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Review

Overview of the signaling pathways involved in metastasis: An intriguing story-tale of the metastatic journey of ovarian cancer cells

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Abstract: Wealth of information has revolutionized our understanding related to the genetics and functional genomics of this heterogeneous disease. Keeping in view the heterogeneity of ovarian cancer, long-term survival might be achieved by translation of recently emerging mechanistic insights at the cellular and molecular levels to personalize individual strategies for treatment and to identify biomarkers for early detection. Importantly, the motility and invasive properties of ovarian cancer cells are driven by a repertoire of signaling cascades, many components of which have been experimentally verified as therapeutic targets in preclinical models as well as in clinical trials. Scientific evidence garnered over decades of research has deconvoluted the highly intricate intertwined network of intracellular signaling pathways which played fundamental role in carcinogenesis and metastasis. In this review we have provided a compendium of myriad of signaling cascades which have been documented to play critical role in the progression and metastasis of ovarian cancer. We have partitioned this multi-component review into different sections to individually discuss and summarize the roles of TGF/SMAD, JAK/STAT, Wnt/β-Catenin, NOTCH, SHH/GLI, mTORC1/mTORC2, VEGFR and Hippo/YAP pathways in ovarian cancer metastasis.

Key words: Cancer; Apoptosis; Signaling; Therapy; Pharmacology.

Introduction

Ovarian cancer is a heterogeneous disease. Groundbreaking discoveries in the past two decades have significantly enhanced our knowledge about the functional characterization of underlying mechanisms of ovarian cancer. Therefore, multistage model proposed by clinical and basic scientists mainly involved genetic/epigenetic changes, clonal expansion, drug resistance, loss of apoptosis and metastasis. This review summarizes latest and landmark research findings which have distilled our understanding about multifaceted and therapeutically challenging nature of ovarian cancer. Excitingly, emerging insights from structural biology and pharmacological research may be game changing mainly in the context of ovarian cancer. Aberrant oncogenic signaling pathways have been uncovered to be tightly woven with many aspects of ovarian cancer, including the onset, progression and metastasis. Deregulation of signaling networks and crosstalk with other transduction cascades resulted in carcinogenesis and metastasis. In the upcoming section, we will critically analyze some of the major research-works which have refined our understanding about role of TGF/SMAD signaling in ovarian cancer metastasis.

TGF/SMAD Signaling

The highly conserved wiring of the SMAD-dependent TGFβ superfamily transduction cascade has been mapped over the last 3 decades after structural characterization of its signaling components. SMAD-dependent signaling is activated by serine phosphorylation of R-SMADs (Receptor-regulated SMADs) by type I receptors. Studies have shown that R-SMADs form a complex with common-mediator SMAD (SMAD4) (1-3). Consequently, R-SMADs/co-SMAD complex moved into the nucleus and interacted with transcriptional factors for regulation of the target genes. Accordingly, "crosstalk" in the nucleus permits SMADs to modulate a wide-ranging array of transcriptional outputs in response to TGFβ stimulation of cancer cells (3-5).

Argonaute-1 (AGO1) promoted ovarian cancer. miR-148a-3p directly targeted AGO1 and downregulated its expression (6). PVT1, a long non-coding RNA blocked miR-148a-3p mediated targeting of AGO1.

ERK1/2 phosphorylation was significantly reduced in AGO1-depleted SKOV3 cancer cells. Additionally, phosphorylated levels of SMAD2 and SMAD4 were also reported to be reduced in AGO1-depleted SKOV3 cancer cells. Importantly, tumor growth was found to be significantly suppressed in mice xenografted with PVT1 or AGO1-silenced-SKOV3 cells (6).

Metformin significantly inhibited p-SMAD2 and p-SMAD3 in ovarian cancer cells (fig.1). Moreover, metformin and cisplatin combinatorially caused shrinkage of the tumor mass in tumor-bearing mice (7).

LY2157299 monohydrate (LY), an inhibitor of TGFβ receptor I inhibited ovarian cancer (8). LY potently inhibited TGFβ1 mediated activation of SMAD2 and SMAD3 in ovarian cancer cell lines. Malignant ascites is an excessive accumulation of fluid in the abdominal cavity and associated with primary and recurrent ovarian cancer. LY significantly inhibited formation of the ascites in tumor-bearing mice (8).

A-83-01, an inhibitor of TGFβ signaling cascade was found to be effective against ovarian cancer (9). Intraperitoneally injected HM-1 cells led to ascites accumulation with diffuse disseminated tumors on the peritoneum in B6C3F1 mice. The ascites contained abundant TGFβ. A-83-01 was injected into the peritoneal cavities of the mice. Importantly, rate of ascites formation was notably slower while survival rate of the mice was reported to be significantly improved in the A-83-01-treated group (9).

TGFβ binds to serine/threonine kinase receptors (TβR1/TβR2) and activates a transduction pathway by phosphorylation of SMAD2 and SMAD3 (10). Phosphorylated SMAD2/3 formed a complex with SMAD4 and translocated into the nucleus to transcriptionally regulate the expression of target genes. Additionally, SMAD7 acts as a bridge protein by recruiting SMURF2 (E3 ubiquitin ligase) to the TGFβ receptor complex, which subsequently results in the proteasomal-mediated degradation of TβR1. TGFβ-activated SMAD3/4 complex positively regulated the expression of FXYD5. FXYD5 blocked the binding of SMAD7 and SMURF2 to TβR1. FXYD5 (FXYD domain-containing ion transport regulator 5) caused disassembly of SMAD7-SMURF2-TβR1 complex, deubiquitinated and stabilized TβR1, consequently enhanced TGFβ signaling and sustained TGFβ driven EMT (fig.1) (10). TGFβ activated SMAD3/SMAD4 complex directly recruited to the promoter region of FXYD5 and promoted transcriptional upregulation of FXYD5. Moreover, FXYD5-overexpressing SKOV3 cancer cells potently enhanced the formation of disseminated nodules in tumor-bearing mice (Bai, 2020). Mice injected with FXYD5-overexpressing cancer cells developed more disseminated nodules. Additionally, immunohistochemistry analysis of the intraperitoneal nodules revealed that FXYD5 tumors exhibited a robust expression of FXYD5 (10).

Disabled homolog 2 DOC-2/DAB2 interacting protein (DAB2IP) has tumor suppressive role in ovarian cancer. E3 ubiquitin ligase SCF^{FBW7} (a SKP1–cullin-1–F-box complex consisting of FBW7 as the F-box protein) targeted proteins by ubiquitylation and degradation. DAB2IP is degraded by different ubiquitin ligases. DAB2IP is degraded by the ubiquitin-proteasome pathway by SCF^{FBW7}. AKT induced phosphorylation of SMURF1 potently enhanced its stability. SMURF1 interacted with DAB2IP and promoted ubiquitination-dependent degradation (fig.1) (11).

Findings from another study clearly indicated that there was a notable reduction in the migratory and invasive potential of SMURF1-depleted SKOV3 and A2780 cancer cells (12). AKT-mediated phosphorylation of SKP2 prevented its degradation. However, DAB2IP blocked AKT-mediated phosphorylation of SKP2 in ovarian cancer cells (12). Overall, these findings suggested that SMURF1 ubiquitinated DAB21P and enhanced the stability of SKP2 for carcinogenesis.

ARHGAP26 (Rho GTPase-activating protein 26) had been shown to negatively regulate Rho family by conversion of GTP-RhoA into its inactive GDP-bound form. SMURF1 overexpression significantly induced ARHGAP26 ubiquitination in SKOV3 cells (fig.1) (13). Moreover, SMURF1 overexpression considerably promoted the migration and invasion of SKOV3 cells. There was an evident reduction in the metastasis in the lung tissues of mice injected with ARHGAP26-expressing-A2780 or HEY cancer cells (13).

Hypoxia- and TGFβ1 also synergistically induced EMT (14). Dual PI3K/mTOR inhibitor NVP-BEZ235 prevented hypoxia-induced EMT and migration of ovarian cancer cells. TGFβ1-induced SMAD2/3 phosphorylation led to transcriptional upregulation of SNAIL in SKOV-3 cancer cells. Moreover, TGFβ1-activated phosphorylation of AKT/GSK-3β prevented SNAIL degradation. Enhanced phosphorylation of AKT and GSK-3β potently stabilized SNAIL. However, NVP-BEZ235 not only inhibited TGFβ1-mediated activation of SMAD2/3 but also inactivated AKT/GSK-3β-driven signaling axis (14).

SMYD3 (SET and MYND domain-containing protein 3) is a histone methyltransferase (15). SMYD3 has been shown to promote ovarian cancer. ITGB6 (Integrin subunit Beta 6) is a membrane-spanning heterodimeric glycoprotein. TGFβ1 is secreted in an inactive form by cancer cells or fibroblasts. SMYD3 and ITGB6 promoted the release and activation of latent TGFβ1 and increased the phosphorylation of SMAD3 in ovarian cancer spheroids. TGFβ1 promoted the expression of ITGB6 and SMYD3 (15).

SMAD4 played central role in the prevention of metastasis of ovarian cancer (16). miR-378 directly targeted SMAD4 and promoted metastasis. circATRNL1 blocked miR-378-mediated targeting of SMAD4. Importantly, the number of tumor nodules and the tumor weights in the abdomen were significantly smaller in mice intraperitoneally injected with circATRNL1-transfected CAOV3 and SKOV3 cancer cells (16). Likewise, miR-205 directly targeted SMAD4 in ovarian cancer cells. miR-205 overexpression promoted the proliferation and invasive potential of ovarian cancer cells (17).

Pioneering research-works have provided compelling evidence that during metastasis ovarian cancer cells use specialized mechanisms for dissemination. Firstly, ovarian cancer cells are exfoliated from the primary tumors, later these cancer cells are disseminated throughout the peritoneal cavity in the serous fluids, and favorably seed in the omental fat band. miR-205 directly targeted SMAD4 and promoted ovarian cancer. There was a marked increase in tumor burden on omentum,

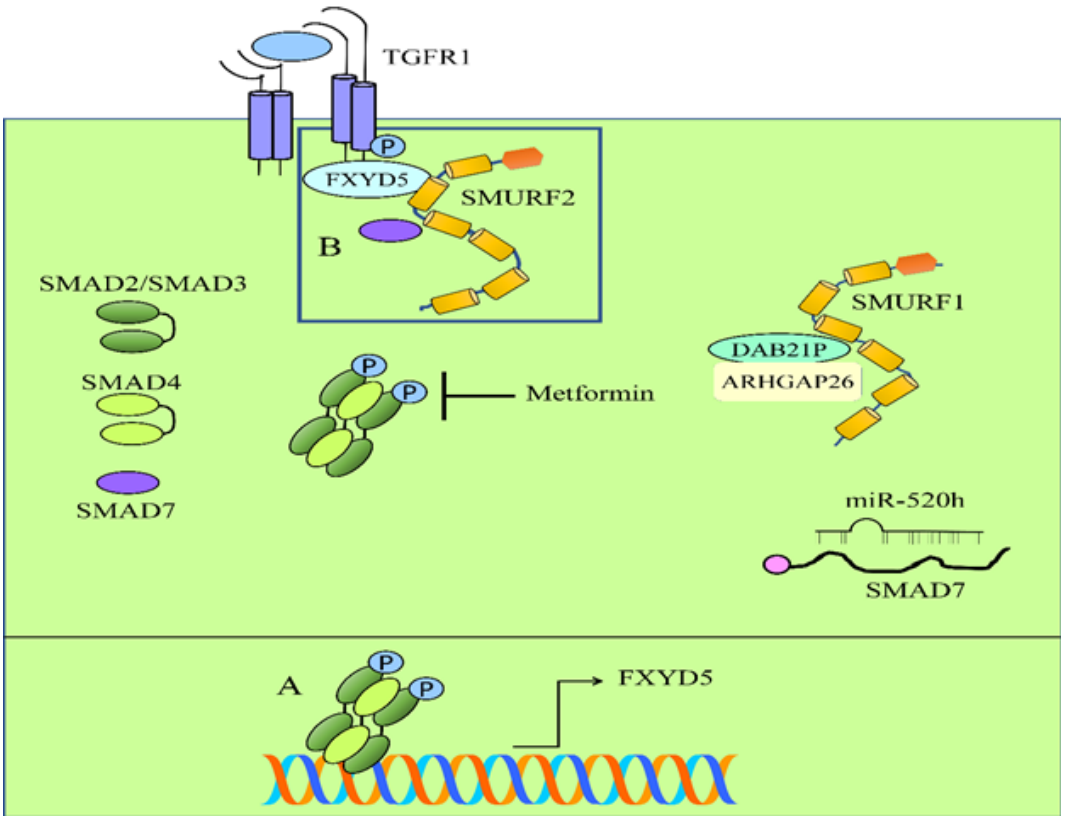

Figure 1. (A-B) TGFβ activated SMAD3/SMAD4 complex promoted transcriptional upregulation of FXYD5. FXYD5 caused disassembly of SMAD7-SMURF2-TβR1 complex, deubiquitinated and stabilized TβR1, consequently enhanced TGFβ signaling. SMURF1 interacted with DAB2IP and ARHGAP26. SMURF1 enhanced the degradation of DAB2IP and ARHGAP26. miR-520h directly targeted SMAD7 and promoted migration and invasion of epithelial ovarian cancer cells. Metformin significantly inhibited p-SMAD2 and p-SMAD3.

bowel mesentery, peritoneal surface, liver and ovary in peritoneal cavities of the mice intraperitoneally injected with miR-205-expressing-HO-8910 cancer cells (18)

DLX1 (Distal-less homeobox-1) has a pro-metastatic role in ovarian cancer (19). FOXM1B and FOXM1C transcriptionally upregulated the expression of DLX1 in ovarian cancer cells. DLX1 interacted with SMAD4 in the nucleus of ovarian cancer cells upon TGFβ1 stimulation and significantly inhibited TGFβ1-induced p21WAF1/Cip1 transcriptional activity. SMAD4 occupancy on promoter region of p21WAF1/Cip1 was attenuated by DLX1 overexpression in ovarian cancer cells which clearly indicated that DLX1 blocked SMAD4 recruitment to the p21WAF1/Cip1 promoter. However, contrarily, expression levels of PAI-1 (plasminogen activator inhibitor-1) and JUNB were found to be upregulated in SMAD4 and DLX1 co-expressing SKOV3 cancer cells. DLX-1-expressing SKOV3 cells rapidly developed tumors. DLX1-expressing SKOV3 cells demonstrated significantly higher capacity of peritoneal dissemination. Even after FOXM1 depletion, DLX1-expressing cancer cells still exhibited ~38% more disseminated tumor nodules in tumor-bearing mice (19).

TGFβ1 enhanced the binding of c-Myb to promoter region of miR-520h (20). miR-520h directly targeted SMAD7 and promoted migration and invasion of epithelial ovarian cancer cells (shown in fig. 1). miR-520h enhanced growth and dissemination of epithelial ovarian cancer cells. Tumors derived from miR-520h-overexpressing-EOC cells were bigger in size while the tumors developed from miR-520h- knockdown-EOC cells were smaller in size. The miR-520h-overexpressing mice developed considerably disseminated nodules in the peritoneal cavity, while miR-520h-silenced mice developed smaller and lighter metastatic nodules. Moreover, SMAD7 overexpression efficiently reduced the number and weight of disseminated nodules (20).

JAK/STAT Signaling

Upon stimulation with cytokine ligands, JAK (Janus kinase) phosphorylates critical tyrosine residues on each other and the cytokine receptor chains. Phosphotyrosyl residues in cytoplasmic tails of these receptors facilitate the recruitment of STAT proteins to the receptor complex, which triggers the activation of STATs. Activated STATs then undergo dimerization and translocate into the nucleus to stimulate the expression of target genes.

Levels of p-JAK and p-STAT3 were noted to be enhanced in cancer cells transfected with miRNA-503-5p inhibitors. However, levels of p-JAK and p-STAT3 were found to be suppressed in MALAT1-silenced OVCAR3 cancer cells (21). MALAT1 acted as a sponge for miR-503-5p.

CD55 is a ligand and transduces the signals through seven-span transmembrane receptor CD97 (22). Stimulation of CD97 with rhCD55 caused weaker induction of JAK2, STAT3, matrix metalloproteinase-2 and -9 in the absence of LPS. However, co-stimulation with LPS and rhCD55 considerably enhanced the migratory and invasive properties of ovarian cancer cells. LPS-induced NF-κB led to downregulation of miRNA-503-5p in cancer cells (fig.2). miRNA-503-5p mediated targeting of CD97-inhibited metastasizing ability of the paclitaxel-resistant ovarian cancer cells (shown in fig. 2). (22).

Alternatively activated macrophages (AAMs) in the

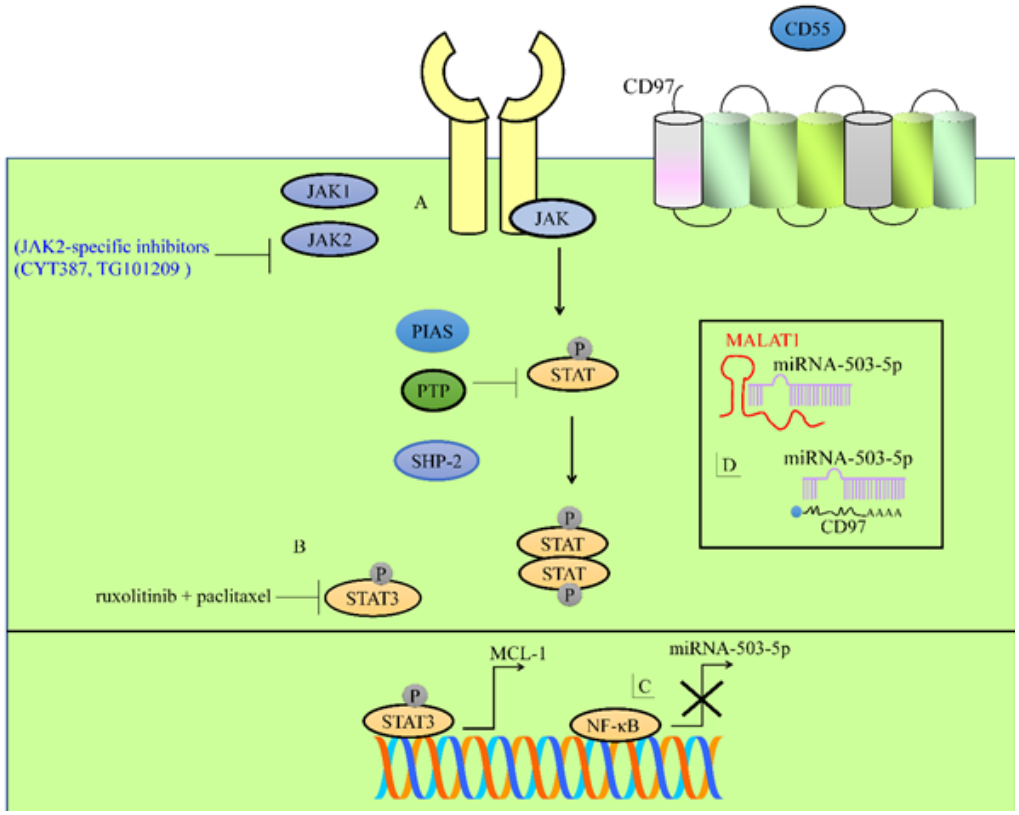

Figure 2. (A) JAK/STAT signaling played central role in metastasis (B) Ruxolitinib and paclitaxel significantly reduced STAT3-mediated increase in the levels of MCL-1. (C) NF-κB led to downregulation of miR-503-5p. miR-503-5p directly targeted CD97. MALAT1 served as a sponge for miR-503-5p.

surrounding ascites fluid have been shown to enhance tumor growth and invasion (23). High-grade serous ovarian cancer (HGSOC) cells were noted to be highly responsive to AAM-derived cytokines. These cytokines activated JAK2/STAT3 transduction cascade leading to MMP9 secretion and spreading of HGSOC spheroids (23).

STAT3 had earlier been shown to enhance the levels of anti-apoptotic protein MCL-1 (24). Combinatorial treatment with ruxolitinib and paclitaxel caused a significant reduction in the levels of MCL-1 in MDAH2774 and OVCAR-8 cancer cells (fig.2). Ruxolitinib and paclitaxel induced tumor regression in mice intraperitoneally injected with OVCAR-8 cancer cells. Levels of p-STAT3 were found to be reduced in the tumor tissues of mice treated with ruxolitinib either alone or in combination with paclitaxel (24).

$CD24^+$ and $CD133^+$ cells have considerable tumor sphere-forming abilities (25). Importantly, basal levels of p-STAT3 were found to be significantly higher in $CD24^+$ cells as compared to $CD24^-$ cells. TG101209, a small molecule JAK2-selective kinase inhibitor has been shown to exert metastasis inhibitory effects. Importantly, vehicle-treated mice demonstrated widespread metastatic disease with metastatic nodules in the intestine, bladder, liver and peritoneum. On the contrary, only 1 of TG101209-treated-xenografted mice demonstrated metastasis (25).

CYT387 (JAK2-specific inhibitor) has been reported to be effective against ovarian cancer (26). Xenografted mice combinatorially treated with paclitaxel and CYT387 developed significantly smaller tumors. Moreover, levels of p-JAK2 and p-STAT3 were noted to be reduced in the tumor tissues of xenografted mice (26).

There is frequent overexpression of TG2 (Tissue transglutaminase) in cancer cells. TG2 promoted metastases and resistance to chemotherapeutic drugs (27). TG2 absence led to reduction in the levels of p-STAT3 in $CD4^+$ and $CD8^+$ T cells by IL-6, IFNγ and TGFβ. STAT3 was less responsive to cytokine stimulation in T cells collected from the ascites of $TG2^{-/-}$ mice. Notably, STAT1 and STAT3 were found to be potently activated in $CD4^+$ cells from $TG2^{+/+}$ in comparison to $TG2^{-/-}$ ascites by IFNγ and TGFβ. STAT3, but not STAT1 was potently phosphorylated by IL-6 in $CD4^+$ cells from $TG2^{+/+}$ in comparison to $TG2^{-/-}$ ascites (27).

SRC, a non-canonical kinase phosphorylated STAT3 at Tyr705 residue in L1CAM-expressing-ovarian cancer cells (28). FGFR inhibition abrogated L1CAM-mediated phosphorylation of both SRC and STAT3. STAT3 inhibition led to a significant shrinkage of Ov90-L1CAM tumors (28).

Oncostatin M is highly expressed by tumor-associated macrophages (29). Binding of oncostatin M to its receptor induced intracellular signaling. Oncostatin M dimerized with IL6ST (interleukin 6 cytokine family signal transducer) and induced activation of STAT3. Human monoclonal antibody clones B14 and B21 directed against extracellular domains of OSMR blocked oncostatin M-induced receptor-IL6ST hetero-dimerization, promoted the internalization and degradation of oncostatin M receptor (29).

Fructose-1,6-bisphosphatase (FBP1) is transcriptionally downregulated by c-Myc in ovarian cancer cells (30). FBP1 interacted with STAT3 and inhibited its

nuclear transportation. STAT3-mediated expression of MMP3, HIF-1α and Bcl-2 in FBP1-expressing ovarian cancer cells. FBP1 overexpression led to inhibition of tumor metastases, reducing both the weight and number of peritoneal disseminated lesions (30).

Levels of IL-6 and p-STAT3 were noted to be suppressed in THOR-knockdown HO8910 and HGSOC cells (31).

ERBB2/ERBB3 signaling enhanced the expression of furin in ovarian cancer cells (32). There was a marked reduction in the migratory and invasive potential of furin-silenced-OVCAR5 and SKOV3 cancer cells. Levels of p-JAK1 and p-STAT3 were found to be reduced in furin-silenced-cancer cells. More prominently, loss of furin expression suppressed tumor growth, the number of tumor nodules in the peritoneum as well as the total volume of ascitic fluid in the peritoneal cavities of experimental mice (32).

Recombinant human Interleukin-17A increased the expression of FABP4 in ovarian cancer cells via STAT3 signaling (33). rhIL-17A enhanced the levels of p-STAT3 and FABP4, but pre-treatment with STAT3 inhibitors significantly blocked rhIL-17A-mediated increase in the levels of FABP4. Moreover, endogenous IL-17A fueled the growth and metastases of ovarian cancer in the peritoneal cavities of animal models. There was an evident increase in the size of tumor nodules in the ovarian tissues of wild-type mice injected with ID8 cancer cells as compared to IL-17A$^{-/-}$ mice. There was a greater abdominal dissemination of tumor cells to the peritoneum in wild-type mice. Particularly, multiple large size tumor nodules were noted throughout the bowel, omentum, mesentery and abdominal wall. However, IL-17A$^{-/-}$ mice developed smaller and fewer tumor masses in the peritoneal cavities (33).

Wnt/β-Catenin Signaling

Pioneering research-works have provided mechanistic insights into Wnt/β-catenin signaling pathway in different cancers and unraveled structural determinants of the functional diversity in ovarian cancer metastasis. Multifaceted roles of Wnt/β-catenin transduction cascade in health and pathology make this cascade an attractive yet intrinsically challenging pharmacological target.

ELF3 (E74 Like ETS Transcription Factor 3) transcriptionally downregulated miR-485-5p. Claudin-4 (CLND4) is directly targeted by miR-485-5p. Accordingly, miR-485-5p targeted CLDN4 for the inactivation of Wnt/β-catenin cascade in ovarian cancer cells (34). Overall, these findings suggested that ELF3 transcriptionally reduced miR-485-5p to potentiate CLDN4 expression. CLDN4 overexpression further enhanced Wnt/β-catenin signaling and metastasis.

Ubiquitin conjugating enzyme E2S (UBE2S) potently promoted the migration and invasion ability of SKOV3 and A2780 cancer cells (35). UBE2S overexpression led to significant reduction in apoptotic death and augmented the olaparib resistance in ovarian cancer cells. Moreover, UBE2S overexpression significantly enhanced Wnt/β-catenin transduction cascade, while the UBE2S knockdown led to the inactivation of the pathway. UBE2S also promoted β-catenin accumulation in nucleus. Anaphase-promoting complex/cyclosome (APC/C) is an E3 ubiquitin ligase. UBE2S has been shown to interact with APC/C complex for the ubiquitination of K19 residue in β-catenin to inhibit its proteasomal degradation (35).

Series of studies have provided convincing proof that exosomes from ovarian cancer cells potently enhanced migration of cancer cells within the tumor microenvironment (36). There was an evident dispersion of tumor nodules throughout the peritoneal cavity in animal models injected with exo-SKOV-3 as compared to the mice injected with exo-OVCAR-3 which developed clustered cell colonies in the proximity or around the injection sites. High levels of p-β-catenin (Serine^{675} and Serine^{552}) were found in the tumor tissues of mice injected with exo-OVCAR-3 (36).

HOXB-AS3 promoted tumorigenic ability of epithelial ovarian cancer cells via activation of Wnt/β-catenin signaling cascade (37).

BRMS1L (Breast cancer metastasis suppressor 1-like) blocked the nuclear accumulation of β-catenin in HeyA8 cells and BRMS1L knockdown enhanced β-catenin transportation from the cytoplasm to the nucleus in EFO27 cells (fig.3) (38).

Ten-eleven translocation 1 (TET1) induces DNA demethylation. TET1 binds to the CpG islands located in the promoter regions of DKK1 and SFRP2, catalyzes the conversion of 5mC to 5hmC and enhances demethylation (39). More importantly, tumors derived from TET1-overexpressing SKOV3 cancer cells were smaller in size (39).

FILIP1L (Filamin A interacting protein 1-like) promoted the proteasomal degradation of p-β-catenin in centrosomes and blocked WNT signaling (Fig.3). There was a marked reduction in the peritoneal metastases of FILIP1L-expressing-OVCA429 and SKOV3 cancer cells (40).

β-catenin downregulated Dicer to fuel pro-metastatic properties (41). There was a marked increase in the levels of Dicer in β-catenin-silenced cancer cells. Highly metastatic- β-catenin-silenced cancer cells demonstrated markedly reduced capacity to form primary tumors and metastatic spread to the peritoneum. These findings clearly indicated that β-catenin enhanced tumor initiation and early seeding of peritoneal metastases in tumor-bearing mice (41).

KAT6A can acetylate non-histone and histones proteins. KAT6A acetylated 294th lysine residue of COP1 (42). KAT6A regulated β-catenin stability by COP1 acetylation. COP1 acetylation abolished COP1 activity as an E3 ubiquitin ligase. Importantly, KAT6A depletion reduced β-catenin levels by inhibition of COP1 acetylation. Knockdown of KAT6A markedly decreased the stability of β-catenin in A2780 and SKOV3 cells. Furthermore, knockdown of KAT6A significantly reduced β-catenin levels in A2780 and SKOV3 cells. Whereas, reduction in the levels of β-catenin was reversed by the KAT6A overexpression. KAT6A-mediated acetylation of COP1 efficiently reduced β-catenin degradation. Subcutaneous xenografts of KAT6A-knockdown A2780 cancer cells were smaller in size. Injection of KAT6A-silenced cancer cells into the abdominal cavity led to significant reduction in the development of peritoneal metastasis. Importantly, KAT6A inhibition led to

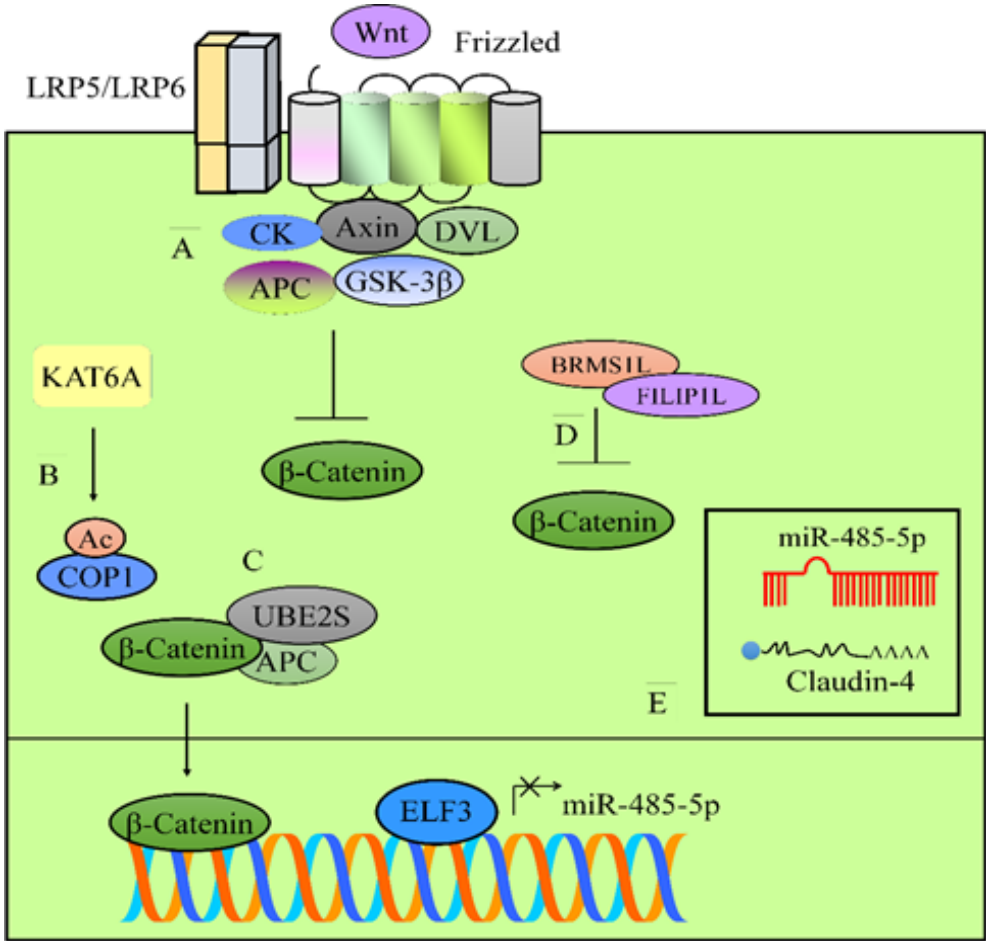

Figure 3. (A) Wnt/β-catenin signaling played central role in metastasis (B-C) KAT6A-mediated acetylation of COP1 efficiently reduced β-catenin degradation. COP1 acetylation abolished COP1 activity as an E3 ubiquitin ligase. UBE2S interacted with APC/C complex for the ubiquitination of K19 residue in β-catenin and inhibited its degradation. (D) BRMS1L blocked the nuclear accumulation of β-catenin and FILIP1L enhanced β-catenin degradation. (E) ELF3 transcriptionally reduced miR-485-5p to potentiate CLDN4 expression.

substantial decrease in the ability of cancer cells to form secondary tumors in the abdominal cavity (42).

Manipulation of Wnt/β-catenin-mediated signaling at the level of its cytosolic or nuclear components harbors high potential for inhibition of carcinogenesis and metastasis. Pharmacological targeting of Wnt/β-catenin pathway might lead to clinically effective therapeutics.

Regulation of NOTCH Signaling

SATB1 (Special AT-rich sequence-binding protein 1) directly upregulated the expression of NOTCH1 (43). Moreover, SATB1 modulated the differentiation of inflammatory dendritic cells by regulation of MHC II (major histocompatibility complex class II) expression through NOTCH1 signaling. RBPJ occupancy at the promoter of H2-Ab1 activated MHC II transcription. Infiltration of inflammatory DCs in ovarian tumors resulted in an immunosuppressive phenotype characterized by rapid increase in the secretion of tumor-promoting IL-6 and Galectin-1. However, SATB1 inhibition in tumor-associated DCs led to reversal of their tumorigenic activity and boosted protective immunity (43).

Levels of NOTCH1 and its target gene HES1 were noted to be reduced in LGR5-silenced cancer cells (44). However, LGR5 overexpression led to increased expression of NOTCH1 and HES1. Tumors derived from LGR5-knockdown SKOV3 cancer cells were significantly smaller in size (44).

Intravenously injected LLC or B16F10 tumor cells infiltrated in the lungs in wild-type C57BL/6 mice (45). Myeloid cells isolated from lung cell suspensions revealed strong expression of JAG1 on their surfaces. Immunohistochemistry analysis of lung sections indicated strong NICD expression in endothelial cells within peritumoral areas. Intraperitoneally injected serous ovarian cancer ID8 cells offer promising and useful approach for the establishment of cancer model for the analysis of cancer-associated inflammation. Importantly, presence of myeloid cells in the peritoneal fluid enhanced tumorigenesis by suppression of T lymphocytes. Intriguingly, dissemination of myeloid cells in peritoneal fluid was severely impaired in RBP-JK-deficient mice implanted with ID8 cells (45).

TIAM1 (T cell lymphoma invasion and metastasis-1) promoted NOTCH pathway and consequently enhanced invasion of ovarian cancer cells (46). miR-1271-5p acted as a tumor suppressor and targeted TIAM1. miR-1271-5p knockdown promoted the proliferative ability of ovarian cancer cells. Levels of HES1 and NOTCH were noted to be reduced in miR-1271-5p-expressing ovarian cancer cells (46).

GATA1 transcriptionally upregulated JAG1 in ovarian cancer cells. Moreover, JAG1 knockdown inhibited GATA1-mediated activation of NOTCH and suppressed proliferation of ovarian cancer cells. JAG1 expression

promoted NOTCH signaling in ovarian cancer cells (47).

Anoikis is a hallmark process in which normal cells undergo a specialized form of apoptosis following detachment from the ECM (Extracellular matrix) (48). COL4A2 (Collagen type IV alpha 2) suppression induced cell death. Levels of COL4A2 were noted to be reduced in NOTCH3-silenced ovarian cancer cells but exogenous collagen IV supplementation reversed the anoikis sensitivity. NOTCH3-silenced cancer cells undergo BIM-induced anoikis but NOTCH3 overexpression promoted anoikis resistance (48).

GClnc1, an lncRNA interacted with FOXC2 and transcriptionally upregulated NOTCH1 in ovarian cancer cells (49). SKOV3 and OVC1 ovarian cancer cells with stable poor expression of GClnc1 were injected into the caudal vein of mice. The number of pulmonary and hepatic metastatic nodules was noted to be significantly reduced in mice injected with GClnc1-silenced ovarian cancer cells (49).

SHH/GLI Signaling

The GLI transcriptional factors are mediators of sonic hedgehog (SHH)-dependent changes in gene expression. In this section we have provided a summary of key findings mainly in the context of SHH/GLI-mediated ovarian cancer metastasis.

BMI1 is a member of the Polycomb repressor complex-1 and regulates gene silencing of target genes. Previous studies had shown that chemotherapeutic drugs induced activation of GLI1-BMI1 signaling axis in ovarian cancer cells (50). However, Berberine inhibited GLI1-BMI1 signaling axis in ovarian cancer cells (51).

Levels of SHH, PTCH1, SMO, and GLI2 were noted to be significantly higher in cisplatin-resistant A2780/DDP cancer cells (52). Findings clearly indicated that SHH/GLI transduction cascade was hyperactive in A2780/DDP cells and induced drug resistance. SMO knockdown promoted apoptotic death of drug-resistant A2780/DDP cancer cells (52).

Rab23 belongs to Ras-related small GTPase superfamily and plays central role in SHH signaling cascade (53). Levels of SHH and GLI1 were reduced in RAB23-silenced ovarian cancer cells. Moreover, EMT-related proteins were reported to be suppressed in RAB23-silenced cancer cells. Proliferation ability of RAB23-silenced-CaoV3 and A2780 cancer cells was noted to be significantly reduced (53).

SHH treatment induced an increase in the levels of MMP7 in cancer cells. Cyclopamine (SMO inhibitor) inhibited SHH signaling in SKOV3 cancer cells (54). MMP7 levels were found to be suppressed in cyclopamine-treated-SKOV3 cancer cells. GANT61 (GLI inhibitor) blocked SHH signaling and reduced MMP7 levels in SKOV3 and ES-2 cancer cells. GLI2 depletion suppressed the levels of MMP7 in SKOV3 cancer cells (54).

SHH significantly upregulated CD24 levels but GANT61 (GLI inhibitor) significantly inhibited CD24 expression in ovarian cancer cells (55). Subcutaneous injections of GANT6 induced tumor regression in mice subcutaneously implanted with SKOV3 cancer cells (55).

In another study it was shown that diindolylmethane and cyclopamine treatment led to marked reduction in the tumor-forming ability of A2780 and OVCAR-429 cancer cells (56).

mTORC1 and mTORC2

Anti-PD-L1 monoclonal antibodies have significant clinical activity against different cancers. Anti-PD-L1 monoclonal antibodies enhanced antitumor activity of PD-1-expressing T cells by blockade of PD-L1/PD-1-driven downstream signaling (57). ID8 ovarian cancer cells generated tumors that mimicked hallmark features of human cancer, including local spread and ascites in syngeneic BL6 mice intraperitoneally injected with cancer cells. Growth rate of the tumors derived from PD-L1^{lo} ID8agg cells was slow after intraperitoneal challenge in NSG and wild-type mice. Levels of RHEB1 (mTORC1 activator) were noted to be significantly reduced in PD-L1^{lo} cells along with marked reduction in mTORC1 signaling. Moreover, Ribosomal protein S6 (RPS6), a downstream signaling protein of mTORC1 was also found to be evidently reduced. PD-L1 promoted basal mTORC1 signaling. There was a paradoxical increase in mTORC1 activity in serum-starved PD-L1^{lo} ID8agg cells. Rapamycin led to effective suppression of mTORC1 in control ID8agg cells and in PD-L1^{lo} ID8agg cells (57).

Ovarian carcinoma has been reported to metastasize to the peritoneal cavity. Intriguingly, role of peritoneal residential macrophages is unclear in ovarian cancers (58). Tim-4 (T cell immunoglobulin and mucin domain containing-4) expressing macrophages have pro-tumorigenic role. Mixture of ID8 cancer cells and Tim-4^{+} TAMs was inoculated in wild-type mice. Intriguingly, addition of Tim-4^{+} TAMs enhanced the development of tumors in xenografted mice. Tim-4^{+} TAMs displayed weak mTORC1 activity. High levels of mitophagy enhanced the survival of Tim-4^{+} TAMs in the tumor microenvironment. Importantly, autophagy deficiency led to loss of Tim-4^{+} tumor-associated macrophages through accumulation of reactive oxygen species. Structural insights had shown that FAK family-interacting protein of 200 kDa (FIP200) interacted with multiple proteins. FIP200, ULK1 and ULK2 are present on autophagic isolation membranes. FIP200 is structural constituent of ULK1-ATG13-FIP200-ATG101 multi-protein machinery and a critical inducer of mammalian autophagy. Inoculation of ID8 tumor cells in *Fip200*$^{-/-}$ mice induced an increase in the accumulation of damaged mitochondria in Tim-4^{+} *Fip200*$^{-/-}$ TAMs. However, accumulation of damaged mitochondria was not reported in Tim-4^{-} *Fip200*$^{-/-}$ TAM. FIP200 deficiency in macrophages retarded the growth of tumors (58).

CCL18 is a chemotactic cytokine expressed by a broader range of lymphocytes. CCL18 overexpression led to activation of mTORC2 cascade including activation of AKT and NDRG1 (59).

Combinatorial treatment with carboplatin and mTORC1 inhibitor (everolimus) was marginally better as compared to single-treatment with carboplatin, but carboplatin and mTORC1/2 inhibitor (PP242) revealed greater tumor regression and considerably suppressed metastases (60). Only the groups treated with PP242

(mTORC1/2 inhibitor) indicated stronger inhibitory effects on p70S6K and 4E-BP1 phosphorylation. Furthermore, PP242-mediated mTORC1/2 inhibition blocked activating AKT phosphorylation and suppressed tumor progression. However, everolimus-mediated mTORC1 inhibition did not block phosphorylation of AKT and tumor progression (60).

VEGF/VEGFR Signaling

Soluble receptors classically lack transmembrane domains as well as intracellular tyrosine kinase segments and are unable to trigger signal transduction (61). Targeting of VEGF pathways by soluble VEGFR1, VEGFR2 and VEGFR3 was substantially efficient against cancers. Tie1 and Tie are cell-surface receptors which also transmit the signals intracellularly. Adenoviral vectors encoding sVEGFR1-IgG (immunoglobulin) fusion protein, sVEGFR3-IgG, soluble Tie1-IgG, soluble Tie2-IgG were used for evaluation of efficacy in the mice inoculated with SKOV-3m cancer cells into the peritoneal cavity. sVEGFR1, sVEGFR3 and sTie2 efficiently reduced the growth rate of intraperitoneal solid ovarian carcinoma. However, there was an increase in the amount of ascites and mice presented edema under the skin after intravenous injections of sVEGFR-1, sVEGFR3 and sTie2 in the tail veins. As Tie2 is involved in growth of lymphatic vessels and VEGFR3 is a central regulator of lymphangiogenesis, therefore, increase in the amount of ascites might be because of combined blockade of these cascades. Pericytes provided strong architectural support to endothelial cells and studies had shown that survival signals from pericytes severely limited the value of anti-angiogenic therapies. There was an increase in the number of vessels without pericytes together with a reduction in pericyte coverage in mice intravenously injected with sVEGFR-1, sVEGFR3 and sTie2 (61).

p-ERK levels were found to be considerably enhanced in FGF9-treated ovarian cancer cells (62). Moreover, mRNA levels of VEGFA and VEGFR2 were also noted to be significantly upregulated FGF9-treated ovarian cancer cells. ETS1 facilitated FGF9-dependent upregulation of VEGFA/VEGFR2 in ovarian cancer cells. Collectively, these findings clearly indicated that FGF9 promoted the binding of ETS1 to the promoter regions of VEGFA and VEGFR2 in ovarian cancer cells (62).

PD-L1 induced tumor angiogenesis and metastases by VEGFR2-mediated transduction cascade in ovarian cancer cells (63). VEGFR2 is a potential receptor of PD-L1 in ovarian cancer. Importantly, overexpression of VEGFR2 led to partial reversal of the inhibition of invasion and migration caused by PD-L1 silencing in ovarian cancer cells. PD-L1 knockdown considerably interfered with the phosphorylation of 951st tyrosine residue in VEGFR2. Durvalumab (PD-L1 inhibitor) and apatinib (VEGFR2 inhibitor) synergistically exerted inhibitory effects on angiogenesis, invasion and migration of ovarian cancer cells. c-JUN, a transcriptional factor triggered the upregulation of PD-L1. PD-L1 morpholinos inhibited zebrafish angiogenesis and these inhibitory effects were found to be rescued by overexpression of VEGFR2. Tumor growth rate was markedly reduced by the silencing of PD-L1 but considerably enhanced uopn VEGFR2 overexpression in mice subcutaneously or intraperitoneally injected with OVCA433 cancer cells (63).

MARCKSL1 (Myristoylated alanine-rich C kinase substrate-like 1) has metastasis-suppressive features (64). MARCKSL1 inhibited phosphorylation of VEGFR2 in ovarian tumorigenesis. Furthermore, MARCKSL1 reduced the levels of VEGF and HIF1α. Additionally, it was reported that MARCKSL1 effectively reduced VEGF-mediated downstream activation of PI3K/AKT signaling pathway components, including PDK1, mTOR, TSC2, p70S6K and GSK-3β (64).

Seminal research works have shown that tumor-associated macrophages (TAMs) enhance the formation of spheroids during early transcoelomic metastases of ovarian cancer (65). There was a notable faction of macrophages in the peritoneal cavities at 2 hours after injection of tumor cells however, macrophages seemingly promoted the growth of tumor cells only after 3 weeks after tumor injections. TAMs undergo polarization to M2-like subtype in the peritoneal cavity microenvironment during ovarian cancer progression. For detailed analysis of the involvement of TAMs in the progression of ovarian cancer, M2 TAMs isolated from the spheroids of OC-bearing donor mice were co-injected with ID8 cells into the peritoneal cavities of new mice. Mice co-injected with TAMs and ID8 group demonstrated marked increase in the tumor growth, tumor weight and accumulation of ascitic fluid. Interestingly TAMs had higher expression levels of EGF and tumor cells displayed an increase in the levels of EGFR. EGF induced the expression of VEGF-C, which further activated VEGFR3 in tumor cells. Certainly, levels of VEGF-C were found to be enhanced in EGF-treated mouse ID8 and SKOV3 cancer cells. VEGFR3 was phosphorylated upon treatment with EGF or VEGF-C. Whereas, these activations were noted to be impaired by erlotinib (EGFR inhibitor) or MAZ51 (VEGFR3 inhibitor). However, EGFR inhibitors failed to inhibit VEGF-C-dependent VEGFR3 activation which clearly suggested that EGF/EGFR cascade operates upstream to VEGF-C/VEGFR3 signaling axis. Interaction of TAMs with tumor cells was inhibited by blockade of EGF/EGFR and VEGF-C/VEGFR3 transduction cascades. Erlotinib was co-injected with SKOV3 cancer cells into the peritoneal cavities of mice and findings indicated that pharmacological targeting of TAMs via liposomal clodronate significantly reduced tumor growth. TAM-secreted EGF played an essential role in spheroid formation and tumor growth at an early stage of transcoelomic metastases of ovarian cancer (65).

Hippo/YAP Pathway

Simvastatin, a lipophilic statin was found to be highly effective against ovarian cancer (66). Cancer-initiating cells-enriched population generated xenografts upon intraperitoneal injections into highly immunodeficient NSG mice. Furthermore, metastatic foci were found to be more vascularized in tumors arising from CICs. There was an evident reduction in the distribution and number of metastatic implants in the mice intraperitoneally administered with simvastatin. Accordingly,

reduction in tumor burden was noted to be correlated with a significant reduction in the volume of ascites. Simvastatin enhanced the accumulation of inactive cytoplasmic form of p-YAP in ovarian cancer cells and chemo-naïve primary cultures (66)

miR-509-3p mimics attenuated migratory capacities of SKOV3 and OVCAR8 cancer cells (67). YAP1 levels were noted to be suppressed in response to miR-509-3p mimics in HEYA8 and OVCAR8 cancer cells (67). miR-199a-3p directly targeted YAP1 and promoted apoptosis of OV90 and SKOV-3 cancer cells (68).

FBXW7γ overexpression reduced the levels of NOTCH1, c-Myc and YAP1 in A2780 and SKOV3 cancer cells. Tumors derived from FBXW7γ-overexpressing A2780 cancer cells were smaller in size (69).

Platelets played contributory role in the metastasis of ovarian cancer in vivo models (70). Co-incubation of OVCAR8 and HEYA8 ovarian cancer cells with platelets potently reduced phosphorylated levels of YAP1(Ser127) and YAP1(Ser397). Moreover, co-incubation of OVCAR8 and HEYA8 cancer cells with platelets induced nuclear accumulation of YAP1 protein. There was a notable infiltration of transfused platelets into tumor tissue which led to an increase in the tumor weight after intraperitoneal injections of cancer cells. Number of metastatic nodules and tumor weights were reported to be increased in mice transfused with platelets. However, these effects of thrombocytosis on the number and total weight of metastatic nodules were abolished completely by YAP1 depletion (70).

Regulation of metastasis by circular RNAs

Studies have shown that circular RNAs acted as competitive endogenous RNAs and antagonized miRNA-mediated targeting of wide-ranging mRNAs. In this section, we have attempted to summarize how different circular RNAs sequestered miRNAs and promoted the expression of mRNAs of wide variety of genes.

Subcutaneous transplantation of hsa_circ_0061140-silenced SKOV3 cells considerably reduced the tumor volume (71). There was an evident increase in the level of miRNA-370 in hsa_circ_0061140-depleted groups. Contrarily, levels of FOXM1 were found to be suppressed in hsa_circ_0061140-silenced cells. Collectively, these findings suggested that hsa_circ_0061140 relieved the inhibitory effects of miRNA-370 on FOXM1 in ovarian cancer cells. However, inhibition of hsa_circ_0061140 facilitated miRNA-370-mediated targeting of FOXM1 (71).

BRD4 (Bromodomain-containing protein-4) is a member of BET family of nuclear proteins (72). Importantly, circCELSR1 enhanced metastasis of ovarian cancer cells by sponging away miR-598 to increase the expression of BRD4. Metastatic ovarian tumor tissues in abdominal cavity were noted to be considerably reduced in the nude mice xenografted with circCELSR1-silenced-ovarian cancer cells (72).

miR-6753-5p and miR-615-5p regulated the expression of MMP2 and NF-κB correspondingly (73). circPUM1 blocked miR-6753-5p and miR-615-5p mediated inhibition of MMP2 and NF-κB. Notably, tumor nodes and metastatic lesions were reported to be reduced in mice intraperitoneally injected with circPUM1-knockdown A2780 cancer cells. Expression of circPUM1 is higher in the exosomes derived from circPUM1-overexpressing CAOV3 cells. To unravel the pro-metastatic role of exosomal circPUM1 on peritoneally disseminated tumors, nude mice were injected with exosomes after intraperitoneal injections of CAOV3 cancer cells. Importantly, number of tumor foci was noted to be substantially enhanced and palpable tumor nodules were thoroughly disseminated in the mesentery, liver as well as peritoneum in the experimental groups treated with circPUM1-loaded-exosomes (73).

circWHSC1 interfered with miRNAs and potentiated the expression of MUC1 and hTERT (74). Nude mice were intraperitoneally injected with CAOV3 cancer cells for tumorigenesis and then injected with circWHSC1-loaded exosomes. Importantly, number of tumor nodules was increased significantly in abdominal cavities of the mice injected with circWHSC1-loaded exosomes (74).

Xenografted mice-based studies

UBR5 (Ubiquitin protein ligase E3 component n-recognin 5) is a HECT domain-containing ubiquitin ligase.

ID8-Luc is a novel syngeneic mouse model developed for the analysis of carcinogenesis and metastasis in orthotopic ovarian cancer. ID8 cells are ovarian surface epithelial cells. For a comprehensive analysis of $Ubr5^{-/-}$ ovarian tumors, ID8 $Ubr5^{-/-}$ cells were intravenously injected into mice (75). At 30 days post injection, there was a rapid increase in metastasis, i.e ~two times more metastatic spread of ID8/$Ubr5^{-/-}$ tumor cells to the lungs. However, there were few liver and pulmonary metastatic nodules in mice bearing ID8/$Ubr5^{-/-}$ at 60 days. Therefore, ID8/$Ubr5^{-/-}$ did not progress from lung micro-metastasis to macro-metastasis. UBR5 loss impaired MET and the colonizing abilities of ID8 tumors in the lungs. Progression of the tumors was inhibited in mice intraperitoneally injected with ID8/$Ubr5^{-/-}$ cells. Mice demonstrated markedly reduced tumor growth, impaired accumulation of the ascites and reduced peritoneal implantation as compared to ID8/GFP-bearing mice. Moreover, reduced number of macrophages in the lungs was reported in the mice intravenously injected with ID8/$Ubr5^{-/-}$ cells. $Ki67^{+}$ cells and $CD68^{+}$ TAMs from peritoneal ascites were noted to be significantly suppressed in ID8/$Ubr5^{-/-}$ bearing mice (75).

Concluding Remarks

Over the last three decades, our conceptual knowledge of the cell-biological and molecular mechanisms that modulate the metastatic cascades and the intricate interaction between cancer cells and tumor microenvironments has grown exponentially. The motility and invasive behavior of ovarian carcinoma cells are controlled by a repertoire of signaling cascades, various components of which have been identified as therapeutic targets in preclinical studies and in clinical trials. Despite substantial advancements related to early detection and conceptual breakthroughs in molecular oncology, efficient targeting of metastasis still remains

an overarching goal as it poses a major challenge in the clinical management of ovarian cancer.

Accordingly, detailed analysis of promising synthetic and natural products having potential clinical value may prove be at a turning point, with greater-than ever opportunities to learn new lessons from previously conducted clinical trials to explore new modalities and to integrate new findings in molecular oncology.

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