

# Epithelial-mesenchymal plasticity and cisplatin resistance in germ cell tumors: Mechanisms and emerging therapeutic strategies (Review)

ELENI SOLANGE DE BRITO GOMES<sup>1,2\*</sup>, TAINÁ MIOTTO DE SOUZA<sup>1,2\*</sup>, ANA LAURA PAIVA OLIVEIRA<sup>1,2</sup>, ANA FLAVIA SOUZA PERES BEZERRA<sup>1,2</sup>, INGRIDY IZABELLA VIEIRA CARDOSO<sup>1,2</sup>, LENILSON SILVA<sup>1,2</sup>, LUIZ FERNANDO LOPES<sup>2,3</sup>, MARCELA NUNES ROSA<sup>1,2</sup> and MARIANA TOMAZINI PINTO<sup>1-3</sup>

<sup>1</sup>Molecular Oncology Research Center, Barretos Cancer Hospital, São Paulo 14784400, Brazil;

<sup>2</sup>Pediatric Oncology Research Group, Molecular Oncology Research Center, Barretos Cancer Hospital,

São Paulo 14784400, Brazil; <sup>3</sup>Children's Cancer Hospital, Barretos Cancer Hospital, São Paulo 14784400, Brazil

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**Abstract.** Germ cell tumors (GCTs) are rare, heterogeneous neoplasms derived from primordial germ cells. Although they typically develop in the gonads, they may also arise in extragonadal locations along the midline of the body. Approximately 90% of patients respond well to cisplatin-based chemotherapy; however, ~30% exhibit treatment resistance. Epithelial-mesenchymal plasticity (EMP), a recognized hallmark of cancer, has been implicated in promoting metastasis and chemoresistance. Nonetheless, studies investigating the specific role of EMP in GCT treatment resistance remain limited. The present review compiles key studies on GCTs, EMP markers and cisplatin resistance using both *in vitro* and *in vivo* models; it highlights the roles of associated genes, transcription factors and proteins, identifying potential therapeutic targets. Advancing our understanding of EMP and identifying novel therapeutic targets may support the development of treatment strategies that complement or replace cisplatin. This, in turn, could improve survival outcomes and create new avenues for molecular research and clinical applications.

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## 1. Introduction

Germ cell tumors (GCTs) are rare, heterogeneous neoplasms derived from primordial germ cells (PGCs). While they typically develop in the gonads, they can also arise in extragonadal midline locations, particularly in children (1). Diagnosis often involves imaging studies and evaluation of serum markers, including  $\alpha$ -fetoprotein,  $\beta$ -human chorionic gonadotropin and lactate dehydrogenase (LDH) (2). GCTs account for ~3% of childhood cancers (3) and represent ~11% of adolescent cancer cases diagnosed post-puberty (4). In adults, although GCTs represent only ~1% of cancers, they are the most common testicular cancer in young adults (5) and account for 20-25% of all ovarian neoplasms (6).

The diverse histologies observed in GCTs arise from the totipotent nature of PGCs and the specific differentiation stage at which genetic alterations occur (7). According to Teilum's classification, germinomas, known as seminomas (SEs) in the testes and dysgerminomas in the ovaries, are undifferentiated cells with pluripotent features that originate directly from PGCs (Fig. 1). Embryonal carcinomas (ECs) arise after early embryonic differentiation and can develop into a variety of histological subtypes. When ECs follow an embryonic developmental pathway, they may differentiate into teratomas (TEs), which contain tissues derived from all three germ layers: Endoderm, mesoderm and ectoderm. Conversely, if cells undergo extra-embryonic differentiation, they may give rise to yolk sac tumors (YSTs), characterized by extra-embryonic mesoblast overgrowth, or choriocarcinomas

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*Correspondence to:* Dr Mariana Tomazini Pinto, Molecular Oncology Research Center, Barretos Cancer Hospital, 1332 Antenor Duarte Vilela Street, Barretos, São Paulo 14784400, Brazil  
E-mail: mariana.pinto49@edu.hospitaldeamor.com.br

\*Contributed equally

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(CC), which display trophoblastic differentiation (7,8). Non-germinomatous GCTs are generally more aggressive than germinomas, exhibiting increased proliferation and a higher metastatic potential (9,10). By contrast, germinomas are associated with a more favorable prognosis due to their high sensitivity to chemotherapy and radiotherapy (11).

Surgical resection is typically the first-line treatment for GCTs (7); however, systemic therapy is generally required for disease control. Etoposide- and cisplatin-based chemotherapy has remained the standard of care for >4 decades, despite the associated toxicity (12). Cisplatin remains the cornerstone of treatment, achieving cure rates of 80-90% (13,14). Nonetheless, ~30% of patients show an incomplete response or develop resistance to cisplatin, leading to poor clinical outcomes and reduced survival (14,15). Alternative treatments demonstrate response rates of only 20-40%, with median survival times of ~6-8 months (16).

The biology of cisplatin resistance is multifactorial (17) and has been linked to several molecular alterations, including changes in tumor protein p53, mouse double minute 2 homolog (12), DNA methylation patterns (18), dysregulation of the platelet-derived growth factor receptor  $\beta$ /AKT signaling pathway (19) and overexpression of ERBB4 oncogenic EGFR-like receptor (20). These resistance mechanisms are often categorized into four types: Pre-target (before DNA-binding); on-target (related to DNA-cisplatin adducts); post-target (cell death signaling pathways induced by cisplatin-mediated DNA damage); or off-target (involving pathways not directly linked to cisplatin-induced signals) (20). Therefore, comprehensive investigations into these resistance mechanisms are essential for identifying novel treatment strategies.

Epithelial-mesenchymal plasticity (EMP) has emerged as a key hallmark of cancer, particularly in its role as a driver of metastasis and chemoresistance (21). EMP is a process characterized by a series of molecular and morphological alterations, accompanied by the expression of specific markers, leading to the suppression of epithelial cell characteristics. During EMP, cells acquire mesenchymal properties, adopting a more malignant phenotype with enhanced invasion, migration and dissemination capabilities (22,23). However, given the paucity of studies investigating the association between EMP and cisplatin resistance in GCTs, the present review was conducted to compile and discuss the available evidence.

## 2. Unraveling the mechanisms and implications of EMP

EMP was initially described as epithelial-mesenchymal transition (EMT), a process in which cells were considered to exist in one of two distinct states, namely an epithelial or mesenchymal phenotype (24). Subsequently, the concept of EMT was redefined as a reversible and transient process, applicable to diverse contexts, and thus renamed EMP (25,26). A further study revealed that the cells can adopt intermediate or hybrid phenotypes, known as partial EMP, displaying characteristics of both epithelial and mesenchymal cells (27). At present, it is widely accepted that these hybrid states are prevalent in the tumor microenvironment (TM), where not all cells complete the full EMP process, meaning they may never fully acquire a mesenchymal phenotype (27).

Epithelial cells are tightly organized with minimal surrounding extracellular matrix (ECM); their distinct characteristics are defined by structural components such as tight junctions, adherens junctions, desmosomes and gap junctions, which maintain strong cell-to-cell adhesion and tissue integrity (28). These junctions are responsible for maintaining both structural and functional integrity. Specifically, they firmly hold cells in place and prevent individual cell displacement. These cell-to-cell interactions involve E-cadherin (CDH1)-mediated junctions and desmosomes, as well as cell-ECM interactions mediated by integrins and other molecules. Together, these interactions confer polarity to epithelial cells with distinct basal and apical functions (29). The polarity of epithelial cells is maintained by tight junctions, which create distinct apical and basal regions and form an effective barrier. This barrier regulates the passage of molecules and ions, thus preserving cell polarity. Adherens junctions, which are mediated by the transmembrane protein CDH1, serve to provide robust cell-to-cell adhesion by connecting to actin filaments, thereby ensuring structural stability (29). Desmosomes function as anchors for cells by interacting with intermediate filaments (30). Additionally, gap junctions, composed of connexins, facilitate direct intercellular communication. Together, these structures promote the integrity and cohesion of epithelial tissue, with key markers including CDH1, desmoplakin, cytokeratins, claudins and occludins (31).

In contrast to epithelial cells, mesenchymal cells display reduced intercellular adhesion and lack apical-basal polarity. These cells interact with ECM components through integrins at focal adhesion sites, enabling cell movement. The cells also feature a cytoplasm rich in vimentin (VIM) filaments, a mesodermal marker, and form irregular structures with enhanced migratory capacity. Other expressed markers include N-cadherin (CDH2), smooth muscle  $\alpha$ -actin ( $\alpha$ -SMA), fibroblast-specific protein 1 (FSP-1), fibronectin (FN1), type I collagen (COL1A1) and matrix metalloproteinases (MMPs) (32).

During EMP, epithelial cells lose cellular polarity by downregulating the expression of cytokeratins and adhesion molecules, such as CDH1. Concurrently, the expression of mesenchymal markers, such as  $\alpha$ -SMA, FSP-1, VIM, CDH2, FN1, COL1A1 and MMPs, is upregulated. Cells with polyhedral morphologies begin to adopt a fibroblast-like morphology (28). This transition is accompanied by enhanced migratory and invasive capacities, resistance to apoptosis and increased production of ECM components (32).

EMP can be triggered in various biological contexts by diverse signaling molecules. These molecules trigger distinct signaling pathways, ultimately activating a specific set of transcription factors (TFs), often referred to as 'master regulators' of EMP. These include members of the Snail family (Snail1 and Snail2/Slug), the zinc finger E-box-binding homeobox proteins (Zeb1 and Zeb2) and the Twist family (Twist1 and Twist2). These TFs suppress the expression of epithelial markers and activate mesenchymal markers (33).

EMP is categorized into three types. EMP type 1 has been associated with a variety of physiological processes, particularly during embryogenesis (Fig. 1), where it plays a key role in the migration and differentiation of cells that form the germ layers (34). These germ layers are the origin of tissue

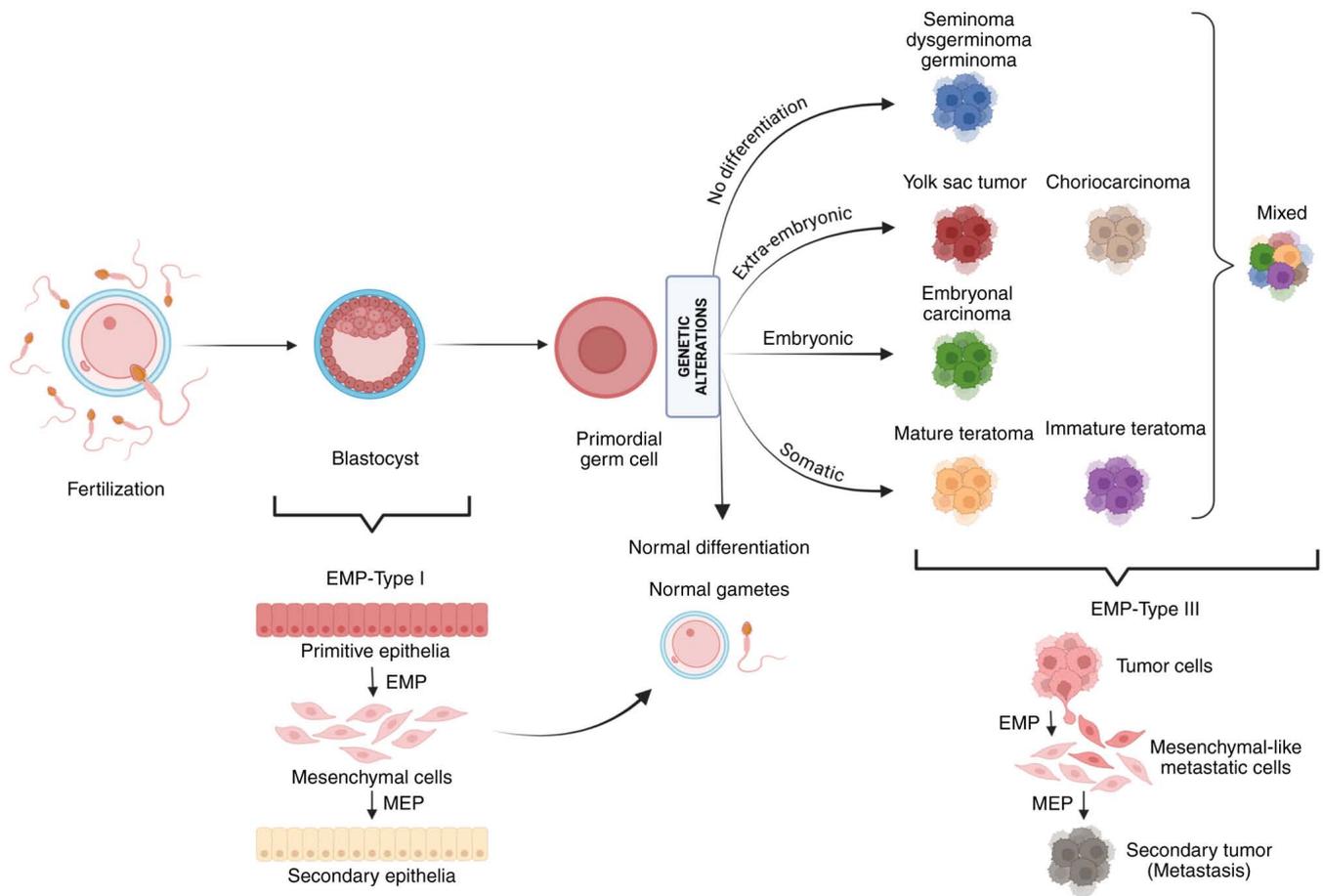


Figure 1. Schematic representation of germ cell tumor development and its association with EMP. Genetic alterations in primordial germ cells lead to different tumor subtypes, with EMP playing a role in organogenesis, tumor progression and metastasis. Created in BioRender. Souza Peres, A. (2025) <https://BioRender.com/g02j275>. EMP, epithelial-to-mesenchymal plasticity; MEP, mesenchymal-to-epithelial plasticity.

and organ development. EMP type 1 has been demonstrated to be non-aggressive and non-invasive; it has been shown to be essential for the proper functioning of physiological processes, including organ and tissue formation, placenta development and embryo implantation (35). EMP type 1 is also involved in the formation of melanocytes from the neural crest, which is essential for pigmentation (22).

EMP type 2 plays an important role in tissue regeneration and fibrosis, including conditions such as renal and pulmonary fibrosis, and can be categorized into physiological (tissue regeneration) and pathological (persistent fibrosis) processes (36). In its physiological form, cells mobilize into fibroblast-like phenotypes, facilitating tissue repair after trauma (37). This regenerative phase is linked to the inflammatory response, which subsides once inflammation is resolved, as observed in wound healing and tissue regeneration (37). However, when inflammation persists, physiological EMP type 2 can progress to a pathological state (31), contributing to chronic fibrosis and organ damage, as observed in the kidney and liver (38). This process is driven by inflammatory cells and fibroblast-like cells, which release various inflammatory signals (39) and contribute to a collagen-rich ECM. Protein markers, such as VIM, serve as molecular targets to identify signs of persistent inflammation that may progress to a chronic state, thereby establishing a link with EMP type 2 (38).

EMP type 3 is closely linked to cancer progression, underscoring the importance of studies exploring the connection between EMP and tumor dissemination (Fig. 1). Such studies can provide an understanding of the molecular mechanisms influencing signaling pathways that regulate EMP activation or its inhibition (40,41), potentially informing novel therapeutic strategies. Furthermore, identifying specific markers associated with EMP is essential for understanding a tumor's metastatic potential, improving patient survival predictions and developing targeted therapies for treatment-resistant cancer (42).

The acquired plasticity of cancer cells through EMP has been widely documented across various cancer types and is strongly associated with resistance to cisplatin (43,44). In the following sections, key studies are reviewed that explore the role of EMP markers in GCTs, focusing on their involvement in cisplatin resistance, drawing insights from both *in vitro* and *in vivo* models.

### 3. EMP analysis *in vitro* models of GCTs

Different cell lines, whose characteristics are presented in Table I, have been used to investigate the molecular mechanisms, genetic alterations and therapeutic responses in GCTs. Most cell lines have been isolated from the testes of adult

Table I. Characteristics of human germ cell tumor cell lines.

Cell lines	Primary tissue	Sex	Age, years	Histologies
NTERA-2	Testis	Male	22	Embryonal carcinoma
2102EP	Testis	Male	23	Embryonal carcinoma
1777N Rpmet	Testis <sup>a</sup>	Male	25	Embryonal carcinoma
NCCIT	Mediastinum	Male	24	Embryonal carcinoma
833KE	Abdomen	Male	19	Embryonal carcinoma
BeWo	Placenta	Male	Fetus	Choriocarcinoma
JAR	Placenta	Male	Fetus	Choriocarcinoma
JEG3	Placenta	Male	Fetus	Choriocarcinoma
TCam-2	Testis	Mal	35	Seminoma
SEM-1	Mediastinum	Male	58	Seminoma
GCT27	Testis	Male	NI	Teratoma
SuSa	Testis	Male	46	Teratoma
NOY-1	Ovary	Female	28	Yolk sac tumor
H12.1	Testis	Male	19	Mixed (embryonal carcinoma and teratoma)
1411HP	Testis <sup>b</sup>	Male	17	Mixed (embryonal carcinoma and yolk sac tumor)

<sup>a</sup>Retroperitoneal metastasis from testis. <sup>b</sup>Mestastatic testicular cancer. NI, not informed.

individuals (aged 22 years or older). Cell lines representing the various histological types of GCTs from children and adolescents are scarce or unavailable, except for choriocarcinoma cell lines from fetal placentas. This lack of pediatric and adolescent cell lines significantly hinders research and molecular studies. Next, the present study will review key studies that have used GCT cell lines to research biomarkers and therapeutic targets, offering insights into future therapeutic strategies.

*ECM remodeling and its role in tumor behavior.* Both normal and transformed mesenchymal and epithelial cells rely on ECM proteins for growth and differentiation *in vitro*. These proteins play a key role in processes such as motility, wound healing and tumor metastasis, mechanisms that are closely linked to EMP. In teratoma cell culture, cells secrete adhesion proteins such as vitronectin, FN1, laminin and type IV collagen (45). In human GCTs, differentiated cells in low-density cultures produce FN1 in a differentiation-dependent manner, with less differentiated cells exhibiting decreased FN1 synthesis (46).

In this regard, some studies indicate that modulation of those ECM proteins influences cell adhesion, plasticity and invasion capacity *in vitro* (47,48). In BeWo cells, the heme oxygenase-1 (*HMOX1*) gene, which is upregulated in various tumor types, has been shown to increase adhesion by modulating laminin and FN1. This process, dependent on peroxidasin homolog, a cell surface peroxidase, occurs at both the gene level and *HMOX1* protein level (Table II) (47). Therefore, the protein network activated by *HMOX1* influences key cellular functions, such as adhesion, signaling and transport, supporting tumor growth and dissemination (47).

The EC 2102EP cell line, derived from a primary tumor, and 1777NRpmet, a differentiated EC cell line with immature teratoma features derived from a metastatic tumor, were

analyzed in a previous study, revealing increased expression of tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein  $\beta$  and  $\gamma$  polypeptide, caldesmon 1, filamin A, *VIM* and vinculin genes in 1777NRpmet compared with the primary tumor, while *PARK7* expression decreased (48). These findings suggest a role for cell adhesion remodeling and ECM crosslinking in testes invasion, EMP and metastasis (48).

ECM remodeling has also been observed in cisplatin-resistant ovarian cancer cell lines, characterized by elevated expression of the collagen type VI  $\alpha$  3 chain gene. Furthermore, these cells demonstrated increased chemoresistance when cultured on collagen IV-coated dishes (49). Similarly, co-culture of GCT cell lines with TM cells has shown an interplay, where exposure to TM-secreted components such as collagen I/IV and FN1 decreased sensitivity to cisplatin (50). These studies demonstrate that several factors contribute to the critical role of ECM remodeling and adhesion molecules in triggering cell plasticity, cisplatin resistance and tumor cell survival.

Beyond ECM proteins, their interaction with other cell components, such as integrins and their signaling pathways, has also been implicated in the EMP process and treatment resistance (51,52). Integrin-linked kinase (ILK) is related to adhesion to the extracellular environment, transduction signaling, and interaction with  $\beta$ -catenin (CTNNB1) and cytoskeletal proteins. BeWo cells exhibit increased ILK activity during the differentiation process, along with the expression of phosphorylated AKT and SNAIL proteins (53).

During EMP, dynamic alterations occur not only in ECM-cell interactions but also in cell-cell adhesion, modulating adhesion and structural proteins. In this setting, CDH2 is upregulated while CDH1 is downregulated, a mechanism that plays a crucial role in malignancies, favoring tumor

cell metastasis and migration. Upregulation of *CDH1* may be linked to decreased migratory ability in cancer cells and increased sensitivity to cell death, likely due to the inhibition of the EMP process (43). These changes are widely associated with TFs such as *TWIST*, *ZEB1/2* and *SNAIL/SLUG* (54).

**EMP and transcriptional regulation.** EMP is governed by specific EMP TFs that orchestrate transcriptional reprogramming to promote the loss of epithelial traits and acquisition of mesenchymal properties.

Expression of *TWIST* and *CDH1* genes was previously evaluated in BeWo cells during differentiation and fusion (55). This differentiation process was accompanied by an increase in *TWIST* expression and a decrease in *CDH1* expression, even in the presence of exogenous *TWIST* expression. However, differentiation failed when *TWIST* was knocked down. Moreover, treatment with 8-Br-cAMP increased *TWIST* levels. This demonstrates the role of these molecules in the differentiation and invasive capacity promoted in the EMP process (55). A study on gestational trophoblastic disease evaluated the expression and influence of *TWIST1* gene silencing in the CC BeWo and JAR cell lines (56). It was revealed that this TF was upregulated, and its knockout significantly inhibited the proliferation, migration and invasion of these cells. Additionally, protein analysis revealed that silencing of the *TWIST1* gene led to increased expression of *CDH1* and decreased expression of *CDH2* and *VIM*. Thus, *TWIST1* silencing promotes an epithelial phenotype, inhibiting EMP and malignant behavior (56). Furthermore, increased *TWIST* protein expression was observed in cisplatin-resistant ovarian cancer cell lines (57).

In the CC BeWo and JEG-3 cell lines, overexpression of *ZEB2* enhanced cell migration and invasion capabilities. Alongside *ZEB2* upregulation, changes were observed in gene expression, cell morphology and protein levels (58). At the gene expression level, alterations occurred in EMP markers, though some divergence was noted among cell clones. Additionally, JEG-3 clones overexpressing *ZEB2* exhibited differential gene expression of other EMP markers, such as decreased *SNAIL* and increased *SLUG* and *TWIST1*, distinct from BeWo clones. This suggests that *ZEB2* overexpression activates cell-specific downstream pathways to promote EMP. Lastly, protein expression of EMP markers was more pronounced in BeWo cells than that in JEG-3 cells (58).

Our group investigated the role of EMP TF *SLUG* in GCTs (21). The analysis revealed distinct expression profiles of EMP markers across different histologies. SEs exhibited lower expression of EMP markers, while ECs and mixed GCTs showed higher expression. *SNAIL* and *SLUG* displayed varying expression levels in each histology, and patients with lower *SLUG* expression had a longer median progression-free survival time. Furthermore, integrated analyses showed that patients expressing low levels of both factors (*SNAIL<sup>low</sup>SLUG<sup>low</sup>*) had a higher progression-free survival rate compared to those with high expression of both TFs (*SNAIL<sup>high</sup>SLUG<sup>high</sup>*) (21).

The role of EMP mediators in enhancing invasiveness is evident across various GCT types, potentially involving different molecular pathways. In human SE, Securin (*PTTG1*) has been identified as an EMP mediator. This gene promotes invasiveness by expressing *MMP2* protein, which facilitates

migration and invasion (59). In the SE SEM-1 cell line, *PTTG1* relies on *ZEB1* to exhibit invasion and cell growth features, and to form an axis that represses *CDH1* expression. Additionally, database analysis shows that in seminoma tumors, where *PTTG1* is more localized in the nucleus compared with the non-seminoma subtype, *CDH1* expression is significantly lower than that observed in non-seminomas (59). Based on these observations, the *PTTG1-ZEB1-CDH1* axis appears particularly relevant in SEs compared with NGGCTs (59). However, further research is needed to elucidate the specific mechanisms through which these mediators influence tumor progression and response to treatment.

**Hypoxia, long non-coding RNAs and emerging mechanisms.** In addition to differentiation-related pathways, external environmental factors, such as hypoxia, influence resistance mechanisms and EMP in GCTs. The role of Notch receptor (NOTCH) signaling was previously investigated in CC, focusing on how it links hypoxia to EMP (60). Overexpression of hypoxia-inducible factor 1- $\alpha$  (HIF-1 $\alpha$ ) protein in JAR and JEG-3 cell lines reduced the expression of epithelial markers (*CDH1*, Cytokeratin 18 and Cytokeratin 19) while enhancing cell migration and invasiveness. HIF-1 $\alpha$  overexpression was positively correlated with NOTCH1 and Hairy and enhancer of split-1 proteins. Inhibiting NOTCH1 with N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester reversed the EMP changes, increasing *CDH1*, CK18 and CK19, while reducing the activities of *MMP2* and *MMP9*. Thus, HIF-1 $\alpha$  promotes CC metastasis through EMP via the NOTCH signaling pathway (60).

Moreover, JEG-3 cells exhibit higher HIF-1 $\alpha$  protein expression compared with control chorionic trophoblast cells. Knocking down *HIF-1 $\alpha$*  decreases cell proliferation and migration. Additionally, it is associated with reduced cell invasion, increased *CDH1*, and decreased *VIM* and  $\alpha$ -*SMA*, thereby suppressing EMP. *HIF-1 $\alpha$*  has been shown to modulate deleted in esophageal cancer 1 (*DECI*). However, when *DECI* is overexpressed, it partially reverses the effects of *HIF-1 $\alpha$*  knockdown, indicating that both *HIF-1 $\alpha$*  and *DECI* regulate EMP mediators (*VIM*,  $\alpha$ -*SMA* and Wnt/CTNNB1 signaling) (61). In one study using BeWo cells and the early placenta, hypoxic conditions induced higher mRNA levels of genes encoding FN1 domains and integrin  $\alpha$ -5 compared with normoxic conditions, while the levels of integrin  $\alpha$ -1 mRNA decreased (62).

Hypoxia is also associated with vasculogenic mimicry (VM), a process whereby cancer cells form tubular channels mimicking blood vessels. These cells may undergo EMP to exhibit an endothelial-like phenotype, enhancing tumor aggressiveness and facilitating metastasis (63). In this context, in a previous study, JAR and JEG-3 cells treated with forskolin, a cAMP activator, exhibited increased VM, migration and invasive capacity. These cells produced *MMP2* and *MMP9* at both the gene and protein levels, increasing the expression of mesenchymal markers via NOTCH1 signaling. NOTCH1 signaling can be triggered under hypoxic conditions and is linked to EMP markers (63).

In GCTs, hypoxia reduces *POU5F1* protein levels in EC cells, contributing to cisplatin resistance. However, overexpression of the sentrin-specific peptidase 1 (*SENPI1*) gene normalizes *POU5F1* protein levels and restores drug sensitivity (64).

Similarly, in EC NTERA-2, 2102EP and NCCIT cell lines exposed to cisplatin under hypoxic and normoxic conditions, cisplatin was less effective under hypoxia. Hypoxic cells displayed a higher IC<sub>50</sub> (NCCIT>2102EP>NTERA-2), indicating that hypoxia reduces cytotoxicity not only for cisplatin but also for other drugs, suggesting it is not exclusively linked to cisplatin resistance in GCTs (65).

Another factor associated with EMP is long non-coding RNAs, such as *SPRY4*, *LINC00467* and *LINC00313*, which regulate gene expression. *LINC00313* was found to be upregulated in TGCT cells, serving as a good biomarker for diagnosis and prognosis. *In silico* analyses revealed that *LINC00313* was associated with lower immune cell infiltration (CD4<sup>+</sup>, CD8<sup>+</sup> and dendritic cells) and an altered immune microenvironment (66). *In vitro* studies demonstrated that *LINC00313* promotes migration and invasion, and acts as an EMP mediator by upregulating VIM, ZEB1, SNAIL, CTNBN1 and CDH2 proteins, potentially through microRNA (miRNA/miR)-138-5p, miR-150-5p, miR-204-5p and miR-205-5p (66). Moreover, long non-coding RNAs are reportedly involved in cisplatin resistance in other tumors (43). Thus, targeting *LINC00313* could be a promising strategy to overcome cisplatin resistance by inhibiting EMP, thereby reducing cancer cell proliferation, migration and invasion.

Despite the scarcity of studies exploring these associations, research on EMP markers in GCT cell lines, primarily BeWo, JAR and JEG-3 (53-56,58,63), has focused on differentiation, invasion and EMP in pregnancy-related phenomena rather than GCTs. Even fewer studies have evaluated EMP in GCTs and its association with cisplatin resistance. In summary, various GCT cell lines have provided valuable insights into the molecular mechanisms underlying EMP. ECM remodeling, integrin signaling and dynamic regulation of key TFs, such as *TWIST*, *ZEB1* and *SLUG* are critical drivers of EMP and tumor progression. However, the limited availability of pediatric and adolescent GCT cell lines poses a significant challenge to understanding the EMP mechanisms in these populations. Additionally, external factors, such as hypoxia and long non-coding RNAs, add complexity to the regulation of EMP and chemoresistance in GCTs.

#### *EMP pathway intersections underlying cisplatin resistance.*

As aforementioned, cisplatin resistance mechanisms can be classified as pre-target, on-target, post-target and off-target (20). A comparison between NTERA-2 cells and their resistant counterparts, NTERA-2R cells, reveals similar behavior in drug uptake, efflux and DNA-binding. However, the resistant lineage exhibits alterations in cell cycle regulation and cell death response. These findings suggest that resistance mechanisms in NTERA-2R are less related to DNA binding and damage induction, and more associated with cellular responses to this damage, implicating post-target or off-target mechanisms (13). Supporting this hypothesis, our group demonstrated that NTERA-2R cells showed upregulation of DNA repair-related genes (O-6-methylguanine-DNA methyltransferase; complex subunit, DNA damage recognition and repair factor; and DNA polymerase delta 4, accessory subunit) compared with NTERA-2 cells (Table III). This overexpression was accompanied by more aggressive cellular behaviors, such as increased proliferation, colony formation

and migration. As expected, NTERA-2R cells exhibited a lower rate of apoptosis following cisplatin treatment compared with NTERA-2 cells. However, combining the proteasome inhibitor MG-132 with cisplatin made the apoptosis rate of the two cell lines comparable, highlighting a potential combinatorial strategy to overcome cisplatin resistance (67).

Regarding DNA repair-related protein expression, a study involving NTERA-2, NCCIT and 2102EP cell lines, and their resistant counterparts, revealed deregulated proteins in at least two resistant cell lines. These included downregulation of cystathionine  $\beta$ -synthase and cystathionine  $\gamma$ -lyase, alongside upregulation of annexin A1 (ANXA1), L-LDH A chain and NADPH-adrenodoxin oxidoreductase. Additionally, transgelin (TAGLN) was upregulated in NTERA-2R cells but downregulated in 2102EP-R cells, while COL1A1 and VIM showed contrasting expression patterns, being upregulated in NTERA-2R cells but downregulated in NCCIT-R and 2102EP-R cells, respectively (Table III). Among these proteins, ANXA1, TAGLN, COL1A1 and VIM are associated with EMP in GCTs or other cancer types (68-70). Gene enrichment analysis detected DNA repair-associated proteins across all resistant lineages, but an EMP gene set was detected in NTERA-2R cells. These results further corroborate the association of cisplatin resistance and EMP in GCTs (14).

Our group also evaluated EMP markers in NTERA-2 and NTERA-2R cells through mRNA quantification, with some findings confirmed at the protein level. After 72 h of cisplatin treatment, mRNA levels of *FNI*, *VIM*,  $\alpha$  smooth muscle actin, *COL1A1*, *TGF- $\beta$*  and *SLUG* were higher in resistant cells compared with those in parental cells (Table III) (21). Additionally, *CDH1* and its corresponding protein exhibited an increasing trend in NTERA-2R, while *CDH2* and its protein displayed a decreasing trend (21). No differences were observed in *SNAIL* expression levels (21). Together, the findings suggest that EMP may be involved in chemoresistance (21).

Upregulation of cancer stem cell (CSC) markers is a well-documented phenomenon in chemoresistant solid tumors, with aldehyde dehydrogenase (*ALDH*) as a key marker (71,72). However, in GCTs, this upregulation is not always consistent. A comparative study of cisplatin-sensitive NTERA-2 cells and their resistant counterpart (NTERA-2R) revealed increased gene expression levels of aldehyde dehydrogenase 1 family member A1 (*ALDH1A1*), *ALDH1A3* and *NANOG*, alongside decreased levels of *ALDH1A2* and *ALDH1B1* in the resistant NTERA-2 cells. However, *NANOG* and *SOX2* protein levels were decreased in the NTERA-2R cells. In another EC cell line, NCCIT-R, *ALDH1A2*, *ALDH1A3*, *NANOG* and *prominin-1* (*CD133*) levels were increased, but protein levels showed no significant changes (Table III). Moreover, treatment with the ALDH inhibitor disulfiram reduced cell viability, which was potentiated by combined cisplatin treatment. This combination also reduced the viability of resistant cells in a 3D spheroid model (73).

Similarly, CSC markers were evaluated in an ovarian YST cell line (NOY-1) and its cisplatin-resistant variant (NOY-1-R). The resistant cells exhibited increased protein expression of CD133, ATP binding cassette subfamily G member 2 (JR Blood Group) (ABCG2) and ALDH3A1. This upregulation was associated with reduced gene promoter methylation, increased expression of ALDH1A3 and higher ALDH enzymatic activity

Table II. Association between EMP and resistance in different germ cell tumor cell lines.

A, Embryonal carcinoma			
First author, year	Cell lines	Association with EMP	(Refs.)
Pulzová <i>et al</i> , 2021	1777N and Rpmet	Cell adhesion remodeling.	(48)
Liu <i>et al</i> , 2022	NCCIT	Long non-coding RNA <i>LINC003131</i> is upregulated in TGCT tissues. Moreover, <i>LINC003131</i> -silencing is associated with decreased migration and invasion in the NCCIT cell line. Potential inhibitor: Panobinostat.	(66)
Abada <i>et al</i> , 2014	NTERA-2	Cisplatin triggers differentiation which leads to resistance. This process is linked with reduced expression of <i>NANOG</i> and <i>POU5F1</i> and increased <i>NES</i> , <i>STMN2</i> and <i>FN1</i> .	(81)
Wu <i>et al</i> , 2012	NCCIT and NTERA-2	Hypoxia reduces levels of POU5F1 protein which triggers drug resistance. <i>SENPI</i> can normalize POU5F1 levels. Potential inhibitor: SENP1.	(64)
Koch <i>et al</i> , 2003	NTERA-2, 2102 EP and NCCIT	Hypoxia reduces drug cytotoxicity. Potential inhibitor: Erythropoietin.	(65)
B, Choriocarcinoma			
First author, year	Cell lines	Association with EMP	(Refs.)
Tauber <i>et al</i> , 2010	BeWo	High expression of <i>HMOX1</i> gene and ECM remodeling.	(47)
Butler <i>et al</i> , 2009	BeWo	Increased ILK, along with the expression of p-AKT and SNAIL proteins.	(53)
Ng <i>et al</i> , 2011	BeWo	Increased invasive capacity with increased <i>TWIST</i> and decreased <i>CDH1</i> .	(55)
Iwaki <i>et al</i> , 2004	BeWo	Hypoxia increases mRNA levels of <i>fibronectin</i> domains and <i>integrin α-5</i> , but not <i>integrin α-1</i> .	(62)
DaSilva-Arnold <i>et al</i> , 2019	BeWo	Overexpression of <i>ZEB2</i> changes the morphology of BeWo and JEG-3 cells towards a mesenchymal phenotype and promotes a gene expression profile consistent with EMP.	(58)
Xu <i>et al</i> , 2022	JEG3	High expression of HIF-1α is found. HIF-1α depends on <i>DEC1</i> to trigger EMP by β-catenin signaling. Potential inhibitor: DEC1 inhibitors.	(61)
Zhang <i>et al</i> , 2024	BeWo and JAR	Transcription factor <i>TWIST1</i> is found to be upregulated compared with a normal extravillous trophoblast cell line.	(56)
Tian <i>et al</i> , 2015	JAR and JEG-3	Overexpression of HIF-1α depends on NOTCH signaling to promote EMP. Potential inhibitor: DAPT.	(60)
Xue <i>et al</i> , 2020	JAR JEG-3	Forskolin promotes invasion, migration, vasculogenic-like network formation and NOTCH-1-mediated EMP.	(63)
C, Seminoma			
First author, year	Cell lines	Association with EMP	(Refs.)
Teveroni <i>et al</i> , 2022	SEM-1	<i>PTTG1</i> depends on <i>ZEB1</i> to repress CDH1. This cooperation facilitates cell invasion and cell growth.	(59)
D, Various cell lines			
First author, year	Cell lines	Association with EMP	(Refs.)
Skowron <i>et al</i> , 2022	TCam-2, 2102EP, JAR and GCT72	ECM remodeling promotes migration and invasion. Secreted collagen I/IV and fibronectin causes decreased sensitivity to cisplatin.	(50)

CDH1, E-cadherin; HMOX1, heme oxygenase-1; EMP, epithelial-mesenchymal plasticity; PTTG1, Securin; NES, Nestin; STMN2, Stathmin 2; FN1, fibronectin; POU5F1, POU Class 5 Homeobox 1; SENP1, sentrin-specific peptidase 1; ECM, extracellular matrix; p-, phosphorylated; CDH1, E-cadherin; HIF-1α, hypoxia-inducible factor 1-α; DEC1, deleted in esophageal cancer 1.

Table III. Additional molecular mechanisms of resistance observed in germ cell tumors.

First author, year	Cell lines	Association with resistance	(Refs.)
Lengert <i>et al.</i> , 2022	NTERA-2 and NTERA-2R	DNA repair genes increased: <i>MGMT</i> , <i>XPC</i> and <i>POLD4</i> . Potential inhibitor: Proteasome inhibitor (MG-132).	(67)
Fichtner <i>et al.</i> , 2022	NTERA-2R, NCCIT-R and 2102EP-R	Upregulated proteins ANXA1, TAGLN, COL1A1 and VIM. Upregulated protein ANXA1; downregulated COL1A1.	(14)
Cardoso <i>et al.</i> , 2024	NTERA-2R	Upregulated protein ANXA1; downregulated TAGLN and VIM. Cisplatin treatment increased <i>FN1</i> , <i>VIM</i> , <i>ACTA2</i> , <i>COL1A1</i> , <i>TGF-<math>\beta</math></i> and <i>SLUG</i> .	(21)
Schmidtova <i>et al.</i> , 2019	NTERA-2	Increase in <i>ALDH1A1</i> , <i>ALDH1A3</i> and <i>NANOG</i> . Decrease in <i>ALDH1A2</i> and <i>ALDH1B1</i> . Decreased proteins NANOG and SOX2.	(73)
Schmidtova <i>et al.</i> , 2019	NCCIT-R	Increase in <i>ALDH1A2</i> , <i>ALDH1A3</i> , <i>NANOG</i> and <i>CD133</i> . No protein expression changes. Potential inhibitor: ALDH inhibitor (Disulfiram).	(73)
Miranda-Gonçalves <i>et al.</i> , 2021	NCCIT-R	Increased VIRMA.	(90)
Port <i>et al.</i> , 2011	NTERA-2R, NCCIT-R and 2102EP-R	Upregulation of hsa-miR-10b and hsa-miR-512-3p.	(91)
Lobo <i>et al.</i> , 2020	NTERA-2R	Increased expression of genes <i>HDAC8</i> , <i>9</i> and <i>11</i> . Only HDAC11 protein was significantly expressed. Potential inhibitor: HDAC inhibitors (Belinostat and Panobinostat).	(17)
Schmidtova <i>et al.</i> , 2020	NOY-1-R	Increase of CD133, ABCG2 and ALDH3A1. Reduced gene and promoter methylation. Increased expression of ALDH1A3. accompanied by higher ALDH enzymatic activity.	(74)
Roška <i>et al.</i> , 2020	H12.1D	Upregulated <i>TRIB3</i> ; <i>IGFBP2</i> , <i>NANOG</i> , <i>POU5F1</i> and <i>SOX2</i> genes downregulated.	(75)
Schmidtova <i>et al.</i> , 2021	TCam-2R and NCCIT-R	Increased gene and protein expression of $\beta$ -catenin.	(84)
Schmidtova <i>et al.</i> , 2021	NTERA-2R	Decreased $\beta$ -catenin and cyclin D1 expression levels. Potential inhibitor: Wnt/ $\beta$ -catenin inhibitor (PRI-724).	(84)
Funke <i>et al.</i> , 2023	JAR-R and 2102EP-R	Overexpression of <i>NAE1</i> . Potential inhibitor: NAE1 inhibitor (MLN4924).	(88)
Skowron <i>et al.</i> , 2020	TCam-2, 2102EP, NCCIT and JAR	Increased gene and protein CDK4/CDK4; CDK6/CDK6 expression weak or absent. Potential inhibitor: CDK4/6 inhibitors (palbociclib and ribociclib).	(92)
Noel <i>et al.</i> , 2010	833K-R, Susa-R and GCT27-R	Upregulation of CCND1.	(93)

VIM, vimentin; FN1, fibronectin; COL1A1, type I collagen; EMP, epithelial-mesenchymal plasticity; VIM, vimentin; FN1, fibronectin; miR, microRNA; MGMT, O-6-methylguanine-DNA methyltransferase; XPC, XPC complex subunit, DNA damage recognition and repair factor; POLD4, DNA polymerase delta 4, accessory subunit; ANXA1, annexin A1; TAGLN, Transgelin; ACTA2, actin alpha 2, smooth muscle; ALDH1A1, aldehyde dehydrogenase 1 family member A1; VIRMA, Vir-like M6A methyltransferase associated; HDAC, histone deacetylase; ABCG2, ATP-binding cassette subfamily G Member 2 (JR Blood Group); ALDH, Aldehyde Dehydrogenase Family Member; TRIB3, tribbles pseudokinase 3; IGFBP2, insulin like growth factor binding protein 2; NAE1, NEDD8 activating enzyme E1 subunit 1; CCND1, cyclin D1.

(Table III). These findings underscore the potential role of the ALDH protein family in cisplatin resistance in refractory YST and suggest cross-resistance to ALDH inhibitors in cisplatin-resistant GCTs (74).

Pluripotency markers have also been examined in cisplatin-sensitive cell lines (H12.1, 2102EP and NTERA-2) and their resistant counterparts (H12.1D, 1411HP and 1777NRpmet). Several genes related to pluripotency, cell metabolism, proliferation and migration were identified. Upregulated genes

included *PCP4*, tribbles pseudokinase 3 (*TRIB3*), *ID2* and *SLC40A1*, while downregulated genes included insulin like growth factor binding protein 2 (*IGFBP2*), *LIT1D1*, *NANOG*, *POU5F1* and *SOX2* (75) (Table III). Among these, *TRIB3* (76), *IGFBP2* (77), *NANOG* (78), *POU5F1* (79) and *SOX2* (80) are associated with EMP-related processes.

Signaling pathways related to differentiation can modulate FN1 expression in cisplatin-resistant NTERA-2 cells. In a previous study, cisplatin treatment reduced the expression of

the TFs *NANOG* and *POU5F1*, which maintain the undifferentiated state, while promoting expression of differentiation markers, such as nestin (*NES*), *Stathmin-2* (*STMN2*) and *FNI*. Cells that did not downregulate *NANOG* and *POU5F1* failed to develop resistance to cisplatin. Overexpression of *NANOG* prevented cisplatin-induced resistance, indicating a link between cellular differentiation and drug resistance (81).

The Wnt/CTNNB1 signaling pathway is known to play a role in GCTs (82,83). The effects of Wnt/CTNNB1 signaling pathway inhibition using PRI-724 were evaluated in GCT cell lines (NTERA-2, JEG-3, TCam-2 and NCCIT) and their resistant variants (NTERA-2R, JEG-3-R, TCam-2-R and NCCIT-R). Gene and protein expression levels of CTNNB1 and cyclin D1 were increased in TCam-2-R and NCCIT-R cells but decreased in NTERA-2R cells. Treatment with PRI-724 induced pro-apoptotic effects (activation of caspases 3/7) in all cell lines, indicating that the Wnt/CTNNB1 pathway contributes to resistance (84).

EMP can be regulated by post-translational modifications (85), which are known to influence cancer aggressiveness and cisplatin resistance in tumors (86,87). One such process is neddylation, which involves conjugation of the ubiquitin-like molecule NEDD8 to a target protein, altering its stability, function or subcellular localization. Neddylated proteins may undergo degradation via the ubiquitin-proteasome system, and aberrant degradation of tumor suppressor proteins through this pathway can contribute to carcinogenesis (88,89).

A genome-scale CRISPR/Cas9 screen showed upregulation of NEDD8-activating enzyme E1 subunit 1 (*NAE1*) gene in cisplatin-resistant colonies of JAR<sub>MPHV2/SAMv2</sub> and 2102EP<sub>MPHV2/SAMv2</sub> cell lines. Inhibition of neddylation with MLN4924 sensitized these cells to cisplatin, resulting in apoptosis, G<sub>2</sub>/M cell cycle arrest,  $\gamma$ H2AX/p27 accumulation and differentiation into mesoderm/endoderm lineages in TGCT cells, while fibroblasts remained unaffected (88). *NAE1* inhibition has also been evaluated in ovarian cancer, in which, both *in vivo* and *in vitro*, exposure to this inhibitor augmented cisplatin activity, showing synergetic capacity, and even resensitized cisplatin-resistant cells/tumors. The drug caused stabilization of NEDD8, and it also promoted apoptosis induced by oxidative stress (86).

Epigenetic alterations, particularly in RNA, have been studied, with N<sup>6</sup>-methyladenosine (m<sup>6</sup>A) being the most common mRNA modification. In GCTs, the protein levels of m<sup>6</sup>A writer complex vir-like m<sup>6</sup>A methyltransferase associated (*VIRMA*) were higher in cisplatin-resistant NCCIT-R cells compared with those in NCCIT cells (88). Knockdown of *VIRMA* reduced m<sup>6</sup>A abundance, increased cisplatin sensitivity, and decreased cell viability, tumor cell proliferation, migration and invasion in NCCIT cells (90).

miRNA expression was also evaluated in the NTERA-2, NCCIT and 2102EP cell lines, and their cisplatin-resistant counterparts (NTERA-2R, NCCIT-R and 2102EP-R). Differentially expressed miRNAs included hsa-miR-10b and hsa-miR-512-3p, which were both upregulated 2-3-fold in all resistant cell lines. Notably, hsa-miR-10b was implicated in breast cancer invasion and metastasis (91).

Histone deacetylases (HDACs), which regulate chromatin accessibility, were investigated in GCT cell lines. Differential expression of HDACs, including HDAC1, HDAC2 and HDAC7,

was observed. TCam-2 cells displayed lower histone expression. In NTERA-2R cells, significant gene-level expression of *HDAC8*, *HDAC9* and *HDAC11* was observed. However, only HDAC11 showed significant protein expression. Treatment with HDAC inhibitors, such as belinostat and panobinostat, reduced cell viability in a time- and dose-dependent manner, inducing cell cycle arrest and apoptosis. Pre-treatment with non-toxic doses of belinostat enhanced cisplatin sensitivity (17).

Cell cycle dysregulation was also analyzed in TGCTs. Gene and protein analysis revealed high expression of *CDK4/CDK4* in TCam-2, 2102EP, NCCIT and JAR cell lines, whereas *CDK6/CDK6* expression was weak or absent (Table III). Treatment with CDK4/6 inhibitors (palbociclib and ribociclib) reduced cell viability and induced cell cycle arrest and apoptosis, suggesting these inhibitors as potential therapeutic options for both cisplatin-sensitive and -resistant GCTs (92). Cisplatin resistance was associated with cyclin D1 (*CCND1*) upregulation in resistant cell lines (833K-R, Susa-R and GCT27-R), encoding cyclin D1, a protein involved in G<sub>1</sub>/S cell cycle transition. Knockdown of *CCND1* reduced cell viability and increased cell death following cisplatin treatment (93).

Cisplatin-resistant GCT cell lines exhibit alterations across multiple biological processes, including DNA repair (94), CSC markers (95), post-translational modifications, epigenetic changes (85), epitranscriptomic alterations and cell cycle dysregulation (96). This current review focuses on how these processes converge on EMP, either as drivers or consequences, providing potential therapeutic targets for treatment.

Despite valuable insights from available studies, critical limitations must be acknowledged. Much of the data on EMP in GCTs are derived from choriocarcinoma-derived cell lines (BeWo, JAR and JEG-3), which, although informative, primarily model placental development and pregnancy-related pathologies rather than non-gestational GCTs. This limits the translatability of findings to the broader spectrum of GCTs, especially those in pediatric and adolescent populations. Moreover, few studies have directly investigated the association between EMP and cisplatin resistance in GCTs, leaving gaps in understanding how these processes intersect and contribute to treatment failure. The reliance on limited *in vitro* models and the scarcity of cell lines representing various histological subtypes and age groups further constrain generalizability. Future research should focus on developing and characterizing additional GCT models, particularly from pediatric cases, and integrating EMP-related mechanisms with chemoresistance pathways to uncover clinically relevant targets.

#### 4. EMP analysis in *in vivo* models of GCTs

Although recent studies have focused on elucidating key insights into plasticity, to the best of our knowledge, few studies demonstrate a specific association between animal experimentation, GCT cellular models and cisplatin resistance. It is evident that numerous studies are limited to standardizing cellular models for xenografts or developing animal models that best represent neoplasia development. These efforts are intended to facilitate future detection in humans through patient-derived xenograft (PDX) models (Fig. 2). Therefore, in this section, the present study will discuss markers of cellular plasticity and approaches in cisplatin resistance models in GCTs.

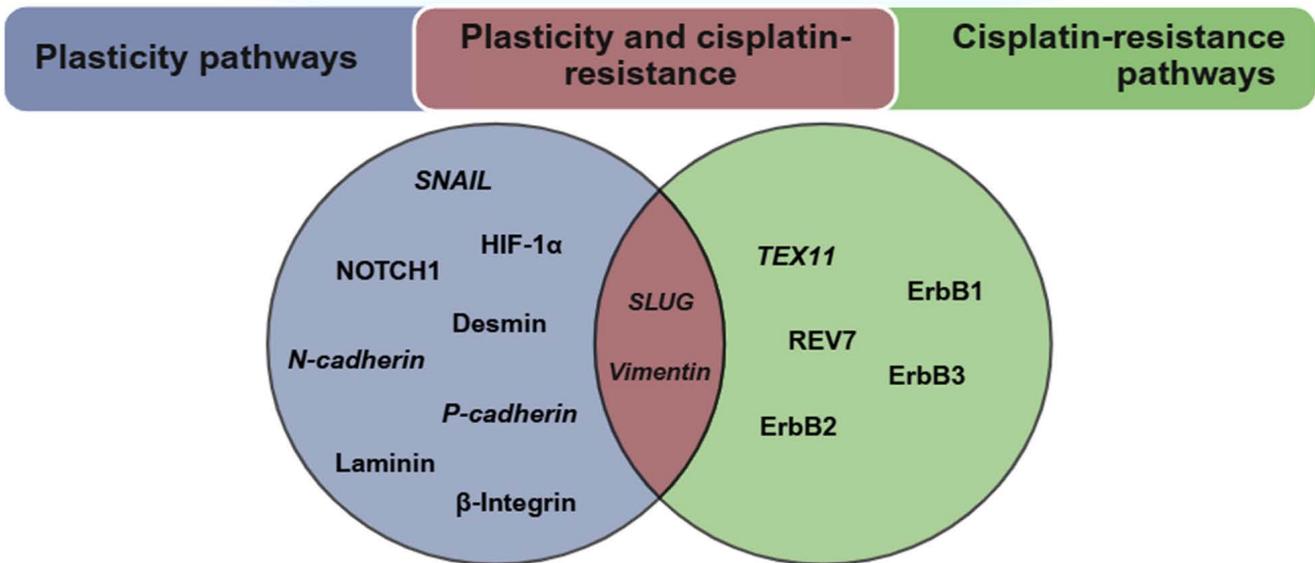
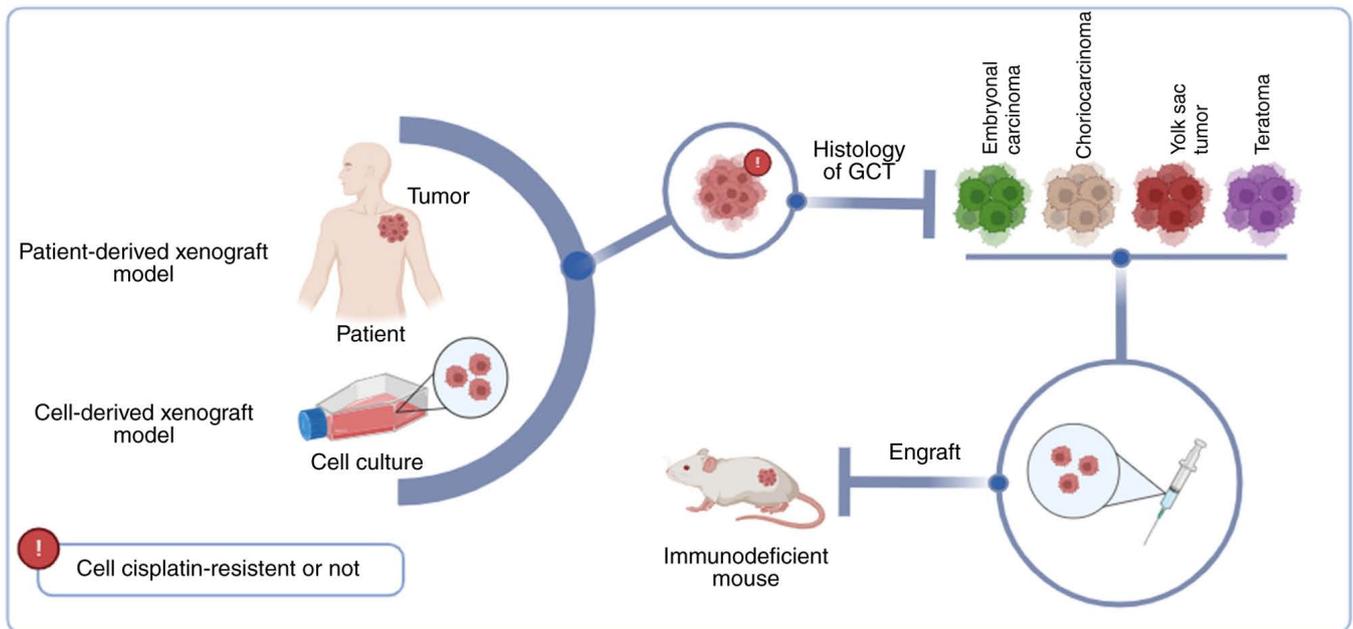


Figure 2. Overview of *in vivo* GCT models and key findings on plasticity and cisplatin resistance. Cellular plasticity pathways, shown in blue, include the genes *SNAIL*, *SLUG*, *HIF-1 $\alpha$* , *N-cadherin* and *P-cadherin* and the proteins NOTCH1, desmin, laminin and  $\beta$ -integrin. Cisplatin resistance pathways, in green, involve the *TEX11* gene and the proteins REV7, ErbB1, ErbB2 and ErbB3. *SLUG* and *vimentin* genes are the only plasticity markers linked to cisplatin resistance in GCTs (in brown). Created in BioRender. Pinto, M. (2025) <https://BioRender.com/z54o788>. GCT, germ cell tumor; HIF-1 $\alpha$ , hypoxia-inducible factor 1- $\alpha$ ; *TEX11*, testis-expressed 11; REV7, reversionless 7.

**Markers of cellular plasticity in GCTs.** Most studies on cellular plasticity in GCTs focus on key TFs, proteins expressed during this phenotypic transition in differentiating cells, and splice variants linked to tumor angiogenesis directly associated with plasticity proteins. The lack of data connecting animal experimentation, GCT cellular models and cisplatin resistance can be attributed primarily to experimental limitations and notable differences between human and animal tumors (97).

In murine EC stem cells, commonly used *in vivo* experiments, the cytoskeleton initially expresses VIM, followed by keratin polypeptides after differentiation (97). By contrast, human EC cells exhibit a divergent pattern of gene expression, initially producing keratin polypeptides and subsequently undergoing spontaneous

or induced differentiation, resulting in the expression of VIM and other intermediate filaments, including neurofilaments (97). For this reason, studies have used xenograft models to address these differences, as discussed below.

Species differences were evident in a study of xenografts of human teratocarcinoma NTERA-2 and 2102EP cells in nude mice, which produced solid tumors (97). Using a polyclonal antibody for human epidermal keratin raised in rabbits and three monoclonal antibodies for specific keratin polypeptides (AE-1, AE-3 and RGE53), researchers analyzed intermediate filament protein expression (97). The results showed that tumors derived from NTERA-2 reacted with all keratin antibodies and exhibited positive cells for neurofilaments and

mesenchymal areas containing VIM and desmin. By contrast, 2102EP-derived tumors expressed only keratin polypeptides. These findings demonstrated differences in intermediate filament expression between human and murine teratocarcinomas (97).

An important aspect of plasticity is the role of TFs such as HIF-1 $\alpha$  and SNAIL (98). HIF-1 $\alpha$  has therapeutic potential in cases where chemotherapy has been unsuccessful, as elevated HIF-1 $\alpha$  levels have been observed in CC. Positive HIF-1 $\alpha$  expression is correlated with NOTCH1 in CC cell lines, suggesting that HIF-1 $\alpha$ -induced plasticity depends on NOTCH1 signaling (98). Reduction of endogenous NOTCH1 signaling was associated with disruption of plasticity, while its activation was linked to increased invasion and metastasis in cells overexpressing HIF-1 $\alpha$  in *in vivo* models of CC. Thus, NOTCH1 is directly related to invasion and metastasis in CC, and its inhibition may be a promising therapeutic target by limiting invasion and metastasis through suppression of plasticity (98).

Another TF, SNAIL, has been found to be strongly associated with plasticity. Evidence shows that both SNAIL and SLUG are present in the germ cells of normal human testes, similar to observations in mice (99,100). Positive regulation of SNAIL has been associated with the induction of metastasis and poor prognosis, while its silencing suppresses tumor growth and invasiveness in breast cancer (101). Although negative regulation of SNAIL has been observed in CC cells treated with a NOTCH1 inhibitor, this evaluation was conducted only *in vitro* (98).

Studies have analyzed the direct or indirect presence of proteins characteristic of the mesenchymal phenotype in *in vivo* models, such as CDH2, laminin and integrin  $\beta$ -1 (102,103). Through the mating of mice with recessive and null N- or P-cadherin mutations, pluripotent embryonic stem cells generated *in vitro* underwent differentiation *in vivo* into TEs (102). The results showed that the differentiation and histogenesis occurred within the TEs, as cells lacking N- and P-cadherin exhibited predominantly adherent structures and significant qualitative and quantitative differentiation. Although the cells were inoculated near the animals' lymph nodes, none metastasized or caused mortality in the host, with the studies noting that some cells likely still expressed *CDH1* (102).

Furthermore, a study using syngeneic 129/Sv male mice examined the impact of the absence of integrin  $\beta$ -1, a protein involved in recognizing various laminins and collagen IV, on teratoma development. Absence of integrin  $\beta$ -1 was found to be efficient for analysis compared with TEs derived from wild-type cells (103). Two stem cell lines, D3 (wild-type) and G201 (integrin  $\beta$ -1-deficient via double knockout of the integrin gene), were inoculated into the mice. After 21 days, tumors were surgically collected. The results showed that animals inoculated with the deficient cell line produced 90% fewer TEs compared with the wild-type, with abundant epithelial cells but losses in cuboidal shape, irregular layer arrangement and reduced fluorescence in laminin  $\alpha$ -1 staining (103). The mutant epithelial cells exhibited a partially widened basal membrane, loop formation and multilayered cells. Additionally, selective negative regulation of laminin-1 was observed, indicating a loss of molecular contacts with cellular receptors and aberrant structural characteristics (103).

It was demonstrated that F9 mouse EC cells have higher affinity for FN1 than for laminin in terms of attachment and dissemination in the animal organism. Laminin is predominantly found in the pulmonary matrix, while FN1 is found in the liver. Thus, the low affinity of these cells for laminin in the lung caused their rapid elimination from that organ. These results were obtained from a study evaluating cell migration after injection into the tail vein of mice, showing that the tumor cell adhesion to organs is necessary but not sufficient for metastasis (104).

A key driver of tumor growth and metastasis is high vascularization due to angiogenesis (105). The splice variant *FN1B* stands out as a specific biomarker of angiogenesis expressed around new blood vessels in various human cancer types, such as glioblastoma and small cell lung cancer. Through culturing mouse embryonal teratocarcinoma cells and inoculating them into 4-week-old female mice, a study observed via microPET imaging, that *FN1B* serves as a promising biomarker for microPET imaging targeting (105).

Taken together, these studies provide significant insights into the molecular and cellular mechanisms underlying plasticity in GCTs, particularly through *in vivo* models and characterization of key proteins, TFs and angiogenesis-related biomarkers. However, distinct differences in intermediate filament expression between human and murine tumors, as well as limited exploration of species-specific pathways, highlight the complexity of translating findings across models. Moreover, while substantial progress has been made in linking plasticity to invasion and metastasis, the role of plasticity in treatment resistance, particularly to cisplatin, remains insufficiently understood. Bridging this knowledge gap will require integrating advanced experimental approaches focusing on the interplay between plasticity mechanisms and therapeutic responses, ultimately paving the way for more precise and effective treatments for GCTs.

*Approaches in cisplatin resistance models.* Several studies on resistance have focused on demonstrating cisplatin resistance in *in vivo* models, particularly in PDX models. However, some of these studies investigated this phenomenon in tumor types other than GCTs, such as ovarian (106,107), lung (108,109), liver (110,111), colorectal (112), gastric (113,114) and brain (115) cancer. Thus, the primary objective of these studies was to characterize cisplatin resistance, analyze genes, proteins and signaling pathways involved in chemoresistance, compare different drugs associated with chemoresistance and establish new targeted drugs for treatment. Nevertheless, few studies have explored the interaction between cisplatin resistance and plasticity, as highlighted below.

A notable study examined the association between plasticity and treatment resistance, using a novel cyclic peptide, MTI-101, in synergy with cisplatin in lung cancer (109). *In vivo* data indicated that the treatment increased CDH1 and decreased VIM expression, suggesting that chronic treatment with MTI-101 could reduce metastatic disease (109). Despite these notable results, a direct association between cisplatin resistance, plasticity and GCT models was not observed. A similar observation was reported using zebrafish xenografts to evaluate the therapeutic benefits of cisplatin and valproic acid in patient-derived laryngeal cancer cell lines (116). Evidence

highlighted that the RK45 cell line activated genes associated with the epithelial phenotype. Following treatment with both chemotherapeutics, upregulation of *CDH1*, which encodes CDH1, and its placental form, *CDH3*, was observed (116). Additionally, negative regulation of the TFs *ZEB1* and *FGFR1*, genes that induce plasticity progression and may be associated with the weak response of RK33 cells to the combination of cisplatin and valproic acid drugs, was noted. Meanwhile, the RK33 cell line exhibited upregulation of mesenchymal phenotype genes, such as *VIM* (116).

Some studies have explored cisplatin resistance in xenograft models using GCT cell lines. An *in vivo* model utilizing the GCT NTERA-2 cell line, including both the parental and cisplatin-resistant strains, was employed to evaluate drug sensitivity. It was reported that both cell lines were sensitive to the DNA methyltransferase inhibitor guadecitabine, suggesting that this agent may offer a potential therapeutic alternative for patients with cisplatin-resistant GCTs. This finding highlights the potential of epigenetic therapies in overcoming resistance and improving treatment outcomes for these patients (117). The same cell line, NTERA-2, was used to investigate the effects of cholecalciferol (inactive form of vitamin D) and 1,25(OH)<sub>2</sub>D<sub>3</sub> on cisplatin treatment in an *in vivo* xenotransplantation mouse model, using tumor growth and tumor size as endpoints. In contrast to the findings with guadecitabine, treatment with the active form of vitamin D showed no antitumor activity *in vivo* (118).

Beyond the use of xenograft models derived from established cell lines, research has focused on developing PDX models. These PDX models preserve essential characteristics of original patient tumor cells, including maintaining high genetic and transcriptional stability. Importantly, key mutations present in primary tumors have been shown to remain consistent across successive PDX passages (119,120). PDX models show greater similarity to human tumors compared with cell lines. Analyses of cisplatin resistance in PDX have shown results resembling those observed in the corresponding patient, highlighting the superiority of PDX models in predicting drug response (119).

A xenograft model of TGCT derived from a patient with cisplatin resistance was used to evaluate potential therapeutic strategies. The findings revealed the efficacy of short interfering RNA targeting testis-expressed 11 (*TEX11*), a gene significantly upregulated in the tumor. *TEX11*, known for its role in meiosis, was implicated in promoting resistance to cisplatin by inhibiting cisplatin-induced double-strand DNA breakage. These results highlight *TEX11* as a promising therapeutic target for addressing cisplatin-resistant TGCTs (121). Additionally, reversionless 7 gene (*REV7*) deficiency is involved in DNA damage repair, cell cycle regulation and gene expression, and it sensitized cisplatin-resistant tumors *in vivo*. This was observed in a tumor xenograft of a GCT (testicular EC) in SCID mice (122). Thus, these studies underscore the potential of targeting key molecular players, such as *TEX11* and *REV7*, in overcoming cisplatin resistance in TGCTs.

A xenograft model was established in immunodeficient mice using cisplatin-resistant cells derived from a previously cisplatin-sensitive YST of a patient with ovarian cancer. This model provided insights into the association between

CSC markers and cisplatin resistance (increased expression of CD133, ALDH3A1 and ABCG2), underscoring potential targets to address chemoresistance (74).

In the context of PDX models for GCTs, primary choriocarcinoma tumors were implanted in mice to evaluate the expression of the ErbB family of receptor tyrosine kinases and assess the effects of their inhibitors (123). The ErbB family is associated with tumor progression, correlating with worse prognosis and resistance to conventional therapies (123). ErbB receptors are naturally expressed in tissues of epithelial, mesenchymal and neuronal origin (123). However, members of the ErbB family (ErbB1, ErbB2 and ErbB3) exhibit distinct expression patterns in tumors, such as CC, EC and YST (120). Specifically, ErbB1 is upregulated in CC, ErbB2 is highly expressed in EC, and elevated levels of ErbB3 are observed in CC, EC and YST (120). Therapeutic inhibition of these markers has shown that targeting a single ErbB family member is insufficient to disrupt tumor growth, as compensatory pathway reactivation contributes to resistance against ErbB-targeted therapies (120,124).

Despite studies identifying important pathways associated with cisplatin resistance or sensitivity in GCTs, little information addresses plasticity. To address this gap, our research group conducted a study analyzing the expression of *SLUG*, a key plasticity TF, in both parental and cisplatin-resistant xenograft tumors (21). Parental and cisplatin-resistant NTERA-2 cells were inoculated into athymic mice, and significant upregulation of *VIM* and *SLUG* expression was observed. This suggests that *SLUG* may serve as an important TF for linking plasticity and cisplatin resistance in GCTs (21). While this study offers insights into the mechanisms of plasticity and cisplatin resistance, murine models do not fully reflect the complexity of human tumors. This is particularly due to species-specific differences in gene expression and treatment response. However, a deeper understanding of the molecular mechanisms underlying EMP and drug resistance presents a promising avenue for the development of innovative therapeutic strategies tailored to target TGCTs, thereby opening new avenues for more effective and personalized treatments.

## 5. Potential inhibitors targeting EMP

Inhibitors targeting EMP have become a key strategy in combating cancer progression, metastasis and treatment resistance. By modulating key signaling pathways and molecular markers associated with plasticity, these inhibitors offer a promising approach to mitigating the aggressive behavior of tumors (125).

In JEG-3 cells, cyclosporin-A (CsA) was shown to promote invasion through reduced CDH1 expression via the EGFR/ERK signaling pathway. This effect was counteracted by U0126, an ERK pathway inhibitor, demonstrating its ability to suppress EMP-related invasive behavior (126). Similarly, the phosphodiesterase-4 (PDE4) inhibitor rolipram has been highlighted as a potential therapeutic agent. Rolipram effectively reduced migration and invasion in JEG-3 and JAR cells by modulating EMP markers at the mRNA and protein levels, including decreased expression levels of MMP9 and TIMP1, and increased expression of CDH1, positioning PDE4 inhibitors as promising candidates for modulating EMP in cancer

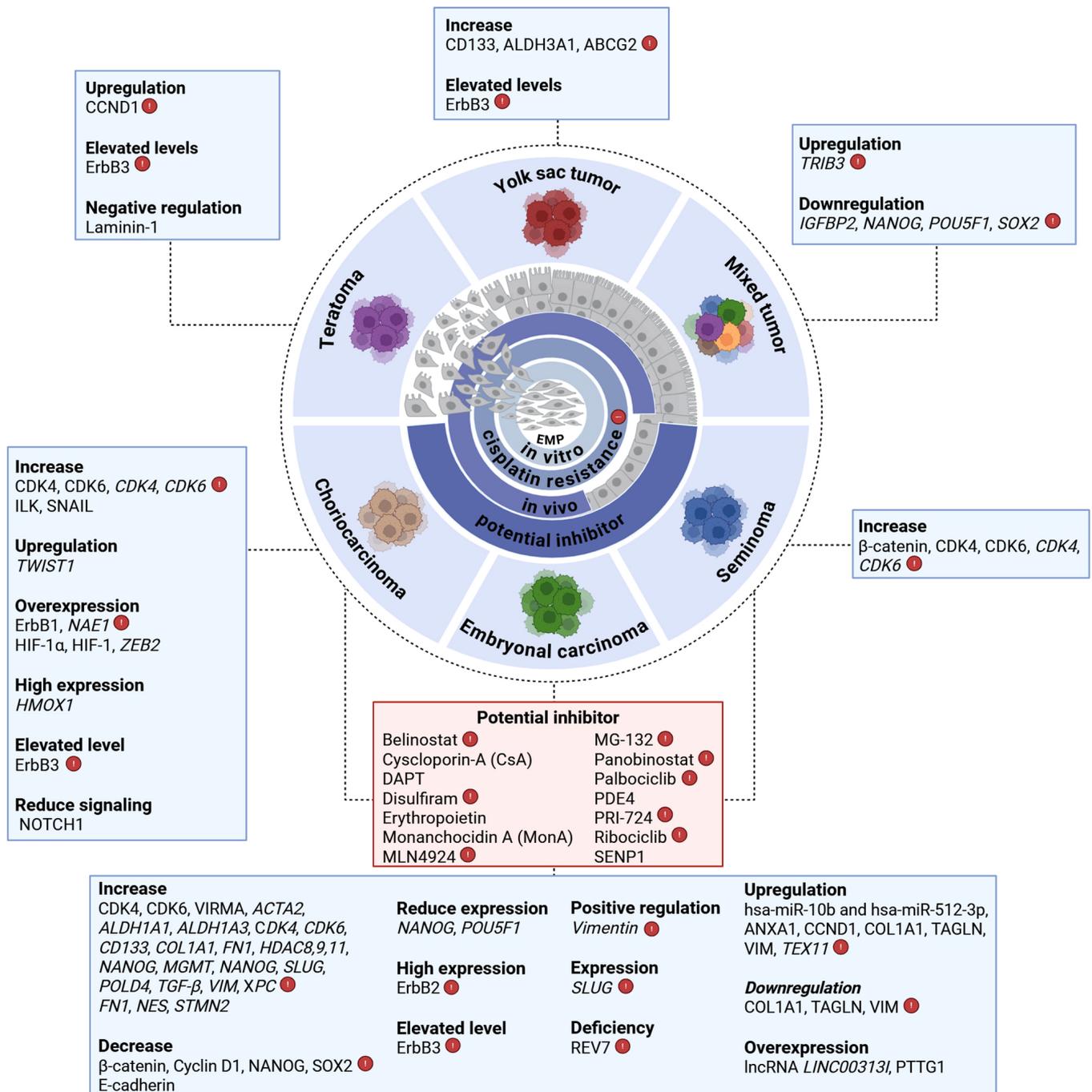


Figure 3. Graphical representation of EMP markers, cisplatin resistance and potential inhibitors across GCT histologies. This figure illustrates the histologies studied both *in vivo* and *in vitro*, including those related to cisplatin resistance and the status of potential inhibitors for targeted therapy. Red circles indicate association with cisplatin resistance. Created in BioRender. Pinto, M. (2025) <https://BioRender.com/x70i558>. EMP, epithelial-mesenchymal plasticity; GCT, germ cell tumor; CCND1, cyclin D1; ErbB, ErbB family; CD133, prominin-1; ALDH3A1, aldehyde dehydrogenase 3 family member A1; ABCG2, ATP binding cassette subfamily G member 2; TRIB3, Tribbles pseudokinase 3; IGFBP2, insulin-like growth factor binding protein 2; NANOG, homeobox protein NANOG; POU5F1, POU class 5 homeobox 1; SOX2, SRY-related HMG-box 2; CDK, cyclin-dependent kinase; VIRMA, Vir-like M6A methyltransferase associated; ACTA2, actin alpha 2; COL1A1, type I collagen; FN1, fibronectin; HDAC, histone deacetylase; POLD4, DNA Polymerase Delta 4, Accessory Subunit; TGF- $\beta$ , transforming growth factor beta; VIM, vimentin; XPC, XPC complex subunit, DNA damage recognition and repair factor; NES, nestin; STMN2, stathmin-2; REV7, reversionless 7; has-miR, Homo sapiens microRNA; ANXA1, annexin A1; TAGLN, Transgelin; TEXT11, testis-expressed 11; LINC003131, long intergenic non-coding RNA; PTTG1, pituitary tumor-transforming gene 1; DAPT, N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; MLN4924, pevonedistat; MG-132, proteasome inhibitor; PDE4, phosphodiesterase 4; SENP1, SUMO specific peptidase 1; ILK, integrin linked kinase; SNAIL, snail family transcriptional repressor 1; TWIST1, Twist-related protein 1; NAE1, NEDD8 activating enzyme E1 subunit 1; HIF-1 $\alpha$ , hypoxia inducible factor 1 Subunit Alpha; ZEB2, zinc finger E-box binding homeobox 2; HMOX1, heme oxygenase 1; NOTCH1, notch receptor 1.

therapy (127,128). Thus, CsA and PDE4 inhibitors demonstrate significant impacts on EMP and invasive behavior in cancer cells.

Further advancements in overcoming cisplatin resistance in GCTs include the use of monanchocidin A (MonA), an alkaloid compound with antitumor properties. MonA demonstrated

the ability to downregulate VIM isoforms, suppress migration and alter cell morphology in cisplatin-resistant NCCIT-R cells, suggesting its potential role in addressing EMP-related resistance mechanisms (129). Additionally, panobinostat, an HDAC inhibitor, has shown potential as an inhibitor of LINC00313, a long non-coding RNA linked to EMP mediation. Although its effects on specific EMP markers require further study, panobinostat represents a promising avenue for therapeutic exploration (65). These inhibitors hold considerable promise for further investigation as potential therapeutic agents for GCT treatment, particularly for addressing EMP-driven cisplatin resistance, a critical unmet clinical need.

*Insights from clinical trials in non-GCT malignancies.* To date, to the best of our knowledge, clinical trials targeting EMP pathways in GCTs remain unavailable. However, investigations into EMP-related targets in other malignancies provide valuable insights. For instance, anecaliximab, a monoclonal antibody inhibiting *MMP9*, a key EMP player, has demonstrated manageable safety profiles and varying efficacy in advanced cancer types, such as gastric and pancreatic adenocarcinoma (130,131). In pancreatic adenocarcinoma, combinations with chemotherapy regimens, such as gemcitabine and nab-paclitaxel, achieved a progression-free survival time of 7.8 months and an objective response rate of 44.4%. In advanced gastric cancer, one study evaluated anecaliximab as monotherapy and in combination with nivolumab, showing a manageable safety profile and clinical activity, with a median progression-free survival time of 1.4 and 4.6 months for monotherapy and combination therapy, respectively (132). Another study assessing anecaliximab combined with nivolumab in pretreated metastatic gastric cancer reported a favorable safety profile but no significant improvement in survival outcomes compared with nivolumab alone (133). A phase I study combining sapanisertib with ziv-aflibercept, a *VEGF* inhibitor, in patients with advanced solid tumors, demonstrated a disease control rate of 78%, with 74% achieving stable disease and 4% achieving a confirmed partial response (134).

Additionally, PRT543, an inhibitor of protein arginine methyltransferase 5, which downregulates NOTCH1 and MYB signaling, achieved a clinical benefit rate of 57% and a median progression-free survival time of 5.9 months in adenoid cystic carcinoma (135). Furthermore, chidamide, an HDAC inhibitor, demonstrated anti-NOTCH1 activity and clinical efficacy in T-cell acute lymphoblastic leukemia, suggesting its utility in EMP-related pathways (136). Moreover, the combination of bevacizumab, an antiangiogenic agent targeting VEGF, and bortezomib, a proteasome inhibitor that suppresses HIF-1 $\alpha$  transcriptional activity, demonstrated clinical activity in a phase I trial involving patients with advanced malignancies. Among the 91 patients treated, 12% achieved either a partial response or stable disease lasting  $\geq 6$  months, highlighting the potential of dual targeting of angiogenesis and HIF-1 $\alpha$  in overcoming resistance to antiangiogenic therapy (137). These findings highlight the potential of EMP-targeting strategies in broader oncology contexts and underscore the need to explore their therapeutic value in GCTs, where emerging evidence suggests a role for EMP in treatment resistance.

To offer a comprehensive summary and highlight connections between all topics discussed in this review, Fig. 3 provides a graphical representation synthesizing the key aspects of the article.

## 6. Conclusion

The present review highlights several critical gaps in the current understanding of EMP and its association with cisplatin resistance in GCTs. A major limitation is the scarcity of studies directly investigating EMP-associated cisplatin resistance in GCTs, with most research focusing separately on EMP or resistance mechanisms rather than their interplay. Additionally, while *in vitro* studies using GCT cell lines have provided some insights, there is a notable lack of *in vivo* research exploring the connection between plasticity and resistance in GCTs. These findings underscore the necessity for integrated studies that combine both EMP and resistance mechanisms to enhance our comprehension of the molecular mechanisms involved. Advancing research in this area could lead to the identification of novel therapeutic targets, ultimately improving outcomes for the 30% of patients with GCT who have a poor prognosis or experience treatment failure. To address the current knowledge gap, future research should focus on longitudinal analysis of EMP markers in patient cohorts, exploring the intricate association between plasticity mechanisms and therapeutic responses. This will help validate their prognostic utility and pave the way for developing more effective and personalized treatments for GCTs.

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## Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

## Authors' contributions

ESDBG, TMDS, ALPO, AFSPB, IIVC, LS, LFL, MNR and MTP contributed to the conception, design, literature search and analysis of the study. ESDBG, TMDS, ALPO, AFSPB, IIVC, LS and MNR drafted and wrote the manuscript. ESDBG, TMDS and AFSPB prepared the figures. MTP supervised the review, critically evaluated and revised the manuscript. Data authentication is not applicable. Data authentication is not applicable. All authors have read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Use of artificial intelligence tools

During the preparation of this work artificial intelligence tools were used to translate the language of the manuscript and, subsequently, the authors revised and edited the content translated by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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