

Review

The Mechanism and Dynamic Regulation of Epithelial to Mesenchymal Transition in Ovarian Cancer

Pande Kadek Aditya Prayudi^{1,2}, I Gde Sastra Winata^{1,2,*}, I Nyoman Bayu Mahendra^{1,2}, I Nyoman Gede Budiana^{1,2}, Kade Yudi Saspriyana^{1,2}, Ketut Suwiyoga^{1,2}

¹Division of Gynecologic Oncology, Department of Obstetrics and Gynecology Prof. Dr. I G.N.G. Ngoerah Hospital, 80113 Denpasar, Bali, Indonesia ²Faculty of Medicine, Udayana University, 80113 Denpasar, Bali, Indonesia

*Correspondence: sastra@unud.ac.id (I Gde Sastra Winata)

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Abstract

Objective: To understand the basic mechanism and dynamic regulation that underlies the epithelial-to-mesenchymal transition (EMT) in ovarian cancer (OC) cells. **Mechanism**: A literature review using evidences from several data bases (i.e., PubMed, EMBASE, Web of Science, Medline, Cochrane, Science Direct, and Google Scholar) were conducted to describe the basic mechanism and dynamic regulation of EMT in OC cells. **Finding in Brief**: EMT is a complex epigenetic reprogramming orchestrated by specific transcription factors (TFs) and multiple upstream activators and regulators, such as transforming growth factor- β (TGF- β), Wnt, Hedgehog, and Hippo signaling pathways. The net result of this cellular reprogramming is the acquisition of mesenchymal phenotypes with increased invasive and metastatic potential, stemness properties and chemoresistance. Recent studies have demonstrated that EMT activation is the result of dynamic and reciprocal interplay between OC cells and their tumor microenvironment (TME). Cellular or non-cellular component of TME, external factors related to TME such as hypoxia, oxidative stress, mechanical forces, as well as exposure to chemotherapy, all play significant role to EMT induction. Current understanding behind the mechanism of EMT induction in cancer cells have proposed the idea that EMT is not merely a binary process involving a complete conversion from epithelial to mesenchymal state, but rather a dynamic process that encompasses a range of hybrid states, a phenotype that has been referred to as "partial EMT". Cells with partial EMT have been known to be more apoptosis-resistant and have more tumor-initiating potential as compared to those with complete EMT. **Conclusions**: Understanding the complex regulatory network that underlies EMT in OC cells is crucial in order to gain insight in developing novel and effective treatment strategies for OC.

Keywords: ovarian cancer; epithelial-to-mesenchymal transition; cellular reprogramming

1. Introduction

In 2020, ovarian cancer (OC) became the third most common gynecologic malignancy with a total of 313,959 new cases, and 207,252 new deaths recorded globally [1,2]. Majority of OC are diagnosed in advance stage due to the ineffective screening, and its silent progression at early stage [3]. OC imposes a significant economic burden with annual average costs being significantly higher in advance stages than early stage OC [4]. Epithelial OC is the commonest histologic type, while only 10% belong to the non-epithelial type. The epithelial subtype has five major histologic types, i.e., serous, mucinous, endometroid, clear cell, and unspecified [5]. Surgical cytoreduction to attain no gross residual disease (R0) followed by adjuvant chemotherapy is the current standard treatment. Recently, maintenance therapies such as poly ADP-ribose polymerase (PARP) inhibitors, bevacizumab, and drugs targeting homologous recombination deficiency (HRD) are incorporated to prolong the survival [6]. However, despite advancement in the treatment of OC, recurrence rate remains high (>45%), and survival for those with advance disease remains dismal. The predictors for recurrence include the extent of carcinomatosis within the peritoneal cavity, the amount of residual disease after cytoreductive surgery, and cellular grade [7].

Epithelial-to-mesenchymal transition (EMT) program has gained popularity among researchers as the responsible cellular mechanism that confers OC cells with increased metastatic potential and drug resistance, thus predisposing to recurrence [8-11]. EMT is a complex epigenetic reprogramming that results in reversible phenotype transition where cancer cells lose their epithelial phenotype and acquire mesenchymal phenotype [12]. The crucial orchestrator in EMT promotion is a well-known group of transcription factors (TFs), which includes SNAI1 (Snail1), SNAI2 (Snail2/Slug), Twist1/2, and zinc-finger E-box binding homeobox 1/2 (ZEB1/2) [13]. These TFs induce epigenetic silencing of epithelial markers (e.g., E-cadherin, Mucin 1 (MUC1), cytokeratin 18) while upregulating the expression of mesenchymal markers (e.g., N-cadherin, vimentin, fibronectin, matrix metalloproteinases (MMPs)) [14]. The upstream regulators of these EMT-transctipion factors (EMT-TFs) are multiple pathways which are mainly involved in embryonic development, such as transforming growth factor- β (TGF- β), Wnt, Hippo and Hedgehog.

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Fig. 1. Schematic illustration of complex regulatory interplays that underlie EMT. Tumor microenvironment (TME) and exposure to chemotherapy are believed to be the activators of EMT. The external factors then drive the expression of specific TFs, and activate signaling transduction pathways (TGF- β , Wnt, Hedgehog, Hippo). Expression of several non-coding RNA also interacts with EMT-TFs and signaling pathways. The net result of this complex regulatory network is the activation of mesenchymal phenotype with increased invasiveness, metastatic potential, stemness, and chemoresistance. EMT, epithelial-to-mesenchymal transition; TGF- β , transforming growth factor- β ; EMT-TFs, EMT-transctipion factors; ADSC, adipose-derived stem cells; CAF, cancer associated fibroblasts; EPC, endothelial progenitor cells; ROS, reactive oxygen species; TAM-M2, tumor associated macrophage-M2; MMPs, matrix metalloproteinases; MUC1, Mucin1.

These pathways can be activated by numerous signals originating from the OC cells itself or their microenvironment.

This literature review will summarize the recent advances in the mechanisms underlying the complex regulatory network of EMT in OCs, with the focus on its key orchestrators such as EMT-TFs, signaling pathways, upstream activator, as well as the dynamics of its regulation.

2. The Key Players of EMT Regulation in OC

EMT is a complex, reversible cellular process orchestrated by multiple activators and signal transduction pathways (Figs. 1,2). The downstream effector in this process is a series of activated TFs (EMT-TFs) that acts as either activator or repressor of the targets gene, with the end result of phenotypic transition from epithelial cells to more invasive mesenchymal cells. Thus, epigenetic reprogramming is at the heart of EMT regulation [15]. By undergoing EMT, OC cells gain characteristics crucial for distant metastasis, resistance to apoptosis, and thus, recurrence after therapy [16].

2.1 Transcription Factors (TFs)

2.1.1 Snail/Slug

Snail and Slug, encoded by the snail family transcriptional repressor 1 (SNAII) and 2 (SNAI2) gene, resis factor alpha (TNF- α), and bone morphogenetic proteins (BMPs) [17-24]. Snail transcriptionally represses the expression of CDH1 gene, which encode E-cadherin. Snail also downregulates other epithelial markers, but upregulates the mesenchymal markers and transcriptional repressor ZEB1 [25,26]. Meanwhile, Slug represses the expression of cell junction components: adherens junction (E-cadherin, β -catenin), tight junction (Occludin, Zona occludens-1 (ZO-1)) and desmosomes (Dsg2) [27]. In OC cell lines, Snail and Slug expressions were mutually exclusive, where Snail downregulates Slug expression [28]. Snail strongly represses epithelial splicing regulatory protein 1 (ESRP1) transcription in OC cells, which resultes in an isoform switching from epithelial spliced variant CD44v to mesenchymal spliced variant CD44s [29,30]. Slug is involved in the ferroptosis regulation in OC cell line through binding to the promoter of Solute carrier family 7 member 11 (SLC7A11) [31]. Slug is also capable of transforming normal fibroblasts to a cancer associated fibroblast (CAF)like state [32]. One study demonstrated that among several

spectively, are transcriptional repressors that play impor-

tant roles in regulating EMT. Snail and Slug activation re-

sults from several signaling pathways, such as receptor tyrosine kinases (RTKs), TGF- β , Notch, Wnt, tumor necro-





Fig. 2. Signaling pathways that regulate the induction of EMT. Several signaling pathways play crucial role in EMT induction. All of these important pathways culminate in the activation of genes encoding the key TFs zinc-finger E-box binding homeobox (ZEB), Snail and Twist. Several miRNAs downregulate the expression of these TFs. SMAD 2/3/4, SMAD family member 2/3/4; GSK, glycogen synthase kinase; APC, adenomatous polyposis coli; AKT, AKT serine threonine kinase; JAK, janus kinase; STAT, signal transducer and activator of transcription; TCF, T cell factor; LEF, lymphoid enhancer factor; mTOR, mammalian target of rapamycin; NF κ B, Nuclear factor kappa B; RTK, receptor tyrosine kinase; AXIN, Axin; PI3K, phosphatidylinositol 3-kinase.

EMT-TFs (Twist, Slug, ZEB1/2), Slug showed the highest expression. Slug is believed to be the master regulator of EMT [33]. Downregulation of Snail and Slug expression greatly suppressed cell invasiveness and promoted cell apoptosis [31,34–37]. Snail inhibition also appears to reduce the expression of C-X-C motif chemokine ligand 1 (CXCL1) and CXCL2, chemokines that attract myeloidderived suppressor cells (MDSCs) [38]. Higher Snail and Slug expression are associated with poorer survival of OC patients [39–41].

2.1.2 Zinc-Finger E-Box Binding Homeobox (ZEB)

ZEB is the zinc finger E-box binding homeobox family of TFs with its two members, ZEB1 and ZEB2. ZEB contain zinc-finger domain that allows binding at the enhancer boxes within the promoter region of target genes [42]. ZEB can interact with several TFs and cofactors, such as Smads (Suppressor of mothers against decapentaplegic), protein 300 (p300)/P300/CBP-associated factor (pCAF), Brahma-related gene-1 (BRG1), Nucleosome remodeling and deacetylation (NuRD) complex, and C-terminal binding protein (CtBP) [43–45]. The interaction determines ZEB role either as transcriptional activator or repressor of the target genes. ZEBs are crucial regulators of TGF- β /BMP signaling pathways [46–48]. ZEB1 synergizes with Smad-mediated transcriptional activation, while ZEB2 represses it [44]. Downregulation of ZEB2 expression in OC decreased the population of cancer stem cells (CSCs)

and reduced the expression of Oct4 and Homeobox protein Nanog (Nanog) [49]. Furthermore, ZEB2 knockdown result in downregulation of N-cadherin and vimentin. ZEB2, but not ZEB1, may regulate the expression of membranous E-cadherin during EMT [50]. ZEB1 is associated with worse overall survival (OS) in patients with solid tumors [51].

2.1.3 Twist

Twist is a member of the basic helix-loop-helix (bHLH) family, and acts as either transcription activator or inhibitor [52]. The two Twist genes, i.e., Twist1 (Twist) and Twist2 (Dermo-1), have 90% similarity [53]. Upregulated Twist expression predicted shorter OS in OC patients [54-56]. Twist1 induce upregulation of Akt upon cisplatin treatment in OC cell lines, which drives resistance [57]. Twist2 also induces chemoresistance in OC cells through induction of Akt serine threonine kinase (Akt)/Glycogen synthas kinase- β (GSK- β) signaling pathways [58]. Twist2 cytoplasmic expression contributes to the maintenance of epithelial characteristics, while nuclear Twist2 induces expression of Vimentin which promotes metastasis. Upregulated expression of Twist2 reduces the expression of Ecadherin while increasing the expression of vimentin [59]. Twist2 also upregulates N-cadherin and β -catenin in human OC cells. Upregulation of β -catenin expression by Twist promotes the activation of Wnt/β -catenin pathway [60]. Under hypoxic conditions, Twist2 is also capable of activating phosphatidylinositol 3-kinase-Akt (PI3K-Akt) pathway, which in turn promotes the survival of cancer cells [61].

2.1.4 Novel TFs

Heat shock transcription factor 1 (HSF1) is a proteotoxic stress-responsive transcription factor that also contribute in EMT. Knockdown of HSF1 expression in OC cell lines impaired TGF- β -induced EMT [62]. Downregulation of HSF1 also results in reduced proliferative activity, and intensified apoptosis [63]. Copy number alteration of HSF1 gene in OC patients is associated with worse outcome [64]. HSF-1 also plays an important role in Aktinduced Slug upregulation [65]. SRY-related HMG-box genes (SOX), a family of pluripotent TFs may also play a role in EMT. Their role in inducing EMT have been demonstrated in several solid cancers [66-69]. Knockdown of SOX2 induced downregulation of vimentin and upregulation of E-cadherin in OC cell lines [70]. Nanog, another important TFs commonly involved in embryonic development, also contributes to EMT. Nanog regulates EMT via 5' AMP-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) signaling pathway [71]. Silencing of Nanog expression in OC cell lines restores expression of Ecadherin [72]. Inhibition of Nanog attenuates the proliferation, migration, and invasion of OC cell lines. Meanwhile, increased expression of Nanog enhances OC cell migration and invasion [73]. Downregulation of Nanog also results in reduced expression of vimentin, β -catenin, and Snail [74].

2.2 Signaling Pathways

2.2.1 Transforming Growth Factor Beta (TGF- β)

TGF- β is a highly pleiotropic cytokine that is capable of inducing EMT. In ovarian surface epithelium (OSE), TGF- β 1 promotes tissue repair after ovulation by induction of EMT [75,76]. TGF- β is overexpressed in the OC microenvironment. TGF- β 1 upregulate the expression of Snail, Slug, Twist, ZEB1, and mesenchymal markers while downregulating E-cadherin [77–80]. TGF- β inhibition results in reduced expression of Smad2, Smad3, Snail, and vimentin, and increased expression levels of Smad4 and E-cadherin, thus blocking the activation of EMT [81,82]. TGF- β 1 also induces overexpression of SOX2, OCT4a, Nanog, CD44, and CD117 in OC cells [83]. TGF- β also induces EMT in peritoneal mesothelial cells, which is associated with cancer-associated mesothelial cells [84]. In promoting EMT, TGF- β expression is mainly mediated by the activity of Smad protein [81]. TGF- β also capable in inducing the expression of long non-coding RNA (lncRNA) activated by TGF- β (lncRNA ATB), which acts as inhibitor to miR-204-3p [85].

2.2.2 Wingless/Integrated (Wnt)

Wingless/Integrated (Wnt) signaling pathway is one of the main orchestrator of EMT in OC. In promoting

EMT, Wnt signaling mainly depends on the activity of β catenin, which is a transcriptional activator to several EMT-TFs (Snail, ZEB, Twist) [34,86–89]. Suppression of the Wnt/ β -catenin result in suppression of EMT in OC cells [86,90–92]. The Wnt/ β -catenin pathway also appears to be activated by Twist, which releases β -catenin from β catenin/E-cadherin complex and thus, leads to nuclear β catenin accumulation [60]. C-X-C motif chemokine ligand 14 (CXCL14) can induce EMT in OC cells via activation of the Wnt/ β -catenin pathway [93]. The bioactive lipid lysophosphatidic acid (LPA) promotes the nuclear translocation of β -catenin and upregulates the expression of Wnt/ β -catenin target genes [94]. The non-canonical Wnt also contributes to EMT via signal transducer and activator of transcription 3 (Stat3) expression [95-97]. Upregulation of Wnt5a, the non-canonical Wnt ligand, increases the transcriptional activation of Snail and induces EMT via protein kinase $C\alpha$ (PKC α) [98,99]. Wnt5a, the activator of noncanonical Wnt pathway, is also capable of inducing EMT in OSE cells [100]. Downregulation of Wnt5a significantly reduces the expression Smad2/3 and Yes-associated protein 1 (YAP1) expression [101].

2.2.3 Hedgehog

Hedgehog (Hh) signaling cascade culminate in a balance between activator or repressor forms of transcription factor Glioma-associated oncogene TFs (Gli) with *PTCH1*, *PTCH2*, and *GL11* being the main target genes. Hedgehog (Hh) ligand, Patched (Ptch), and Smoothened (Smo) are proteins involved in the activation of Hh signaling cascade [102]. Hh signaling plays an important role in the regulation of invasiveness, chemoresistance, as well as maintenance of CSCs characteristics [103–108]. A crosstalk between Sonic Hh-Gli1 signals and PI3K-Akt pathway regulates EMT induction in OC cells [109]. Inhibition of Hh signaling results in inhibition of EMT [110,111]. Gli1 also regulates the expression of Snail1, Slug, and Twist. Gli1 and Gli2 repress the expression of E-Cadherin [112].

2.2.4 Hippo

The Hippo pathway is a tumor suppressive pathway involved in regulating tissue growth, and their component comprises a pair of related serine/threonine kinases, macrophage stimulating 1 and 2 (MST1 and MST2), large tumor suppressor kinase 1 and 2 (LATS1 and LATS2), and lastly Salvador family WW domain containing protein 1 (SAV1), and Mps one binder 1 (MOB1A and MOB1B) [113]. This pathway downregulates the activity of YAP/TAZ. Following Hippo inactivation, YAP and transcriptional enhanced associate domain (TEAD) form complex within the nucleus to direct transcription of target genes. EMT-TFs are capable of complex formation with YAP/TEAD, which in turn upregulate the expression of YAP target genes in inducing EMT in OC cells [114– 116]. High YAP and TEAD expression is associated with



OC progression. Hippo pathway is also involved in regulating chemoresistance in OC cells [117–121]. Hippo signaling interacts with other EMT-inducing pathways, such as TGF- β and Wnt [101].

2.3 Non-Coding RNA

2.3.1 Micro-RNA

MicroRNA (miRNA) is a single-stranded non-coding RNA that acts as antisense RNA to downregulate expression of the target genes at the post-transcriptional level. Several miRNAs are involved in the regulation of EMT in OC cells. miR-200 family of miRNAs is a well-known EMT suppressor. Low level of miR-200 expression is demonstrated in normal OSE, but the increased expression is present in OC [122]. Overexpression of miR-205 and/or miR-200 family result in downregulation of ZEB1 transcription factor and Wnt5a [123]. miR-200c upregulation induced downregulation of ZEB1 and vimentin, and upregulation of E-cadherin [124]. In OC cells line, miR-200c overexpression decrease Snail, increase E-cadherin, and significantly reduce the invasiveness and tumorigenic potency of OC cell lines [125]. miR-203 expression can attenuate TGF- β pathway in OC cells [126]. Let-7 miRNA family is another EMT suppressor. The overexpression of Let-7g in OC cell lines reduce vimentin, Snail, and Slug expression [127]. miR-16 promotes the inactivation of the Wnt/ β catenin signaling pathway, thus inhibiting EMT. miR-16 upregulates the expression of Cadherin-1 and downregulates the expression of Snail, Slug, Twist 1, vimentin, and Cadherin-2 in OC cell lines [128]. miR-30d represses EMT by targeting Snail [129]. Expression of miR-30d reversed the TGF- β 1-induced EMT phenotypes in OC cell lines. Expression of miR-186 in OC cells downregulates the expression of Twist1 and subsequent reversal of EMT [130]. miR-141 overexpression upregulates E-cadherin, and decreases cell invasiveness in OC cell line [131]. However, several miRNAs also act as an EMT inducer. miR-1301 upregulates Snail, Slug, and N-cadherin expression, while downregulating E-cadherin [132]. The miR-150-5p also plays important roles in EMT regulation [133]. miR-27a promotes EMT via activation of Wnt/ β -catenin signaling pathway [134].

2.3.2 Long Non-Coding RNA

Long non-coding RNA (lncRNA) is non-protein coding RNA with length longer than 200 nucleotides that regulates target gene expression at both transcriptional and posttranscriptional level [135]. lncRNA are capable of direct binding to DNA or RNA to affect the transcription process. lncRNA H19 expression in the OC cells promotes migration and EMT-related activity [136]. Silencing of lncRNA in colon cancer associated transcript 2 (CCAT2) results in EMT inhibition. Knockdown of lncRNA CCAT2 also inhibits the expression of β -catenin and the activity of Wnt signaling pathway [137]. TGF- β treatment in OC cell lines results in the upregulation of lncRNA H19 and downregulation of miR-370-3p. H19 overexpression or miR-370-3p knockdown are capable of promoting TGF- β -induced EMT [138]. lncRNA-ATB downregulation results in EMT suppression [139]. By regulating the expression of LATS2, lncRNA ASAP1 Intronic Transcript 1 (ASAP1-IT1) induces downregulation of YAP1 expression and thus, preventing EMT in OC cells [140].

3. Activation of EMT Program in OC

EMT activation is the result of dynamic and reciprocal interplay between OC cells and their tumor microenvironment (TME). Cellular or non-cellular component of TME can act either as activator or inhibitor to certain signaling transduction pathways associated with EMT. On the other hand, OC cells can induce differentiation of cellular component of TME into certain phenotypes that favor metastatic potential of drug resistance of OC cells. These complex regulations are also affected by external factors related to TME, such as hypoxia, oxidative stress, as well as mechanical forces.

3.1 The Role of Tumor Microenvironment

3.1.1 Cellular Components

TME is the niche or environment in which the cancer cells closely interact with the host stroma, including cellular and non-cellular component of TME. The cellular components include immune cells, endothelial cells and fibroblast, while the non-cellular components include extracellular matrix and cellular metabolites. The cellular components play a more dominant role in promoting EMT. Cancer associated fibroblasts (CAF) are one of the key cellular components of TME that play important role in EMT induction. CAF are more proliferative and have higher metabolic states as compared to normal fibroblasts [141]. CAF-derived exosomes are rich in TGF- β as compared to normal fibroblasts, and are capable of inducing EMT in OC cell lines [142]. CAF highly secretes interleukin 6 (IL-6) and promotes TGF- β -mediated EMT via the Janus kinase 2 (JAK2)/Signal transducer and activator of transcription 3 (STAT3) pathway [143]. CAFs also secretes periostin, which functions as a ligand for integrin $\alpha v\beta 3$. Periostin is also capable of inducing EMT, mediated by TGF- β in OC cells [144]. The increased expression of fibroblast growth factor-1 (FGF-1) in CAFs induces the phosphorylation of fibroblast growth factor receptor-4 (FGFR-4) in OC cell line, which then induces the activation of mitogen-activated protein kinase (MAPK)/Extracellular signal-regulated kinase (ERK) pathway and EMT-associated gene Snail1 and matrix metalloproteinase 3 (MMP3) expression [145]. Stanniocalcin-1 expressed by CAF, is capable of upregulating the expression of fibronectin, vimentin, and Slug [146].

Tumor associated macrophage (TAM) plays a critical role in the interaction between TME and OC cells. TAMs are capable of differentiating into two disctinct phenotypes: M1, which has pro-inflammatory with anti-tumor activity, and M2, which has anti-inflammatory with pro-tumor activity. The path of TAMs is determined by the local TME [147]. In a mouse model of OC, TAM require ZEB1 expression to activate the tumor-promoting functions [148]. OC cells secretes macrophage colony-stimulating factor (M-CSF) to drive the differentiation of M2-TAM [149]. M2-TAMs are capable of inducing EMT of OC cells by releasing chemokine (C-C motif) ligand 18 (CCL18). On the other hand, CCL18 induces M-CSF transcription in OC cells through the activity ZEB1 transcription factor. Thus, a CCL18-ZEB1-M-CSF interacting loop exists between OC cells and TAMs that regulate the tumor progression and metastasis through EMT [149]. OC-derived exosomes containing miR-222-3p and miR-940 are also capable in inducing TAM polarization into the M2 phenotype [150,151].

Adipose-derived stem cells (ADSCs) are mesenchymal stem cells obtained from adipose tissues. ADSCs have been shown to affect the proteomic profile of OC cells via paracrine mechanism in favour of OC progression [152]. Secretion of TGF- β from ADSC result in activation of the TGF- β pathway in OC cells and subsequent EMT activation [153]. The relationship between ADSC and OC cells seems to be reciprocal. OC cells are capable of inducing the expression of CAF markers in ADSC, including alpha-smooth muscle actin (α -SMA) and fibroblast activation protein, via the TGF- β 1 signaling pathway [154,155]. The addition of ADSCs into the medium of OC culture significant increase of the paired box 8 (PAX8) level in OC cells [156]. Overexpression of PAX8 lead to upregulation of Snail, Twist and Zeb2 [157].

Endothelial progenitor cells (EPCs), the bone marrowderived stem cells, play a significant role in tumor angiogenesis and growth. EPCs are recruited into the neovascular bed of the tumor in response to certain signals or cytokines secreted by tumor cells [158–160]. EPCs are able to invade into the OC cell clusters, whereas normal human microvascular endothelial cells are not capable of invading OC cell clusters [161]. Circulating levels of EPCs are significantly increased in OC patients and correlate with tumor stage and residual tumor size [162]. OC cells cultured in EPC-conditioned media (EPC-CM) demonstrate an increase in TGF- β . EPC-CM also induce loss of cell junctions, reduced expression of E-cadherin, increased expression of N-cadherin, and development of a fibroblastic phenotype in OC cells, which are the consistent feature of EMT [163].

3.1.2 Non-Cellular Components

The non-cellular components of TME also participate in promoting EMT. Collagen I enhance OC cells motility and invasiveness through the increased expression of MMPs and $\alpha 5\beta 1$ integrin. Collagen I matrix upregulate the expression of N-cadherin, vimentin, fibronectin, and transcriptional factors Snail and Slug [164]. Collagen I also upregulates the activity of TGF- β 1/Smad4 and Wnt5b/ β catenin signaling cascade [165]. IL-6 treatment downregulates the expression of epithelial markers, and upregulates the expression of mesenchymal markers in OC cell line. Overexpression of IL-6 in OC cells significantly increases the expression of MMP-2 and MMP-9 and thus, enhancing their migration ability [166].

Hypoxia is one of the most important non-cellular factors in inducing EMT. Hypoxia influences cellular processes such as angiogenesis, acquisition of stem cell-like features, chemoresistance, as well as EMT [167-170]. Hypoxia is the characteristic of the peritoneal environment. Hypoxia induces the stabilization of hypoxia-inducible factor-1 α (HIF-1 α), which then translocate into the nucleus, to bind to HIF-1 β and forming HIF-1 heterodimer. HIF-1 heterodimer acts as transcription factor targeting genes with the hypoxic responsive elements (HRE). Under hypoxic conditions, OC cells presents morphological changes consistent with EMT [171]. HIF-1 expression in OC stem cells results in the induction of Twist1 and E12 expression [172]. Downregulation of HIF-1 α expression leads to upregulation of E-cadherin and downregulation of the vimentin [173]. Hypoxia downregulates the expression of miR-210, the EMT repressor [174]. Hypoxia also upregulates the expression of C-X3-C motif chemokine receptor (CX3CR), increasing the chemotactic response to C-X3-C motif chemokine ligand 1 (CX3CL1), and thus leading to tumor progression and metastasis [175]. Hypoxia also upregulates the expression of signal transducer and activator of transcription 4 (STAT4), which contributes to the regulation of EMT [176]. Hypoxic stress downregulated the expression of Sirtuin (silent mating type information regulation 2 homolog) 1 (SIRT1), a negative regulator of HIF-1 α [177].

Reactive oxygen species (ROS), such as hydroxyl free radicals, superoxide, and hydrogen peroxide can accumulate within the TME due to active metabolic patterns of OC cells and tumor stromal cells. ROS accumulation can lead to lipid peroxidation, antioxidants deprivation, and ultimately programmed cell death which depend on iron, also known as ferroptosis. However, OC cells develop several mechanisms that confer resistance to ferroptosis [178]. ROS are involved in the regulation of EMT. ROS accumulation induced the increased expression of HIF-1 α and subsequent transcriptional induction of lysyl oxidase (LOX), which then decreases the expression of E-cadherin [179]. ROS scavenging negatively affect migration and invasion of OC cells through reversing EMT [180].

OC cells endure several mechanical forces from their TME. The presence of ascites and interstitial fluid confer the shearing force to the OC cells, while tumor expansion against the extracellular matrix and TME exert tension on the tumor periphery. Furthermore, tumor expansion and the hydrostatic pressure of ascites also exert internal compression forces to the OC cells [181]. Those forces give impact

into biochemical regulation and signaling pathways of OC cells, including the regulation of EMT. Oscillatory tension significantly decreases the expression of E-cadherin while increasing the expression level of Snail [182]. Tissue stiffness, recapitulated by substrate stiffness *in vitro*, promotes OC cells proliferation and nuclear translocation of the oncogene YAP. Substrate softening has been demonstrated to promote changes consistent with EMT [183,184].

3.2 The Role of Chemotherapy

Results from in vitro studies have demonstrated that chemoresistant OC cells express markers of EMT [8,185-190]. On the other hand, exposure to chemotherapy has been demonstrated to induce EMT in OC cells. Cisplatin induced EMT-associated morphological changes in OC cells [191–193]. Receptor cells co-cultured with carboplatin- or etoposide-treated feeder cells in Transwell co-culture system exhibited increased expression of EMT markers vimentin and Snail. The altered microenvironment of either carboplatin- or etoposide-16-treated feeder OC cells also significantly increased the migration of the OC cells [194]. OC cells treated with carboplatin exhibited phenotypic changes consistent with EMT [195]. Clinical studies in OC patients revealed that treatment with platinum-based chemotherapy increased the proportion of EMT-like circulating tumor cells (CTC), accompanied by the "de novo" emergence of PI3K α +/Twist+ EMT-like CTCs [196]. Continuous exposure to increasing doses of paclitaxel lead to the establishment of OC cell lines that are resistant to paclitaxel and exhibit phenotypic changes consistent with EMT [197]. However, the EMT-inducing effect of chemotherapy, whether it is direct or indirect, remains unclear. The proposed mechanisms are that chemotherapy may directly trigger intracellular signaling, such as orchestrated cellular defense response against platinum toxicity. On the other hand, while killing the tumor cells, chemotherapy indirectly influences the EMT of the remaining cancer cells. Chemoterapy is also believed to induce oxidative stress that is capable of inducing EMT.

4. The Dynamics of EMT Regulation

The traditional concept viewed EMT as a binary process in which the phenotype of cancer cells can change completely into either epithelial or mesenchymal cells. However, a newer concept of "partial EMT" has been proposed, in which cancer cells can undergo phenotypic changes with both epithelial and mesenchymal phenotype [198,199]. Tumours in a partial EMT state exhibit low expression of EMT-TFs, and co-express both epithelial and mesenchymal genes. A recent study in pancreatic ductal adenocarcinoma cells reported that partial EMT results from different mechanisms underlying complete EMT. Cancer cells lose their epithelial phenotype through an alternative posttranslational process of protein relocalizations, which lead to "partial EMT". In that study, E-cadherin protein was

found to be confined to intracellular foci in delaminated cells exhibiting a mesenchymal morphology. Furthermore, cancer cells that exhibit partial EMT, migrate and form circulating tumor cell clusters, rather than disseminate as single cell as in "complete EMT" [200]. However, it remains unclear whether partial EMT represents an intermediate stage where cancer cells are in a paused transitional state within the mesenchymal differentiation continuum, or it is a final fate of the cancer cells. Interestingly, one study support the later hypothesis, in which they found no evidence that complete and partial EMT co-exist within the same tumor [50]. This finding implicate that the tendency of cancer cells to use either a complete or partial EMT program is a specific and stable feature of an individual tumor. Cells with partial EMT have been known to be more resistant to apoptosis and have greater tumor-initiating potential, as compared to those with complete EMT. EMT interconversions are also dynamically regulated during the development and progression of ovarian tumors [50,201].

Transcription Factor Balance in Partial EMT

A review by Jolly et al. [198] stated that the core regulatory network for EMT or MET (Mesenchymal-to-Epithelial Transition) acts as a "three-way", which give rise to three distinct phenotypes, i.e., epithelial (E), mesenchymal (M), and hybrid, or partial EMT (pEMT). The core regulatory network depends on two mutually inhibitory loops, i.e., miR-34/SNAIL and miR-200/ZEB. E phenotype is defined as high miR-200/miR-34, low ZEB/SNAIL; M phenotype is defined as low miR-200/miR-34, high ZEB/SNAIL; and partial EMT is defined as low miR-34/ZEB, high SNAIL/miR-200. Another theory proposed that miR-200/ZEB, with input from SNAIL, behaves as a three-way switch allowing for the existence of three phenotypes, i.e., E (high miR-200, low ZEB), M (low miR-200, high ZEB), and E/M or partial EMT (medium miR-200, medium ZEB). Therefore, ZEB activation is a necessary requirement for the acquisition of a complete EMT. However, results from experimental studies observing partial EMT appear to be more consistent with medium miR-200, medium ZEB theory. One study demonstrated that difference in expression and subcellular localization of transcription factor 21 (Tcf21) and Slug (Tcf21/Slug balance) is associated with phenotypic plasticity [202]. Downregulation of Slug by Tcf21 is known to maintain the epithelial properties of high grade serous carcinoma (HGSC). The study identified the association of Tcf21/Slug balance with additional intermediate phenotypic states (i.e., iE or iM), supporting the proposed hypothesis of a multistep EMT program [202]. Tcf21 high-Slug low expression was identified in E phenotype, Tcf21 low-Slug high expression was identified in M phenotype, and Tcf21 moderate-Slug moderate expression (E-M or pEMT) was identified as stable phenotypes. Intermediate E or M represents a metastable state associated with phenotypic switches. Intermediate E



Fig. 3. Schematic illustration of dynamic regulation underlying phenotypic plasticity in EMT. A newer concept of EMT introduces the concept of partial EMT in which cancer cells exhibit both mesenchymal and epithelial characteristics. This phenotypic plasticity are determined by the balance of expression from certain TFs and their inhibitors (miRNA), such as the balance between miR-34/SNAIL and miR-200/ZEB. The expression and subcellular location of Tcf21 and Slug also influence the phenotype of cancer cells. Cancer cells undergoing partial or hybrid EMT tend to be more invasive, more resistant to apoptosis, and have greater tumor initiating potential.

(iE) expressed epithelial markers, Snail, Twist1, but lacked Tcf21, while the tumor group with low EMT-TF expression was identified as intermediate M (iM). The dynamic regulation of EMT, which encompasses a range of phenotypic plasticity, is summarized in Fig. 3.

5. Conclusions

Epithelial-to-mesenchymal transition or EMT is a form of epigenetic cellular reprogramming governed by complex regulatory networks that confers OC cells with increased invasiveness and drug resistance. EMT is orchestrated by multiple TFs, upstream activators, and regulators that result in the acquisition of mesenchymal phenotypes with increased metastatic potential, stemness properties, and chemoresistance. EMT activation is the result of dynamic and reciprocal interplay between OC cells and their tumor microenvironment. EMT is a dynamic process of phenotypic plasticity, which encompasses a range of hybrid states. Understanding this complex regulatory network is crucial in order to gain insight in developing novel and effective treatment strategies for OC.

Abbreviations

ADSC, Adipocyte-derived stem cell; AKT, AKT serine threonine kinase; AMPK, 5' AMP-activated protein kinase; ASAP1-IT1, ASAP1 Intronic Transcript 1; ATB, Activated by TGF- β ; bHLH, basic helix-loop-helix; BMP, Bone morphogenetic protein; BRG1, Brahma-related gene-1; CAF, Cancer associated fibroblast; CCAT2, Colon canCluster of differentiation standard isoform; CD44v, Cluster of differentiation 44 variant isoform; CDH1, Cadherin 1; CSCs, Cancer stem cells; CtBP, C-terminal binding protein; CTC, Circulating tumor cells; CX3CL, C-X3-C motif chemokine ligand ; CX3CR, C-X3-C motif chemokine receptor; CXCL1, C-X-C motif chemokine ligand 1; CXCL14, C-X-C motif chemokine ligand 14; CXCL2, C-X-C motif chemokine ligand 2; EMT, Epithelial-tomesenchymal transition; EPC-CM, Endothelial progenitor cell-conditioned media; ERK, Extracellular signalregulated kinase; ESRP1, Epithelial splicing regulatory protein 1; FGF-1, Fibroblast growth factor-1; GLI, Gliomaassociated oncogene transcription factors; GSK- β , Glycogen synthase kinase- β ; HIF-1 α , Hypoxia-inducible factor 1-alpha; HRD, Homologous recombination deficiency; HRE, Hypoxia responsive element; HSF1, Heat shock transcription factor 1; iE, Intermediate epithelial; IL, Interleukin; iM, Intermediate mesenchymal; JAK, Janus kinase; LATS, Large tumor suppressor kinase 1; lncRNA, Long non coding ribonucleic acid; LOX, Lysyl oxidase; LPA, Lysophosphatidic acid; M-CSF, Monocyte-colony stimulating factor; MAPK, Mitogen-activated protein kinase; MDSC, Myeloid-derived suppressor cells; miRNA, Micro ribonucleic acid; MMP, Matrix metalloproteinase; MOB1, Mps one binder 1; MST, Macrophage stimulating; mTOR, Mammalian target of rapamycin; MUC1, Mucin 1; NANOG, Homeobox protein Nanog; NFkB, Nuclear fac-

cer associated transcript 2; CCL18, C-C motif chemokine

ligand 18; CD117, Cluster of differentiation 117; CD44s,

tor kappa B; NuRD, Nucleosome remodeling and deacetylation; OC, Ovarian cancer; OCT4, Octamer-binding transcription factor 4; OSE, Ovarian surface epithelium; p300, Protein 300; PARP, Poly (ADP-ribose) polymerase ; PAX, Paired box; pCAF, P300/CBP-associated factor; pEMT, Partial epithelial-to-mesenchymal transition; PI3K, Phosphatidylinositol 3-kinase; PKC α , Protein kinase C alpha; PTCH, Protein pathced homolog; R0, No gross residual disease; ROS, Reactive oxygen species; RTK, Receptor tyrosine kinase; SAV, Salvador family WW domain containing protein 1; SIRT1, Sirtuin (silent mating type information regulation 2 homolog) 1; SLC7A11, Solute carrier family 7 member 11; SMAD, Suppressor of mothers against decapentaplegic; SMO, Smoothened; SNAI1, Snail family transcriptional repressor 1; SNAI2, Snail family transcriptional repressor 1; SOX, SRY-related HMG-box genes; STAT3, Signal transducer and activator of transcription 3; TAM, Tumour associated macrophage; TAM-M2, Tumour associated macrophage-M2 phenotype; Tcf21, Transcription factor 21; TEAD, Transcriptional enhanced associate domain; TF, Transcription factor; TGF- β , Transforming growth factor- β ; TME, Tumour microenvironment; TNF- α , Tumor necrosis factor alpha; TWIST, Twist-related protein; WNT, Wingless/Integrated; YAP, Yes-associated protein; ZEB, Zinc-finger E-box binding homeobox; ZO-1, Zona occludens-1; α -SMA, Alpha-Smooth muscle actin.

Author Contributions

PKAP performed conception and design, acquisition of data, analysis and interpretation of data and drafting the manuscript. INBM and INGB performed conception and design, analysis and interpretation of data, and reviewing the manuscript. IGSW and KYS performed acquisition of data, analysis and interpretation of data and drafting the manuscript. KS performed conception and design, analysis and interpretation of data, reviewing and final approval of published version. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.



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