 2013; Urick and Bell 2019; Carlson and McCluggage 2019).

## Notch Signaling in the Endometrial Cancer

**Expression of Notch Signaling Components** Deregulated expression of Notch receptors and Notch ligands has been found in an increasing number of human solid tumors. However, there are only a limited number of reports of Notch signaling in the endometrial cancer. Moreover, as in the case of normal endometrium/endometriosis, the role of Notch signaling in endometrial cancer is ambiguous and seems to depend on analyzed Notch receptor/ligand as well as on the analysis methods. For Notch signaling component expression analysis, in most cases immunohistochemical staining was used, and the expression in the endometrium was compared to normal tissue from separate patients. Suzuki et al. have published the first report about Notch signaling in endometrial cancer in 2000 (Suzuki et al. 2000). They reported that endometrial cancer cells express a significantly lower level of NOTCH4 compared to normal endometria proposing the role of NOTCH4 in endometrial cancer development. Cobellis et al. studied normal (n = 60) and pathological (n = 60) endometrium samples by immunohistochemical analysis. They detected Notch signaling changes in different normal endometrium phases. The elevated expression of NOTCH1 in hyperplasia and carcinoma compared to polyps was found, whereas NOTCH4 and JAG1 expression decrease correlated to histological grade. In support, the expression of NOTCH1 and NOTCH4 correlated to p21 and cyclin D expression level (Cobellis et al. 2008). Mitsuhashi et al. demonstrated, using immunohistochemistry, the expression of NOTCH1, NOTCH3, JAG1, and DLL4 proteins was higher in endometrial cancer ($n$ = 76) versus normal endometrium ($n$ = 37) from non-cancer patients. Additionally, the elevated level of NOTCH1 correlated with cancer aggressiveness such as invasion into the myometrial layer and metastasis. High expression of NOTCH1 and JAG1 was associated with poor patient's prognosis (Mitsuhashi et al. 2012). Mori et al. using immunohistochemistry staining also obtained similar results that NOTCH1 expression in endometrial adenocarcinoma ($n$ = 21) was significantly higher than in normal endometrium (Mori et al. 2012). When the amount of proteins NOTCH1, NOTCH3, NOTCH4, and JAG2 was determined by Western blot analysis of endometrial cancer and adjacent nontumor tissue from the same patient, the level of proteins NOTCH1 and NOTCH3 was unchanged. Meanwhile the relative amount of proteins NOTCH4 and JAG2 was decreased in the majority of stage I endometrial cancer samples, compared to nontumor endometrium of the same patient (Sasnauskiene et al. 2014).

Gene expression analysis between normal and malignant patient samples showed significant elevation of *JAG2* level in endometrial cancer tissues, but it has no impact on cancer patient survival (Townsend et al. 2019). Jonusiene et al. found the decreased expression of Notch receptors (*NOTCH1*, *NOTCH2*, *NOTCH3*, and *NOTCH4*), ligands (*JAG1*, *JAG2*, and *DLL1*), and Notch target gene *HES1* in endometrial cancer tissue compared to normal endometrium of the same patient at mRNA level. For this analysis the samples of endometrial cancer and adjacent nontumor endometrial tissue from the same woman were used ($n$ = 50) (Jonusiene et al. 2013). This study supports the notion that Notch signaling is less active in endometrial cancer and supposes

that it can function as tumor suppressor. In Williams et al. investigation, it was discovered that early endometrial cancer cells lose the apicobasal polarity in the low-grade endometrial cancer. In addition, it was observed the mislocalization of Notch receptor. The decreased expression of *NOTCH4* receptor, ligand *JAG1*, and downstream targets *HES1* and *HEY1* in low-grade endometrial cancer was detected by RT-PCR analysis, indicating that overall Notch signaling is suppressed in low-grade endometrial cancer, while the expression of *NOTCH1* and *NOTCH2* was unchanged (Williams et al. 2017).

Polychronidou et al. analyzed the expression of NOTCH2, NOTCH3, and JAG1 in endometrial carcinoma tissue ($n = 204$) by immunostaining. It was found that more than 70% of tumor were negative for all three proteins. The study has been performed only using cancer tissues. The analysis showed that expressions of NOTCH2 and JAG1 have opposite prognostic impacts. The expression of NOTCH2 has the unfavorable prognostic impact in endometrial cancer, while JAG1 expression reflects a favorable prognosis. Moreover, expression of JAG1 was favorable in the absence NOTCH2/3 and was very similar to patients with low or undetectable expression of all three markers. The expression of NOTCH3 did not yield significant results possibly, due to small number of NOTCH3-positive patients (Polychronidou et al. 2018).

The data of a retrospective study obtained from The Cancer Genome Atlas (545 tumor and 35 adjacent tissues) concluded that expression of Notch ligand DLL3 was upregulated in endometrial cancer tissues. Evaluation depends on patient age, FIGO stage, and grade. It was also discovered that upregulation of DLL3 expression was associated with the shortest overall survival in patients with endometrial cancer (Wang et al. 2018).

All available data concerning Notch signaling member's expression in endometrial cancer are summarized in Table 4.1. All investigations coincide on the only one point: the expression levels of NOTCH4 protein and mRNA are decreased in endometrial cancer. Meanwhile, the expression status of other Notch signaling members is scarce and sometimes contradictory, and more data are needed to draw the conclusions (Table 4.2).

**Table 4.2** Expression analysis of Notch signaling members in endometrial cancer

| | Protein expression | mRNA expression |
|---|---|---|
| NOTCH1 | ↑[b,c,d] ↕[e] | ↓[g] ↕[h] |
| NOTCH2 | ND | ↓[g] ↕[h] |
| NOTCH3 | ↑[c] ↕[e] | ↓[g] |
| NOTCH4 | ↓[a,b,e] | ↓[g,h] |
| JAG1 | ↓[b] ↑[c] | ↓[g,h] |
| JAG2 | ↓[e] | ↓[g] ↑[f] |
| DLL1 | ND | ↓[g] |
| DLL3 | ND | ↑[i] |
| DLL4 | ↑[c] | ND |
| HES1 | ND | ↓[g,h] |
| HEY1 | ND | ↓[h] |

[a]Suzuki et al. (2000); [b]Cobellis et al. (2008); [c]Mitsuhashi et al. (2012); [d]Mori et al. (2012); [e]Sasnauskiene et al. (2014); [f]Townsend et al. (2019); [g]Jonusiene et al. (2013); [h]Williams et al. (2017); [i]Wang et al. (2018); *ND* not determined

**Stem Cells in Endometrial Cancer** A small population of adult stem cells, including epithelial progenitors, mesenchymal stem cells, and side-population cells, have been identified in the human endometrium. These cells contribute to regenerative capacity of the endometrium (Evans et al. 2016). Cancer stem cells (CSCs) are cells with stem-like properties crucial for generation of neoplastic cell population; they are responsible for invasiveness and formation of drug resistance (Hanahan and Weinberg 2011; Carvalho et al. 2015). Different molecules were studied as markers of CSC in endometrial cancer, including CD133, CD44, CD117(c-kit), and aldehyde dehydrogenase 1 (ALDH1) (Tempest et al. 2018; Giannone et al. 2019). Different signaling pathways regulate stemness in EC, including Notch signaling.

The cell surface marker CD133 is known as prominin; it identifies stem-like cell population. $CD133^+$ cells have exhibited a more aggressive proliferation in vitro and higher resistance to chemotherapeutic drugs cisplatin and paclitaxel (Elbasateeny et al. 2016). Analysis of endometrial cancer Ishikawa cells, separated into two

$CD133^+$and $CD133^-$ subpopulations, demonstrated the increased level of NOTCH1 protein in cancer stem-like cells $CD133^+$. The blockade of the Notch signaling with γ-secretase inhibitor DAPT suppressed CSC proliferation. Moreover, a treatment of Ishikawa cells with DAPT and other therapeutic target, EGFR inhibitor, was more efficient than treating with any compound alone. Authors concluded that Notch signaling seems to be a promising therapeutic target for CSCs (Shang et al. 2018).

Another stem cells marker in EC is RNA-binding protein Musashi-1. Gotte et al. found an increased protein level of Musashi-1 in endometriosis and endometrial cancer. siRNA silencing of Musashi-1 resulted in decreased expression of NOTCH1 protein and its downstream target HES1 in Ishikawa cells. At the functional level, these changes promote reduced cell proliferation and apoptosis induction (Gotte et al. 2011). It was also shown that patients with upregulated Musashi-1 expression have poor survival rate, which may be an independent prognostic factor for endometrial cancer (Ma et al. 2015).

**Crosstalk of Notch and Obesity Signals** Increasing body mass index is associated with a significant increase in the risk of endometrial cancer (Reeves et al. 2007; Renehan et al. 2008). It has been demonstrated that in comparison with all obesity-related cancers, increasing body mass index is most strongly associated with endometrial cancer incidence and mortality (Schmandt et al. 2011). Although the correlation between obesity and cancer incidence is identified, the molecular mechanisms linking these processes remain the area of intensive studies. Obesity is characterized by excess of adipose tissue, which drives the dysregulation of complex metabolic and endocrine activities (Crean-Tate and Reizes 2018). Leptin is an adipose tissue-secreted hormone, which correlates with the level of adiposity and body mass index in women. Leptin signaling has been shown to induce breast cancer growth and progression (Ando et al. 2014). It was demonstrated that Notch, IL-1, and leptin crosstalk outcome (NILCO) is involved in the induction of breast cancer cell proliferation and migration, where leptin upregulates Notch ligands, receptors, and target genes (Guo and Gonzalez-Perez 2011). In analogy to breast cancer, the group of Gonzalez-Perez hypothesized that NILCO could be a link between obesity and endometrial cancer progression (Daley-Brown et al. 2015). This group has demonstrated that leptin is an inducer of Notch receptors (NOTCH1–4), ligands (JAG1 and DLL4), and downstream effectors (survivin, HEY2) and leptin (OB-R) and IL-1 (IL-1R tI) receptors in endometrial cancer cells (Daley-Brown et al. 2019). The impact of leptin was higher for the poorly differentiated and more aggressive cell lines An3Ca and KLE, resembling type II endometrial cancer. Leptin also upregulated the expression of NOTCH1, NOTCH3, and NOTCH4 receptors in the more differentiated HEC-1A and Ishikawa cells, resembling more differentiated type I endometrial cancer. Moreover, it was demonstrated that leptin induces cell cycle progression and proliferation of endometrial cancer cells. The importance of leptin signaling for endometrial cancer has to be proved using animal models.

**Mutations in Notch-Related Genes** DNA repair system plays a crucial role in recognition and repairing of insertions or deletions in microsatellites – the repeated sequences of DNA. Abnormal function of a repair system causes the creation of novel microsatellite fragments resulting in microsatellite instability (MSI). Some cancer types, including endometrial cancer, exhibit higher rates of MSI (16.5%). Higher rates of MSI tumor selectively share alterations in genes of common pathways including Notch and Wnt proposing possibilities of pathway-targeted therapies (Trabucco et al. 2019). Mutations of *NOTCH1* and *NOTCH2* genes were identified in endometriosis-associated ovarian cancer, and it may predispose endometriotic lesion to malignant transformation (Er et al. 2016).

**miRNAs** A class of a small noncoding RNAs, miRNAs, are important for gene regulation, and they are differentially expressed in various malignant tissues. It was identified that 138 miRNAs

are differently expressed in endometrial cancer in comparison to the normal endometrium. Among deregulated miRNAs was miR-34a, regulating members of Notch family *NOTCH1*, *NOTCH2*, *JAG1*, and *DLL*. In addition, *NOTCH1* was regulated by miR-34* and miR-27b* (Jurcevic et al. 2014). Upregulation of miR-34 led to a significant decrease of *NOTCH1* and *DLL1* at mRNA level, while downregulation led to a significant increase in this mRNA (Jurcevic et al. 2016). Devor et al. reported a significant downregulation of miRNA-181c in endometrial cancer. The decrease of miRNA-181c was in part attributed to upregulation of NOTCH2 (Devor et al. 2017).

## Conclusion

The aberrant expression of Notch signaling receptors and ligands suggests that this pathway is important for changes in cycling endometrium and in disorders such as endometriosis or endometrial cancer. There are controversial suggestions concerning the role of Notch signaling in the endometrium that are supported by sometimes contradictory results about expression changes of Notch molecules. Therefore, additional functional studies are required to reveal the importance of Notch signaling for endometrial cancer progression. Future challenges in the field include choosing of the right methods and approaches to analyze the importance of Notch signaling for endometrial cancer. It is necessary to understand how this pathway interacts with other signaling pathways, including Wnt and Hedgehog. These new studies may offer new potential markers for endometrial cancers molecular classification and prognostic or therapeutic targets.

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