

## The EMT signaling pathways in endometrial carcinoma

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**Abstract** Endometrial cancer (EC) is the most common gynecologic malignancy of the female genital tract and the fourth most common neoplasia in women. In EC, myometrial invasion is considered one of the most important prognostic factors. For this process to occur, epithelial tumor cells need to undergo an epithelial to mesenchymal transition (EMT), either transiently or stably, and to differing degrees. This process has been extensively described in other types of cancer but has been poorly studied in EC. In this review, several features of EMT and the main molecular pathways responsible for triggering this process are investigated in relation to EC. The most common hallmarks of EMT have been found in EC, either at the

level of E-cadherin loss or at the induction of its repressors, as well as other molecular alterations consistent with the mesenchymal phenotype-like L1CAM and BMI-1 up-regulation. Pathways including progesterone receptor, TGF $\beta$ , ETV5 and microRNAs are deeply related to the EMT process in EC.

### Introduction

Endometrial cancer (EC) is made up of a biologically and histologically diverse group of neoplasms characterized by their distinct pathogeneses. These differential characteristics

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36 have been classified into a dualistic model, first proposed  
 37 by Bokhman [1]. Nowadays, this model is being chal-  
 38 lenged, since tumors seen in daily practice occasionally  
 39 show overlapping or combined morphologic and molecular  
 40 characteristics between both types, in addition to ambigu-  
 41 ous features [2]. Nevertheless, we still follow this classi-  
 42 fication as it is currently used in the clinical practice. Type  
 43 I, or estrogen-dependent endometrioid ECs, represent the  
 44 most common subtype. This type usually develops in peri-  
 45 and early postmenopausal women. It is an estrogen-asso-  
 46 ciated lesion often seen in conjunction with endometrial  
 47 hyperplasia. The histological subtypes that correspond to  
 48 endometrioid adenocarcinoma and its variants, as well as  
 49 mucinous adenocarcinoma, are allocated to this group.  
 50 Type II, or non-endometrioid ECs, tend to affect older,  
 51 postmenopausal women and are a non-estrogen-associated  
 52 lesion. These cancers are not preceded by endometrial  
 53 hyperplasia, though they can occasionally arise in endo-  
 54 metrial polyps or from precancerous lesions, endometrial  
 55 intraepithelial carcinoma, or in the vicinity of atrophic  
 56 endometrium [3]. The most virulent histologic subtypes,  
 57 such as papillary serous, clear cell carcinomas, and carci-  
 58 nosarcomas are to be found in this group [4]. The clini-  
 59 copathological differences between the two types are  
 60 paralleled by specific genetic alterations, with type I  
 61 showing microsatellite instability and mutations in PTEN,  
 62 PIK3CA, K-RAS, and CTNNB1 (beta-catenin) and type II  
 63 exhibiting TP53 mutations and chromosomal instability  
 64 [5].

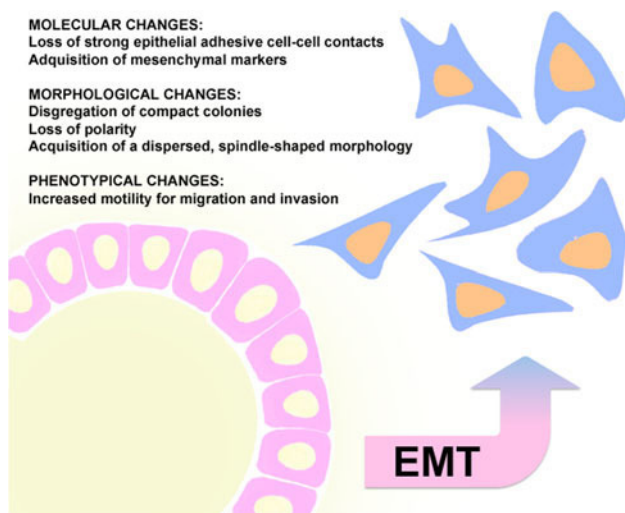
65 Epidemiologically, EC is ranked as the most common  
 66 gynecologic malignancy of the female genital tract and the  
 67 fourth most common neoplasia in women after breast,  
 68 colorectal and lung cancer. Recent data from the US esti-  
 69 mates that 47,130 new cases of EC will be diagnosed in  
 70 2012, but only 8,010 deaths are expected [6]. According to  
 71 these statistics, EC is considered to be a good prognosis  
 72 cancer, since it is usually detected in its initial stages by the  
 73 presentation of disease-related symptoms. In its early  
 74 stages, EC is confined to the endometrium and can be  
 75 treated by hysterectomy with or without adjuvant treatment  
 76 resulting in survival rates around 96 % at 5 years. How-  
 77 ever, 30 % of all EC cases are still diagnosed at regional or  
 78 distant stages, and are related to lower survival rates, 67  
 79 and 16 %, respectively. When comparing the epidemiol-  
 80 ogic data of both EC types, type I lesions are generally  
 81 diagnosed at an earlier stage and possess a more favorable  
 82 prognosis than those of type II. Generally, type II are more  
 83 aggressive tumors that are commonly diagnosed in  
 84 advanced stages and often recur, despite aggressive  
 85 treatment [7].

86 In order to tackle the causes of mortality associated with  
 87 EC, it is necessary to understand how this cancer dissemi-  
 88 nates. EC spreads by direct extension through the

89 myometrium, in an exfoliation of cells that are shed  
 90 through the fallopian tubes, lymphatic dissemination, and/  
 91 or hematogenous dissemination [8]. The most common  
 92 route of spread is the direct extension of a tumor to the  
 93 myometrium. In this review, we will focus on the mecha-  
 94 nisms that initiate this local invasion, as this invasion is  
 95 considered one of the most important prognostic factors for  
 96 type I and type II ECs, usually correlating with lymphatic  
 97 spread, risk of recurrence, and overall survival rate. In the  
 98 multistep process that characterizes myometrial invasion,  
 99 the initial events are delineated by the dissociation of  
 100 tumor cells from the epithelial layer of the endometrial  
 101 glands and the penetration through the basement membrane  
 102 into the adjacent connective tissue, i.e., the myometrium  
 103 [7]. This process is similar in other types of cancer, and  
 104 many authors [8, 9] have pointed out that for this process to  
 105 occur, epithelial tumor cells need to undergo an epithelial  
 106 to mesenchymal (EMT) transition, either transiently or  
 107 stably, and to differing degrees.

## Epithelial to mesenchymal transition (EMT) 108

109 EMT is a well-described process whereby epithelial cells  
 110 lose their polarity and cell–cell contacts, undergoing a  
 111 dramatic remodeling of the cytoskeleton and acquiring a  
 112 migratory phenotype, which activates a mesenchymal-like  
 113 gene expression program. The epithelial cells form a sheet  
 114 or layer of cells that are tightly connected laterally by  
 115 specialized junction structures, including adherens junc-  
 116 tions, desmosomes, hemidesmosomes, tight junctions and  
 117 gap junctions. Among these, adherens junctions play a  
 118 particularly important role in assembling and constructing  
 119 lateral epithelial cell–cell adhesions. The epithelial cells  
 120 also establish an aligned, apical–basal polarity through  
 121 their association with a lamina layer at their basal surface,  
 122 which ensures that the cells are only able to migrate lat-  
 123 erally, maintaining their position within the epithelium  
 124 [10]. Upon receiving specific signals, EMT occurs. Mes-  
 125 enchymal cells are defined by three major characteristics in  
 126 their cellular phenotype and their behavior (Fig. 1): (1) a  
 127 loss of strong epithelial adhesive cell–cell contacts and the  
 128 acquisition of a dispersed, spindle-shaped morphology with  
 129 migratory protrusions, (2) changes in the differentiation  
 130 markers from cell–cell junction proteins, i.e., E-cadherin  
 131 and cytokeratin intermediate filaments (specific to epithe-  
 132 lial cells) to vimentin filaments and fibronectin, and (3) an  
 133 increased motility for invasion through the extracellular  
 134 matrices. All three changes are not necessarily observable  
 135 during an EMT; however, the single cell acquisition of the  
 136 ability to migrate and invade the extracellular matrices is  
 137 considered to be a functional hallmark of EMT. Hence,  
 138 mesenchymal cells are able to detach, penetrate through the



**Fig. 1** General features of epithelial to mesenchymal transition (EMT). Schematic diagram of the main molecular, morphological and phenotypical changes occurring in EMT

139 basement membrane and infiltrate surrounding tissues to  
 140 then metastasize at secondary sites [11, 12]. To reach  
 141 successful dissemination, EMT must be triggered in epi-  
 142 thelial cells through an appropriate signaling pathway.  
 143 Cancer cells must also develop complex interactions to  
 144 integrate stimuli from their surrounding microenviron-  
 145 ments [13, 14].

146 Several transcription factors have emerged in recent  
 147 years that trigger the down-regulation of E-cadherin and,  
 148 consequently, produce EMT in different types of cancer.  
 149 These transcription factors include the transforming growth  
 150 factor- $\beta$  (TGF $\beta$ ), epidermal growth factor (EGF), insulin  
 151 growth factor 1 (IGF-1), interleukin, vascular endothelial  
 152 growth factor (VEGF), platelet-derived growth factor  
 153 (PDGF), integrin/integrin-linked kinase (ILK), notch,  
 154 fibroblast growth factor (FGF) and Wnt/ $\beta$ -catenin signaling  
 155 pathways [15–18]. Most of these signals exert their action  
 156 on E-cadherin repression through the modulation of a set of  
 157 pleiotropically acting transcription factors, including  
 158 members of Snail (Snail and Slug) and basic helix-loop-  
 159 helix (E47 and Twist) families, as well as two double zinc  
 160 finger and homeodomain (Zeb1 and Zeb2) factors [9].  
 161 These repressors are expressed in various combinations in a  
 162 number of malignant tumor types and have been shown in  
 163 experimental models of carcinoma formation to be causally  
 164 important for programming invasion [10, 19]. Hence, these  
 165 repressors are recognized as key inducers of EMT.

## 166 EMT in EC invasion

167 In EC, hallmarks of EMT have been reported either at  
 168 the level of E-cadherin loss or at the induction of its

repressors. On one side, Hurt et al. [20] and Singh et al. 169  
 [21] have described high levels of Zeb1 in relation to 170  
 gynecologic carcinoma progression, and Kyo et al. [22] 171  
 have observed a pattern of twist expression preferentially 172  
 at the ‘marginal regions’ of ECs, which was inversely 173  
 correlated with E-cadherin expression. Snail was also 174  
 proposed to play a role in EC progression and was 175  
 correlated with reduced estrogen-receptor  $\alpha$  expression 176  
 [23]. On the other side, several reports [24, 25] have 177  
 associated negative E-cadherin expression, increased 178  
 nuclear  $\beta$ -catenin, and loss of  $\alpha$ -catenin with the acqui- 179  
 sition of aggressive biological behavior, especially in 180  
 high-grade tumors. Furthermore, some studies have 181  
 demonstrated a correlation between reduced E-cadherin 182  
 and the presence of lymph node metastasis and/or 183  
 adverse patient outcomes [26–30]. 184

Independently of the most common features of EMT 185  
 described above, other molecular alterations have been 186  
 consistent with this phenotype. For example, L1CAM 187  
 expression was inversely correlated to the expression of 188  
 hormone receptors and E-Cadherin and was found to be 189  
 associated with poor prognosis [31]. L1CAM up-regula- 190  
 tion was mimicked in EC cell lines treated with the EMT 191  
 inducer TGF $\beta$ 1 and blocked when Slug was depleted. In 192  
 line with this result, L1CAM presents two functionally 193  
 active promoter sites containing  $\beta$ -catenin/TCF-LEF and 194  
 E-boxes, respectively, indicating that its expression can be 195  
 regulated by TGF $\beta$ 1, but also by Wnt/ $\beta$ -catenin [32]. 196  
 Conversely, the over-expression of a self-renewal gene, 197  
 called BMI-1, has been found in multiple human cancers, 198  
 including gastric cancer [33], lung cancer [34], breast 199  
 cancer [35], prostate cancer [36], ovarian cancer [37], and 200  
 EC [38]. BMI-1 up-regulation has been found to con- 201  
 tribute to the EMT-derived invasive phenotype in EC 202  
 cells, and its silencing reverts EMT and reduces EC cell 203  
 invasion [39]. 204

Interestingly, Stewart and Little [40] were the first to 205  
 approach an immunohistochemical identification of the 206  
 distinctive morphological alterations consistent with EMT 207  
 features in EC. They managed to characterize the presence 208  
 of microcystic, elongated and fragmented glands within EC 209  
 tissues, which they named “MELFs”. MELFs are specific 210  
 tumor alterations, which, through immunohistochemical 211  
 study, can be identified by strong CK7 expression, showing 212  
 a reduction of E-Cadherin and hormone receptor and 213  
 increased snail expression. Loss of hormone receptors and 214  
 decreased E-cadherin immunoreactivity have been reported 215  
 previously in EC [23, 41–44] and in breast carcinoma 216  
 [45, 46]. Although EMT has been broadly described in EC, 217  
 the molecular pathways responsible for triggering this 218  
 process are still poorly delineated for this type of cancer. 219  
 Here, we will review current literature pertaining to the 220  
 main effectors of EMT in EC. 221

222 **Progesterone receptor**

223 Presence of progesterone receptors (PR) has been described  
224 as an important asset in the prognosis and treatment of EC  
225 [47, 48]. In well-differentiated EC, PR expression is usu-  
226 ally maintained and treatment with medroxyprogesterone  
227 acetate (MPA) is usually successful. In contrast, a loss of  
228 PR expression is a negative prognostic factor and is asso-  
229 ciated with the development of a more invasive phenotype,  
230 in which MPA treatment is only occasionally successful in  
231 15–20 % of the cases [43, 49].

232 Recently, Van der Host et al. [44] have postulated that a  
233 loss of progesterone signaling in progressive disease may  
234 play a role in the induction of EMT, as well as diminished  
235 T-cell infiltration. In that study, PR modulated cell lines in  
236 the presence of MPA resulted in an inhibition of migration  
237 and a down-regulation of mesenchymal markers. An  
238 assessment of the pathways involved in EMT showed that  
239 progesterone modulated cell lines presented a down-regu-  
240 lation of EGF, IGF-1, IL-6, integrin/ILK, PDGF, TGF $\beta$ ,  
241 VEGF and Wnt/ $\beta$ -catenin signaling. These altered signal-  
242 ing pathways were also modulated in a gene expression  
243 study comparing progressive and non-progressive EC tis-  
244 sue samples. To date, a link between progesterone and the  
245 Wnt/ $\beta$ -catenin signaling pathway has been broadly  
246 described [41, 43, 50, 51], though links between proges-  
247 terone and other altered pathways remain unclear.

248 **TGF $\beta$** 

249 TGF $\beta$  signals are largely known as tumor promoters of  
250 cellular responses, such as proliferation, survival, migra-  
251 tion and invasion, and are related to EMT induction in  
252 other types of cancer [52]. TGF $\beta$  exerts its effect by  
253 binding to a heteromeric complex of transmembrane ser-  
254 ine/threonine kinase, the type I (RI) and type II (RII)  
255 receptors. Following ligand binding to the receptors com-  
256 plex, RI phosphorylates Smad2 and Smad3. The phos-  
257 phosphorylated Smads form a heteromeric complex with Smad4  
258 and translocate into the nucleus to regulate TGF $\beta$ -respon-  
259 sive gene transcription [53].

260 As relates to EC, the disruption of TGF $\beta$  signaling has  
261 been observed at the early stages of carcinogenesis, leading  
262 the endometrium to divert from normal growth control  
263 [54]. In contrast, increased levels of RII and Smad4 were  
264 described in infiltrating ECs, when compared to non-infil-  
265 trating tissues at the protein level, while Smad2 and Smad4  
266 mRNAs were down-regulated. A comparison between the  
267 molecular profiles of high risk and low risk of recurrence  
268 phenotypes for EC also pointed to a prominent role for  
269 TGF $\beta$  signaling in the acquisition of an aggressive phe-  
270 notype [55]. In vitro studies confirmed that TGF $\beta$  plays a

principal role at the initiation of EC invasion, through  
271 promotion of the EMT that leads to the acquisition of an  
272 invasive phenotype in Hec1a and RL95-2 cells [55, 56].  
273

**ETV5** 274

ETV5 belongs to the PEA3 subfamily of Ets transcription  
275 factors, characterized by a sequence of 85 amino acids in  
276 an evolutionarily conserved DNA-binding domain that  
277 regulates the expression of a variety of genes by binding to  
278 a central A/GGAA/T core motif, in cooperation with other  
279 transcriptional factors and cofactors [57, 58]. Up-regulation  
280 of the ETV5 gene in EEC was described by Planagumà  
281 et al. [59] with a specific and significant increase restricted  
282 to those tumor stages associated with myometrial infiltra-  
283 tion. Recently, ETV5 has arisen as an inducer of EMT in  
284 EC through a main regulatory effect on the Zeb1 promoter,  
285 and has also been found to induce EMT in ovarian cancer  
286 cell lines [60]. Along with ETV5 over-expression in EC  
287 cell lines, the main features of EMT were also observed.  
288 These included a modulation of cell adhesion, cell–cell  
289 contact and cellular junctions, and actin cytoskeleton  
290 reorganization. At the same time, main cellular functions  
291 were altered, as well as cell-to-cell signaling and interac-  
292 tion and cellular movement, as the principal modified  
293 biological processes [61].  
294

295 Concomitant with EMT, the up-regulation of ETV5 in  
296 EC also regulated the MMP2 [62] and HEP27 [63] pro-  
297 moter regions. The former protein is a matrix metallopro-  
298 teinase, which is primarily responsible for the degradation  
299 of the helical domains of type IV collagen, i.e., the prin-  
300 cipal collagen of basement membranes that allows tumor  
301 cells to infiltrate surrounding tissues. The latter is a  
302 member of the superfamily of short-chain dehydrogenases/  
303 reductases that protect tumor cells against apoptosis, which  
304 is induced by oxidative stress generated during the invasive  
305 process.

306 In our work, we have also identified a number of pro-  
307 teins involved in the acquisition of invasive capabilities by  
308 ECs [64], such as lipoma-preferred partner (LPP), which  
309 acts as a novel co-regulatory partner of ETV5 in the tran-  
310 scriptional regulation of the EMT process. LPP is reorga-  
311 nized from cell–cell contacts to focal adhesions when EMT  
312 is induced by ETV5, and it translocates to the nucleus  
313 ahead of external stimuli, establishing cross-talk between  
314 the tumor cells and their surrounding microenvironments  
315 [61].

316 Interestingly, our group linked TGF $\beta$  and progesterone  
317 receptor pathways with ETV5 by means of a proteomic  
318 analysis that compared EC cells stably over-expressing  
319 ETV5 with both control, non-transfected cells and cells that  
320 had been transfected with the empty vector [63] (Fig. 2).



321 **MicroRNA**

322 Several reports have shown the importance of certain mi-  
 323 croRNAs in modulating EMT. These microRNAs represent  
 324 a class of small, non-coding RNAs with important regu-  
 325 latory functions in diverse biological processes [65, 66],  
 326 including cancer [67]. In EC, and specifically in the car-  
 327 cinosarcoma histology from type II EC, Castilla et al. [68]  
 328 managed to identify specific microRNA signatures that  
 329 distinguished epithelial from mesenchymal areas. Among  
 330 the 377 microRNAs assayed, 14 microRNAs were found  
 331 differentially expressed. The most strongly up-regulated  
 332 microRNA was miR-155, indicating that this miRNA  
 333 participated in EMT. The role of this microRNA in EMT  
 334 had been previously suggested in vitro, in relation to  
 335 TGFβ. TGFβ-treated normal murine mammary gland epi-  
 336 thelial cells underwent EMT, and miR-155 was found to be  
 337 significantly up-regulated. The ectopic expression of miR-  
 338 155 also disrupted tight junction formation and promoted  
 339 cell migration and invasion [69]. Another key finding of  
 340 this study in carcinosarcomas was the marked down-reg-  
 341 ulation of all five members of the miR-200 family in the  
 342 mesenchymal cells. Several studies have demonstrated the  
 343 miR-200 family to be a powerful marker and determining  
 344 factor of the epithelial phenotype of cancer cells. In EC cell  
 345 lines, a member of this family, miR-200c, has been con-  
 346 firmed to maintain the epithelial phenotype by targeting  
 347 Zeb1 and Zeb2 and, moreover, to actively repress a pro-  
 348 gram of mesenchymal and neuronal genes involved in cell

349 motility and anoikis resistance [70, 71]. Regarding mi-  
 350 croRNAs regulation in other types of EC, Dong et al. [39]  
 351 unveiled that miR-194 inhibited the EMT of EC cells by  
 352 targeting the oncogene BMI-1.

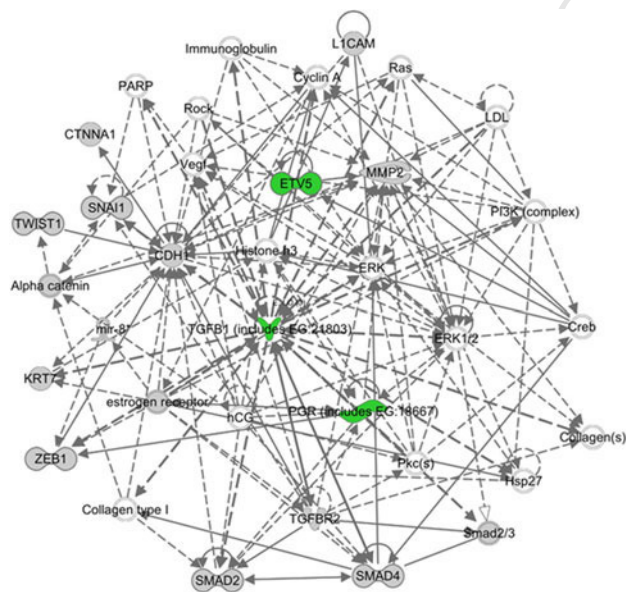
353 Only seven of more than 24,000 entries for “endometrial  
 354 cancer” in PUBMED correspond to “endometrial cancer”  
 355 and “EMT.” This data underscores the necessity of more  
 356 research on this topic. It is crucial for us to understand the  
 357 molecular mechanisms of myometrial invasion, as it repre-  
 358 sents one of the most important prognostic variables of EC.

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374 **References**

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375 **Fig. 2** Epithelial to mesenchymal transition (EMT) networks in  
 376 endometrial carcinoma (EC). To obtain the interaction network, we  
 377 selected genes differentially expressed in EC that have been related to  
 378 EMT, and we used Ingenuity Pathways Analysis software to plot  
 379 direct and indirect interactions

Author Proof

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