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

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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.

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.
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 KRA52, FGFR2, and p53 genes (Ignar-Trowsebridge 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...
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 membranocyttoplasmic (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...
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. 2014a).

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 2015a).

**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
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(S) 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 (Pieczynska 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 (Bleichschmidt 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 (Pieczynska 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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<td>tumorsogenesis. Gli induces SNAIL which suppresses E-cadherin and</td>
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<td>phospho-Akt is responsible for the increase of COX2 gene expression</td>
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<td>Receptor activator of NFKB (RANK) and its ligand promote EC cell</td>
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<td>assay which is effectively blocked by pharmacological inhibitors of</td>
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<td>Hypoxia signalling</td>
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<td>Tissue samples from AH and ECC ((n = 152))</td>
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<td>phenotype in EEC</td>
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<td>HIF1 pathway and its target genes, particularly</td>
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<td>DEC2 (SHARP1) play an important role in endometrial carcinogenesis and</td>
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<td>tumor phenotype development</td>
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\(^*\) AH, EAH, and EEC; normal, EAH, EC (\(n = 120\)); Ishikawa, KLE, AN3CA, RL 95-2, and HEC18 cell lines.
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<td>TrkB signaling</td>
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<td>ETV5-related proteomic approach reinforces role of this transcription factor in regulation of the migratory and invasive tumor behavior</td>
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SWI/SNF signaling

Loss of ARID1A expression observed exclusively in EEC and co-occurs with alterations in the PI3K-Akt pathway

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

ARID1A loss more common in high-grade EEC and associates with mismatch protein deficiency and normal p53 expression

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

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

SWI/SNF complex is involved in the pathogenesis of dedifferentiation of EEC in a subset of cases and correlates with aggressive rhabdoid phenotype

Epigenetic modifications and miRNA regulation

DNA methylation changes regulate gene expression not only by affecting proximal promoters but also distant enhancers and transposable elements

Gene hypomethylation 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

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

CDH13, RASSF1A, and GSTP1 are the most frequently methylated genes in endometrial hyperplasia and carcinoma

Epigenetic inactivation of EFEMP1 inhibits tumor growth and invasion. Ectopic EFEMP1 expression is associated with EMT, most likely by perturbing extracellular matrix

Downregulation of tumor suppressor EMX2 is a critical factor in the carcinogenesis and progression of EC

Loss of tumor suppressor ARID1A is associated with deep myometrial invasion and is an early event in the carcinogenesis of EEC

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

Table 1 Continued

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<td>Gene hypomethylation 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</td>
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<td>CDH13, RASSF1A, and GSTP1 are the most frequently methylated genes in endometrial hyperplasia and carcinoma</td>
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<td>Epigenetic inactivation of EFEMP1 inhibits tumor growth and invasion. Ectopic EFEMP1 expression is associated with EMT, most likely by perturbing extracellular matrix</td>
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<td>Qiu et al. (2013)</td>
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<td>Loss of tumor suppressor ARID1A is associated with deep myometrial invasion and is an early event in the carcinogenesis of EEC</td>
<td>FFPE tissues of primary EC (n = 535), metastatic lesions (n = 77), hyperplasia (n = 38), fresh frozen primary tumors (n = 122)</td>
<td>Werner et al. (2013)</td>
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<td>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</td>
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MicroRNA regulation

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<td>EZH2 expression</td>
<td>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. EZH2 may regulate EC migration along with FAK through modulation of E-cadherin. The entire miR-200 family is up-regulated in EEC, implicated in the EMT process by negatively regulating ZEB1 and ZEB2. Alterations in endometrial miR-200c observed during transformation into cancerous states and target the expression of ZEBs, VEGFA, FLT1, IKKB, KLF9, and FBLN5. Up-regulated expression of all members of the miR-200 family observed in all stages of EEC. miR-206 overexpression inhibits ER-A dependent cell proliferation, impairs invasiveness and induces cell cycle arrest in EEC cell line. 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. 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. miR-205 promotes cellular proliferation, migration, and invasion of EEC through targeting estrogen-related receptor gamma. miR-194 expression was lower in EEC patients with more advanced stage. It regulates EMT by suppressing expression of BMI-1. miR-31 overexpression promotes anchorage-independent growth in vitro and increases the tumor forming potential in vivo. 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 miR-199a-3p inhibits EEC cell proliferation through negative regulation of mTOR expression. miR promotes cell apoptosis and senescence, suppresses EMT and CSC properties of aggressive EC cells. hsa-miR-181a plays an oncogenic role in endometrial tumorigenesis and is a critical regulator of tumor metastasis in advanced EC. Tissue samples (normal, SH, CH, AH, and EO) (n = 92). HEC1A and Ishikawa cell lines. FFPE tumor samples (type 1 n = 141 and type 2 n = 61). FFPE samples of CAH, EEC, and PE (n = 34). Endometrial samples from EC (n = 17), normal (n = 35), perimenopausal (n = 3), postmenopausal (n = 2), Depo-Provera (n = 5), Ishikawa cell line. Tissue samples (EAC, FIGO stages I–III, n = 30; PE and SE, n = 20). RL95-2, Ishikawa and KLE cell lines; tissue samples (EEC, n = 30; SE and PE, n = 20). Tumor samples (n = 75); RL95-2, AN3CA, and KLE cell lines; mouse xenograft model. Tissue samples (normal n = 22 and EEC n = 53); Ishikawa, KLE, and AN3CA cells. HHUA, HOUA-I, and HEC50B cell lines. Tumor samples (stages I–IV) (n = 34); HEC50B, HEC1A, and HEC108 cell lines. Tissue samples (EAC, FIGO stages I–III, n = 30; PE and SE, n = 20).</td>
<td>Jia et al. (2014)</td>
<td>Zhou et al. (2013)</td>
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<td>Tumor micro-environment</td>
<td>CAFs promote EEC cell proliferation, in part by modulating PI3K/Akt and MAPK/ERK pathways. Silencing of miR-148a in CAFs from EC patients results in WNT10B-mediated stimulation of tumor motility.</td>
<td>EEC and paired adjacent nontumor tissue (n = 10); Ishikawa cells. HEC50, HOUA-I, SPAC-1-L, SPAC-1-S, and EM cell lines. Tumor samples (grade 3 EEC, n = 50). Primary epithelial and stromal cells from human endometrial tissues (EC and EH, n = 47); ECC1, Hec1A, and T-HESC cells. Tissue samples (EC n = 4; EH n = 1). Primary endometrial fibroblast cells; ACI-15B, EC1, and ACI-98 cell lines.</td>
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initiation and progression through mechanisms such as cell growth and cell survival (Dong et al. 2014b).

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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**Table 1 Continued**

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<td>MELF pattern</td>
<td>CAFs stimulate progression of malignancy through the release of SDF1. Increased expression of SDF1 was associated with a more aggressive phenotype of the tumor. MELF invasion represents an active cellular event during EC invasion. Upregulation of cyclin D1 and p16, together with loss of membranous B-catenin expression observed in tumor foci composed of MELF glands. KRAS mutations are more frequent in MELF positive than MELF negative tumors. MELF changes represent areas of active tumor invasion. MELF pattern of myometrial invasion shows significant association with lymph node metastasis. Cases with MELF-pattern show an increased rate of lymph node metastasis even within the subset of EEC with lymphvascular space invasion.</td>
<td>Tumor samples (n=92) (stages 1A, IB, II, IIIA, IIIC, and IV)</td>
<td>Walentowicz-Sadlecka et al. (2014)</td>
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<td><strong>Tumor samples EEC (n=21)</strong></td>
<td><strong>Tumor samples with MELF type of myometrial invasion (n=22)</strong></td>
<td>Stewart &amp; Little (2009) Stewart et al. (2009)</td>
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<td>MELF positive (n=33) and MELF negative (n=33) low grade EECs</td>
<td>Tumor samples (n=351)</td>
<td>Stewart et al. (2010) Stewart et al. (2011)</td>
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<td>T1 stage EEC with LVI and associated lymph node dissection (n=80)</td>
<td>Tumor samples</td>
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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, CTNNB1 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.

McCaspel 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 (TGFβ) 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).
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 anchored 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. 2010a). 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
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 grade 1 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 (Ptc). Binding of Hh ligand with Ptc 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 relocation 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 (Li et al. 2009). Feng et al. (2007) observed significant step wise increase in the expression of Shh, Ptc, 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, Ptc, and Smo was significantly downregulated as compared to hyperplastic endometrium.
**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 TNFα, IL11, bacterial products, e.g., lipopolysaccharide and reactive oxygen species. In the cytoplasm, NFKB complex is rendered inactive by inhibition of IK (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 (Bassères & 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 p14ARF/p53/p21 WAF1 pathway. Consistent with these findings, upregulation of NFKB activity was observed in human EC cells expressing phosho-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 HIF1α, and the constitutively expressed HIF1β. 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. (2014a) 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.
Inactivation of p53 and PTEN TSGs has been proposed as one of the mechanisms that activates HIF1 pathway in endometrial carcinogenesis (Horlée 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. 2013a). 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. 2013b).

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. 2014b). 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. 2010b, 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 FOXA1 expression from primary to metastatic lesions (Tangen et al. 2014). Wang et al. (2014) observed that FOXA1 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 PPAR expression in a PTEN-null endometrial cell line resulted in decreased p-Akt expression (Nickhomi-Amiry et al. 2012). The PPAR 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).
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 carcinoma. 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. (2014a) 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 (2015b) 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. 2014b). 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...
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. 2013). 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 EFEMP1, 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 ZEB1 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 ZEBs, 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 ECC 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 tumorogenesis 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.
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.
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 EEC 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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