Tumor progression, metastasis, and modulators of epithelial–mesenchymal transition in endometrioid endometrial carcinoma: an update

Annu Makker and Madhu Mati Goel

Post Graduate Department of Pathology, King George's Medical University, Lucknow 226003, Uttar Pradesh, India

Correspondence should be addressed to A Makker
Email
annumakker@gmail.com

Abstract

Endometrioid endometrial carcinoma (EEC), also known as type 1 endometrial cancer (EC), accounts for over 70–80% of all cases that are usually associated with estrogen stimulation and often develops in a background of atypical endometrial hyperplasia. The increased incidence of EC is mainly confined to this type of cancer. Most EEC patients present at an early stage and generally have a favorable prognosis; however, up to 30% of EEC present as high risk tumors, which have invaded deep into the myometrium at diagnosis and progressively lead to local or extra pelvic metastasis. The poor survival of advanced EC is related to the lack of effective therapies, which can be attributed to poor understanding of the molecular mechanisms underlying the progression of disease toward invasion and metastasis. Multiple lines of evidence illustrate that epithelial–mesenchymal transition (EMT)-like events are central to tumor progression and malignant transformation, endowing the incipient cancer cell with invasive and metastatic properties. The aim of this review is to summarize the current knowledge on molecular events associated with EMT in progression, invasion, and metastasis of EEC. Further, the role of epigenetic modifications and microRNA regulation, tumor microenvironment, and microcystic elongated and fragmented glands like invasion pattern have been discussed. We believe this article may perhaps stimulate further research in this field that may aid in identifying high risk patients within this clinically challenging patient group and also lead to the recognition of novel targets for the prevention of metastasis – the most fatal consequence of endometrial carcinogenesis.

Key Words
- endometrioid endometrial carcinoma
- progression
- invasion
- metastasis
- EMT modulators

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Introduction

Endometrial cancer (EC) is the most common gynecological malignancy worldwide, with an estimate of more than 288 000 women developing the cancer annually (Jemal *et al.* 2011). Of significance, the incidence and mortality rates for EC have been rising in the developed and developing countries and are projected to rise further with the increasing aging population and prevalence of obesity (Bakkum-Gamez *et al.* 2008).

Based on epidemiology, conventional histopathology, and clinical behavior EC are divided into two subtypes.

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Type 1 ECs are usually endometrioid in histology, present with early stage disease at diagnosis, are well differentiated with respect to grade and are frequently associated with hyper estrogenic milieu. It has been proposed that most of these carcinomas represent the end point of a continuum of morphologically distinctive hyperplastic lesions that cover a range from endometrial hyperplasia without atypia to endometrial hyperplasia with atypia, simple or complex. Atypical endometrial hyperplasia (AEH) has been reported to be associated with invasive EC in 62% endometrial biopsy specimens, suggesting that AEH may be the direct precursor to endometrioid EC (EEC; Amant *et al*. 2005, Trimble *et al*. 2006). EEC usually exhibits a high incidence of loss of function alterations in the *PTEN* tumor suppressor gene (TSG) as well as defects in DNA mismatch repair resulting in microsatellite instability (Samarnthai *et al*. 2010). These tumors may also contain activating mutations of the *CTNNB1*, *PIK3CA*, and *PIK3R1* genes, and less frequently *KRAS2*, *FGFR2*, and *p53* genes (Ignar-Trowbridge *et al*. 1992, Kobayashi *et al*. 1999, Hayes *et al*. 2006, Pollock *et al*. 2007). The type 2 EC usually have non-endometrioid histology (e.g. papillary serous or clear cell), are poorly differentiated, and are often advanced stage at the time of diagnosis. Non-EECs are more likely to harbor *p53* mutation, and are characterized by widespread aneuploidy (Risinger *et al*. 2013).

In addition, undifferentiated carcinoma of the endometrium (UEC) has been identified as a poorly differentiated subtype of EC, characterized by a solid growth pattern. UEC represent 9% of all ECs and are often classified as FIGO grade 3 EEC, although they have a more aggressive phenotype. Occasionally, these tumors develop as undifferentiated areas associated with grade 1 or 2 EEC, and such cases are referred to as dedifferentiated carcinomas (Silva *et al*. 2006). Mutations in *CTNNB1*, *PPP21A*, and *p53* genes have been suggested to contribute to tumor progression from EEC to UEC (Kuhn *et al*. 2014).

Although the overall 5-year survival rate in patients with stage I EEC approaches 90%, ~30% of these tumors are high risk tumors (stage IC–grade 3 or more advanced), which have invaded deep into the myometrium at diagnosis and progressively lead to local or extra pelvic metastasis with a 5-year survival ranging from 16 to 66% (Ries *et al*. 2007). Several treatment options, such as hysterectomy, hormonal therapy, and combinations of radiation and chemotherapy are effective for early stage EC; however, only limited options remain if the tumors metastasize (Rauh-Hain & del Carmen 2010).

Increased cell invasion and migration are defining characteristics of metastatic cancer cells. Recent studies have documented that cell invasion during tumor progression may be critically dependent on the acquisition of epithelial–mesenchymal transition (EMT) features. EMT is a complex, stepwise phenomenon which is vital for morphogenesis during embryonic development and is also reinitiated during cancer progression leading to a more invasive and metastatic phenotype. During the EMT process epithelial cells lose basal apical polarity, become more spindle shaped, and acquire the motile and cancer stem cell (CSC) phenotypes which are capable of both tumor initiation and sustenance of tumor growth and exhibit a heightened propensity to metastasize to distant organs. In addition, acquisition of EMT phenotype has been associated with drug resistance, which could give rise to recurrence and metastasis after standard chemotherapeutic treatment (Weinberg 2008).

The molecular and cellular mechanisms underlying EMT are complex and can be initiated by multiple extracellular signals and many secreted soluble factors that finally activate different signaling pathways and transcription factors (Fig. 1). EMT inducing signals overlap significantly with pathways ensuring other carcinogenesis processes such as proliferation, resistance to apoptosis, angiogenesis, self sufficiency for survival and invasion. Further, dynamic interactions between tumor microenvironment and cancer cells are also known to facilitate EMT induction and drive metastatic progression (Thiery 2002).

Disassembly of cell–cell junction together with down-regulation of epithelial protein E-cadherin (CDH1) is an important hallmark of EMT. Progressive loss of E-cadherin is often coupled with the expression of non-epithelial cadherins, such as the mesenchymal N-cadherin and cadherin-11, a process known as 'cadherin switching'. In addition cells, acquire markers such as smooth muscle actin, fibronectin, or vimentin, as well as increased activity of matrix metalloproteinases, e.g., MMP2, MMP3, and MMP9 (Thiery & Sleeman 2006). Transcription factors like TWIST, members of the SNAIL, SLUG, and *ZEB1* protein families orchestrate the EMT program and function as EMT core regulators. An association between EMT like cellular phenotype as revealed by changes in expression of marker proteins and tumor aggressivity has been well proven in various human malignancies including EC (Valdes *et al*. 2002).

The aim of this review is to summarize current knowledge on molecular events associated with EMT in progression, invasion, and metastasis of EEC. We focus on the role of EMT effectors and core regulators, and molecular pathways associated with EMT in EEC progression and metastasis. In addition, the role of epigenetic

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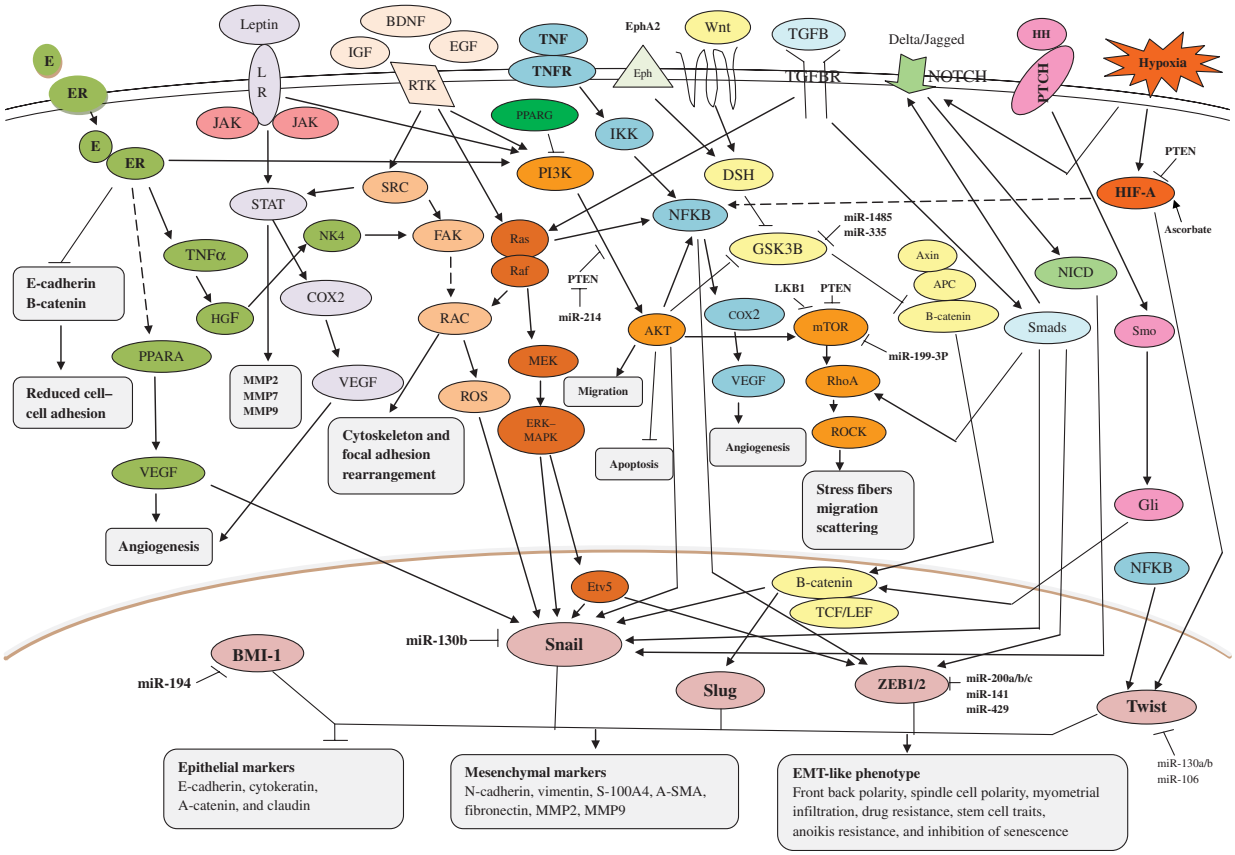

Figure 1

Schematic illustration of the major molecular pathways and transcription factors associated with EMT program in EEC. Several microRNAs regulate the EMT inducing signaling pathways and the core EMT regulators. Sharp arrows denote activation and blunt arrows indicate inhibition. Dotted lines indicate the presence of intermediate signaling molecules (not shown). End points of the EMT program are boxed (see text for details). A full colour version of this figure is available at http://dx.doi.org/10.1530/ERC-15-0218.

modifications and microRNA (miRNA) regulation, tumor microenvironment, and microcystic elongated and fragmented (MELF) like invasion pattern has been discussed. A systematic search using PubMed and Google Scholar was conducted for publications between January 2005 and September 2015 with the text phrases 'endometrial cancer', 'endometrial hyperplasia', 'epithelial mesenchymal transition', and 'molecular pathways'. Articles were read, analyzed, and screened with a focus on EEC. Primary reference lists from these manuscripts as well as PubMed's 'Related Articles' feature were used to identify additional relevant articles.

EMT effectors and core regulators in EEC progression and metastasis

Loss of epithelial cell–cell contacts through inhibition of E-cadherin is important for the development of invasion and metastatic capacity in human malignancies. Loss of E-cadherin expression has been demonstrated to be critical for the progression of EC and a steady decrease in E-cadherin histoscores was observed as the tumor became more invasive. Malignant transformation of endometrial glands was associated with change in E-cadherin expression from pure membranous to membrano-cytoplasmic (Ahmed & Muhammad 2014). Decreased E-cadherin expression in EC has been associated with tumor dedifferentiation and deep myometrial invasion (Sakuragi *et al*. 1994). Combined positive E-cadherin, A-catenin, and B-catenin expression was reported to be an independent and positive prognostic factor for survival in patients with grade 1–2 carcinomas, whereas negative E-cadherin expression was found to be associated with histologic grade 3 and with non-EEC and decreased cancer specific survival (Scholten *et al*. 2006, Abal *et al*. 2007). These observations are corroborated by the findings of

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Yang *et al*. (2014) who observed significant downregulation of epithelial markers (E-cadherin and A-catenin) and upregulation of mesenchymal markers (N-cadherin and vimentin) in the tumors of patients with late stage and high grade EC.

A role for the SNAIL, one of the most prominent transcriptional E-cadherin repressors, in endometrial progression and dedifferentiation was proposed by Blechschmidt *et al*. (2007). The authors observed an increase in SNAIL protein expression and its inverse correlation with E-cadherin expression in both primary tumors and metastasis of EEC. Montserrat *et al*. (2011) investigated the activation status of EMT program in early stages of EEC and observed SNAIL upregulation in non-invasive (stage IA) and myoinvasive (stages IB and IC) tumors. As compared to normal endometrium, stage IC tumors overexpressed the whole set of E-cadherin repressors viz. SNAIL, TWIST, *ZEB1*, HMGA2, and SLUG, whereas tumors of stages IA and IB overexpressed only SNAIL. The authors suggested that not only is EMT involved in myometrial invasion but also in intra-endometrial EEC and also that there is a progression in the expression of EMT markers with tumor invasiveness. An association of lymph node metastasis and death risk with reduced E-cadherin and nuclear SNAIL expression has also been reported (Tanaka *et al*. 2013). Supernat *et al*. (2013) found association between decreased SLUG expression and shorter overall survival. SLUG expression showed correlation with SNAIL and was suggested to serve as a prognostic factor in EC. However, the authors could not find correlation between expression of EMT and CSCs markers, which might suggest absence of association between EMT and CSC phenotype in EC or additional markers should be examined.

TWIST1 promotes EMT in EEC either by directly repressing E-cadherin or upregulating the expression of BMI-1, an EMT inducer (Dong *et al*. 2011). Owing to its antiapoptotic functions, TWIST facilitates EMT and provides EEC cells more infiltrative phenotypes, leading to deep myometrial invasion and poor patient survival. Though not statistically significant, an association between TWIST and pelvic node metastasis was found (Kyo *et al*. 2006). An oncogenic role for Kruppel like factor 17 (KLF17), a member of KLF transcription factor family was recently demonstrated during EEC progression via initiating EMT through regulation of TWIST1 (Dong *et al*. 2014*a*).

In vitro findings have provided direct evidence for the role of *ZEB1* in controlling EC cell motility. Forced *ZEB1* expression in Ishikawa cells (a type 1 non-aggressive, *ZEB1* negative cell line) resulted in reduced E-cadherin expression and increased migration (Singh *et al*. 2008). While *ZEB1* overexpression was observed in tumor associated stroma of low grade EEC, in grade 3 EEC and uterine papillary serous carcinomas, *ZEB1* was expressed in both stroma and epithelial derived carcinoma cells (Spoelstra *et al*. 2006). Recently, Feng *et al*. (2014) reported significantly higher *ZEB1* expression in endometrial biopsies from patients with lymph node metastasis as compared to those without lymph node metastasis. *ZEB1* expression was also significantly associated with histological subtype, grade and myometrial invasion. A critical role for ETV5, a member of Ets transcription factor, in early stages of invasion of EC through the promotion of EMT has been proposed. ETV5 activates *ZEB1* resulting in E-cadherin repression and complete reorganization of cell–cell and cell substrate contacts (Colas *et al*. 2012).

Although the endometrioid and non-EECs follow different pathogenetic pathways, loss of E-cadherin has been associated with adverse prognosis in both tumor subtypes. Negative or reduced E-cadherin expression has been reported in 62–87% of serous and clear cell cancers respectively. *ZEB1* expression was found to be associated with loss of E-cadherin in type 2 EC. However, it was observed that even substantial reduction in *ZEB1* could not restore E-cadherin levels in Hec50co cells (an aggressive type 2 cell line; Singh *et al*. 2008).

An EMT molecular phenotype has been described as a characteristic feature of undifferentiated ECs. *ZEB1* overexpression and downregulation of miR-200s in these tumors was associated with absent or downregulated E-cadherin expression (Romero-Perez *et al*. 2013). Upregulation of fascin, an actin binding protein, was recently suggested to represent an EMT like process in UECs and also contribute toward the invasive character of this aggressive EC subtype (Stewart & Crook 2015*a*).

Molecular factors associated with EMT in progression and metastasis of EEC

Steroid hormones and their receptors

The significance of steroid hormones and their receptors (ER and PR) in endometrial carcinogenesis is well accepted. Women with EC have higher estrone and estradiol levels as compared to healthy women (Allen *et al*. 2008). It has been proposed that estrogen promotes EC invasion via induction of humoral interactions between the cancer and stromal cells. Estrogen was

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found to stimulate tumor necrosis factor alpha (TNFα) expression from EC cells, which in turn induced stromal hepatocyte growth factor (HGF) expression resulting in enhanced NK4 (an HGF antagonist/angiogenesis inhibitor) sensitive invasion of EC cells. In addition, a correlation was observed between EC cell invasion and the expression and dimerization of integrin α(v)β(5) as well as activation of focal adhesion kinase (FAK) molecular events known to be associated with induction of EMT (Choi *et al*. 2009).

ER exists in two main forms, ER-A and ER-B encoded by separate genes ESR1 and ESR2 respectively. An important role of ER-A in the development of endometrial hyperplasia has been demonstrated (Pieczyńska *et al*. 2011). While early stage, well-differentiated EC usually retain expression of both ER-A and ER-B, loss of either or both these receptors is often observed in advanced stage and poorly differentiated tumors (Gehrig *et al*. 1999). An association between ER-A loss, increased EMT and an aggressive clinico-pathologic phenotype has been demonstrated in EC. A significant correlation was observed between ER-A negative tumor status and expression of E-cadherin, B-catenin, catenin p120, and P-cadherin as well as vascular invasion and deep myometrial infiltration (Wik *et al*. 2013). ER-A loss has previously been associated with increased expression of SNAIL in these tumors (Blechschmidt *et al*. 2007).

Recently, Kreizman-Shefer *et al*. (2014) proposed loss of ER as an advanced molecular pathology of EC with deregulation of molecular pathways. Common deregulation events include *PTEN* inactivation by mutation, *de novo* methylation of ER-A gene and aberrant methylation of CpG islands. A previous study examining role of ER-A in the development of EC in a *PTEN*$^{-/-}$; *ER-A*$^{-/-}$mouse model has shown that absence of ER-A leads to an increased incidence of *in situ* and invasive carcinoma (Joshi *et al*. 2012). Upregulated expression of NPMI, a nucleolar phosphoprotein, was recently suggested to play a role via ER-A in the effects of estrogen on malignant progression of EEC (Zhou *et al*. 2014).

ER-B is highly expressed in EC with severe myometrial invasion and an important role of this receptor isoform in the progression of myometrial invasion has been suggested (Mylonas 2010). Pertinently an extensive myometrial invasion may be a progeny of EMT which has been implicated in invasive characteristics of endometrial tumors (Montserrat *et al*. 2011).

Progesterone regulates endometrial function by antagonizing estrogen mediated cell proliferation and inducing cellular differentiation (Graham & Clarke 1997). Indeed, progesterone therapy has been used of late to impede development of EC associated with unopposed estrogen (Yahata *et al*. 2006). Expression of either or both isoforms of PR (PR-A and PR-B) was found to be reduced or absent in AEH (Pieczyńska *et al*. 2011) and in both glands and stroma of EC biopsies in comparison to non-malignant endometrial tissue (Kreizman-Shefer *et al*. 2014). In addition to its growth inhibiting effects, progesterone plays a significant role in regulating invasive properties of EC cells. Van der Horst *et al*. (2012) assessed progesterone signaling in non-progressive and progressive primary EC tissues. Progression of disease was characterized by loss of PR expression which was associated with loss of immunosupression and increased transition from an epithelial to a more mesenchymal, more invasive phenotype. In addition, progesterone inhibited cancer cell migration in Ishikawa cell line due to inhibition of EMT. In high grade EECs showing more widespread myometrial invasion, loss of PR was strongly associated with increased expression of CD44 (a CSC marker) and decreased expression of E-cadherin. The findings indicate that the molecular circuitries underlying EMT and cancer stemness may be closely interlinked during EEC progression (Hanekamp *et al*. 2003, Saito *et al*. 2004).

Overexpression of PR-B isoform has been demonstrated in highly malignant forms of endometrial, cervical, and ovarian cancers (Fujimoto *et al*. 1997). It has been suggested that PR-B status controls EMT in ECs. PR-B activation by progesterone resulted in tumor suppression by inhibiting cell growth and invasiveness via suppression of the expression of MMPs (1, 2, 7, and 9) and Ets1 transcription factor (Saito *et al*. 2004). The relative overexpression of PR-B without transcriptional repression by PR-A was related to the metastatic potential in ECs (Kreizman-Shefer *et al*. 2014).

In a recent study, Berg *et al*. (2015) reported preserved ER-A and PR expression in both premalignant endometrial lesions as well as grade 1 EEC. Significant reduction in receptor levels as well as increase in EMT score was detected from grades 2 to 3, suggesting EMT as a late event in endometrial carcinogenesis linked to loss of hormone receptors (Table 1).

Cell signaling pathways

Phosphatidylinositol 3-kinase/Akt/mTOR signaling Accumulating genetic and cancer biology evidence have demonstrated that phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR pathway is a central mechanism controlling EMT/CSC features, besides its role in cancer

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Table 1 Summary of molecular events associated with EMT in progression, invasion, and metastasis of EEC

Factors	Mechanism	Clinical material/model[a]	References
Steroid hormones and their receptors	ER-A loss in EC is associated with increased EMT, vascular invasion, and deep myometrial invasion	Tumor samples FIGO stages I/II and III/IV ($n=76/155/286/111$)	Wik *et al*. (2013)
	ER-A negative EC associated with increased expression of SNAIL, reduced E-cadherin expression and tumor progression	Primary tumors ($n=87$) and metastases ($n=26$) of EEC	Blechschmidt *et al*. (2007)
	Absence of ER-A leads to an increased incidence of *in situ* and invasive carcinoma	Pten $^{+/-}$ ER$\alpha^{-/-}$ mouse model Pten deleted Hec1A cells	Joshi *et al*. (2012)
	NPM1 overexpression may play a role in the effects of estrogen on the malignant progression of EEC via ER-A signalling	Tumor samples FIGO stages I–IV ($n=56$)	Zhou *et al*. (2014)
	Absent or reduced ER-A during EC progression may be due to loss of FOXA1 function	Tissue samples from AH and EC ($n=86$); Ishikawa, RL95-2, Hec1B, and AN3CA cell lines	Wang *et al*. (2014)
	ER-B is important in progression of myometrial invasion	Tumor samples FIGO stages I–IV ($n=214$)	Mylonas (2010)
	Loss of PR is associated with increased CD44 and decreased E-cadherin expression	Ishikawa PRAB-36 cells	Hanekamp *et al*. (2003)
	Progression of EEC characterized by loss of PR, increased EMT, loss of immunosuppression, and more invasive phenotype	Non-progressive ($n=9$) and progressive ($n=9$) primary tumor tissues, Ishikawa cells	Van der Horst *et al*. (2012)
	Loss of PR particularly in stroma indicates invasive characteristic of tumor; relative PR-B overexpression related to metastatic potential	Grades 1 and 2 FFPE tumor samples ($n=15$)	Kreizman-Shefer *et al*. (2014)
	PR-B status controls EMT. PR-B activation inhibits cell growth and invasiveness via suppression of expression of MMPs and Ets1	Ishikawa cell line	Saito *et al*. (2004)
	EMT a late event in endometrial carcinogenesis, linked to loss of hormone receptors	Tissue from CAH, primary EEC and metastatic lesions from EECs ($n=729$)	Berg *et al*. (2015)
Cell signaling pathways			
PI3K/Akt/mTOR signaling	EGFR acting upstream of PI3K/Akt plays initiating role to stimulate EMT via SNAIL upregulation and E-cadherin downregulation	FFPE primary tumor samples ($n=17$), two Ishikawa cell lines	Hipp *et al*. (2009)
	EGFR shows negative correlation with epithelial markers and a positive correlation with mesenchymal markers in EC cells transfected with EGFR	Ishikawa cell line	Yang *et al*. (2014)
	IR/IGF1R induce EH and promote EEC cell growth through activation of PI3K/Akt/mTOR signalling	FFPE samples of CAH and grade 1 EEC ($n=44$)	McCampbell *et al*. (2006)
	Anti-invasive and anti-metastatic effect of metformin in EC cells associated with MMP2/9 and Akt, NFKB, and ERK1/2 signaling	ECC1 cells	Tan *et al*. (2011)
	Transcriptional changes in the PI3K pathway are early events in the invasive step to grade 1 EEC	Tissue from CAH, primary EEC and metastatic lesions from EECs ($n=729$)	Berg *et al*. (2015)
	Loss of PTEN expression is an early event in endometrial tumorigenesis	FFPE endometrial tissue of PE, SH, CAH, and EEC ($n=87$)	Sarmadi *et al*. (2009)
	Loss of *Cdh1* promotes aggressive EC phenotypes when cells are initiated by ablation of *Pten*	Cdh$^{d/d}$, Pten$^{d/d}$, and Cdh$^{d/d}$Pten$^{d/d}$ mouse models	Lindberg *et al*. (2013)
	Pten $^{+/-}$ ER $^{-/-}$ mice show a higher incidence of *in situ* and invasive carcinoma	Pten $^{+/-}$ ER $^{-/-}$ mouse model	Joshi *et al*. (2012)
	Progression of EC is associated with *PTEN* mutation, estrogen treatment induces more severe endometrial tumorigenesis	PR$^{cre/+}$ Pten$^{f/f}$ and Pten$^{d/d}$ mouse models	Kim *et al*. (2013)

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Table 1 *Continued*

Factors	Mechanism	Clinical material/model[a]	References
	Low ER-A associated with markers for EMT, stathmin (a marker associated with PTEN loss) and a high PI3K activation signature	Tumor samples FIGO stages I/II and III/IV ($n=76/155/286/111$)	Wik *et al*. (2013)
	Lkb1 inactivation promotes development of invasive EC	Sprr2f-Cre transgenic mouse model	Contreras *et al*. (2010)
	Concomitant loss of *pten* and *lkb1* leads to rapid development of advanced EEC with 100% penetrance	$Pten^{loxp/loxp}Lkb1^{loxp/loxp}$ mouse model	Cheng *et al*. (2014)
Ras/Raf/MEK/MAPK/ERK signaling	Raf1/MAPK mediates EGF action; stimulates EpCAM cleavage and internalization of EpICD into the nucleus to activate mesenchymal cadherins accompanied by loss of E-cadherin and upregulation of SNAIL	Normal and malignant endometrial cells; EC samples	Hsu *et al*. (2014)
	EGFR via Akt and ERK1/2 pathways regulates expression of SNAIL in Ishikawa cells	FFPE primary tumor samples ($n=17$), two Ishikawa cell lines	Hipp *et al*. (2009)
	MAPK/ERK pathway is involved in the progression and invasion of EEC mediated by EMT	Tumor samples ($n=42$) stage I (IA, IB, and IC)	Montserrat *et al*. (2011)
	Up-regulated expression of kinase suppressor of RAS1 is responsible for endometrial carcinogenesis as well as anchorage-independent cell growth	Tumor samples (grades 1–3 and stages I–IV) ($n=24/26$); Ishikawa 3-H-12, KLE, RL-95, and HEC1A cell lines	Llobet *et al*. (2011)
	GPR30 signaling via the MEK/MAPK/ERK pathway promotes cell proliferation and the invasion potential of EC cells through its action on MMP2 and MMP9	Tumor samples ($n=50$), RL95-2 and KLE cells	He *et al*. (2009)
	Conditional *Pten* ablation and *K-ras* mutation significantly accelerates development of EC	Double mutant mouse model $PR^{cre/+}Pten^{f/f}K\text{-}ras^{G12D}$; $Pten^{d/d}K\text{-}ras^{G12D}$	Kim *et al*. (2010)
	PI3K and KRAS signaling activation suggested as early events in the development from CAH to EEC	Tissue from CAH, primary EEC and metastatic lesions from EECs ($n=729$)	Berg *et al*. (2015)
Wnt/B-catenin signalling	Conditional ablation and activation of B-catenin leads to aberrant epithelial structures and EH formation	$K5Cre;Catnb^{(ex3)fl/+}$ mouse model, Ishikawa and ECC1 cell lines. UE2AA – a mouse derived endometrial cell line. Human specimens of eutopic endometrium ($n=3$), CAH ($n=28$), EACs (G1 and G3, $n=41$)	Villacorte *et al*. (2013)
	LEF1, cyclin D1, and MMP7 play a role in endometrial gland formation and carcinogenesis	Lef1 knockout mice and WT controls; human tumor samples ($n=99$)	Shelton *et al*. (2012)
	B-catenin expression associated with loss of E-cadherin is involved in acquisition of aggressive biological behavior, especially in high grade ECs	Tumor samples (FIGO stages I–IV) ($n=73$)	Shih *et al*. (2004)
	Inhibition of Wnt signaling by Dickkopf-3 is accompanied by decreased proliferation, reduced anchorage independent growth and decreased invasiveness	Tumor samples ($n=27$) stages I/II and II/IV; T-HESC, ECC1, HEC-iA, and RL95-2 cell lines; mouse xenograft model	Dellingera *et al*. (2012)
	Wnt/B-catenin and PTEN pathways have a synergistic effect on EC onset, progression and acquisition of a more aggressive malignant behaviour	$Pgr^{Cre/+}$, $Apc^{15lox/+}$, and $Pten^{ex5lox/-}$ mouse models	Van der Zee *et al*. (2013)
TGFB signalling	TGFB1 plays important role in the initial steps of EC invasion by promoting EMT leading to acquisition of an invasive phenotype	Tumor samples ($n=51$); HEC1A and RL95-2 cell lines	Muinelo-Romay *et al*. (2011)
	Abrogation of TGFBR signaling induces apoptosis and reduces invasive and metastatic potential by reversal of autocrine TGFB-induced EMT	HEC1A cells	Lei *et al*. (2009)

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Table 1 *Continued*

Factors	Mechanism	Clinical material/model[a]	References
	Progesterone inhibits basal and TGFB1 induced cancer cell viability and invasion accompanied by increased E-cadherin and decreased vimentin expression	Ishikawa, HEC1B, and RL95-2 cell lines	Bokhari *et al*. (2014)
	TGFB upregulates prostate apoptosis response-4 expression with simultaneous induction of EMT	KLE and HEC1A cell lines	Chaudhry *et al*. (2014)
	Emmprin knockdown leads to inhibition of cell proliferation, migration, and invasion through TGFB, EGF, NFKB, VEGF, MMP2, and MMP9 resuting in increased levels of E-cadherin and reduced levels of vimentin and SNAIL	Endometrial tissue normal, hyperplasia, adenocarcinoma, and carcinosarcoma ($n=164$); HEC50B and KLE cells	Nakamura *et al*. (2012)
Hedgehog signalling	Expression of signaling elements of Hedgehog pathway increase stepwisely in EH and EC; activation of this pathway is involved in malignant transformation of EC	Tissue from EH (simple, complex, and atypical) and EC (stages I–IV) ($n=112$); Ishikawa, HEC1A/B, HHUA, KLE, and RL95-2 cell lines	Feng *et al*. (2007)
	Over expression of Gli1 proposed as an early event in endometrial tumorigenesis. Gli induces SNAIL which suppresses E-cadherin and displaces B-catenin from adherens junctions	FFPE samples EC ($n=80$; stages I–IV), normal endometrium (PE, SE, and interval, $n=15$), EH (simple and complex, $n=14$), CAH ($n=37$); Hec1A, Hec1B, and RL95-2 cell lines	Liao *et al*. (2009)
NFKB signalling	Upregulation of NFKB activity observed in human EC cells expressing phospho-Akt is responsible for the increase of *COX2* gene expression closely associated with parameters of tumor aggressiveness	Hec1A, RL 95-2, and Ishikawa cell lines	St-Germain *et al*. (2004)
	COX2 and NFKB signaling mediates the progression of hyperplasia to cancer	Tissue samples of benign endometrial polyps, EH, endometrial intra-epithelial neoplasia, and EEC	Faloppa *et al*. (2014)
	Blockade of NFKB activity by Sunitinib reduces cell viability, proliferation, clongenicity, and induces apoptotic cell death	Ishikawa 3-H-12, RL-95, and Hec1A cell lines	Sorolla *et al*. (2012)
	NFKB activation is a novel target of oncogenic KRAS signals in endometrial carcinogenesis	*In vitro* carcinogenesis model with human endometrial epithelial cells	Mizumoto *et al*. (2011)
	Receptor activator of NFKB (RANK) and its ligand promote EC cell proliferation, migration, and invasion via the MAPK pathway	Endometrial tissue normal, EAH and EC ($n=120$). Ishikawa, KLE, AN3CA, RL 95-2, and HEC1B cell lines	Wang *et al*. (2015)
JAK/STAT signalling	Leptin potently induces invasion of EC cells in matrigel invasion assay which is effectively blocked by pharmacological inhibitors of JAK/STAT and PI3K	Ishikawa and ECC1 cell lines	Sharma *et al*. (2006)
	Functional activation of COX2 in EC cells is induced by leptin through JAK2/STAT3, MAPK/ERK, and PI3K/Akt pathways	Ishikawa, ECC1, Hec1A, Hec1B, RL95-2, and AN3CA cell lines	Gao *et al*. (2009)
	Leptin promotes EC growth and invasiveness by activating STAT3 and ERK1/2 signaling pathways which is effectively blocked by inhibitors of STAT3 (AG490) and ERK1/2 (PD98059)	Ishikawa cell line	Liu *et al*. (2011)
Hypoxia signaling pathway	A critical role for HIF1a/TWIST/E-cadherin system has been suggested in malignant progression and acquisition of metastatic phenotype in EEC	Tissue samples from AH and EEC ($n=152$)	Feng *et al*. (2013)
	HIF1 pathway and its target genes, particularly DEC2 (SHARP1) play an important role in endometrial carcinogenesis and tumor phenotype development	Tissue samples from CAH and EC ($n=86$); Ishikawa, HEC1, SNG-II, and SNG-M cell lines	Yunokawa *et al*. (2007)

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Table 1 *Continued*

Factors	Mechanism	Clinical material/model[a]	References
	SHARP1 has a tumor suppressive function during EC progression especially in the regulation of angiogenesis	Tumor samples ($n=110$); Ishikawa and RL95-2 cell lines	Liao *et al*. (2014*a*)
	Higher HIF1A expression has been suggested to be associated with the higher risk of recurrence	Tumor samples, stages IA, IB, II, IIIA, IIIC, and IV ($n=92$)	Sadlecki *et al*. (2014)
TrkB signaling	TrkB and BDNF are involved in EC tumorigenesis and metastasis. TWIST expression is modulated by TrkB, and is essential for TrkB induced EMT like transformation and anoikis resistance	Tissue samples from AH and EC ($n=130$); Ishikawa, RL95-2, HEC1B, KLE, AN3CA, and SPEC2 cell lines	Bao *et al*. (2013*a*)
	TrkB–STAT3–miR-204-5p circuitry regulates clonogenic growth, migration, and invasion of EC cells. Reduced miR-204-5p expression correlates with tumor stage and lymph node metastasis	Tumor samples ($n=110$); Ishikawa and HEC1B cell lines; mouse xenografts	Bao *et al*. (2013*b*)
	BDNF demonstrates a principal role coordinating ETV5-mediated EMT in EC. Impairment of BDNF/TrkB/ERK axis in EC cells reversed the aggressive and invasive phenotype promoted by upregulation of ETV5 at the invasive front	Tumor samples (EEC stage IB, $n=13$); HEC1A and HEC1A–ETV5 cell lines; *in vivo* mouse model of tumor dissemination	Alonso-Alconada *et al*. (2014)
	ETV5-related proteomic approach reinforces role of this transcription factor in regulation of the migratory and invasive tumor behavior	Hec1A cell line; FFPE sections from human ECs and tumors originated from Hec1A and Hec1A GFP–ERM/ETV5 cells orthtopically implanted in mice	Monge *et al*. (2009)
Notch signaling	SHARP1 regulates Notch/EMT pathway in EC. Positive correlation detected between SHARP1 and E-cadherin levels; negative correlation between SHARP1 and vimentin, SNAIL and JAG1	Tumor samples ($n=15$); Ishikawa and HEC1B cell lines	Liao *et al*. (2014*b*)
	Notch pathway has a tumor-suppressive role in human EC cells	Tumor (stage 1) and adjacent non tumor tissue ($n=22$)	Sasnauskiene *et al*. (2014)
	Notch1–JAG1 axis enhances the invasiveness and motility of EC cells	Tumor samples (stages I, III, and IV, $n=76$); Ishikawa, HHUA, Hec1A, Hec1B, and KLE cell lines	Mitsuhashi *et al*. (2012)
	FOXA1 transcription factor activates Notch pathway; a functional role for FOXA1 in mediating migration and invasion in EC cells has been suggested	Tissue samples from AH and EC ($n=87$); AN3CA, RL95-2, and HEC1B cell lines; mouse tumor xenograft model	Qiu *et al*. (2014)
	Switch in FOXA1 expression from primary to metastatic lesion associates with EC progression and correlates with CDKN2A expression in metastasis	Tissue samples from primary ($n=529$) and metastatic ($n=199$) lesions	Tangen *et al*. (2014)
PPAR/RXR pathway	PPAR/RXR pathway contributes to endometrial carcinogenesis by control of PTEN expression and modulating VEGF secretion	Tissue samples from benign and EC (grades 1–3) ($n=20$); Ishikawa and HEC1A cell lines	Nickkho-Amiry *et al*. (2012)
	PPARg agonist rosiglitazone inhibits proliferation and induces apoptosis in a mouse model of endometrial hyperplasia	HEC1A and Ishikawa cell lines; PTEN heterozygote murine model	Wu *et al*. (2008)
Eph receptor signaling	Role for EphA2 as a regulator in relation to EMT in EC has been suggested		Hwang (2014)
	EphA2 overexpression observed in EEC correlates with advanced disease, lack of hormone receptor expression and poor prognosis	Tissue samples (EEC, $n=139$ and benign, $n=10$)	Kamat *et al*. (2009)
	Over expression of EphA2 is associated with markers of angiogenesis and correlates with aggressive clinical features	Tumor samples ($n=85$); HEC1A, HEC1B, and Ishikawa cell lines; orthotopic mouse model	Merritt *et al*. (2010)

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Table 1 *Continued*

Factors	Mechanism	Clinical material/model[a]	References
SW1/SNF signaling	Loss of ARID1A expression observed exclusively in EEC and co-occurrs with alterations in the PI3K–Akt pathway	FFPE samples of primary ECs ($n=146$)	Bosse *et al*. (2013)
	Loss of ARID1A an early event in carcinogenesis of EEC and correlates with deep myometrial infiltration. No relation between gene signatures for EMT and ARID1A expression observed	Tissue samples from primary EC ($n=535$), metastatic lesions ($n=77$), and EH ($n=38$)	Werner *et al*. (2013)
	ARID1A loss more common in high-grade EEC and associates with mismatch protein deficiency and normal p53 expression	Tissue samples from high grade endometrial cancers ($n=190$)	Allo *et al*. (2014)
	ARID1A expression is associated with the differentiation status, ER and p53 but not clinical stage, depth of myometrial invasion, lymph node metastasis and overall patient survival	Tissue samples, EC ($n=74$; stages I–IV), CH ($n=20$), AH ($n=20$), and normal endometrium ($n=20$)	Zhang *et al*. (2014*a*,*b*)
	Role of SWI/SNF subunit alterations in the progression/dedifferentiation of EC suggested. SWI/SNF and MMR protein deficiencies may act synergistically in deregulating DNA repair mechanisms	22 undifferentiated EC out of which 17 were dedifferentiated	Stewart & Crook (2015*a*)
	SWI/SNF complex is involved in the pathogenesis of dedifferentiation of EEC in a subset of cases and correlates with aggressive rhabdoid phenotype	Poorly differentiated (grade 3) and undifferentiated tumor samples ($n=26$)	Strehl *et al*. (2015)
Epigenetic modifications and miRNA regulation			
DNA methylation/ demethylation; histone acetylation/deacetylation	DNA methylation changes regulate gene expression not only by affecting proximal promoters but also distant enhancers and transposable elements	Primary endometrial tumor samples (EAC1, EAC2, EAC3, UPSC1, UPSC2, and UPSC3)	Zhang *et al*. (2014*a*,*b*)
	Gene hypermethylation may be an early event in endometrial endometrioid tumorigenesis. While ER-A, PR, hMLH1, CDKN2A/P16, CDH1/E-CADHERIN, SFRP1, SFRP2, and SFRP5 show promoter methylation status in EEC, SFRP4 shows demethylation	Benign, premalignant and malignant endometrial lesions ($n=39$)	Di Domenico *et al*. (2011)
	Loss of PR-B and progesterone responsiveness leads to methylation of HOXA10 promoter, activation of SNAIL, inhibition of E-cadherin, increased invasion and tumor dissemination	SPEC2 and KLE cell lines; tumor samples (grades 1–3 EEC, $n=121$ and UPSC, $n=30$)	Yoshida *et al*. (2006)
	CDH13, *RASSF1A*, and *GSTP1* are the most frequently methylated genes in endometrial hyperplasia and carcinoma	Tissue samples (normal, SH, CH, CAH, and EC) in mutation positive/ negative, EC positive/negative, sporadic groups ($n=172$)	Nieminen *et al*. (2009)
	Epigenetic inactivation of EFEMP1 inhibits tumor growth and invasion. Ectopic EFEMP1 expression is associated with EMT, most likely by perturbing extracellular matrix	FFPE samples (normal, AH, and EC, $n=134$), fresh frozen EC tissues ($n=97$); HEC1B, RL95-2, ISK, SPEC2, AN3CA, and KLE cell lines	Yang *et al*. (2013)
	Downregulation of tumor suppressor EMX2 is a critical factor in the carcinogenesis and progression of EC	Tissue samples (EC $n=122$ and normal $n=25$); Ishikawa, KLE, AN3CA, and SPEC2 cell lines	Qiu *et al*. (2013)
	Loss of tumor suppressor ARID1A is associated with deep myometrial invasion and is an early event in the carcinogenesis of EEC	FFPE tissues of primary EC ($n=535$), metastatic lesions ($n=77$), hyperplasia ($n=38$); fresh frozen primary tumors ($n=122$)	Werner *et al*. (2013)
	EZH2 overexpression is associated with EC invasion and metastasis. Inhibition of EZH2 decreases proliferation, migration, and invasion either by upregulation of E-cadherin or inactivation of Wnt/B-catenin signalling	ECC1, RL95-2, HEC1A, and T-HESC cell lines; FFPE EC tissue ($n=40$)	Eskander *et al*. (2013)

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Factors	Mechanism	Clinical material/model[a]	References
	EZH2 expression is an early event in EC carcinogenesis. Overexpression of EZH2 correlates with deep myometrial invasion, LVSI and enhanced cell proliferation of EC cells	Tissue samples (normal, SH, CH, AH, and EC) ($n=92$). HEC1A and Ishikawa cell lines	Jia *et al*. (2014)
	EZH2 may regulate EC migration along with FAK through modulation of E-cadherin	FFPE tumor samples (type 1 $n=141$ and type 2 $n=61$)	Zhou *et al*. (2013)
MicroRNA regulation	The entire miR-200 family is up-regulated in EEC, implicated in the EMT process by negatively regulating ZEB1 and ZEB2	FFPE samples of CAH, EEC, and PE ($n=34$)	Snowdon *et al*. (2011)
	Alterations in endometrial miR-200c observed during transformation into cancerous states and target the expression of ZEBs, VEGFA, FLT1, IKKB, KLF9, and FBLN5	Endometrial samples from EC ($n=17$), normal ($n=35$), perimenopausal ($n=3$), postmenopausal ($n=2$), Depo-Provera ($n=5$); Ishikawa cell line	Panda *et al*. (2012)
	Up-regulated expression of all members of miR-200 family observed in all stages of EEC	Tissue samples (EAC, FIGO stages I–III, $n=30$; PE and SE, $n=20$)	Jurcevic *et al*. (2014)
	miR-206 overexpression inhibits ER-A dependent cell proliferation, impairs invasiveness and induces cell cycle arrest in EEC cell line	RL95-2, Ishikawa and KLE cell lines; tissue samples (EEC, $n=30$; SE and PE, $n=20$)	Chen *et al*. (2012)
	Elevated miR-222-3p expression promotes proliferation, invasion, G1 to S phase transition and increases raloxifene resistance by suppressing ER-A expression in EC cells	Tumor samples ($n=75$); RL95-2, AN3CA, and KLE cell lines; mouse xenograft model	Liu *et al*. (2014)
	Aberrant expression of miR-200b, miR-130a/b, miR-625, and miR-222 associated with tumorigenesis and metastasis. Ectopic expression of miR-130b and knockdown of DICER1 increased the expression of vimentin, ZEB2, N-cadherin, Twist, and Snail	Ishikawa and AN3Ca cell lines	Li *et al*. (2013)
	miR-205 promotes cellular proliferation, migration, and invasion of EEC through targeting estrogen-related receptor gamma	Tissue samples (normal $n=22$ and EEC $n=53$); Ishikawa, KLE, and AN3CA cells	Su *et al*. (2013)
	miR-194 expression was lower in EEC patients with more advanced stage. It regulates EMT by suppressing expression of BMI-1	HHUA, HOUA-I, and HEC50B cell lines	Dong *et al*. (2011)
	miR-31 overexpression promotes anchorage-independent growth *in vitro* and increases the tumor forming potential *in vivo*	Tumor samples (stages I–IV) ($n=34$); HEC50B, HEC1A, and HEC108 cell lines	Mitamura *et al*. (2014)
	miR-214 is differentially expressed in FIGO stage I and controls PTEN expression. miR-18a is differentially expressed in FIGO stage II and regulates KRAS. miR-148b and miR-335 regulate members of the Wnt pathway. miR-17 and miR-34a regulate BCL2 and CCND1 genes involved in PI3K/Akt signalling	Tissue samples (EAC, FIGO stages I–III, $n=30$; PE and SE, $n=20$)	Jurcevic *et al*. (2014)
	miR-199a-3p inhibits EEC cell proliferation through negative regulation of mTOR expression	EEC and paired adjacent nontumor tissue ($n=10$); Ishikawa cells	Wu *et al*. (2013)
	miR promotes cell apoptosis and senescence, suppresses EMT and CSC properties of aggressive EC cells	HEC50, HOUA-I, SPAC-1-L, SPAC-1-S, and EM cell lines. Tumor samples (grade 3 EEC, $n=50$)	Konno *et al*. (2014)
	hsa-miR-181a plays an oncogenic role in endometrial tumorigenesis and is a critical regulator of tumor metastasis in advanced EC	Primary epithelial and stromal cells from human endometrial tissues (EC and EH, $n=47$); ECC1, Hec1A, and T-HESC cells	He *et al*. (2015)
Tumor microenvironment	CAFs promote EC cell proliferation, in part by modulating PI3K/Akt and MAPK/ERK pathways	Tissue samples (EC $n=4$; EH $n=1$)	Subramaniam *et al*. (2013)
	Silencing of miR-148a in CAFs from EC patients results in WNT10B-mediated stimulation of tumor motility	Primary endometrial fibroblast cells; ACI-158, EC1, and ACI-98 cell lines	Aprelikova *et al*. (2013)

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Factors	Mechanism	Clinical material/model[a]	References
	CAFs stimulate progression of malignancy through the release of SDF1. Increased expression of SDF1 was associated with a more aggressive phenotype of the tumor	Tumor samples ($n=92$) (stages 1A, IB, II, IIIA, IIIC, and IV)	Walentowicz-Sadlecka *et al*. (2014)
MELF pattern	MELF invasion represents an active cellular event during EC invasion	Tumor samples EEC ($n=21$)	Stewart & Little (2009)
	Upregulation of cyclin D1 and p16, together with loss of membranous B-catenin expression observed in tumor foci composed of MELF glands	Tumor samples with MELF type of myometrial invasion ($n=22$)	Stewart *et al*. (2009)
	KRAS mutations are more frequent in MELF positive than MELF negative tumors	MELF positive ($n=33$) and MELF negative ($n=33$) low grade EECs	Stewart *et al*. (2010)
	MELF changes represent areas of active tumor invasion		Stewart *et al*. (2011)
	MELF pattern of myometrial invasion shows significant association with lymph node metastasis	Tumor samples ($n=351$)	Pavlakis *et al*. (2011)
	Cases with MELF-pattern show an increased rate of lymph node metastasis even within the subset of EEC with lymphvascular space invasion	T1 stage EEC with LVSI and associated lymph node dissection ($n=80$)	Hertel *et al*. (2014)

SH, simple hyperplasia; AH, atypical hyperplasia; EH, endometrial hyperplasia; CH, complex hyperplasia; CAH, complex atypical hyperplasia; CAFs, cancer-associated fibroblasts; EACs, endometrial adenocarcinomas; EEC, endometrioid endometrial carcinoma; FFPE, formalin-fixed paraffin embedded; LVSI, lymphvascular space invasion; PE, proliferative endometrium; SE, secretory endometrium.
[a]Tissue/tumor samples mentioned in the table were human samples unless otherwise specified.

initiation and progression through mechanisms such as cell growth and cell survival (Dong *et al*. 2014*b*).

Activation of PI3K may occur through growth factor receptors with tyrosine kinase activity (RTKs), G-protein coupled receptors, and oncogenes such as Ras, resulting in an accumulation of phosphatidylinositol-P3 at cell membranes, and subsequent activation of Akt, which in turn leads to upregulation of downstream targets including mTOR (Weigelt *et al*. 2013). Dysregulation of the PI3K/Akt pathway has been observed in all subtypes of EC and associated with more aggressive disease (Zhang *et al*. 2012). Furthermore, PI3K and mTOR inhibitors are in phase I/II trials in advanced EC based on molecular alterations reported in aggressive ECs (Salvesen *et al*. 2012, Shoji *et al*. 2012).

Epidermal growth factor receptor (EGFR), an RTK, acts upstream of PI3K/Akt pathway and is overexpressed in EC as compared to normal cycling endometrium (Lelle *et al*. 1993). An initiating role of EGFR in stimulating EMT via upregulation of SNAIL coupled with downregulation of E-cadherin was observed in EC cells. Inhibition of Akt in these cells resulted in SNAIL downregulation and thereby acquisition of invasive motility (Hipp *et al*. 2009). EGFR showed a negative correlation with epithelial markers and a positive correlation with mesenchymal markers in Ishikawa cells (Yang *et al*. 2014). A previous study demonstrated increase in expression of epithelial marker proteins and decrease in expression of mesenchymal proteins MMP9 and MMP2 on treatment of EC cells with EGF inhibitor AG1478 (Yan *et al*. 2012).

Insulin and insulin-like growth factors (IGFs) have been reported to play a significant role in the development of EC. Overexpression of insulin receptor (IR) or IGF1 receptor (IGF1R) induces endometrial hyperplasia and promotes EEC cell growth through activation of PI3K/Akt/mTOR signaling (McCampbell *et al*. 2006). IGF1R and IGF2 levels were much higher in advanced stage (stages III–IV) malignant tissue as compared to stages I–II or endometrial hyperplasia (Pavelic *et al*. 2007). Elevated levels of circulating insulin and endometrial IGF1 were found to increase the aggressiveness of EC (Gunter *et al*. 2008). Levels of phospho-IR, phospho-IRS1, and phospho-Akt were significantly higher in patients with high grade, advanced stage, deep myometrial invasion, and lymph node metastasis (Wang *et al*. 2012).

An anti-invasive and anti-metastatic role for metformin, an insulin sensitizer, was observed in EC cells. All these effects were associated with nuclear factor kappa-light-chain-enhancer of activated B cells (NFKB), MMP2/9, as well as Akt and ERK1/2 (Tan *et al*. 2011).

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Recently, metformin was reported to suppress EC cell growth via cell cycle arrest and concomitant autophagy (Takahashi *et al*. 2014).

Inactivation of *PTEN*, a major negative regulator of the PI3K pathway has been associated with the development of EC in mouse knockout and human observational studies (Mutter 2001, Kandoth *et al*. 2013). Loss of *PTEN* expression was suggested to be partly associated with the ECs through a premalignant phase and described as an early event in endometrial tumorigenesis (Sarmadi *et al*. 2009). Interestingly, high dose progestin therapy has been shown to reverse pre-existing *PTEN* inactivated endometrial latent precursors and endometrial hyperplasia in some women (Orbo *et al*. 2006).

Lindberg *et al*. (2013) generated a mouse model in which *PTEN* and *Cdh1* were conditionally ablated in the uterus. Deletion of both the genes induced EMT phenotype and accelerated features of neoplastic transformation in the uterus by inducing myometrial invasion, proliferation, massive angiogenesis, and loss of steroid hormone receptors as well as Akt activation. Cell adhesion molecules, *CTNNB*1 and *CLDN*, were also suppressed. The findings suggest that loss of *Cdh1* promotes aggressive EC phenotypes when cells are initiated by ablation of *PTEN*. These findings are corroborated by the results of Berg *et al*. (2015) who observed molecular changes in *PTEN* and PIK3CA in AEH and described transcriptional changes in the PI3K pathway as early events in the invasive step to grade 1 EEC.

In an oophorectomized mouse model, Joshi *et al*. (2012) observed that *PTEN* mutation, independent of estrogen, can initiate the development of complex atypical hyperplasia (CAH). Prolonged exposure to high levels of unopposed estrogen promoted progression of CAH to carcinoma in the setting of *PTEN* loss. In addition, absence of ER-A led to an increased incidence of *in situ* and invasive carcinoma suggesting that loss of ER-A may be a mechanism by which tumors become more aggressive. These results were corroborated by the findings of Kim *et al*. (2013) who observed association of EC progression with *PTEN* loss in a genetically engineered mouse model. Estrogen treatment induced more severe endometrial tumorigenesis in these animals. Later, Wik *et al*. (2013) demonstrated association of low ER-A with markers for EMT, stathmin (a marker associated with *PTEN* loss), and high PI3K activation status. Interestingly, PI3K and mTOR inhibitors are amongst the top ranked drug signatures negatively correlated with ER-A negative tumors.

McCampbell *et al*. (2010) observed mTOR activation early in progression of endometrial hyperplasias and in some histologically normal epithelial cells. Treatment with WAY-129327, an mTOR inhibitor, decreased hyperplasia incidence and proliferative indices significantly confirming the dependence of development and growth of these lesions on mTOR signaling. In a previous study, Milam *et al*. (2007) reported reduced progression of endometrial hyperplasia with oral mTOR inhibition in the *PTEN* heterozygote murine model.

Loss of tumor suppressor *LKB1* has been reported in 21% of primary endometrial tumors and is associated with activation of the mTOR pathway (Lu *et al*. 2008). Role of *LKB1* inactivation in enhancing cell motility and invasiveness, and triggering EMT through the induction of *ZEB1* has been suggested (Roy *et al*. 2010). Mice with homozygous endometrial *LKB1* inactivation underwent diffuse malignant transformation of the entire endometrium with rapid extra uterine spread and death suggesting that *LKB1* inactivation was sufficient to promote the development of invasive EC (Contreras *et al*. 2010). In a mouse model of EC, dual loss of *PTEN* and *LKB1* in the endometrial epithelium led to rapid development of advanced EEC with 100% penetrance and short host survival (Cheng *et al*. 2014). Co *et al*. (2014) analyzed *LKB1* gene expression in low and high grade EECs and found that *LKB1* is a direct transcriptional target of p53. Loss of WT p53 in high grade EEC may contribute to the *LKB1* loss observed in these aggressive tumors.

Ras/Raf/MEK/MAPK/ERK pathway The Ras/Raf/MEK/MAPK/ERK pathway represents a major signaling cascade that is activated by RTKs in response to growth factors. ERK activation has been suggested to be important for various key features of EMT including downregulation of adherens junctions and their associated proteins, increased MMP activity, induction of actin stress fibers, and acquisition of motile and invasive properties. Activation of MAPK/ERK pathway is also one of the Smad-independent events necessary for transforming growth factor beta (TGFB) mediated EMT (Edme *et al*. 2002).

Alterations in MAPK/ERK signaling during endometrial carcinogenesis have been reported at different levels of the pathway including mutations in *K-RAS*, *B-RAF*, or hypermethylation of *RASSF1A* TSG. These mutations were observed in all grades of EEC and have also been reported in AEH, suggesting a relatively early role for these mutations in endometrial carcinogenesis (Kim *et al*. 2010). *B-RAF* mutation, however, was shown to be important for malignant transformation, rather than the premalignant stages of EEC (Feng *et al*. 2005).

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In a recent study by Hsu *et al*. (2014), EGF was found to induce EMT in EC cells via Raf1/MAPK signaling pathway. EGF stimulated epithelial cell adhesion molecule (EpCAM) signaling determined the extent of invasiveness and disease progression. Cleavage of EpCAM and internalization of its intracellular domain EpICD into the nucleus activated mesenchymal cadherins associated with loss of E-cadherin and upregulation of SNAIL. Involvement of EMT in tumor aggressiveness was confirmed by the presence of permissive transcriptional epigenetic profile of mesenchymal cadherins in ECs with poor survival as compared to silenced epigenetic signature in less aggressive tumors. The results are in agreement with those of Hipp *et al*. (2009) who showed regulation of SNAIL by activation of EGFR via Akt and ERK1/2 pathways in Ishikawa cells. Activation of EGFR/MAPK pathway via overexpression of EGFR and its contribution to reduced PR-B expression and increased progestin resistance in EC has been reported (Ai *et al*. 2010).

The involvement of MAPK/ERK pathway in the progression and invasion of EEC mediated by EMT was explored by Montserrat *et al*. (2011). The myoinvasive front of EEC strongly expressed p-ERK, indicating a preferential activation of the MAPK pathway in this area of the tumor. The observation was further confirmed in Ishikawa cell line infected with lentiviruses carrying the V600E mutation of *BRAF*, wherein loss of B-catenin, E-cadherin, and cytokeratin and increase in expression of vimentin and SNAIL protein correlated with p-ERK1/2 at the tumor myoinvasive front. Further, the MEK1/2 inhibitor U0126 reversed the mesenchymal phenotype.

Upregulated expression of kinase suppressor of Ras 1 (KSR1) was demonstrated in ECs compared with normal endometrial tissue. Upregulation of KSR1 as responsible for carcinogenesis was compatible with its scaffolding function in regulation of Raf/MEK/ERK signaling. Further, KSR1 inhibition using shRNA not only blocked EC cell proliferation but also anchorage independent cell growth, a predictor for tumorigenicity, and metastatic potential (Llobet *et al*. 2011).

He *et al*. (2009) showed that activation of GPR30 signaling via the MAPK pathway was responsible for the aggressiveness of both ER-negative and ER-positive EC by modifying tumor proliferation and invasion through its action on MMP2 and MMP9 as well as increased interleukin 6 (IL6) secretion. Zhou *et al*. (2011) confirmed a key role of cross talk between MAPK signaling and ER status in the development and progression of EC.

Reports on coordinate mutation of *KRAS* and members of PI3K pathway are available. Conditional *PTEN* ablation and *K-ras* mutation in mouse uterus dramatically accelerated development of EC as compared to single mutation of either gene (Kim *et al*. 2010). *PTEN* mutations and loss, mutations in *PIK3CA*, as well as PI3K and KRAS signaling activation have been suggested as early events in the development from CAH to EEC, while hormone receptor loss and EMT occur during dedifferentiation (Berg *et al*. 2015).

Wnt/B-catenin pathway Wnt/B-catenin signaling is often activated in endometrial hyperplasia and cancer and has been associated with unopposed estrogen signaling and loss of PRs (Wang *et al*. 2009, Chandra *et al*. 2014). B-catenin, the hallmark protein of the canonical Wnt signaling pathway, plays a central role in the activation of transcription factors belonging to the TCF/lymphoid enhancing factor (LEF) family. In the absence of Wnt signaling, B-catenin is found in the cytoplasm either as a component that binds cadherins to A-catenin and the cytoskeleton or in a complex with scaffold protein axin, the tumor suppressor adenomatous polyposis coli (APC) and glycogen synthase kinase 3B (GSK3B). Activation of frizzled receptors by Wnt ligands inhibits GSK3B activity, which promotes nuclear localization of activated B-catenin, resulting thereby in EMT development through SNAIL accumulation and E-cadherin downregulation (Clevers 2006).

Nuclear B-catenin immunopositivity is a molecular feature of type 1 ECs. B-catenin activating mutations at its GSK3B binding consensus site were identified in 15–40% of endometrial tumors, whereas loss of heterozygosity at the APC locus was found in 24% of cases with nuclear B-catenin staining (Wang *et al*. 2010*a*). While overall B-catenin levels correlated negatively with EC grade, a positive correlation was seen between nuclear B-catenin accumulation in cells at the invasive front of the tumor and tumor stage, grade, and poor prognosis (Saegusa & Okayasu 2001, Saegusa *et al*. 2001). B-catenin expression associated with loss of E-cadherin expression was found to be involved in the acquisition of aggressive biological behavior, especially in high grade ECs (Shih *et al*. 2004). In ECC1 cells inhibition of Wnt signaling by Dickkopf-3 (Wnt antagonist) was accompanied by decreased proliferation, reduced anchorage independent growth and decreased invasiveness (Dellingera *et al*. 2012).

Augmented B-catenin and forkhead box A2 (FOXA2) transcription factor expression has been suggested as an essential feature during the formation of endometrial hyperplasia. Conditional ablation and activation of B-catenin in the mouse uterine epithelia resulted in

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aberrant epithelial structures and endometrial hyperplasia formation respectively (Villacorte *et al*. 2013). LEF1, a Wnt-pathway target gene, and its downstream targets cyclin D1 and MMP7 were found to have a role in endometrial gland formation and carcinogenesis (Shelton *et al*. 2012).

Van der Zee *et al*. (2013) demonstrated a synergistic effect of Wnt/B-catenin and *PTEN* pathways in EC. While loss of *PTEN* function was described as the *conditio sine qua non* for EC onset, constitutive activation of the Wnt/B-catenin pathway was suggested to promote EEC rather than initiating the disease. Recently, S100P (a member of family of S100 calcium binding proteins) was found to promote endometrial cell proliferation by increasing nuclear translocation of B-catenin (Guo *et al*. 2014).

TGFB signaling Members of the TGFB family have been identified as important inducers of EMT during development as well as carcinogenesis (Zavadil & Bottinger 2005). TGFB exerts its effect through heterotrimeric complex of transmembrane serine/threonine kinase, the type I (RI) and type II (RII) receptors. Following ligand binding, RI phosphorylates Smad2 and Smad3 (R-Smads). Phosphorylated R-Smads form a complex with Smad4 and translocate into the nucleus to regulate TGFB-responsive gene transcription.

Muinelo-Romay *et al*. (2011) suggested an important role of TGFB1 in the initial steps of EC invasion through the promotion of EMT, leading to acquisition of an invasive phenotype in EC cell lines. Treatment with SB-431542, a specific TGFB1 inhibitor, precluded persistent EC invasion. In a previous study, abrogation of TGFB receptor signaling was found to induce apoptosis and reduce invasive and metastatic potential of EC cells by reversal of autocrine TGFB induced EMT, supporting its role in promoting cell survival and tumor progression (Lei *et al*. 2009). Immunoreactivity of Nodal (a member of TGFB superfamily) and its co-receptor Cripto was found to increase dramatically in the transition from grade1 to grades 2 and 3 ECs, suggesting association of these molecules with tumor progression (Papageorgiou *et al*. 2009).

Recently, progesterone was shown to inhibit basal and TGFB1 induced cancer cell viability and invasion, which was accompanied by increased E-cadherin and decreased vimentin expression (Bokhari *et al*. 2014). Chaudhry *et al*. (2014) observed that TGFB upregulates the expression of prostate apoptosis response-4, a tumor suppressor protein, with simultaneous induction of EMT in endometrial and cervical cancer cells. Prolonged TGFB3 treatment disrupted epithelial cell morphology, promoted cell motility and induced upregulation of SNAIL, vimentin, *ZEB1* and N-cadherin, and downregulation of claudin-1 and E-cadherin. Monge *et al*. (2009) linked the TGFB1 pathway with increased invasive ability promoted by ETV5 transcription factor during the initial steps of EC dissemination.

Extracellular MMP inducer (Emmprin) was recently shown to play a key role in endometrial tumor progression and metastasis. Emmprin knockdown by siRNA resulted in significant decrease in the expression of TGFB, EGF, VEGF, MMP2, and MMP9 in EC cells. Transfection of the emmprin siRNA caused significant increase in the expression of E-cadherin and decreased expression of vimentin and SNAIL. Significant inhibitory effects on cell proliferation, migration, and invasion were observed in EC cells after transfection with the emmprin siRNA (Nakamura *et al*. 2012).

Hedgehog signaling Hedgehog (Hh) signaling is transduced by a transmembrane protein, Smoothened (Smo) the activity of which is suppressed by the membrane receptor (Ptch). Binding of Hh ligand with Ptch releases Smo which leads to the activation and nuclear translocation of Gli transcriptional factors, resulting in the transcription of genes like bone morphogenetic protein 2 (*BMP2*; King *et al*. 2008). Overexpression of Gli1 was related to relocalization of B-catenin from cytoplasm to nucleus and proposed as an early event in endometrial tumorigenesis. This effect was associated with the induction of SNAIL by Gli, which downregulates E-cadherin and thus displaces B-catenin from adherens junctions (Liao *et al*. 2009). Feng *et al*. (2007) observed significant step wise increase in the expression of Shh, Ptch, Smo, and Gli1 in endometrial hyperplasia and carcinoma and suggested that activation of this pathway is involved in malignant transformation of a subset of ECs. Further, treatment of Ishikawa and HHUA cells with cyclopamine, a specific inhibitor of Hh pathway suppressed growth of these cell lines by 56 and 67% respectively. Later, Kim *et al*. (2009) reported an overall increase in expression of Hh signaling molecules in hyperplastic endometrium as compared to normal endometrium. In carcinoma samples extensive alterations in the expression pattern of signaling molecules were observed. While, nuclear Gli2, cytoplasmic Gli3 and Su(Fu) were overexpressed, expression of Shh, Ptch, and Smo was significantly downregulated as compared to hyperplastic endometrium.

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NFKB signaling NFKB is a family of transcription factors comprising five structurally related subunits: p50, p52, c-Rel, RelB, and p65. In the canonical pathway, NFKB is induced by various inflammatory stimuli, such as TNFA, IL11, bacterial products, e.g., lipopolysaccharide and reactive oxygen species. In the cytoplasm, NFKB complex is rendered inactive by inhibition of KB (IKB). Phosphorylation of IKB activates NFKB pathway, resulting in release and translocation of NFKB to the nucleus, where it binds to KB-responsive elements in NFKB target genes (Basséres & Baldwin 2006). NFKB binds to *ZEB1/2* and TWIST1 promoter resulting in regulation of EMT phenotype (Chua *et al*. 2007, Li *et al*. 2012).

Multiple signaling pathways activate NFKB in EC cells. Loss of function mutation in *PTEN* and activating mutation in *PIK3CA* have been reported as putative activators of NFKB through Akt expression in EC and precursor lesions (Hayes *et al*. 2006). Saegusa *et al*. (2012) demonstrated upregulation of SOX9 transcription factor in EC. An association was observed between SOX9 and NFKB signaling as well as Akt status, which may modulate cell proliferation through alteration in the $p14^{ARF}$/p53/$p21^{WAF1}$ pathway. Consistent with these findings, upregulation of NFKB activity was observed in human EC cells expressing phospho-Akt and was responsible for the increase of *COX2* gene expression closely associated with parameters of tumor aggressiveness (St-Germain *et al*. 2004). In a multistep model of EEC carcinogenesis, the activation of COX2 and NFKB signaling was hypothesized to mediate the progression of hyperplasia to cancer (Faloppa *et al*. 2014). Blockade of NFKB activity by RTK inhibitor sunitinib reduced cell viability, proliferation, clonogenicity, and induced apoptotic cell death in EC cell lines (Sorolla *et al*. 2012).

Mizumoto *et al*. (2011) observed NFKB as a critical target of KRAS-induced endometrial carcinogenesis and suggested potential utility of NFKB inhibitors for EC chemoprevention especially with KRAS mutation. Recently, receptor activator of NFKB (RANK) and its ligand RANKL has been demonstrated to play a pivotal role in EC progression via the MAPK pathway. Higher RANK/RANKL expression levels were observed in ECs with myometrial invasion, lymph node metastasis, and lymphovascular space involvement, the key parameters linked to EMT program in EC (Wang *et al*. 2015).

JAK/STAT pathway Obesity is an established risk factor for EC, due in part to adipokines such as leptin and adiponectin (Acrp30). The JAK/STAT and Akt pathways have been implicated as critical mediators of leptin action. Constitutive activation of STAT3, a proto-oncogenic transcription factor, has frequently been detected in various human cancers including EC (Lay *et al*. 2012). In most epithelial cell types, STAT3 activity promotes cell cycle progression, cell survival induces MMPs (2, 7, and 9), and through the associated breakdown of extracellular matrix can facilitate EMT (Yu *et al*. 2009). In a recent study, treatment of EC cells with HO-3867 was shown to reduce the high levels of pSTAT3 Ser727 by inducing cell cycle arrest and apoptosis (Tierney *et al*. 2014).

Leptin induces functional activation of COX2, a critical factor of endometrial carcinogenesis in obesity, through JAK2/STAT3, MAPK/ERK, and PI3K/Akt pathways (Gao *et al*. 2009). A few studies have reported leptin induced growth and invasiveness of EC cell lines through activation of STAT3 and ERK1/2 signaling pathways. Acrp30 effectively reversed leptin stimulated cell proliferation (Liu *et al*. 2011, Wu *et al*. 2012). Pharmacological inhibitors of JAK/STAT (AG490) and PI3K (LY294002) have previously been reported to block leptin induced invasion of EC cells in matrigel invasion assay (Sharma *et al*. 2006).

Hypoxia signaling pathway Hypoxia and EMT have been identified as key events in tumor invasion and metastasis, and hypoxia inducible factor 1 (HIF1) stabilization directly or indirectly controls the expression of EMT regulators such as SNAIL, SIP, and *ZEB* (Evans *et al*. 2007). HIF1 is a heterodimeric complex composed of two subunits, the oxygen sensitive HIF1A, and the constitutively expressed HIF1B. Under hypoxic conditions, the two subunits associate to form a functional transcriptional complex, thereby activating the transcription of many target genes through direct binding to the hypoxia response element (Masson & Ratcliffe 2014). A critical role for the HIF1A/TWIST/E-cadherin system has been suggested in malignant progression and acquisition of metastatic phenotype in EEC. An intrinsic positive association was observed between HIF1A overexpression and high levels of TWIST in the endometrial carcinogenesis spectrum represented by the normal endometrium, atypical hyperplasia and EEC (Feng *et al*. 2013).

Among the HIF1 target genes, DEC2 expression was found to be differentially regulated and play an important role in endometrial carcinogenesis (Yunokawa *et al*. 2007). Liao *et al*. (2014*a*) presented a mechanistic link between SHARP1 (or DEC2) and HIF1A, and provided clinical and functional evidence suggesting exploitation of this pathway during EC progression, especially in the regulation of angiogenesis.

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Inactivation of *p53* and *PTEN* TSGs has been proposed as one of the mechanisms that activates HIF1 pathway in endometrial carcinogenesis (Horĺee *et al*. 2007). An association between low tissue ascorbate levels, increased HIF1 activity, and an aggressive tumor phenotype was found in EC (Kuiper *et al*. 2010). Higher HIF1A expression has recently been suggested to be associated with the increased risk of recurrence in EC (Sadlecki *et al*. 2014).

Tropomyosin related kinase B signaling Tropomyosin related kinases (Trks) are RTKs, which when stimulated by brain derived neurotrophic factor (BDNF) induce activation of various downstream pathways including Akt, Src, or MAPK resulting in cell proliferation, apoptosis resistance, and metastasis in human cancer models (Lee *et al*. 2012). Both TrkB and BDNF are highly expressed in human EC as compared to the normal endometrium. Overexpression of TrkB or stimulation by BDNF resulted in altered expression of the mediators of EMT. RNA interference mediated depletion of TWIST blocked TrkB induced EMT transformation and tumorigenesis *in vitro*. Additionally, TrkB depleted EC cells underwent mesenchymal to epithelial transition and anoikis *in vivo* (Bao *et al*. 2013*a*). The authors recently found that a TrkB–STAT3–miR-204-5p circuitry regulates proliferation and invasion of EC cells. Reduced expression of miR-204-5p showed association with lymph node metastasis (Bao *et al*. 2013*b*).

ETV5 transcription factor has been linked to the promotion of EMT and EC dissemination. Interestingly, BDNF demonstrated a principal role coordinating ETV5 mediated EMT in EC. Impairment of BDNF/TrkB/ERK axis in EC cells reversed the aggressive and invasive phenotype promoted by the upregulation of ETV5 at the invasive front (Alonso-Alconada *et al*. 2014). ETV5 related proteomic approach performed in Hec1A cell line reinforced a role of this transcription factor in regulation of the migratory and invasive tumor behavior (Monge *et al*. 2009). A cooperative role of transcription factor RUNX1/AML1 and ERM/ETV5 in association with MMP2 and MMP9 has been proposed during early steps of myometrial invasion (Planaguma *et al*. 2011).

Notch signaling The Notch pathway may act as an oncogene or as a tumor suppressor and can thus promote or inhibit tumor cell growth. In mammals, it consists of four transmembrane receptors (NOTCH 1–4) and five transmembrane ligands: three Delta proteins (DLL1, DLL3, and DLL4) and two Jagged proteins (JAG1 and JAG2). Ligand binding to its cognate receptor initiates proteolytic cleavage of the receptor by TACE metalloproteinase and γ-secretase, resulting in the release and translocation of the intracellular receptor domain into the nucleus where it induces transcriptional activation of target genes (Wang *et al*. 2008).

A link between hypoxia and activation of NOTCH has been demonstrated in solid tumors. Notch signaling is required to convert the hypoxic stimulus into EMT, increased motility and invasiveness (Chen *et al*. 2010). Pertinently, SHARP1 was found to play a critical role in malignant progression and acquisition of metastatic phenotypes in EC. A positive correlation was detected between SHARP1 and E-cadherin levels and negative correlation between SHARP1 and levels of JAG1, SNAIL, and vimentin (Liao *et al*. 2014*b*). While a few investigators have reported downregulated expression of Notch signaling molecules (Jonusiene *et al*. 2013, Sasnauskiene *et al*. 2014), others found a significantly higher expression of Notch related molecules in EC as compared to normal endometrium (Mitsuhashi *et al*. 2012).

The NOTCH1–JAG1 axis was suggested to enhance the invasive properties of EC (Wang *et al*. 2010*b*, Sasnauskiene *et al*. 2014). An increase in NOTCH1 expression was observed in tumors with invasive properties such as vessel or lymph node involvement and myometrial invasion. Consistent with this, inhibition of Notch signaling by DAPT treatment suppressed the invasiveness and motility of EC cells (Mitsuhashi *et al*. 2012).

The FOXA1 transcription factor activates Notch pathway by influencing androgen receptor expression and promotes EC cell proliferation. A functional role for FOXA1 in mediating migration and invasion in EC cells was suggested (Qiu *et al*. 2014). A recent study demonstrated association of EC progression with a switch in FOXOA1 expression from primary to metastatic lesions (Tangen *et al*. 2014). Wang *et al*. (2014) observed that FOXOA1 suppresses the progression of EC via crosstalk with ER-A.

PPAR/RXR pathway PPARs are ligand activated transcription factors that belong to the nuclear hormone receptor family. The PPAR/RXR pathway was shown to contribute to endometrial carcinogenesis by control of *PTEN* expression and modulating VEGF secretion. Reducing PPARG expression in a *PTEN*-null endometrial cell line resulted in decreased p-Akt expression (Nickkho-Amiry *et al*. 2012). The PPARG agonist rosiglitazone was found to inhibit proliferation and induce apoptosis in a mouse model of endometrial hyperplasia, suggesting a potential for chemoprevention (Wu *et al*. 2008).

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Furthermore, overexpression of VEGF has been associated with poor prognostic factors in EC including deep myometrial invasion and lymph node metastasis (Hirai *et al.* 2001). Pertinently, Bevacizumab, a monoclonal antibody targeting VEGF-A has been studied in a phase II trial of recurrent EC (Morotti *et al.* 2012).

Ephrin receptor signaling The ephrin (Eph) receptors are the largest family of tyrosine kinases and are classified as EphA and EphB based on interaction with their ligands. Available reports have shown that high levels of EphA2 promote various aspects of malignant phenotype, including cell growth, migration, invasion, angiogenesis, and survival of cancer cells. A recent study in gastric cancer cells has shown that EphA2 promotes EMT through activation of Wnt/B-catenin signaling (Huang *et al.* 2014). Based on reports demonstrating association between EphA2 and EMT in various cancers, a role for EphA2 as a regulator in relation to EMT in EC has been suggested (Hwang 2014).

EphA2 overexpression was observed in EEC and correlated with advanced disease, lack of hormone receptor expression and poor prognosis (Kamat *et al.* 2009). Using an orthotopic uterine cancer mouse model, Merritt *et al.* (2010) found overexpression of EphA2 in over half of ECs and was associated with markers of angiogenesis, aggressive clinical features and was predictive of poor clinical outcome. EphA2 targeted chemotherapy using an antibody drug conjugate resulted in significant growth inhibition of EC cells both *in vitro* and *in vivo* (Lee *et al.* 2010).

Switch/sucrose non-fermenting chromatin remodeling complex The role of Switch/sucrose non-fermenting (SWI/SNF) complex, also known as the Brg1, associated factors complex in the initiation and progression of cancer is emerging. AT-rich interactive domain 1A gene (ARID1A), a subunit of the SWI/SNF complex, has been reported as a novel tumor suppressor of gynecologic cancer and one of the driver genes in endometrial carcinogenesis. While loss of ARID1A has been found exclusively in EEC, no loss of expression of other subunits viz. SMARCD3 or SMARCB1 was detected. Loss of ARID1A usually occurs simultaneously with alterations in the PI3K/Akt pathway and has been associated with sporadic MSI (Bosse *et al.* 2013). Liang *et al.* (2012) previously reported frequent co-occurrence of mutations in ARID1A gene with mutations in the PIK3CA gene and with PI3K/Akt pathway activation in EC.

Similar ARID1A loss was observed in endometrial hyperplasia and in primary endometrioid tumors suggesting that loss of ARID1A may be an early event in the carcinogenesis of EEC. A correlation with deep myometrial infiltration supported importance of ARID1A loss for development of early invasiveness. No relation, however, was identified between two gene signatures for EMT and ARID1A mRNA and protein expression levels (Werner *et al.* 2013). Zhang *et al.* (2014*a*) observed significant association of BAF250 (or ARID1A) expression with differentiation status of EC. However, no association with clinical stage, the depth of myometrial invasion, lymph node metastasis, and overall survival of patients with EC was seen. Further, the expression of BAF250 was positively correlated with ER and negatively correlated with p53 in poorly differentiated EC. In another study, loss of BAF250 was found to be more common in high grade EECs and was associated with mismatch protein deficiency and normal p53 expression (Allo *et al.* 2014).

Recently, Stewart & Crook (2015*b*) suggested role of SWI/SNF subunit alterations in the progression/dedifferentiation of EC and that SWI/SNF and MMR protein deficiencies may act synergistically in deregulating DNA repair mechanisms in undifferentiated EC. Strehl *et al.* (2015), reported involvement of SWI/SNF complex in the pathogenesis of dedifferentiation of EEC in a subset of cases and highlighted correlation of SWI/SNF alterations with the aggressive rhabdoid phenotype.

Role of epigenetic modifications and miRNA regulation

Epigenetic modifications The methylation/demethylation of DNA and acetylation/deacetylation of histones are the most common and important epigenetic modifications associated with the development, progression, and metastasis of EC (Ma & Gao 2014).

DNA methylation changes have been found to be an important signature of EC and regulate gene expression by affecting not only proximal promoters but also distal enhancers and transposable elements (Zhang *et al.* 2014*b*). APC hypermethylation in EC has been associated with endometrioid phenotype and microsatellite instability (Moreno-Bueno *et al.* 2002). Nieminen *et al.* (2009) emphasized early and widespread tumor suppressor promoter methylation changes in endometrial tumorigenesis. Among the 24 TSGs studied, *CDH13*, *RASSF1A*, and *GSTP1* were the most frequently methylated genes in endometrial hyperplasia and carcinoma. A recent study corroborated the findings of Nieminen *et al.*, aberrant CpG methylation of the promoter region of *GSTP1* and

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RASSF1A was found to be an important event in endometrial carcinogenesis and suggested to have an impact on tumor aggressiveness (Fiolka *et al*. 2013).

A clear tendency of increasing methylation of steroid receptors (ER-A and PR), DNA mismatch repair (hMLH1), tumor suppressors (CDKN2A/p16 and CDH1/E-cadherin) and Wnt pathway inhibitors (SFRP1, SFRP2, and SFRP5) was observed from benign to malignant endometrial lesions, highlighting the possible role of aberrant methylation in the initiation and progression of EC (Domenico *et al*. 2011). Loss of PR-B and progesterone responsiveness in EC cells was previously found to inactivate HOXA10 gene expression by promoter methylation and promote EMT by activation of SNAIL and inhibition of E-cadherin expression, followed by increased tumor invasiveness and dissemination (Yoshida *et al*. 2006).

Promoter hypermethylation was reported to be a major mechanism for inactivation of *EFEMPI*, a candidate TSG in EC. EFEMP1 inhibited tumor cell proliferation, metastasis, and invasion *in vitro* and suppressed tumorigenesis in nude mice. Ectopic EFEMP1 expression in EC was associated with EMT, most likely through disturbing ECM such as E-cadherin, vimentin, MMP2, and MMP9 (Yang *et al*. 2013). Downregulation of tumor suppressor EMX2 (the human homologue of *Drosophila* empty spiracles gene 2) has been suggested to be a critical factor in the carcinogenesis and progression of EC. Reduced EMX2 expression was correlated with tumor stage, grade, and the depth of myometrial invasion (Qiu *et al*. 2013).

Enhancer of zeste homolog 2 (EZH2), a master regulatory gene, has a critical role in cancer development through its ability to epigenetically silence TSGs owing to its intrinsic histone methyl transferase activity. shRNA mediated EZH2 inhibition in EC cells was found to decrease proliferation, migration, and invasion via either upregulation of E-cadherin or inactivation of Wnt/B-catenin signaling (Eskander *et al*. 2013). EZH2 expression was observed in the precursor lesions of EC, suggesting high EZH2 expression as an early event in EC carcinogenesis. Further, decreased tumor cell proliferation, migration and invasion observed in EC cell lines as a result of EZH2 inhibition was parallel to an increased expression of Wnt pathway inhibitors sFRP1 and DKK3, and concomitant decrease in B-catenin levels (Jia *et al*. 2014). Zhou *et al*. (2013) have suggested that EZH2 may regulate EC migration along with FAK through E-cadherin modulation.

Inhibiting DNMT activity has been suggested as a valuable target for epigenetic therapy of EC. A combination of histone deacetylase inhibitors and DNMT inhibitors was found to suppress growth of EC, which was likely mediated by upregulation of E-cadherin and downregulation of Bcl2 (Yi *et al*. 2012).

miRNA regulation Alterations in miRNA expression levels have been implicated in oncogenesis of nearly all cancers including the EC subtypes. They serve as important regulators of EMT and metastasis by regulating EMT-related genes. Snowdon *et al*. (2011) identified 43 miRNAs that were dysregulated in CAH and EEC as compared to normal controls. The entire miR-200 family including miR-200a/b/c, miR-141, and miR-429 was upregulated in EEC. The miR-200 family has been implicated in the EMT process and negatively regulates *ZEB*1 and *ZEB2* transcription factors, which in turn negatively regulate E-cadherin. Alterations in endometrial miR-200c were demonstrated during transformation into cancerous states and were shown to target the expression of *ZEB*s, VEGFA, FLT1, IKKB, KLF9, and FBLN5 (Panda *et al*. 2012). Jurcevic *et al*. (2014) observed upregulated expression of all members of miR-200 family in all stages of EEC.

Role for miR-206 has been demonstrated in ER-A positive EEC cell line. miR-206 overexpression inhibited ER-A dependent cell proliferation, impaired invasiveness and induced cell cycle arrest (Chen *et al*. 2012). Liu *et al*. (2014) demonstrated miR-222-3p overexpression in ER-A negative EC tumors which was associated with high grade, late stage and nodal metastasis.

Aberrant expression of miR-200b, miR-130a/b, miR-625, and miR-222 was found to be associated with tumorigenesis and metastasis in EC. Silencing of miR-130b induced E-cadherin expression, while ectopic expression of miR-130b and knockdown of DICER1 increased the expression of vimentin, *ZEB2*, N-cadherin, TWIST, and SNAIL in EC cells (Li *et al*. 2013). Frequent upregulation of miR-205 has also been demonstrated in EEC. Inhibition of miR-205 reduced cellular proliferation, migration, and invasion (Su *et al*. 2013). miR-194 was found to regulate EMT by suppressing BMI-1 in EC. The miR-194 expression was significantly low in EEC patients with more advanced stage (Zhai *et al*. 2013). The expression levels of miR-31 were found to increase significantly in patients with a high risk of recurrence. miR-31 overexpression promoted anchorage independent growth *in vitro* and increased the tumor forming potential *in vivo* (Mitamura *et al*. 2014).

Jurcevic *et al*. (2014) identified 138 miRNAs that expressed differentially between normal and malignant endometrium. miR-214 was differentially expressed in FIGO stage I and controls *PTEN* expression. miR-18a was correlated with FIGO stage II and regulates KRAS.

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miR-148b and miR-335 regulate members of the Wnt pathway. miR-17 and miR-34a regulate BCL2 and CCND1, which are involved in PI3K/Akt signaling. miR-199a-3p inhibits EEC cell proliferation through negative regulation of mTOR expression (Wu *et al*. 2013).

miR-101 was found to suppress the EMT and CSC properties of aggressive EC cells at least in part by attenuating EZH2 expression, followed by elevation of BAX, p21, epithelial markers and TIMP3, downregulation of mesenchymal markers and suppression of Wnt/B-catenin pathway (Konno *et al*. 2014). A recent study suggested oncogenic role for hsa-miR-181a in endometrial tumorigenesis and its critical role in tumor metastasis of advanced EC (He *et al*. 2015).

Role of tumor microenvironment

The tumor microenvironment refers to the complex milieu of supporting cells, i.e., stromal cells, which co-exist with the primary tumor. The stroma includes cancer-associated fibroblasts (CAFs), inflammatory cells and endothelial cells, which facilitate EMT induction and drive metastatic progression through interaction with cancer cells (Vong & Kalluri 2012). Arnold *et al*. (2002) observed that secretions from normal endometrial fibroblast cells can inhibit proliferation of Ishikawa cells. A recent study corroborated these findings and the anti-proliferative effect was attributed to inhibition of PI3K signaling (Shi *et al*. 2011). Deletion of APC activity in murine stroma cells resulted in their trans-differentiation to a more myofibroblastic phenotype accompanied by reduced ER-A expression and was sufficient to induce endometrial hyperplasia and cancer (Tanwar *et al*. 2011). Aprelikova *et al*. (2013) demonstrated that silencing of miR-148a in CAFs from EC patients results in WNT10B-mediated stimulation of tumor motility.

A pro-tumorigenic role of fibroblasts in EC progression has been suggested. EC cells demonstrated increased cell motility and invasiveness in response to CAF secretion. CAFs promoted EC cell proliferation, in part by modulating PI3K/Akt and MAPK/ERK pathways. In fact, targeting CAFs was suggested to be the mode of action by which rapamycin and its analogues control EC progression in clinical setting. (Subramaniam *et al*. 2013). Chung *et al*. (2014) recently demonstrated suppression of CAF mediated EC proliferation by specific inhibitors for PI3K/Akt (LY294002) and MAPK/ERK (U0126).

An important role for stromal derived factor 1 (SDF1) and its receptors in regulating the process of metastasis formation has been suggested. CAFs were found to stimulate the progression of malignancy through the release of SDF1 and an increased expression of this molecule was associated with a more aggressive phenotype of the tumor. Recent studies have confirmed a major role of SDF1 in EC invasion and metastasis. SDF1 induced invasion was found to be inhibited by treatment with Kisspeptin-10 (Schmidt *et al*. 2014, Walentowicz-Sadlecka *et al*. 2014).

MELF type invasion

Some EEC display a distinctive pattern of invasion characterized by the presence of MELF glands. The MELF pattern includes loss of conventional glandular architecture, attenuation of the neoplastic epithelium and infiltration of stroma by small nests of cells and individual tumor cells which are often associated with a prominent fibromyxoid stromal alteration and represent a specific tumor stromal reaction similar to epithelial mesenchymal interactions observed in other tumors (Zaino 2014). Stewart *et al*. (2009) observed upregulation of cyclin D1 and p16, together with loss of membranous B-catenin expression in tumor foci composed of MELF glands. The authors found that the MELF type invasion was characterized by strong CK7 expression. MELF areas were usually negative for hormone receptors and showed reduced E-cadherin, a pattern consistent with EMT and supporting the hypothesis that MELF invasion represents an active cellular event during EC invasion (Stewart & Little 2009). Tumors with MELF pattern of myometrial invasion showed more frequent vascular invasion and focal mucinous differentiation. *KRAS* mutations were more frequent in MELF positive than MELF negative tumors (Stewart *et al*. 2010). The neoplastic epithelium in MELF-type invasion usually showed strong fascin immunoreactivity. The localized increase in fascin expression suggests that the MELF changes represent areas of active tumor invasion (Stewart *et al*. 2011).

Pavlakis *et al*. (2011) demonstrated significant association between MELF pattern of myometrial invasion and lymph node metastasis which could be considered as an additional factor for advanced stage disease. Hertel *et al*. (2014) observed that cases with MELF pattern carry an increased rate of lymph node metastasis even within the subset of endometrioid tumors with lymphvascular space invasion. The findings have implications in routine clinical practice as it signals the importance of recognizing MELF pattern myoinvasion.

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Conclusion

To conclude, results of the studies summarized in the present review support a critical role of EMT related processes in progression and metastasis of EEC. EMT inhibition is thought to be a promising approach to treat invasive cancer; however, current treatment modalities for EEC, exploiting the use of EMT process as a pharmacological target remain underexplored owing in part to potential drug resistance in this population of cells and also to the lack of sufficient *in vivo* data. Continued research in this field will help in identifying appropriate targets in the core EMT program that may offer new therapeutic opportunities for controlling EC progression, metastasis and possibly preventing cancer recurrence in the clinical setting.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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