

## REVIEW

# Epithelial–mesenchymal transition: The history, regulatory mechanism, and cancer therapeutic opportunities

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## Abstract

Epithelial–mesenchymal transition (EMT) is a program wherein epithelial cells lose their junctions and polarity while acquiring mesenchymal properties and invasive ability. Originally defined as an embryogenesis event, EMT has been recognized as a crucial process in tumor progression. During EMT, cell–cell junctions and cell–matrix attachments are disrupted, and the cytoskeleton is remodeled to enhance mobility of cells. This transition of phenotype is largely driven by a group of key transcription factors, typically Snail, Twist, and ZEB, through epigenetic repression of epithelial markers, transcriptional activation of matrix metalloproteinases, and reorganization of cytoskeleton. Mechanistically, EMT is orchestrated by multiple pathways, especially those involved in embryogenesis such as TGF $\beta$ , Wnt, Hedgehog, and Hippo, suggesting EMT as an intrinsic link between embryonic development and cancer progression. In addition, redox signaling has also emerged as critical EMT modulator. EMT confers cancer cells with increased metastatic potential and drug resistant capacity, which accounts for tumor recurrence in most clinic cases. Thus, targeting EMT can be a therapeutic option providing a chance of cure for cancer patients.

**Abbreviations:** bHLH, basic helix–loop–helix; ceRNA, competing endogenous RNA; circRNA, circular RNA; CSC, cancer stem cell; CTC, circulating tumor cell; DTP, drug-tolerant persister; E-cadherin, epithelial cadherin; ECM, extracellular matrix; EGF, epidermal growth factor; EMT, epithelial–mesenchymal transition; EMT-TF, EMT-associated transcription factor; EPSC, EMT-promoting Smad complex; EZH2, enhancer of zeste homolog 2; F-actin, filamentous actin; FGF, fibroblast growth factor; G-actin, globular actin; GAP, GTPase-activating protein; GDI, guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; HDAC, histone deacetylase; HGF, hepatocyte growth factor; IL, interleukin; lncRNA, long noncoding RNA; LSD1, lysine-specific histone demethylase 1; m6A, N6-Methyladenosine; M-cadherin, muscle cadherin; MDR, multidrug resistance; MET, mesenchymal-epithelial transition; miRNA, microRNA; MMP, matrix metalloproteinase; NAC, N-acetylcysteine; N-cadherin, neuronal cadherin; ncRNA, noncoding RNA; OS, overall survival; P-cadherin, placental cadherin; PRC1/2, polycomb repressive complex 1/2; R-cadherin, retinal cadherin; ROS, reactive oxygen species; Snail1/2, zinc finger protein SNAI1/2; TAM, tumor-associated macrophage; TGF, transforming growth factor; Twist1/2, Twist-related protein 1/2; USP, ubiquitin-specific protease; ZEB1/2, zinc finger E-box-binding homeobox 1/2; ZO, Zona occludens.

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Here, we introduce a brief history of EMT and summarize recent advances in understanding EMT mechanisms, as well as highlighting the therapeutic opportunities by targeting EMT in cancer treatment.

#### KEYWORDS

cancer progression, embryogenesis, EMT, redox signaling, targeted therapy

## 1 | INTRODUCTION

Epithelial–mesenchymal transition (EMT) describes a reversible transition process during which epithelial cells reduce their epithelial properties and gain mesenchymal characteristics.<sup>1</sup> In the reverse process, MET, the transdifferentiated mesenchymal cells can revert back to epithelial state.<sup>1,2</sup> EMT was initially identified as an embryogenesis event, which is now recognized to be ubiquitous throughout every aspect of life activity, including wound healing, fibrosis, and tumor metastasis.<sup>3–8</sup> During EMT, epithelial cells lose their junction proteins, among them the epithelial cadherin (E-cadherin) is the most known glue.<sup>9</sup> Down-regulation of E-cadherin renders cells to be separated with each other, acquiring mesenchymal morphology and invasive capability. Besides, cytoskeleton is also reorganized in this process, which is associated with the formation of pseudopodia and consequent enhanced mobility as well as metastatic capacity.<sup>10–12</sup> Moreover, matrix metalloproteinases (MMPs) secreted during EMT lead to destruction of matrix barrier, making cells ready to move.<sup>13–16</sup> Generally, these programs are orchestrated by a group of EMT-associated transcription factors (EMT-TFs), including zinc finger protein SNAI1 (Snail1), zinc finger protein SNAI2 (Snail2, also known as Slug), Twist-related protein 1/2 (Twist1/2), and zinc finger E-box-binding homeobox 1/2 (ZEB1/2).<sup>17–21</sup> These EMT-TFs induce epigenetic silencing of epithelial marks such as E-cadherin while activating mesenchymal marks such as N-cadherin, vimentin, and MMPs, which proceeds EMT program.<sup>22,23</sup> The upstream signals regulating EMT can be various, many of which are embryogenesis-related, including TGF $\beta$ , Wnt, Hedgehog, and Hippo.<sup>3,24–31</sup> This fact addresses the intrinsic crosstalk between embryonic development and cancer metastasis.

In terms of cancer biology, EMT is one of the most notable hotspots for its crucial role in the regulation of metastasis, metabolic reprogramming, stemness, inflammation, chemoresistance, and other hallmarks of cancer.<sup>2,20,32–37</sup> In the clinic perspective, EMT is frequently observed in high-grade tumor cases with poor prognosis.<sup>27,38–40</sup> It is well known that EMT leads to detachment of cancer cells from extracellular matrix

(ECM) and subsequent entering into blood, resulting in the generation of circulating tumor cells (CTCs) thus promoting tumor metastasis.<sup>41</sup> Interestingly, many EMT-TFs can also activate the transcription of genes related with metabolic reprogramming, stemness, and inflammatory responses, indicating that EMT and many other hallmarks of cancer are inter-connected.<sup>42–45</sup> Furthermore, given that EMT is a highly reversible process, a part of cancer cells is in an intermediate state between epithelial and mesenchymal phenotype (intermediate EMT, also known as hybrid EMT, partial EMT, or incomplete EMT) thus exhibiting plasticity and heterogeneity, which contribute to cancer progression and drug resistance.<sup>2,33,46–49</sup> These facts indicate that EMT promotes more aggressive behaviors of tumor but also implicate therapeutic opportunity. For example, loss of epithelial junctions indeed enhances invasive ability, but also makes cancer cells vulnerable to ferroptosis.<sup>50,51</sup> Therefore, EMT acts as a double-sword in cancer, which implicates a promising anticancer strategy via selectively inhibiting prometastasis effect or boosting proferroptosis effect of EMT.<sup>52–55</sup>

Here, we briefly review the history of EMT research and provide several prospects or visions for the future in this field. We summarize recent advances in understanding EMT phenotypes and mechanisms including the loss of junction proteins, reorganization of cytoskeleton, activation of EMT-TFs, and signal transduction of multiple embryogenesis and redox pathways. We also discuss the impact of EMT on the metabolic reprogramming, stemness acquisition, and inflammatory microenvironment of tumors, and highlight the therapeutic intervention targeting EMT so as to provide new insights into the treatment of cancer.

## 2 | A BRIEF RESEARCH HISTORY OF EMT

Elizabeth D Hay is the pioneer who discovered EMT in 1958, though this term was not formally used at that time.<sup>56,57</sup> She found that during the forelimb regeneration of *Amblystoma* larvae, the blastema cells can

dedifferentiate, proliferate, and then redifferentiate into cartilage, thus contributing to the development of the limb.<sup>56</sup> Three years later, she used an autoradiography method to label epidermis before amputating limbs, where she observed epidermis cells can migrate over the wound surface which is required for limb regeneration.<sup>58</sup> These findings show similarity with EMT program and implicate EMT as an important development event during wound healing and tissue biogenesis. In 1966, Elizabeth D Hay and her colleagues reported that the tight junctions are upregulated at the advanced stages of development in chick embryogenesis, making the cells connected with each other thus functioning as a single tissue, rather than separated cell populations.<sup>59,60</sup> Now we know her observation resembles a MET process in which epithelial marks are reexpressed. The concept of EMT was not so popular until 1968, when Elizabeth D Hay was present at the 18th Hahemann symposium in Baltimore. At this meeting, she introduced how epithelial cells transformed into mesenchymal cells during the development of neural tube. This speech addressed the importance of the epithelial–mesenchymal interactions during embryonic development and attracted attention of researchers of this field, leading to a rapid evolution of this field. As a result, a variety of studies were conducted by different research groups to elucidate the roles of epithelial–mesenchymal interactions in organ biogenesis, including the development of heart valve, neural crest, Mullerian duct, intestinal brush border membrane, embryonic lungs, and so on.<sup>61–65</sup> In 1982, Elizabeth D Hay used the term “epithelial–mesenchymal transformation” to describe the transformation into mesenchymal cells from epithelial cells under the three-dimensional collagen gel condition, which repressed the apical–basal polarity of epithelial cells and enhanced their mobility.<sup>66</sup> However, Elizabeth D Hay used another term “epithelial–mesenchymal transition” in 1995, when she summarized several EMT-promoting genes and the reversed process MET in a review.<sup>67</sup> After that, “epithelial–mesenchymal transformation” and “epithelial–mesenchymal transition” were both referred to EMT program with no substantial differences. In 2003, the term “epithelial–mesenchymal transition” was confirmed as the official name to describe EMT after the meetings of the EMT International Association, in order to distinguish EMT from malignant transformation used in oncology.<sup>1</sup>

After the extensive research of EMT phenotypes, scientists were curious as to which factors can induce EMT. In 1985, hepatocyte growth factor (HGF) was reported to act as the “scatter factor” to dissolve the junction proteins between epithelial cells, resulting in their morphologic changes and migration.<sup>68,69</sup> Besides, fibroblast growth factor (FGF) and transforming growth factor (TGF) were both found to induce EMT in rat bladder carcinoma cells

in 1990, suggesting the role of EMT in cancer.<sup>70,71</sup> Another growth factor, epidermal growth factor (EGF), was also demonstrated to promote EMT in rat neonatal hepatocytes by upregulating the expression of vimentin, a crucial mesenchymal mark.<sup>72</sup> These observations indicated that a variety of growth factors, at least including HGF, FGF, TGF, EGF, are potent inducers of EMT. Among them, TGF $\beta$  is probably the most investigated growth factor in EMT research. In 1990, TGF $\beta$  was found to dynamically express in mouse endocardial cells according to different embryonic stages, contributing to cardiac development via regulation of EMT.<sup>73</sup> In the same year, TGF $\beta$  was also reported to alter morphology and activate migration in the chicken chorioallantoic membrane, resulting in microvascular angiogenesis.<sup>74</sup> Given the secretory nature of TGF $\beta$ , it is not surprising that the EMT-promoting function of TGF $\beta$  requires its receptor on cell membrane. In 1994, TGF $\beta$  was demonstrated to induce EMT in mouse mammary gland NMuMG cells, as evidenced by the decrease of epithelial markers, increase of mesenchymal marks and reorganized cytoskeleton.<sup>75</sup> Importantly, truncation of Tsk7L type I receptor abolished these EMT phenotypes, indicating that this receptor is indispensable for the EMT-promoting function of TGF $\beta$ .<sup>75</sup> In addition to the receptor, the activation of TGF $\beta$  signaling also involves Smad proteins, suggesting that Smads play certain roles in TGF $\beta$ -induced EMT.<sup>76</sup> As expected, in 1999, TGF $\beta$  was found to promote the nuclear translocation of Smad2/3/4, leading to EMT in NMuMG cells.<sup>77</sup> These findings indicate that various growth factors, especially TGF $\beta$ , are crucial signals activating EMT. Importantly, since 1990s, the roles of EMT in oncology were gradually noted. For instance, TGF $\beta$ -induced EMT was demonstrated to enhance invasiveness of cancer cells, leading to tumor metastasis, which can be abrogated by its neutralizing antibodies.<sup>78</sup> This observation is in line with the concept that cancer is to some extent a type of developmental disease, given the fact that they share common features such as aberrant EMT program.

Along with the rapid evolution of this field, the mechanisms of EMT were gradually uncovered in 1990s. Indeed, EMT is largely driven by several EMT-TFs, which initiate complex transcriptional program to regulate EMT mark expression, cytoskeleton organization, pseudopodia formation, MMP secretion, as well as consequent cell migration and invasion. Snail is the first EMT-TF identified in 1992, when it was found to be involved in the gastrulation during murine development.<sup>79</sup> And after 2 years, Slug, also known as Snail2, was documented.<sup>80</sup> Using the antisense oligonucleotides against Slug, EMT events were abolished in early chick embryos, indicating its key roles in vertebrate development.<sup>80</sup> In 2001, Twist was identified as another EMT-TF, facilitating the palatogenesis in embryonic rats.<sup>81</sup> This finding provided an explanation for why mutations of

Twist gene result in Saethre-Chotzen syndrome, a developmental disease in human. In the same year, the zinc finger E-box-binding homeobox (ZEB) was demonstrated as an important EMT-TF.<sup>82</sup> In this study, ZEB was found to bind with the promoter of E-cadherin, repressing its transcription. This effect mitigates E-cadherin-mediated intercellular adhesion, leading to cell invasion thus being involved in tumor progression.<sup>82</sup> Actually, the epigenetic silencing of E-cadherin was found to be a common mechanism shared by all these EMT-TFs in 2000s, suggesting the central roles of EMT-TFs in the loss of cell junctions during EMT.<sup>83–86</sup> Before long, EMT-TFs were also reported to be capable of regulating the cytoskeleton organization, pseudopodia formation, and MMP secretion.<sup>87–91</sup> These evidences indicate EMT-TFs underlie the molecular basis of EMT program. In addition to their roles in embryonic development, these EMT-TFs were also investigated in the context of cancer in 2000s. For example, Snail was found to be associated with the progression of poorly differentiated breast carcinoma in 2002.<sup>92</sup> Two years later, the metastasis-promoting role of Twist was demonstrated.<sup>9</sup> Since then, the connection between EMT and cancer has been greatly appreciated.

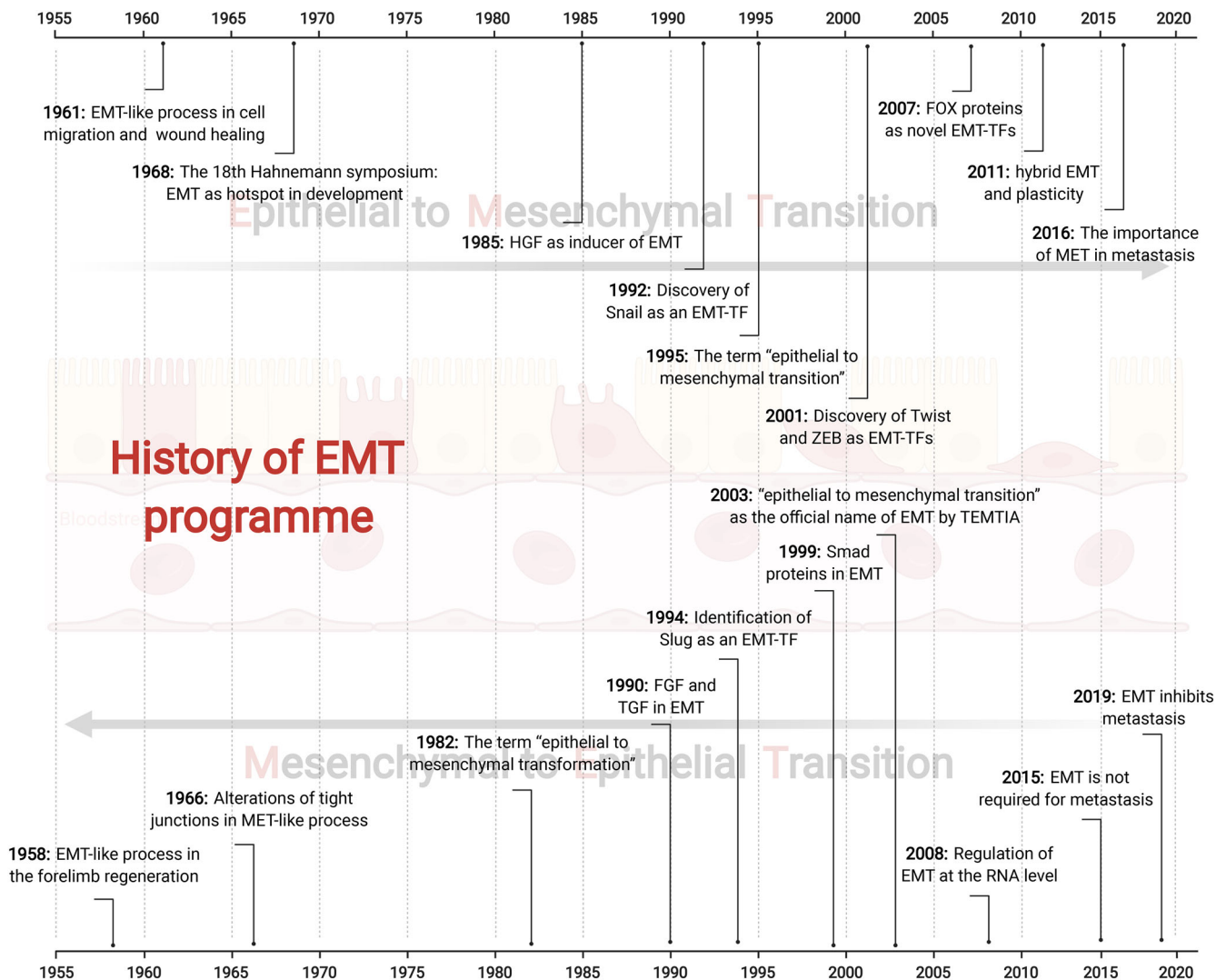
In recent years, the studies focused on EMT and cancer have been more comprehensive, in which several novel mechanisms and concepts were elucidated.<sup>2,20</sup> First, novel EMT-TFs were characterized. In 2007, the Forkhead box (FOX) transcription factor FOXC2 was shown to promote breast cancer metastasis through activating EMT.<sup>93</sup> This finding was followed by the characterization of other FOX family members during EMT, including FOXO3a, FoxF1, FOXA1, FOXA2, and FOXQ1 in the next years.<sup>94–97</sup> In addition to FOX proteins, a GATA transcription factor Serpent (Srp) was also found to regulate EMT through repressing E-cadherin in *Drosophila* in 2011, and the similar function was also observed in the mammalian orthologs of Srp, GATA4, and GATA6.<sup>98</sup> Moreover, other novel EMT-regulating transcription factors, such as PRRX1 and Sox protein family members, were also reported.<sup>99–102</sup> Second, EMT was found to be regulated at the RNA level. In 2008, the RNA alternative splicing of p120 was reported to promote invasiveness via regulating EMT.<sup>103</sup> Three years later, a high-throughput analysis revealed the alternative splicing signature during EMT, and the key splicing mediators such as RBFOX, MBNL, CELF, hnRNP, and ESRP were also identified in this process.<sup>104</sup> Moreover, microRNAs (miRNAs) were also demonstrated to regulate EMT either positively or negatively. For instance, miR-200 and miR-205 were shown to inhibit EMT through targeting ZEB1 and ZEB2, whereas miR-9 downregulated the expression of E-cadherin thereby facilitating EMT.<sup>105,106</sup> More recently, the circular RNAs (circRNAs) and their alternative splicing factor, Quaking, were presumed to be involved in the regulation of EMT.<sup>107</sup> Third, EMT was realized

as a hybrid process in which epithelial and mesenchymal characteristics coexist, instead of a binary process, in most cases.<sup>108</sup> In 2011, the intermediate stages of EMT were defined in trophoblast stem cells. These cells expressed both epithelial and mesenchymal marks and acquired higher metastatic potential compared with cells in the beginning epithelial state and ending mesenchymal state.<sup>109</sup> Following studies revealed that cells in hybrid EMT state were associated with stemness, plasticity, distant colonization, and anoikis resistance, contributing to drug resistance, immune suppression, and tumor recurrence.<sup>110–113</sup> Moreover, it has been recently shown that the loss of Fat1 promotes hybrid EMT state of cancer cells via CAMK2–CD44–SRC axis and EZH2–SOX2 axis, which upregulates the mesenchymal properties and maintains the epithelial characteristics, respectively.<sup>114</sup> This finding provided new insights for understanding mechanisms underlying intermediate EMT state. Fourth, EMT is probably not required for metastasis. In 2015, two independent groups found that inhibition of EMT did not abrogate cancer metastasis, but improved drug sensitivity of tumors.<sup>115,116</sup> Their observations suggest that EMT is dispensable for metastasis; however, the combinational treatment of chemotherapies with EMT inhibition could be potential strategy for overcoming cancer drug resistance. Fifth, the importance of MET in tumor metastasis was gradually appreciated. In 2016, researchers found that mesenchymal cells arriving at distant organ readily underwent MET program to enter into an epithelial state, which is required for colonization in the final stage of metastasis.<sup>117</sup> This observation was further supported by the finding in 2019, which described that E-cadherin facilitates breast cancer metastasis.<sup>118</sup> Together, these evidences indicate that EMT is a rapidly evolving field (Figure 1).

### 3 | REORGANIZATION OF CELL JUNCTIONS AND CYTOSKELETON: KEY CHARACTERISTICS OF EMT

The integrity of epithelial tissues and the morphology of epithelial cells are maintained by specialized surface proteins and cytoskeleton. Surface proteins form cell–cell junctions and cell–matrix junctions, making epithelial cells as a whole thus restricting individual mobility. Deconstruction of cell junctions, including adherent junctions, tight junctions, desmosomes, and gap junctions, leads to separation of epithelial cells with each other and disassociation with basement membrane, as well as the loss of cell contact inhibition.<sup>119</sup> These events result in loss of apical–basal polarity, thus facilitating metastasis. In addition to their well-known metastasis-suppressive functions, several junction proteins can also promote tumor





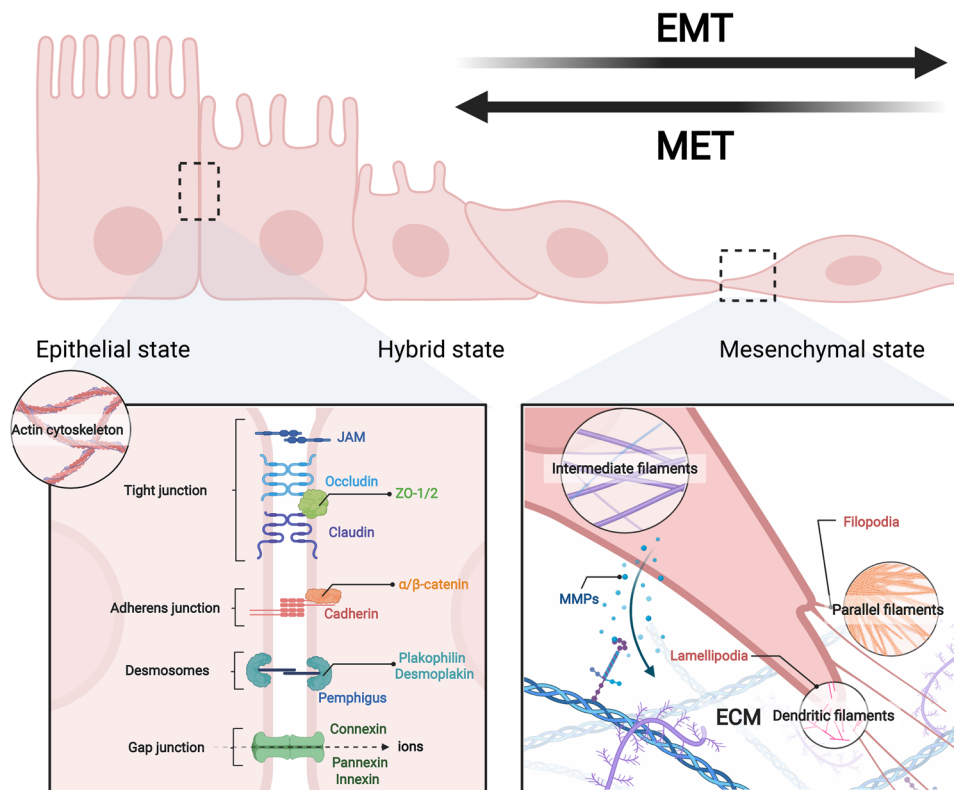
**FIGURE 1** A brief history of EMT. The EMT phenotype was firstly discovered by Elizabeth D Hay, who is the pioneer for this field. From 1950s to 1980s, EMT was largely investigated in the context of developmental biology with few mechanistic studies. During 1980s to 2010, the connection between EMT and cancer metastasis was intensively documented, and mechanistic studies revealed the central roles of EMT-TFs in EMT program. From 2010s to now, new concepts of EMT is increasingly developed, which include novel EMT regulators, the hybrid EMT state, multiple cancer hallmarks induced by EMT, the tumor-suppressive effects of EMT, and EMT-based cancer therapeutic strategies

dissemination, such as claudin-11.<sup>120</sup> This observation suggest that junction proteins play dual roles in tumor metastasis. Moreover, crosstalk exists among these different types of cell junctions. For instance, desmoplakin, one of the components of desmosomes, is able to maintain gap junctions through regulating Ras/MAPK signaling.<sup>121</sup> As transmembrane proteins, junction proteins are commonly associated with Rho GTPases through their cytoplasmic domains, thereby regulating cytoskeleton organization.<sup>122</sup> Cytoskeleton controls the morphology and mobility of cells, reorganization of which promotes morphological change of epithelial cells into a spindle-like mesenchymal shape. During this process, pseudopodia is elongated to enable directional motility and consequent cell movement. In addition to these physical effects, emerging evi-

dence showed that junction proteins and cytoskeleton can also function as signaling molecules to regulate signal transduction, thereby affecting invasiveness of cells.<sup>123,124</sup> Actually, cell junctions mediate cell-cell communications mediating nonautonomous behaviors of cells, whereas cytoskeleton might serve as scaffold to facilitate biochemical reactions via providing reaction places. Therefore, reorganization of cell junctions and cytoskeleton are key characteristics during EMT (Figure 2).

### 3.1 | Altered junctional components

There are four common junction types that comprise the epithelial connection, namely adherent junctions,



**FIGURE 2** An overview of cellular phenotype changes during EMT. EMT is a highly reversible process with epithelial, hybrid, and mesenchymal states. In epithelial state, cells are held together to preserve epithelial integrity via several junction structures, namely adherens junctions, tight junctions, desmosomes, and gap junctions, which is composed of several epithelial proteins including E-cadherin, claudins, occludins, connexins, and many others. Disruption of these junctions leads to the entry of cancer cells into hybrid state and following mesenchymal state, in which cells express mesenchymal marks such as N-cadherin, Vimentin, and MMPs. In addition, cells reorganize their cytoskeleton networks to support the formation of pseudopodia thereby facilitating metastasis

tight junctions, desmosomes, and gap junctions. One of the most known adherent junctions is the transmembrane protein E-cadherin (epithelial cadherin, encoded by CDH1 gene), whose cytoplasmic region binds with  $\beta$ -catenin and p120 catenin, thereby being associated with cytoskeleton. The extracellular fragment of E-cadherin provides intercellular adhesion between opposing epithelial cells in a calcium-dependent manner.<sup>125</sup> In normal condition, E-cadherin is one of the most important epithelial marks. Given the fact that most tumors originate from epithelial tissues, deregulation of E-cadherin has been regarded as a hallmark of tumorigenesis. Indeed, in the context of neoplasm, E-cadherin has long been proved as a tumor-suppressor gene inhibiting cancer initiation and progression. Loss of E-cadherin, often caused by epigenetic silencing or genetic mutations, is a common event in a wide range of tumors, including breast cancer, gastric cancer, colorectal cancer, liver cancer, lung cancer, and so on.<sup>30,126–129</sup> Interestingly, E-cadherin has been also shown considerable expression level in tumor metastases.<sup>130</sup> Moreover, a recent study indicated that E-cadherin is required for tumor metastasis by buffering

oxidative stress in breast cancer.<sup>118</sup> One possible explanation is that E-cadherin has to be reexpressed in MET process, which facilitates the distal colonization in the late stage of metastasis. Other adherent junctions, such as N-cadherin (neuronal cadherin, CDH2), P-cadherin (placental cadherin, CDH3), R-cadherin (retinal cadherin, CDH4), and M-cadherin (muscle cadherin, CDH15), share similar structure but their functions are distinct.<sup>131</sup> N-cadherin is a mesenchymal mark promoting tumor metastasis, which is opposite to E-cadherin.<sup>132</sup> R-cadherin is an epithelial mark resembling E-cadherin, loss of which has been shown to facilitate EMT and tumor progression.<sup>133</sup> Interestingly, the role of P-cadherin can be either tumor promoting or tumor suppressive, depending on particular context.<sup>134</sup> For instance, the high expression of P-cadherin was correlated with the progression of lung cancer and ovarian cancer,<sup>135,136</sup> whereas other reports showed that P-cadherin preserves epithelial barrier to inhibit the metastasis of melanoma.<sup>137,138</sup> These dual functions of P-cadherin in regulating cancer metastasis might be attributed to the distinct characteristics of different cancer types. To be specific, in response to

gonadotropin-releasing hormone (GnRH), P-cadherin induces the activation of IGF-1R in a ligand-independent manner, which phosphorylates p120 catenin, thereby promoting metastasis in ovarian cancer.<sup>136</sup> However, other tumor types such as melanoma might not be relevant to GnRH, IGF-1R, or p120 catenin. In other words, GnRH, IGF-1R, or p120 catenin might be with very low basal level or loss of function in other cancers, leading to the disruption of this pro-metastatic signaling, therefore P-cadherin failed to activate metastasis in such cancers. In this context, P-cadherin may serve as intercellular glue to prevent metastasis. Certainly, this postulation needs to be validated by substantial evidence.

Apart from adherent junctions, the epithelial integrity is also maintained by tight junctions, which consist of claudins, zonula occludens, and others. These tight junction proteins contain several family members. For instance, 27 members are characterized in claudin family, whereas only three members are found in zonula occludens family.<sup>139</sup> Similar to adherent junctions, tight junctions also play crucial roles, either positive or negative, in the EMT phenotype and cancer progression. Claudin-1 was shown to promote EMT and invasion of colorectal cancer through upregulating ZEB-1, while inhibiting tumor metastasis in gastric cancer via mediating the tumor-suppressive function of RUNX3.<sup>140,141</sup> Besides, it has been reported that claudin-2 promotes tumor metastasis in breast, lung, and colorectal cancer, but is negatively associated with the high-grade pancreatic cancer.<sup>142–146</sup> Therefore, the specific roles of claudin proteins in EMT status and tumor progression are largely context dependent. Another tight junction proteins, Zona occludens (ZO), are also key components of epithelial tissue. There are three members found in ZO family, namely ZO-1, ZO-2, and ZO-3. Among them, ZO-1 is the most investigated one in the neoplastic context. For example, loss of ZO-1 was reported to promote metastasis of breast cancer, colorectal cancer, liver cancer, pancreatic cancer, and so on.<sup>147–150</sup> These observations indicate that ZO-1 mainly serves as a tumor suppressor, in contrast to the diverse functions of abovementioned claudin proteins. However, a recent study provided exceptional evidence describing that ZO-1 can activate Rac-1-mediated cytoskeletal organization, thereby promoting metastasis in colorectal cancer.<sup>151</sup> More intriguingly, different tight junction proteins can be associated with each other, thereby forming complex junction architecture. For instance, the cytoplasmic region of claudins is able to bind with the PDZ domain of zonula occludens.<sup>152</sup> Though physical association is observed, the functional link between claudins and ZO proteins remains elusive.

Desmosomes represent another form of cell junctions, which consist of desmosomal cadherins (includ-

ing desmogleins and desmocollins), armadillo proteins (including plakoglobins and plakophilins), and desmoplakin. These proteins form complex structure anchoring the intermediate filaments to the plasma membrane between neighboring cells, thus maintaining epithelial integrity.<sup>153</sup> As tumor suppressors, downregulation of desmosomal components plays vital roles in EMT program and consequent cancer progression.<sup>154</sup> For example, impaired desmosomes were shown to promote EMT and consequent tumor progression in invasive breast cancer.<sup>155</sup> Besides, loss of desmosomes was observed in invasive pancreatic neuroendocrine tumors (PNET), and genetic deletion of desmoplakin enhanced tumor metastasis in the PNET mouse model.<sup>156</sup> Interestingly, the proper functions of desmosomes require particular posttranslational modifications on several components. Palmitoylation of plakophilin was shown to play critical roles in the assembly of desmosomes, and dephosphorylation of plakophilin-1 is able to promote epidermal carcinogenesis.<sup>157,158</sup> In fact, the tumor-suppressive roles of desmosomes were also reported in several other tumor types, including liver cancer, breast cancer, and lung cancer.<sup>159,160</sup> Even so, the oncogenic roles of desmosomal components have been also reported. For instance, desmoglein-3 is overexpressed in human head and neck cancer and associated with advanced tumor stage, and inhibition of desmoglein-3 is sufficient to mitigate tumor progression both in vitro and in vivo.<sup>161</sup> In addition to desmoglein-3, the tumor-promoting functions of several other desmosomal components were recently summarized elsewhere.<sup>162</sup> This evidence suggest that desmosomes might not be simply regarded as tumor suppressive molecules, but rather multifunctional structure during tumor development.

Gap junctions are ion channels formed by connexin, pannexin, and innexin proteins, which provide both adherence and direct intercellular communication between neighboring cells.<sup>163</sup> Among them, connexins are the most investigated channels. Similar to other junction proteins, connexins play dual roles in tumorigenesis. It has been reported that connexins are overexpressed in tumors, such as connexin-26 in pancreatic cancer and colorectal cancer.<sup>164,165</sup> However, it has been also shown that connexins can serve as tumor suppressors, including connexin-43 and connexin-45 in colorectal cancer.<sup>166,167</sup> Moreover, high expression of connexins can predict either better or poor prognosis in cancer patients. For instance, overexpression of connexin-43 prolongs the survival of patients with prostate cancer, breast cancer, and colorectal cancer,<sup>166,168,169</sup> whereas accelerating death of patients with bladder cancer, esophageal squamous cell carcinoma, and oral squamous cell carcinoma.<sup>170–172</sup> Not surprisingly, gap junction-regulated EMT and tumor progression are largely dependent on their ion channel function, which

provide communication between nearby cells, in addition to their adherent effect. It has been reported that gap junctions among U2OS cells failed to inhibit EMT, but gap junctions between U2OS cells and osteoblasts did, suggesting that gap junctions inhibit EMT through U2OS-osteoblast communication rather than merely intercellular glue.<sup>173</sup> Moreover, gap junction was shown to amplify potassium currents, thereby establishing electrochemical communication between neuron and glioma. This effect significantly promotes glioma progression, which can be abrogated by gap junction antagonists.<sup>174</sup> Thus, targeting gap junctions can be a potential therapeutic strategy for cancer treatment.

### 3.2 | Cytoskeleton reorganization

As mentioned above, cell–cell junctions are linked with actin cytoskeleton to form epithelial architecture, suggesting the vital roles of cytoskeleton organization during EMT program. Indeed, junction proteins are associated with Rho GTPases, which are dominantly responsible for the organization of actin cytoskeleton.<sup>10,175</sup> The activity of Rho GTPases is finely tuned by three classes of regulators, namely guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and guanine nucleotide dissociation inhibitors (GDIs).<sup>176</sup> In response to adherent or growth factor signals, Rho GTPases are activated by the exchange of GDP with GTP, and this process is catalyzed by GEFs. In contrast, Rho GTPases can be inactivated by GAP-mediated hydrolyzation of GTP into GDP. GDIs controls the subcellular localization of Rho GTPases by forming protein complex. To date, 20 Rho GTPase family members are identified, among which RhoA, Rac1, and Cdc42 are the most documented, especially in neoplastic context.<sup>177,178</sup> For example, RhoA has been found to be overexpressed in colorectal cancer, breast cancer, lung cancer, ovarian cancer, gastric cancer, and so on.<sup>179–181</sup> The upregulation of Rac1 can be observed in prostate cancer, gastric cancer, breast cancer and leukemia.<sup>179,181–183</sup> Cdc42 was reported to be overexpressed in colorectal cancer, breast cancer, lung cancer, and melanoma.<sup>179,184–186</sup> Interestingly, RhoA gene was shown to be rarely amplified but frequently deleted in a wide range of tumors according to The Cancer Genome Atlas dataset, suggesting its tumor suppressive role which is somewhat contradictory to most literatures.<sup>177</sup> Rho GTPases regulate actin polymerization through complex mechanisms, thereby organizing cytoskeleton. In this process, globular actin (G-actin) is polymerized to form filamentous actin (F-actin), whereas Arp2/3 complex is one of the key molecular machines enabling this.<sup>187</sup> For

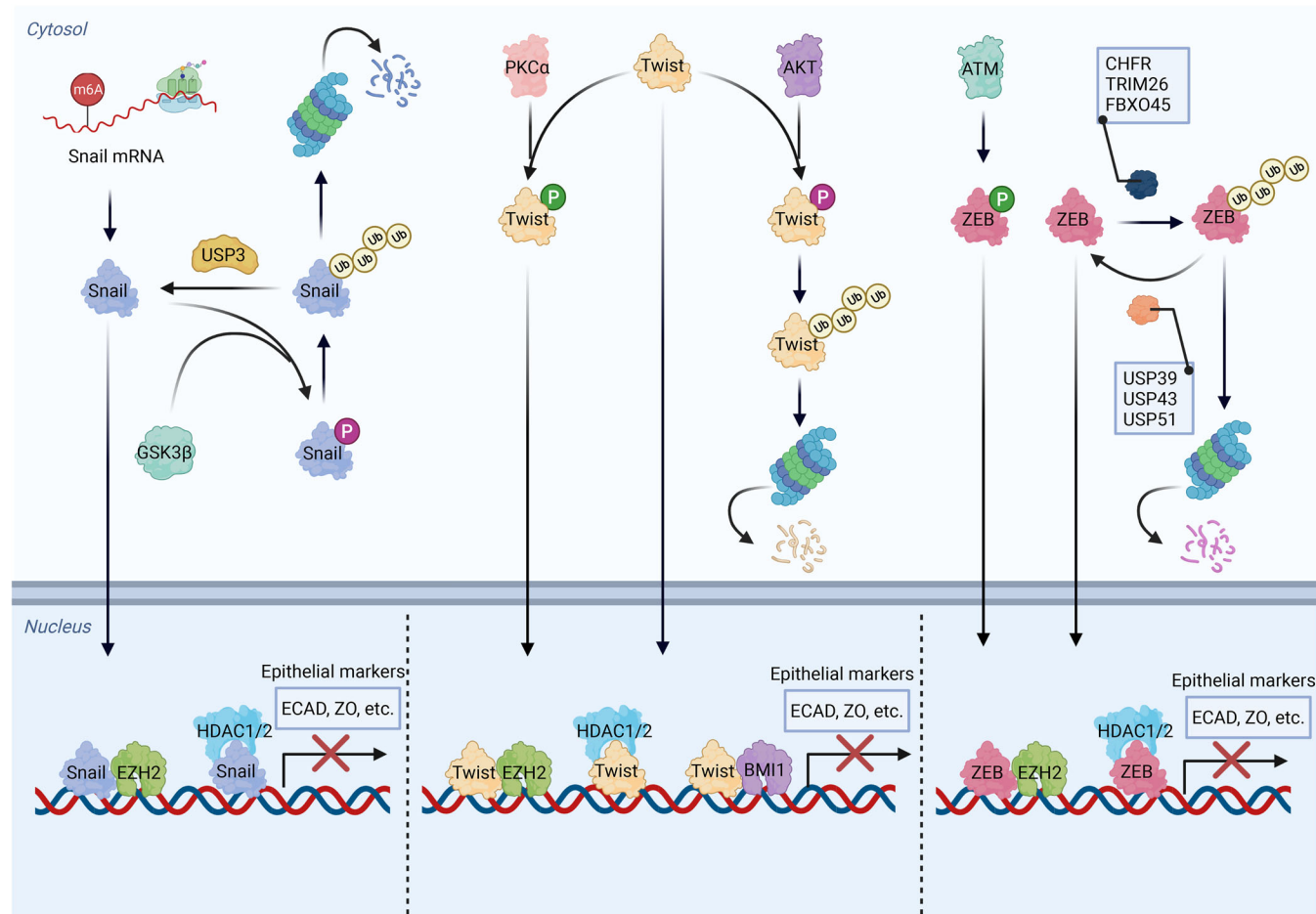
example, Rac1 can bind with the nucleation promoting factor WAVE, which activates Arp2/3 to generate branched actin networks.<sup>187,188</sup> Similarly, Cdc42 interacts with N-WASP, leading to the activation of Arp2/3 thus playing critical role in actin polymerization.<sup>189,190</sup> Aberrant formation of F-actin in cancer cells is closely correlated with EMT and metastasis of a variety of tumors, including hepatocellular carcinoma, glioblastoma, pancreatic cancer, bladder cancer, breast cancer, and so on.<sup>30,191–197</sup> Besides, the antagonists for actin polymerization, latrunculin A/B, have been shown anticancer effects through disrupting the formation of F-actin.<sup>30,198–200</sup> This evidence indicates that the organization of actin cytoskeleton network profoundly affects EMT and tumor progression.

Cytoskeleton reorganization frequently leads to the formation of membrane protrusions, namely lamellipodia, filopodia, invadopodias, and podosomes, thus contributing to the migration and invasion of tumor cells. Lamellipodia and filopodia are defined as sheet-like and spike-like extensions, respectively. Both protrusions are present on the leading edge of migrating cells, which determine the movement direction of cells.<sup>201</sup> Invadopodia appears on the ventral surface of membrane, and often involved in the degradation of ECM via MMPs.<sup>202</sup> Podosomes are similar to, but less effective in ECM degradation than invadopodia.<sup>203,204</sup> These membrane protrusions can be visualized from microscopy, thus being useful marks for EMT. Compelling evidence suggests the crucial roles of membrane protrusions in EMT and tumor progression. For instance, formation of filopodia encouraged by EMT program facilitates both initiation and metastatic colonization in breast cancer.<sup>205</sup> Besides, invadopodia is correlated with the EMT program and consequent metastasis in a variety of tumors, including hepatocellular carcinoma, breast cancer, bladder cancer, and so on, suggesting invadopodia as a potential prognostic marker for tumor metastasis.<sup>206–210</sup> Given their critical roles in tumor metastasis, inhibitors targeting these protrusions can be of therapeutic value. For example, lidocaine has been shown to reduce metastatic dissemination of breast cancer by inhibiting the formation of invadopodia.<sup>211</sup> However, the formation and turnover of these membrane protrusions are highly dynamic and the duration ranges from minutes to hours, targeting these structures might be with off-target effects thus waiting for further investigation.

## 4 | ACTIVATION OF KEY TRANSCRIPTION FACTORS

Cells that undergo EMT program are characterized by a global change in gene expression, resulting in loss of epithelial marks and gain of mesenchymal properties. This





**FIGURE 3** EMT-TFs are key drivers of EMT program. The EMT program is controlled by several EMT-TFs, including Snail, Twist, and ZEB. These EMT-TFs are regulated at transcriptional and posttranslational levels, such as protein phosphorylation, ubiquitination, and RNA m6A modification. The m6A modification on EMT-TF mRNAs can be recognized by different m6A readers, which facilitate the translation or promote RNA decay. The protein phosphorylation is coordinated by kinases and phosphatases, whereas the ubiquitination can be balanced via E3 ligases and deubiquitinases. When translocated into nucleus, EMT-TFs bind with different epigenetic modifiers such as EZH2, HDAC1/2, BMI1 to form transcriptional complexes, thereby regulating EMT program

process is largely regulated at the transcription level by several EMT-TFs, including Snail (encoded by *SNAI1*), Slug (*SNAI2*), Twist-related proteins, zinc-finger E-box-binding (ZEB) proteins, and others (Figure 3). During EMT, these EMT-TFs are activated at the transcriptional level (e.g., transcribed by other TFs) and posttranslational level (e.g., phosphorylation and ubiquitination), which have been observed in various cancer thus can be potential therapeutic targets. One of the most known target genes of EMT-TFs is *CDH1*, which encodes the important epithelial mark E-cadherin. Nearly all EMT-TFs can repress the transcription of E-cadherin through similar mechanisms, suggesting the functional redundancy between them.<sup>212</sup> However, these EMT-TFs differ from each other in many aspects, such as individual structure, size, tissue specificity, binding partner, and target preference. Therefore, the specific, nonredundant functions of EMT-TFs are gradually appreciated.<sup>213</sup> Indeed, EMT-TFs are spatiotempo-

rally regulated thus contributing to distinct expression patterns in different cancer types, which is correlated with specific characteristics of different tumors such as drug sensitivities.<sup>214</sup> The idea of nonredundant functions of EMT-TFs can be supported by plenty of evidence. First, an EMT-TF can be either oncogenic or tumor suppressive. For instance, the tumor-promoting role of ZEB1 has been widely reported.<sup>17,215,216</sup> However, ZEB1 can also function as a tumor suppressor to inhibit the progression of acute myeloid leukemia (AML).<sup>217</sup> Moreover, in *KRAS*-mutated lung cancer, ZEB1 was shown to inhibit tumor progression via repressing *ERBB3*.<sup>218</sup> Given the fact that EMT-TFs are oncogenic in most cases, those tumor suppressive effects directly demonstrate their nonredundant functions. Second, different EMT-TFs can exhibit diverse expression pattern in the same cancer type. For example, *Twist1* and ZEB1 were reported to be respectively overexpressed and downregulated in lung cancer.<sup>218</sup> This

finding indicates that Twist1 and ZEB1 regulate lung cancer through contrary ways. Third, different members in the same EMT-TF protein family can play opposite roles. For instance, ZEB1 was shown to promote the progression of melanoma, whereas ZEB2 functions as a tumor suppressor to inhibit this process.<sup>4,219</sup> Fourth, different members in the same EMT-TF protein family can be selectively activated by the same upstream signal. In response to proangiogenic factor SDF1 $\alpha$ , endothelial Slug, but not Snail, is activated thus contributing to pathological angiogenesis and tumor growth.<sup>220</sup> Moreover, one EMT-TF can be regulated by another EMT-TF. It has been shown that Snail can transiently repress the transcription of Twist1 in response to TGF $\beta$  stimuli, which is followed by Snail degradation, Twist1 reexpression, and consequent EMT as well as tumor metastasis in breast cancer.<sup>221</sup> This evidence suggest that different EMT-TFs can form complex regulatory network to coordinate the EMT program, and a single EMT-TF may not be sufficient to dictate cancer metastasis in some circumstances.

#### 4.1 | Snail

Snail proteins include Snail1 (Snail) and Snail2 (Slug), which promote EMT and metastasis in a variety of cancer through the epigenetic regulation. Briefly, Snail harbors four zinc-finger motifs in its carboxy-terminal region, which bind with the E-box DNA sequence of target genes.<sup>20</sup> This event facilitates the recruitment of the polycomb repressive complex 2 (PRC2), which contains the methyltransferase enhancer of zeste homolog 2 (EZH2). The recruitment of PRC2 leads to DNA methylation as well as repressive histone modifications such as H3K9me2, H3K9me3, and H3K27me3 on the promoter of target genes, resulting in epigenetic silencing.<sup>222</sup> A variety of junction proteins are repressed through this mechanism, including E-cadherin, occludin, ZO-1, claudin-3, claudin-5, claudin-7, and so on.<sup>83,223–226</sup> Interestingly, PRC2 also induces active marks, such as H3K4me3.<sup>227</sup> This mark might be associated with the MET program, or the increase of mesenchymal proteins including N-cadherin and MMP-9.<sup>228,229</sup> In addition to PRC2, Snail also recruits other epigenetic modifiers such as histone deacetylases (HDACs) and lysine-specific histone demethylase 1 (LSD1), thereby regulating EMT program.<sup>230–232</sup> For example, Snail has been found to form protein complex with HDAC1 and HDAC2 at the promoter of E-cadherin, leading to the deacetylation and consequent repression of E-cadherin, which results in the EMT and migration of breast cancer, pancreatic cancer, and nasopharyngeal cancer.<sup>233–236</sup> To date, the mechanisms underlying the preference of Snail to different epigenetic modifiers remain unclear.

The expression and function of Snail can be regulated at transcriptional level, posttranscriptional level, and posttranslational level. The transcription of Snail involves other transcription factors, such as NF- $\kappa$ B, YY1, and even Snail itself.<sup>237–239</sup> Particularly, several transcription factors capable of regulating Snail are the components of embryogenesis-related pathways, including YAP (component of Hippo pathway), Gli1 (Hedgehog), Smad (TGF $\beta$ ), and many others.<sup>240–242</sup> This connection between EMT and embryonic development signaling will be discussed in the following section. The regulation of Snail at the posttranscriptional level is evidenced by N6-Methyladenosine (m6A) modification on its mRNA.<sup>243</sup> Briefly, the methyltransferase-like 3 (METTL3) induces m6A modification in Snail CDS, which can be recognized by YTH domain-containing family protein 1 (YTHDF1).<sup>243</sup> This event facilitates polysome-mediated translation of Snail, leading to EMT and metastasis of tumor cells.<sup>243</sup> Besides, it has been reported that UDP-glucose enhances the stability of Snail mRNA, resulting in the overexpression of Snail and the metastasis of lung cancer.<sup>244</sup> In terms of its posttranslational regulation, the most known mechanisms are phosphorylation and ubiquitination. For instance, GSK-3 $\beta$ -mediated phosphorylation of Snail at its first motif promotes its ubiquitination and subsequent degradation, whereas phosphorylation at its second motif leads to its cytoplasmic retention.<sup>245</sup> In contrast, phosphorylation of Snail at Ser249 by the PAR-atypical protein kinase C (aPKC) inhibits the ubiquitination of Snail, thus promoting tumor metastasis.<sup>246</sup> Besides, the ubiquitin-specific protease 3 (USP3) has been shown to stabilize Snail via deubiquitination.<sup>247</sup> Interestingly, aforementioned transcription factor, NF- $\kappa$ B, also regulates the ubiquitination of Snail.<sup>248</sup> Another member of Snail protein family, Slug, is also regulated by ubiquitination. It has been shown that several deubiquitinases, including USP5, USP10, USP20, counteract the ubiquitination of Slug therefore enhancing its protein stability.<sup>249–251</sup> Moreover, recent study showed that Slug can be SUMOylated through the interaction with Ubc9 and SUMO-1, which enhances the transcriptional repression activity of Slug and promotes the progression of lung cancer.<sup>252</sup>

#### 4.2 | Twist

Twist protein family consists of two members Twist1 and Twist2, which belong to the basic helix–loop–helix (bHLH) transcription factors.<sup>253</sup> Based on their structural similarities and functional redundancy, we mainly discuss Twist1 here although the differences between them have been reviewed elsewhere.<sup>254</sup> As one of important EMT-TFs, Twist1 has long been associated with cancer progression.

For instance, knockout of *Twist1* was shown to abrogate tumor metastasis in breast cancer.<sup>255</sup> Similar to *Snail*, *Twist1* represses the epithelial marks such as E-cadherin,  $\alpha$ -catenin,  $\gamma$ -catenin and upregulates mesenchymal marks including vimentin, fibronectin, N-cadherin, leading to EMT and cancer progression.<sup>9,256</sup> Aforementioned epigenetic repressing complex, PRC2, can be also recruited by *Twist1* to induce the H3K27me3 modification at *Ink4A/Arf* locus, thus preventing the senescence of mesenchymal stem cells.<sup>257</sup> Besides, *Twist1*-mediated EMT program involves other epigenetic modifiers, such as the methyltransferase SET8, the PRC1 component BMI1, and the NuRD transcriptional repressive complex. For example, during breast cancer metastasis, *Twist1* interacts with SET8 thus inducing a H4K20 monomethylation mark at the promoter of E-cadherin and N-cadherin, which downregulates and upregulates the transcription, respectively.<sup>258</sup> Besides, *Twist1* recruits BMI1 to repress the transcription of E-cadherin, and this effect is correlated with the poor prognosis of head and neck cancers.<sup>259</sup> Furthermore, it has been reported that *Twist1* can recruit NuRD complex, which contains HDAC1/2, to decrease the acetylation of H3K9 at *Foxa1* promoter, thereby repressing the expression of *Foxa1*.<sup>260</sup> This event contributes to the *Twist1*-induced metastasis of breast cancer, but less responsible for *Twist1*-induced EMT phenotype of breast cancer cells, which is interesting.<sup>260</sup>

Compelling evidence has suggested that *Twist1* can be regulated at transcriptional level and posttranslational level. In neuroblastoma, both N-Myc and c-Myc proteins physically associate with the promoter of *Twist1*, thus activating its transcription.<sup>261</sup> Other transcription factors, such as *Sox12* and *Sox13*, can also transcribe *Twist1* thus leading to EMT and metastasis of HCC cells.<sup>262,263</sup> In contrast, the bHLH transcription factors BHLHE40 and BHLHE41 inhibit the transcription of *Twist1*.<sup>264</sup> The posttranslational modifications on *Twist1* include phosphorylation and ubiquitination. For example, PTEN with K27-linked polyubiquitination (PTEN<sup>K27-polyUB</sup>) can dephosphorylate *Twist1* at Ser123, resulting in the nuclear translocation of *Twist1* and subsequent EMT phenotype.<sup>265</sup> In terms of the ubiquitination, the E3 ligase Pirh2 is able to prime *Twist1* for degradation.<sup>266</sup> Interestingly, the RING-finger E3 ligase RNF8 promotes the K63-linked ubiquitination of *Twist1* at K38, which enhances, but not decreases, the protein stability of *Twist1*, leading to its nuclear localization thereby playing critical roles in cancer drug resistance.<sup>267</sup> It is worth noting that there is causal relationship between the phosphorylation and ubiquitination of *Twist1*, as evidenced by the facts that protein kinase C $\alpha$  (PKC $\alpha$ )-mediated phosphorylation of *Twist1* at Ser144 diminishes the ubiquitination of *Twist1*, whereas AKT1-mediated phosphorylation of *Twist1* at Ser42, Tyr121,

and Ser123 enhances its ubiquitination.<sup>268,269</sup> Sometimes, these posttranslational modifications regulate *Twist1* activity through affecting protein–protein interaction. For instance, LYN-mediated phosphorylation of *Twist1* leads to the dissociation between *Twist1* and its cytoplasmic anchor G3BP2, resulting in the nuclear translocation of *Twist1*.<sup>270,271</sup> This event underlies the nature of *Twist1* as a mechanomediator in response to mechanical cues such as matrix stiffness.<sup>270,271</sup> Besides, the association between *Twist1* and another binding partner TGIF1 is able to inhibit *Twist1*, whereas this inhibitory effect can be abolished by the phosphorylation of TGIF1 in pancreatic ductal adenocarcinoma.<sup>272</sup> Interestingly, *Twist1* can bind with itself to form a homodimer, regulating fibroblast activation, cell migration, and embryonic development.<sup>273,274</sup>

### 4.3 | ZEB

ZEB is potent EMT inducer, which represses epithelial marks including E-cadherin, ZO-1, claudin-1, desmoplakin, and is implicated in various type of cancer such as gastric cancer, leukemia, squamous cell carcinoma, liver cancer, colorectal cancer, and so on.<sup>82,140,150,159,275–278</sup> There are two members in vertebrate ZEB protein family, namely ZEB1 and ZEB2, which share structural and functional similarities but are also distinguished by considerable differences. For example, a switch from ZEB2 to ZEB1 promotes the progression of melanoma, suggesting these two ZEB proteins function in an opposite manner under this condition.<sup>4</sup> These diverse effects of ZEB1 and ZEB2 can be explained by different epigenetic modifiers recruited by them. ZEB1 binds p300 to activate transcription, whereas ZEB2 interacts with C-terminal-binding protein (CTBP) to silence the target genes.<sup>279</sup> In addition, ZEB1 has been shown to recruit the SWI/SNF chromatin-remodeling protein BRG1, thereby repressing E-cadherin independently of CTBP.<sup>280</sup> Moreover, ZEB1 can also recruit NuRD complex, which contains HDAC1/2, to promote the EMT and tumor progression in pancreatic cancer and lung cancer.<sup>281,282</sup> Similarly, the NuRD complex can also recruited by ZEB2, thus regulating the metastasis of breast cancer and the differentiation of neural cells.<sup>283,284</sup>

A variety of transcription factors have been found to activate ZEB1, such as *Snail1*, *Twist*, *ETS1*, and myocyte enhancer factor 2A (MEF2A).<sup>285,286</sup> In addition to this transcriptional regulation, ZEB1 is also regulated at the protein level, such as phosphorylation. For instance, ERK phosphorylates ZEB1 at Thr867, which inhibits its nuclear translocation, DNA binding, and function of transcriptional repression.<sup>287</sup> This ERK–ZEB1 axis is involved in the progression of various tumors, including lung

cancer, breast cancer, liver cancer, prostate cancer, ovarian cancer, and glioblastoma.<sup>288–293</sup> Interestingly, ERK also activates ZEB1 through upregulating aforementioned transcription factor ETS1, suggesting that ERK can regulate ZEB1 in either direct or indirect manner.<sup>294</sup> Moreover, ZEB1 has been also reported to be phosphorylated by ATM at S585, which enhances its protein stability in breast cancer cells.<sup>295</sup> Similar to other EMT-TFs, ZEB1 can also be regulated by ubiquitination. For example, the E3 ligase tripartite motif-containing 26 (TRIM26) downregulates ZEB1 through ubiquitination-mediated protein degradation, whereas USP39 acts as a deubiquitinase to stabilize ZEB1.<sup>296</sup> Thus, TRIM26 and USP39 function in an antagonistic manner to coordinate the fate of liver cancer.<sup>296</sup> Besides, the ubiquitination of ZEB1 involves additional E3 ubiquitin ligases such as checkpoint with Forkhead and ring finger domains (CHFR), F-box only protein 45 (FBXO45), and deubiquitinases including USP51 and USP43.<sup>297–300</sup> As to ZEB2, the regulation of protein stability is associated with E3 ubiquitin ligases FBXW7 and TRIM14.<sup>301,302</sup> These observations indicate that the regulation of ZEB proteins is rather complex in tumor progression.

#### 4.4 | Novel EMT-regulating transcription factors

As a rapidly evolving field, more transcription factors have been identified as novel regulators of EMT, such as PRRX1 and Sox. PRRX1 cooperates with Twist1 to induce EMT during embryogenesis and tumor invasion, but overexpression of PRRX1 is associated with a favorable prognosis in breast cancer patients.<sup>99</sup> Mechanistically, loss of PRRX1 induces MET, which is required for metastatic colonization of breast cancer cells.<sup>99</sup> Moreover, PRRX1 loss-mediated MET also confers cancer cells with stemness, leading to drug resistance thus further explaining why PRRX1 overexpression predicts good prognosis.<sup>99,303</sup> However, opposite finding showed that upregulated PRRX1 induces EMT, cancer stemness, metastasis, and poor prognosis in colorectal cancer, suggesting the role of PRRX1 is highly context-dependent in different tumor types.<sup>100</sup> This contradiction might be attributed to the diverse functions of PRRX1 isoforms, namely PRRX1b that activates EMT, whereas PRRX1a that activates MET, and this isoform switching underlies tumor invasion and metastatic colonization in pancreatic cancer.<sup>304</sup> Sox protein family consists of over 20 members in vertebrates, many of which are involved in tumor initiation and progression.<sup>305</sup> Numerous studies indicated that Sox proteins regulate EMT via classical EMT-TFs. For instance, Sox13 transcriptionally activates Twist, thereby promoting EMT in liver cancer.<sup>263</sup>

Interestingly, several Sox proteins have been shown to modulate EMT through either direct or indirect way. Sox4 binds with the promoter of Slug to activate its transcription, leading to EMT in uterine carcinosarcoma.<sup>306</sup> Alternatively, Sox4 can also directly transcribe N-cadherin, thus inducing EMT independent of classical EMT-TFs.<sup>101</sup> Another Sox protein, Sox9, can directly bind with the promoters of claudin-1 and ZEB1 to modulate their transcription, suggesting both direct and indirect regulation of EMT by Sox9.<sup>102</sup>

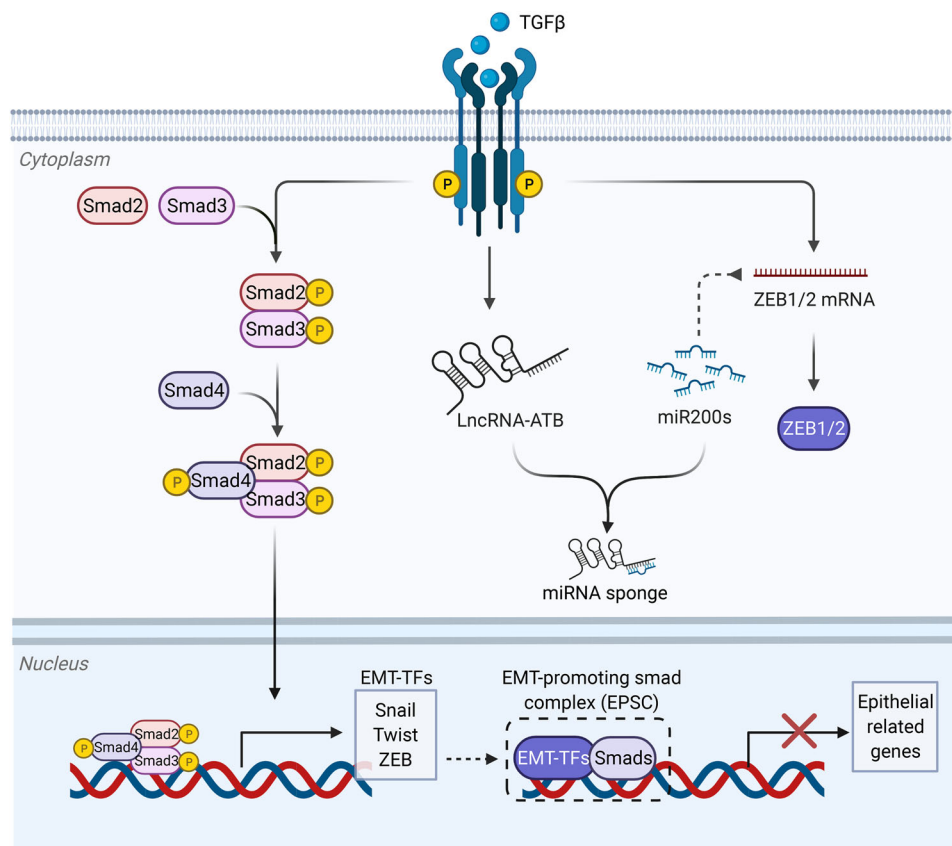
## 5 | SIGNALINGS IN EMBRYONIC DEVELOPMENT LINK TO EMT

It is well acknowledged that carcinogenesis and embryonic development share remarkable similarities. A series of features of embryogenesis, including EMT, angiogenesis, ECM remodeling, cell differentiation, and migration, are also important hallmarks of cancer. Indeed, EMT is fine-tuned during embryonic development for morphogenesis of organs, whereas tumor cells hijack this program for cancer progression. Therefore, tumor is to some extent considered a problem in the field of developmental biology. For instance, proregenerative glia progenitors perform spinal cord repair via EMT in response to spinal cord injury in zebrafish, whereas dysregulated brain development such as excessive interneuron generation promotes the formation of brain tumors in human.<sup>307,308</sup> Aforementioned EMT-TFs that widely studied in the context of neoplasia, actually play critical roles in embryonic development. For example, Snail and PRRX1a govern the internal left–right (L/R) asymmetry, which is fundamental to the proper function of organs (e.g., the heart laterality) during the development of vertebrates.<sup>309,310</sup> Generally, embryonic development is regulated by several evolutionarily conserved signaling pathways, including TGF $\beta$ , Wnt, Hedgehog, and Hippo.<sup>311–314</sup> These signalings have to be restricted when developmental processes are completed, which is the prerequisite for the proper morphology and function of organisms. Aberrant reactivation of these pathways in a well-mature organ, however, frequently contributes to tumor development. Compelling evidence indicate that EMT program in cancer cells are largely regulated by embryogenesis-related signalings, suggesting EMT as an intrinsic link between embryonic development and tumor progression.

### 5.1 | TGF $\beta$

TGF $\beta$  signaling is activated by the interaction between TGF $\beta$  ligands and receptors (type I and type II) on





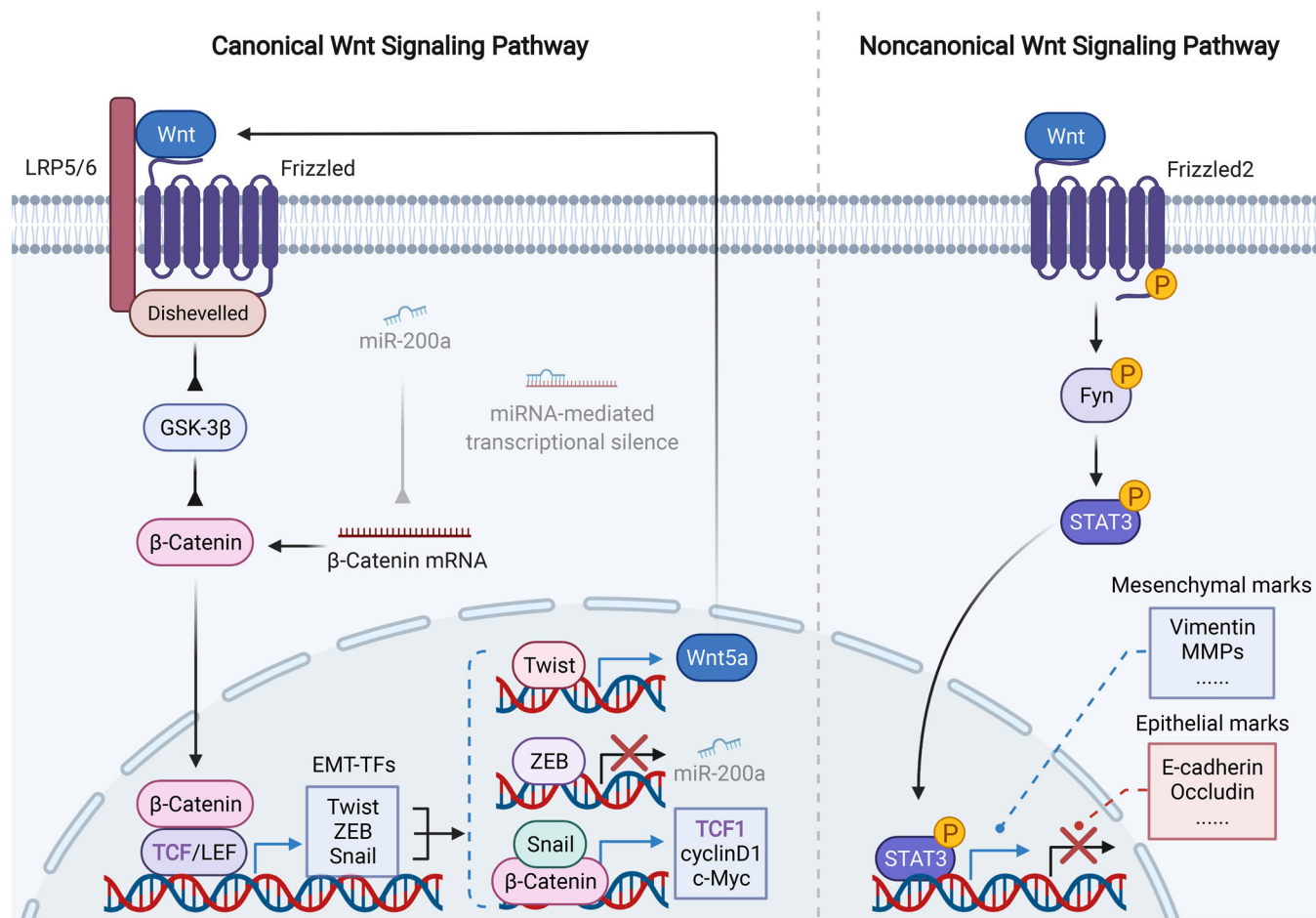
**FIGURE 4** The crosstalk between EMT and TGF $\beta$  signaling. Activated TGF $\beta$  signaling leads to the nuclear translocation of Smad2/3/4 complex, which directly recognizes the promoters of EMT-TFs to initiate their transcription. In turn, EMT-TFs can bind with Smad proteins to form EMT-promoting Smad complex (EPSC), thus regulating the transcription of epithelial and mesenchymal marks. Besides, TGF $\beta$  signaling also regulate the expression of EMT-TFs through noncoding RNAs

the cell membrane. Next, type I receptor is phosphorylated by type II receptor, which then phosphorylates Smad proteins, the key transcription factors mediating the biological consequence of this signaling. There are eight members in Smad protein family with different roles. Briefly, Smad1/2/3/4/5/8 positively regulate TGF $\beta$  signaling through the formation of activated Smad complexes, which translocate into nucleus to initiate gene transcription. In contrast, Smad6/7 negatively regulate TGF $\beta$  signaling via preventing the formation of activated Smad complexes and facilitating the degradation of TGF $\beta$  receptors.<sup>315</sup> In the context of neoplasm, although TGF $\beta$  signaling can be both oncogenic and tumor suppressive, a variety of tumors benefit from activated TGF $\beta$  signaling, and targeting TGF $\beta$  signaling can be a promising therapeutic strategy for cancer treatment.<sup>316,317</sup> One of the mechanisms is that TGF $\beta$  signaling regulates EMT program of tumor cells, thus promoting cancer progression (Figure 4). For example, TGF $\beta$ -Smad signaling has been shown to activate the expression of Snail1, leading to EMT and proliferation of lung cancer cells.<sup>24</sup> Not surprisingly, Smad proteins play central roles in TGF $\beta$ -mediated

EMT program. Indeed, Smads physically associate with EMT-TFs such as Snail, Twist, ZEB to form an EMT-promoting Smad complex (EPSC; e.g., Snail1-Smad3/4 complex), which represses the transcription of epithelial marks while increasing the expression of mesenchymal marks.<sup>224,318</sup> Besides, TGF $\beta$  induces long noncoding RNA (lncRNA)-ATB, which acts as a competing endogenous RNA (ceRNA) to competitively bind with miR-200s. This effect increases the expression of ZEB1/2, leading to EMT and metastasis of liver cancer.<sup>319</sup> In contrast, several tumor suppressors exhibit their antimetastatic function through inhibiting TGF $\beta$  signaling, including circular RNA circPTK2 and lncRNA SMASR.<sup>320,321</sup> Interestingly, TGF $\beta$  signaling has been also demonstrated tumor-suppressive via a lethal EMT.<sup>322</sup> This observation is in line with the context-dependent roles of TGF $\beta$  signaling.

## 5.2 | Wnt

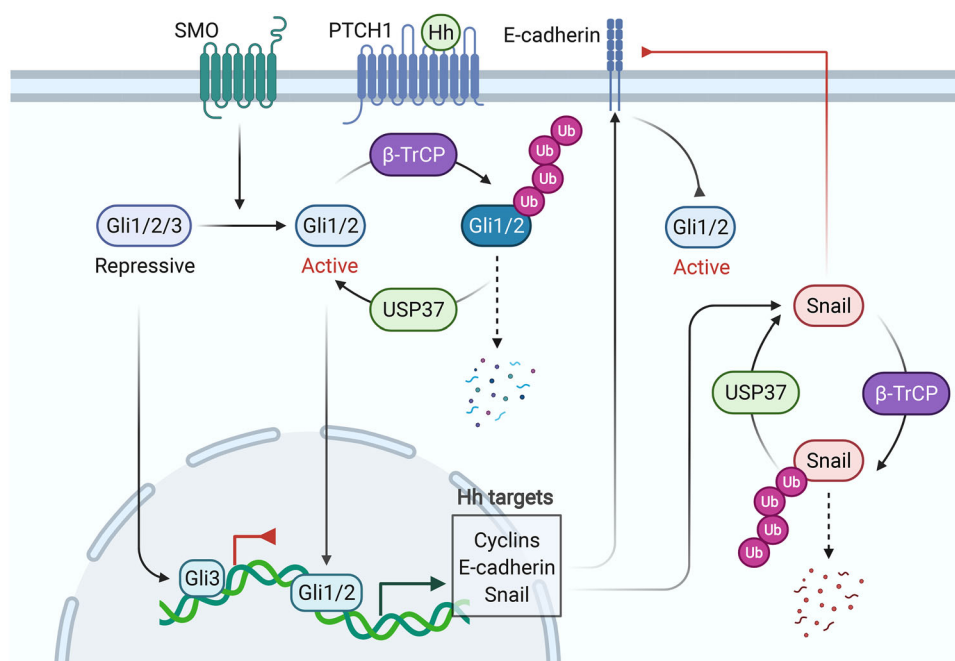
Wnt signaling exert its biological effects mainly through the control of transcription cofactor  $\beta$ -catenin. Briefly, in



**FIGURE 5** Regulation of EMT via canonical or noncanonical Wnt pathway. In canonical Wnt pathway, nuclear  $\beta$ -catenin/LEF/TCF transcriptional complex activates the transcription of EMT-TFs, thus regulating EMT. In turn, EMT-TFs affect Wnt signaling to form feedback loops. For example, Snail/ $\beta$ -catenin complex activates transcription of TCF1, which is the key transcription factor of Wnt signaling. ZEB represses the transcription of miR-200a, leading to the reactivation of  $\beta$ -catenin. Twist can transcribe Wnt5a, which activates Wnt receptor Frizzled. In noncanonical Wnt pathway, activation of Frizzled2 leads to the phosphorylation and consequent nuclear translocation of STAT3, which upregulates mesenchymal marks and represses epithelial marks independent of  $\beta$ -catenin

the quiescent state,  $\beta$ -catenin is ubiquitinated and inhibited by GSK-3 $\beta$  complex. When activated, Wnt proteins bind with the Frizzled receptor, leading to the recruitment of coreceptor LRP5/6. This event leads to the activation of Dishevelled (Dvl), which inhibits GSK-3 $\beta$  thus protecting  $\beta$ -catenin from degradation. Then, stabilized  $\beta$ -catenin translocates into nucleus, where it interacts with transcription factor LEF/TCF to activate transcription.<sup>323–325</sup> In terms of oncology, Wnt signaling is overall oncogenic, which allows malignant proliferation and metastasis of cancer cells, and this process involves the induction of EMT program<sup>326</sup> (Figure 5). For instance, Her2 positive early disseminated cancer cells can enter into a partial EMT state via activation of Wnt signaling, thereby initiating metastasis in breast cancer.<sup>327</sup> Mechanistically, the EMT-promoting function of Wnt pathway is largely attributed to  $\beta$ -catenin/LEF/TCF-mediated transcription control. This is supported by the fact that  $\beta$ -catenin/TCF4

can bind to the promoter of ZEB1 and increase its transcription, leading to the EMT and metastasis of colorectal cancer.<sup>328</sup> Similarly,  $\beta$ -catenin/TCF3 and  $\beta$ -catenin/LEF1 interact with and activate the promoter of Snail and Twist, respectively.<sup>329–331</sup> As a consequent, Snail can physically associated with  $\beta$ -catenin to form a transcription complex, which activates Wnt target genes independent of TCFs.<sup>332</sup> In contrast, autophagic degradation of  $\beta$ -catenin has been shown to inhibit EMT and abrogate the metastasis of colorectal cancer.<sup>333</sup> Interestingly, Wnt signaling can also promote EMT through a noncanonical way independent of  $\beta$ -catenin. For example, the Wnt receptor Frizzled2 drives EMT and tumor metastasis in liver cancer via Fyn and Stat3, and this process is not affected by pharmacological inhibition of  $\beta$ -catenin.<sup>27</sup> In addition, EMT-TFs can in turn activate Wnt signaling, forming a positive feedback loop to proceed EMT program. It has been shown that ZEB1 can repress the expression of miR200A, leading



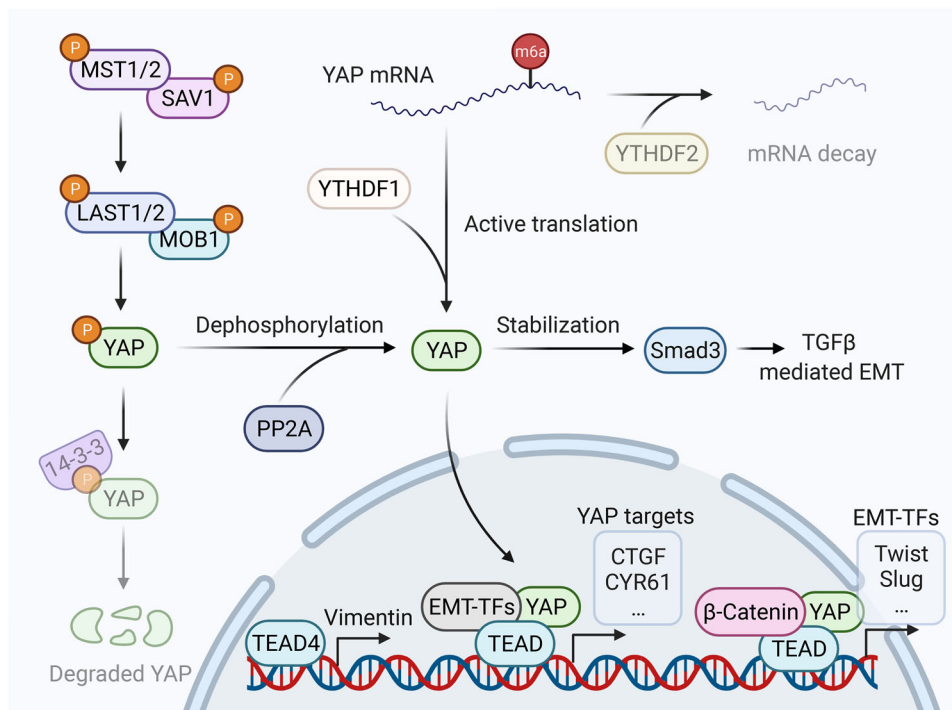
**FIGURE 6** Hedgehog signaling regulates EMT and cancer progression. Hedgehog (Hh)-mediated inhibition of PTCH1 leads to the activation of SMO, which promotes the activation of the transcription factor Gli1/2. The target genes of Gli1/2 include several EMT associated proteins such as Snail and E-cadherin. Besides, Snail and Gli share the same protein turnover system, namely  $\beta$ -TrCP-mediated protein degradation and USP37-induced protein stabilization. In turn, cellular EMT status affects Hedgehog signaling, as evidenced by loss of E-cadherin has been shown to activate Gli1/2

to the reactivation of  $\beta$ -catenin.<sup>334,335</sup> Besides, Twist binds with the promoter of Wnt5a to increase its transcription, which activates Wnt signaling thus promoting breast cancer metastasis.<sup>336</sup>

### 5.3 | Hedgehog

Hedgehog signaling is activated by the binding of hedgehog ligands with the receptor Patched (PTCH1). This binding leads to the activation of membrane GPCR-like protein Smoothed (SMO). In the absence of activated SMO, the transcription factor complex Gli1/2/3 is processed by proteasome, during which the active Gli1/2 is degraded, whereas the repressive Gli3 is preserved, resulting in transcriptional repression. When SMO is activated in response to Hedgehog signals, Gli1/2/3 is differentially processed to yield an active Gli1/2, which translocates into nucleus to initiate transcription.<sup>337,338</sup> Similar to other developmental pathways, Hedgehog signaling is tightly correlated with EMT program (Figure 6). For instance, aberrant activation of Hedgehog signaling promotes EMT of immature ductular cells, the accumulation of which results in fibrosis, cirrhosis and other liver diseases.<sup>29,339,340</sup> In the field of oncology, Hedgehog sig-

naling has long been implicated in cancer progression, at least partially due to its critical role in the regulation of EMT program of cancer cells.<sup>341–343</sup> In human cholangiocarcinoma tissues, Hedgehog ligand is highly expressed to repress E-cadherin, leading to a EMT phenotype and elevated viability of tumor cells.<sup>344</sup> Besides, Hedgehog signaling-mediated EMT, characterized by the overexpression of vimentin, Snail, N-cadherin, and repression of E-cadherin, ZO-1, has been also reported in the metastasis of bladder cancer and breast cancer.<sup>345,346</sup> Inhibition of Hedgehog signaling with the administration of Vismodegib, the antagonist for PTCH1, is able to suppress EMT and cell proliferation of castration-resistant prostate cancer.<sup>347</sup> Compelling evidence suggest that Hedgehog signaling promotes EMT via the key transcription factor Gli. Indeed, the EMT-TF Snail is a transcriptional target of Gli.<sup>241</sup> Stabilization of Gli1, which is mediated by the deubiquitinase USP37, has been shown to activate EMT and increase invasiveness in breast cancer.<sup>348</sup> Interestingly, USP37 also catalyzes the deubiquitination of Snail.<sup>349</sup> Moreover, both Gli and Snail can be ubiquitinated by E3 ligase  $\beta$ -TrCP.<sup>245,350</sup> Therefore, EMT program and Hedgehog signaling are connected via sharing protein turnover machinery. In turn, loss of E-cadherin or activation of EMT-TFs can activate Gli, thus probably forming a positive



**FIGURE 7** The connection between EMT and Hippo signaling. When Hippo signaling is inactivated, YAP and TEAD form complex in nucleus to direct transcription. EMT-TFs can bind with YAP/TEAD complex, leading to a functional switch of EMT-TFs from epigenetic repressors into activators, resulting in the overexpression of YAP target genes to facilitate tumor progression. Besides, Hippo signaling can regulate EMT via the interplay with other developmental pathways, such as TGF $\beta$  signaling and Wnt signaling. For instance, YAP can stabilize Smad3, the component of TGF $\beta$  signaling, thus promoting TGF $\beta$ -mediated EMT. Besides, YAP/TEAD complex can be associated with  $\beta$ -catenin to form a novel transcriptional complex, which activates the transcription of EMT-TFs. Moreover, TEAD4 can transcriptionally activate the mesenchymal protein vimentin independent of YAP

signaling circuit to sustain EMT phenotype.<sup>351,352</sup> Briefly, EMT-TFs activate Six1, which stimulate Hedgehog signaling in neighboring tumor cells, but how E-cadherin deficiency activates Gli remains elusive.<sup>351,352</sup> Although most literatures indicate that Hedgehog signaling positively regulates the EMT program and tumor progression, the contrary findings were also reported that describe that Gli1 binds with the promoter of E-cadherin to activate its transcription, thereby inhibiting EMT.<sup>353</sup> Besides, downregulation of Gli1 results in the disassembly of adherens junctions, leading to EMT and cell migration in pancreatic ductal adenocarcinoma.<sup>353</sup> This observation suggests a context-dependent role of Hedgehog signaling in EMT and cancer progression.

## 5.4 | Hippo

The core of Hippo signaling is a kinase cascade that negatively regulates the activity of transcription cofactors YAP/TAZ. Given the oncogenic nature of YAP/TAZ, the Hippo signaling is generally regarded as a tumor-suppressive pathway. Briefly, active Hippo signaling

is characterized by phosphorylation of MST1/2–SAV1 complex, which phosphorylates LATS1/2–MOB1 complex. Then, phosphorylated LATS1/2 induce the phosphorylation of YAP/TAZ, leading to their cytoplasmic retention or ubiquitin-mediated degradation. When Hippo signaling is inactivated, dephosphorylated YAP/TAZ can translocate into nucleus, where they interact with transcription factors TEAD1/2/3/4 to initiate transcription.<sup>354,355</sup> YAP/TAZ have been demonstrated potent EMT inducers promoting the progression of a variety of cancer (Figure 7). For instance, YAP is able to promote EMT and tumor metastasis by downregulating E-cadherin and remodeling cytoskeleton in renal cancer and nasopharyngeal carcinoma.<sup>356,357</sup> Mechanistically, YAP interacts with several EMT-TFs, including ZEB1, Snail, and Slug to form a transcriptional complex. This event results in a functional switch of EMT-TFs from transcriptional repressors to transcriptional activators, which activates tumor-promoting genes involved in EMT, tissue regeneration, and cancer metastasis.<sup>216,358–361</sup> During EMT, the activation of YAP can be attributed to multiple mechanisms. For example, the catalytic subunit of protein phosphatase 2A (PP2Ac)-mediated dephosphorylation of YAP facilitates YAP nuclear



translocation, leading to EMT and metastasis of HCC.<sup>362</sup> Besides, excessive formation of filamentous actin (F-actin) leads to the dephosphorylation of LATS1, resulting in YAP stabilization and consequent liver cancer metastasis.<sup>30</sup> Interestingly, YAP can either positively or negatively regulate the formation of F-actin, suggesting this process is highly context-dependent.<sup>363,364</sup> Besides, the activity of YAP can also be regulated at the RNA level. N6-methyladenosine (m6A) modification on the YAP mRNA can be recognized by YTHDF1 and YTHDF2, which facilitates the translation and decay of YAP mRNA, respectively.<sup>365</sup> Moreover, Hippo signaling can be cross-linked with other developmental pathways, such as TGF $\beta$  and Wnt, to coordinate EMT program. It has been shown that YAP can prevent GSK3 $\beta$ -mediated Smad3 degradation, thereby promoting Smad3-induced EMT.<sup>366</sup> Furthermore, YAP is physically associated with  $\beta$ -catenin to form a transcriptional complex with TEAD4 in nucleus, leading to the overexpression of EMT-TFs including Slug and Twist in breast cancer.<sup>367</sup> It is worth noting that TEAD4 can regulate EMT and promote metastasis by directly transcribing vimentin without the binding with YAP in colorectal cancer, which is interesting.<sup>368</sup>

## 6 | REGULATION OF EMT BY REDOX SIGNALING

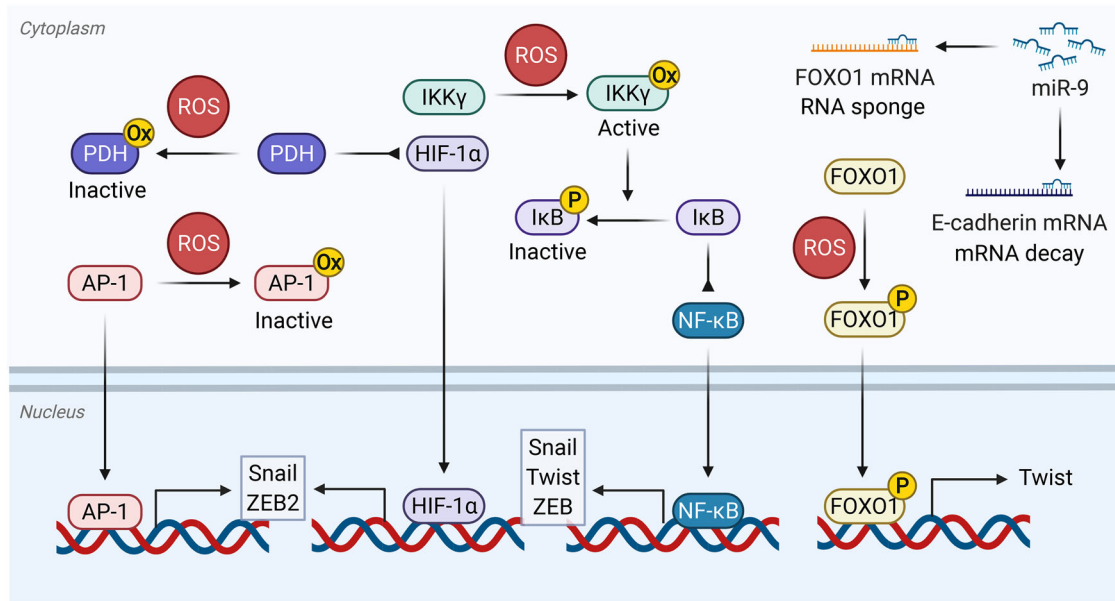
In cancer cells, active metabolic patterns result in accumulation of reactive oxygen species (ROS), including hydroxyl free radicals, superoxide, and hydrogen peroxide, leading to oxidative stress. On one hand, ROS promote malignant transformation via inducing DNA damage and genomic instability, therefore being regarded as oncogenic molecules.<sup>369</sup> Besides, ROS also sustain the proliferation of tumor cells and drive more aggressive phenotype.<sup>370,371</sup> However, excessive accumulation of ROS leads to cell death, suggesting that oxidative stress regulates cancer in contradictory ways.<sup>372</sup> For example, oxidative stress can promote ferroptosis in melanoma to inhibit tumor metastasis, whereas administration of antioxidants has been shown to facilitate the metastasis of lung cancer.<sup>373,374</sup> In order to preserve rapid proliferation and aggressive behavior while avoiding death, tumor cells achieve redox equilibrium via increasing their antioxidant capacity. This process is enabled by the activation of antioxidant transcription factors (e.g., NRF2, NF- $\kappa$ B, p53, FOXO, etc.),<sup>375–378</sup> the expression of antioxidant enzymes (e.g., SODs, CAT, PRDXs, TRXs, GPXs, etc.),<sup>379–383</sup> and the production of small antioxidant molecules (e.g., GSH, vitamin C, vitamin E, etc.).<sup>384–386</sup> Therefore, targeting oxidative stress is a potential anticancer strategy.<sup>387–389</sup> Particularly, oxidative stress regulates cancer initiation and progression at least partially through modulating EMT program. For

instance, ROS stimulate the expression of Snail, thus promoting EMT in breast cancer.<sup>13</sup> Similarly, ROS accumulation induced by lipid peroxidation, GSH depletion, and SLC7A11 deficiency can initiate EMT in lung cancer cells.<sup>390</sup> In consistence with these, treatment of antioxidants, such as N-acetylcysteine (NAC), curcumin, resveratrol, has been shown to inhibit EMT in a variety of tumor cells.<sup>391–393</sup> It is worth noting that ROS also promote MET in cancer cells. For example, 2-deoxyglucose-induced ROS accumulation promotes the phenotype transition of mesenchymal breast cancer stem cells (CSCs) into epithelial breast CSCs.<sup>394</sup> This evidence suggests that EMT program is profoundly affected by cellular redox status.

Mechanistic studies have revealed that ROS are not only toxic molecules that randomly cause damages, but also serve as secondary messengers to regulate signaling transduction.<sup>395</sup> This is enabled by a number of ROS-sensitive proteins, termed redox sensors.<sup>396</sup> In response to the stimulation of ROS, certain cysteine residues on redox sensors can be oxidized at their sulfhydryl to generate cysteine sulphonate or to form disulfide bonds, leading to the conformational changes, formation of protein complex, and consequent acquisition of new biological functions.<sup>397</sup> These processes can be reversed by antioxidant machinery, thus fine-tuning cell behaviors called redox signaling.<sup>398</sup> Compelling evidence suggest the critical roles of redox signaling in cancer progression. For example, redox modification of pyruvate kinase M2 (PKM2) at Cys358 causes a decrease of PKM2 enzymatic activity, leading to a metabolic reprogramming of cancer cells which supports tumor growth under oxidative stress.<sup>399</sup> In terms of EMT program, redox signaling regulates the functions of junctions proteins, actin cytoskeleton, and EMT-TFs, thereby affecting cancer progression (Figure 8).<sup>400</sup> Aforementioned developmental pathways that control EMT program are actually crosslinked with redox signaling. For instance, TGF $\beta$ -induced EMT can be enhanced or abolished by the treatment of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) or NAC, respectively.<sup>401</sup> Besides, ROS has been shown to activate Wnt signaling and subsequent EMT during wound healing.<sup>402</sup> Furthermore, the Hippo component TAZ is a typical redox sensor, whose cysteines can undergo S-glutathionylation in response to ROS.<sup>403</sup> This redox modification improves the protein stability of TAZ, which facilitates its nuclear translocation and subsequent transcription of target genes.<sup>403</sup> Together, these evidence suggest that redox signaling plays critical roles in EMT.

### 6.1 | Redox regulation of cell junctions and cytoskeleton

ROS increase endothelial permeability through disrupting the integrity of cellular junctions between endothelial



**FIGURE 8** Regulation of EMT by oxidative stress and redox signaling. Redox signaling modulates EMT program via the regulation of redox sensors, including AP-1, PHD, IKK $\gamma$ , FOXO1, and many others. Briefly, these redox sensors can be either activated or inactivated via cysteine oxidation, thereby positively or negatively regulate the transcription of EMT-TFs. Moreover, FOXO1 mRNA acts as ceRNA which binds with miR-9, protecting E-cadherin mRNA from miR-9-mediated degradation. This effect inhibits EMT program independent of EMT-TFs

cells. For instance, ROS induced by the metabolic intermediate, 4-hydroxy-2-nonenal, has been shown to modulate adherens junctions, tight junctions and integrins, leading to dysfunction of endothelial barrier.<sup>404</sup> In line with it, another study showed that protein tyrosine phosphatase SHP2 is a critical factor preserving the integrity of endothelial barrier.<sup>405</sup> In response to lipopolysaccharide (LPS)-induced oxidative stress, SHP2 is oxidized at its Cys459, leading to its inactivation.<sup>405</sup> This event activates Fyn-related kinase, which phosphorylates several junction proteins, resulting in the disruption of endothelial adherens junction.<sup>405</sup> Besides, ROS-mediated endothelial permeability also involves the S-glutathionylation of Rac1, a small Rho GTPase that regulates cytoskeleton.<sup>406</sup> Metabolic stress-induced S-glutathionylation of Rac1 at Cys81 and Cys157 can inactivate Rac1, leading to the reorganization of cytoskeleton network and consequent vascular permeability in the aorta.<sup>406</sup> Interestingly,  $\beta$ -actin can be oxidized at Cys374, suggesting a direct regulation of cytoskeleton by redox signaling.<sup>407</sup> In intestinal epithelial cells, the protein level of E-cadherin can be decreased in response to TNF- $\alpha$ -induced oxidative stress, while its mRNA level is not changed.<sup>408</sup> This observation suggests that ROS-mediated loss of E-cadherin might be at least partially independent of conventional epigenetic mechanisms by EMT-TFs.<sup>408</sup> In addition to E-cadherin, many other EMT marks have been shown to be regulated by oxidative

stress, including claudins, occludin, zonula occludens,  $\alpha$ -SMA, and vimentin.<sup>409,410</sup>

## 6.2 | Regulation of EMT-TFs by redox sensors

As mentioned above, EMT-TFs play crucial roles in EMT program. Though most EMT-TFs do not seem to undergo direct redox modification, they are actually regulated by other redox sensors. The transcription factor, activator protein-1 (AP-1), is a typical redox sensor involved in EMT program. In response to oxidative stress, cysteines between its subunits are oxidized to form intermolecular disulfide bond, which decreases its DNA binding affinity.<sup>411–413</sup> AP-1 has been shown to directly bind with the promoter of Snail and ZEB2, thus activating their transcription, leading to EMT and metastasis of skin cancer, cervical cancer, and breast cancer.<sup>414–417</sup> Besides, AP-1 can also physically interact with Snail and Twist, forming a transcriptional complex to induce EMT.<sup>418,419</sup> Another redox sensor, prolyl hydroxylase (PHD), can be inactivated by the oxidation of cysteines in its catalytic domain.<sup>420</sup> This event leads to the activation of HIF-1 $\alpha$ , which transcribes ZEB1 and Twist to induce EMT and promote tumor metastasis.<sup>421–423</sup> HIF-1 $\alpha$  can also improve the protein stability of Snail, thereby promoting cancer metastasis.<sup>424</sup> In addition, the

redox sensor IKK $\gamma$  (also known as NEMO) can be activated via ROS-induced disulfide bond formation between Cys54 and Cys347.<sup>425</sup> Activated IKK $\gamma$  phosphorylates I $\kappa$ B, leading to the dissociation between I $\kappa$ B and NF- $\kappa$ B. This effect results in the activation of NF- $\kappa$ B, which promotes the transcription of several EMT-TFs.<sup>426</sup> Moreover, FOX, class O (FOXO) proteins are a group of redox sensitive transcription factors, which play vital roles in cellular antioxidant defense.<sup>378,427</sup> In response to ROS, FOXO4 and transportin-1 can form protein complex through intermolecular disulfide bond, promoting the nuclear translocation of FOXO4, which is required for the transcription of SOD2 and subsequent ROS elimination.<sup>428</sup> Simultaneously, FOXO proteins are largely involved in cancer progression, notably the EMT program.<sup>429</sup> For example, hypoxia-induced oxidative stress promotes the phosphorylation and activation of FOXO1, which directly binds with the promoter of Twist to increase its transcription, leading to EMT and metastasis of prostate cancer.<sup>430</sup> Besides, FOXO1 can also upregulate ZEB1 during EMT process.<sup>431</sup> Another FOXO family member, FOXO3a, has been shown to inhibit  $\beta$ -catenin via upregulating miR-34 or directly protein binding.<sup>432</sup> This effect abolishes the  $\beta$ -catenin-mediated transcription of ZEB1, thus repressing EMT in prostate cancer.<sup>432</sup> Interestingly, FOXO proteins might regulate EMT independent of classical EMT-TFs. For instance, FOXO1 mRNA serves as a competitive endogenous RNA (ceRNA), whose 3'UTR region can be targeted by miR-9.<sup>433</sup> This event protects E-cadherin mRNA from miR-9-mediated degradation, resulting in maintenance of E-cadherin expression thus inhibiting breast cancer metastasis.<sup>433</sup>

## 7 | EMERGING ROLES OF EMT IN HALLMARKS OF CANCER

Cancer cells acquire distinct hallmarks in different stages during progression, including rapid proliferation, resisting cell death, deregulating cellular energetics, tumor-promoting inflammation, acquiring stemness, inducing angiogenesis, activating metastasis, and many others.<sup>434</sup> Among them, tumor metastasis is the canonical consequence of EMT program which has been widely reported. Even so, new concepts have also emerged in recent years. For instance, tumor cells in hybrid EMT state, but not completed mesenchymal state, possess highest metastatic capability.<sup>108</sup> In line with this, expression of the epithelial mark E-cadherin, which is conventionally thought to inhibit metastasis, actually promotes metastasis in some circumstances.<sup>118</sup> Moreover, it has been reported that EMT is not required for metastasis.<sup>115,116</sup> This evidence indicate that EMT-regulated tumor metastasis is

highly context-dependent. Here, we mainly focus on the contribution of EMT on other cancer hallmarks, including metabolic reprogramming, CSC property, and tumor-promoting inflammation.

### 7.1 | Metabolic reprogramming

Cancer cells undergo metabolic reprogramming to meet the energy and nutrient demand, which enables the sustaining proliferation in adverse condition. One of the well-known metabolic reprogramming is the “Warburg effect” describing that tumor cells utilize glycolysis rather than oxidative phosphorylation even in a normoxia condition, termed as “aerobic glycolysis.”<sup>435</sup> During EMT process, tumor cells reprogram their glucose metabolism and lipid metabolism to maintain aggressive behaviors. Specifically, the key EMT-TF Snail can regulate the transcription of enzymes in glucose metabolism. For instance, Snail represses the expression of phosphofructokinase PFKP, thus switching the glucose flux toward pentose phosphate pathway.<sup>436</sup> Besides, Snail-mediated silencing of fructose-1,6-biphosphatase induces glycolysis, which maintains stemness and invasiveness of basal-like breast cancer cells.<sup>437</sup> However, it has been also reported that metastatic cancer cells prefer oxidative phosphorylation to glycolysis, which might be due to the decrease of glucose uptake and consequent energy crisis in CTCs.<sup>438</sup> Therefore, tumor cells probably tend to utilize oxidative phosphorylation to achieve more efficient ATP production rather than glycolysis during EMT.<sup>439</sup> This concept is supported by the finding that nuclear phosphoglycerate kinase 1 drives EMT and metastasis by oxidative phosphorylation in Smad4-deficient pancreatic ductal adenocarcinoma cells.<sup>440</sup> Notably, aberrant glucose metabolism in turn affects EMT through regulating EMT-TFs, such as Snail. It has been shown that hyperglycemic condition induces an O-GlcNAc modification at Ser112 on Snail1, thereby enhancing its protein stability to facilitate EMT program in breast cancer cells.<sup>441</sup>

Lipid metabolism is closely related with cancer metastasis, as evidenced by the finding that the metabolic shift toward fatty acid oxidation is critical for tumor metastasis to lymph nodes.<sup>442</sup> Besides, the formation of lipid droplets has been found to promote metastasis of tumor cells.<sup>443</sup> Given the direct connection between EMT and metastasis, it is not surprising that lipid metabolism plays vital roles during EMT program. Indeed, EMT-like morphological changes require the alterations in membrane lipid composition, which is enabled at least partially through the fatty acid translocase CD36.<sup>444,445</sup> In addition, the formation of invadopodia, a classical EMT-like morphological alteration, is induced by the reprogramming of

lipid metabolism during the progression of liver cancer.<sup>446</sup> Apart from regulation of membrane dynamics, lipid metabolism also regulates the fate of EMT cells through ferroptosis, a cell death form induced by lipid peroxidation. Cancer cells in completed mesenchymal state that lack of E-cadherin expression largely suffer from severe oxidative stress, making them highly vulnerable to ferroptosis.<sup>118</sup> Nevertheless, lymph protects those metastatic cancer cells from ferroptosis by relieving oxidative stress, which is dependent on the abundant oleic acid, a kind of unsaturated fatty acid.<sup>373</sup> This evidence suggests that metabolism with different lipid substrates can diversely regulate ferroptosis, cell survival, and metastasis during EMT. Furthermore, different lipid metabolism enzymes also regulate metastasis in contrary ways. For example, the lipid metabolism enzyme ATP-citrate lyase can promote the metastasis of colorectal cancer and liver cancer.<sup>447,448</sup> In contrast, another lipid metabolism enzyme, acyl-CoA synthetase long-chain family member 4, sensitizes cancer cells to ferroptosis by esterifying arachidonic acid and adrenic acid into phosphatidylethanolamine.<sup>449</sup> According to the profound impacts of lipid metabolism in EMT and metastasis of cancer, targeting lipid metabolism can be a potential therapeutic strategy for cancer treatment. For instance, inhibiting lipid metabolism by simvastatin-based nanomedicine has been shown to reverse EMT and overcome cancer drug resistance.<sup>450</sup>

## 7.2 | Cancer stem cell property

The capacity of tumor initiation is partly driven by the activation of the EMT program because active EMT-TFs confer numerous properties of stemness on those with cell plasticity. Indeed, aberrant expression of EMT-TFs, such as Twist1, SIP-1, and Snail, in cancer cells contributes to the acquisition of stem-cell features, as evidenced by the increased self-renewal ability *in vitro* and tumor propagation potential *in vivo*.<sup>42,437,451,452</sup> Moreover, ZEB1, as an EMT activator, has been identified to enhance tumorigenic and colonization capacity by stabilizing the stem cell marker Sox2 in a miR-200c-mediated manner in pancreatic cancer derived from a KPC model with mutant Kras and p53.<sup>17</sup> Similar results have been demonstrated in glioblastoma, where ZEB1 contributes to tumor initiation by orchestrating a series of stemness effectors, including SOX2, OLIG2, and CD133/PROM1.<sup>452</sup> Additionally, the results from breast cancer indicate that the EMT regulators ZEB1/2 and Bmi1 promote stemness toward tumorigenesis and metastasis via TET-Family-dependent epigenetic modulation. Mechanistically, miR-22 induces chromatin remodeling by decreasing 5hmC levels by inhibiting the TET family, therefore leading to epigenetic silenc-

ing of miR-200, which is involved in the expression of ZEB1/2 and Bmi1.<sup>453</sup> Recently, a novel signaling axis, the basic helix-loop-helix (bHLH) transcription factor E2A with Snail1, was confirmed to participate in the maintenance of breast cancer stemness, facilitating tumor initiation, metastasis, and drug resistance.<sup>454</sup> It has also been reported that deletion of the protocadherin FAT1 induces a hybrid EMT state in skin squamous cell carcinoma and lung tumors by triggering not only CAMK2-CD44-SRC axis-mediated YAP1 activation and ZEB1 expression but also EZH2-regulated SOX2 expression, thus resulting in maximal stemness and accelerated cancer progression.<sup>114</sup>

In addition to the involvement of tumorigenesis, EMT-mediated stemness is also identified as a key driver of acquired drug resistance and subsequent tumor relapse after drug holidays.<sup>35</sup> Accumulated evidence reveals that cancer cells in EMT activation seem to undergo dedifferentiation similar to stem cells, showing upregulation of antiapoptotic and drug efflux proteins.<sup>455,456</sup> For instance, multidrug resistance (MDR), especially to gemcitabine, in pancreatic cancer is closely correlated with the activation of the EMT-like transcription factor ZEB1.<sup>457</sup> In detail, solute carrier family 39 member 4, which functions to modulate the intracellular zinc concentration, induces the expression of the zinc-dependent EMT protein ZEB1 by stimulating STAT3 activation. Consequently, ZEB1-induced ITG A3/B1 triggers integrin  $\alpha3\beta1$  signaling and subsequent c-Jun-N-terminal kinase signaling, therefore inhibiting the expression of gemcitabine transporter ENT1.<sup>457</sup> Similarly, SRF/MRTF-mediated signaling is involved in the mesenchymal transition of melanoma with RAC1P29S by upregulation of the EMT-TFs Snai2 and Jun, driving a low apoptosis rate and BRAFi resistance.<sup>458</sup>

Of note, various cancer therapies, including chemo-, radio-, and immunotherapy, can also result in an EMT-mediated stress adoption mechanism, which was recently defined as one feature of the drug-tolerant persister (DTP) state.<sup>459</sup> These residual cells with EMT characteristics govern the nongenetic mechanisms to enter the dormant state against a wide spectrum of antiproliferation treatments.<sup>460</sup> For example, Matthew and colleagues indicate that high-mesenchymal persister cancer cells derived from drug treatments hold more resistance to tyrosine kinase inhibitor therapy and chemotherapy.<sup>461</sup> Furthermore, the features of EMT activation, upregulation of vimentin, and downregulation of E-cadherin have also been examined in MDR persister cells, which develop DTP phenotypes after canonical MDR reversal treatment.<sup>462</sup> Notably, observations in patients with breast cancer or mice with lung cancer suggest a strong underlying connection among EMT, stemness, and drug resistance by analyzing the expression of EMT markers in posttherapy



specimens, showing an activated EMT program and elevated chemoresistance-related proteins.<sup>115,463</sup>

### 7.3 | Tumor-promoting inflammation

The progression of cancer is largely dependent on the interaction between tumors and tumor microenvironment, especially local inflammatory status. Inflammatory microenvironment is composed by a variety of cells, including tumor cells, stromal cells, tumor-associated macrophages (TAM), cancer-associated fibroblasts, myeloid-derived suppressive cells, T cells, monocytes, dendritic cells, adipocytes, and so on. Inflammatory response is required for immunosurveillance to kill cancer cells; however, it can also promote cancer progression. For example, macrophages can be polarized into M2 tumor-promoting ones. During cancer progression, EMT program can regulate the function of inflammatory and immune cells, whereas several inflammatory cytokines are potent inducers of EMT.<sup>45</sup> As such, EMT program and inflammatory response are affected by each other thus forming complex network, which leads to cancer regression or progression.

Mounting evidence indicate that EMT can be regulated by inflammation. For instance, pancreatitis has been found to promote EMT of premalignant pancreatic epithelial cells, leading to their dissemination to liver, and this process can be abolished by the administration of immunosuppressive agent dexamethasone.<sup>464</sup> Generally, inflammation-induced EMT is largely attributed to proinflammatory cytokines, such as NF- $\kappa$ B, interleukins (ILs), interferons, chemokines, and others. The level of IL-22 is increased during pancreatitis, which promotes EMT of pancreatic ductal adenocarcinoma cells and subsequent tumor progression.<sup>465</sup> Another IL, IL-6, facilitates the transformation of normal liver stem cells into metastatic CSCs by inducing EMT.<sup>466</sup> Interestingly, IL-35 derived from M2 TAMs has been reported to promote MET of metastatic tumor cells, thus resulting in distant colonization.<sup>467</sup> Similar tumor-promoting effects were also observed in other proinflammatory factors, such as M2 TAM-derived chemokine CCL-22 and natural killer cell-derived interferon IFN- $\gamma$ , both of which promote the progression of liver cancer via inducing EMT.<sup>468,469</sup> Mechanistically, these cytokines might regulate the expression of EMT-TFs, thus affecting EMT program. For instance, the pro-inflammatory cytokine NF- $\kappa$ B is able to inhibit the ubiquitination and degradation of Snail, thereby mediating inflammation-induced tumor metastasis.<sup>248</sup>

In contrast to the large amount of literature describing inflammation-regulated EMT, the investigation of EMT-regulated inflammation and immune response is rather

limited. It has been shown that EMT can change the antigens present in tumor surface, recruit M2 TAMs instead of M1 macrophages, thus contributing to the immunosuppression in breast cancer, and this process is probably enabled by the release of cytokine granulocyte-macrophage colony-stimulating factor.<sup>470,471</sup> Besides, the EMT-TF ZEB2 can regulate cytokine signaling thus playing critical role in hematopoietic differentiation and myeloid diseases.<sup>276</sup> Interestingly, tumor cells undergoing EMT have been reported to increase susceptibility to natural killer cells, which enhance immunosurveillance to inhibiting metastasis in lung cancer.<sup>472</sup> This observation suggests that inflammatory or immune responses are not always tumor promoting, but can provide beneficial for cancer patients in some circumstances if not being hijacked by tumor cells.

## 8 | EMT-BASED CLINICAL APPLICATIONS IN ONCOLOGY

### 8.1 | Clinical diagnosis by identifying EMT markers

Given the crucial role of the EMT program in tumorigenesis and development, the identification of EMT-involved biomarkers has accordingly emerged as a diagnostic approach, which is used to match personalized therapies or adjust the treatment intensity. Indeed, an increasing number of clinical cases have indicated that EMT multi-marker combination analysis by immunohistochemistry or immunofluorescence is a key part of tumor stratification or companion diagnostics for patients with different tumor types.<sup>473,474</sup> (Table 1)

In colorectal cancer, miR-200c, a known EMT mediator that has been investigated in preclinical models, is examined in 45 pairs of primary tumor tissues and corresponding matched liver metastases. Expectedly, altered expression of miR-200c, which has low expression in the invasive front of primary cancer tissues and high expression in liver metastasis tissues, showed a strong association with various EMT-related proteins, such as ZEB1, E-cadherin, and vimentin, suggesting a potential metastatic indicator for patients.<sup>475</sup> In line with this, the results from a longitudinal follow-up study based on 185 stage II/III colorectal cancer patients solidly demonstrated a relationship between a high combined EMT score and poor prognosis.<sup>476</sup> Furthermore, several EMT genes, including CDH11, MMP2, and ZEB1, have been identified with higher expression in invasive breast cancer than in pure ductal carcinoma in situ, indicating that EMT activation is related to a high risk of invasive disease in all subtypes of breast cancer, especially in ER-negative disease.<sup>477</sup> A study inves-

TABLE 1 Implications of several EMT markers in the diagnosis of cancer

EMT markers	Sample types	Synergetic markers	Clinical symptoms	Cancer types
miR-200c	Tissue biopsy	ZEB1, E-cadherin, and vimentin	Liver metastases	Colorectal cancer
ZEB1	Tissue biopsy	CDH11, MMP2	Invasive disease	Breast cancer
E-cadherin	Tissue biopsy	N-cadherin, vimentin	Biochemical recurrence	Prostate cancer
$\beta$ -catenin	Tissue biopsy	E-cadherin, vimentin, CD133, CD44v6, and so on	Metastases and therapy adaptation	Prostatic, colorectal, breast cancer, and so on
Twist1	Liquid biopsies	Akt2, PI3K $\alpha$ , and ALDH1	Metastases and therapy adaptation	Breast cancer
PI3K $\alpha$ and/or Akt-2	Liquid biopsies	ALDH1	Metastases and therapy adaptation	Colorectal cancer

tingating prostate cancer patients treated pre- and post-radiotherapy revealed that downregulation of E-cadherin combined with upregulation of N-cadherin and vimentin was correlated with biochemical recurrence, providing an independent predictive factor for treatment response to radiotherapy.<sup>478</sup> Recently, Navas and colleagues proposed a validated, high-resolution digital microscopic immunofluorescence assay for assessing the EMT phenotype of various human tumor specimens. Their study revealed that  $\beta$ -catenin+ cancer cells accompanied by the induction of E-cadherin and vimentin exist in various metastatic patients. Notably, the expression of stemness markers, such as CD133, CD44v6, ALDH1, and NANOG, is also examined in some cases, implying a standardized assessment for clinical monitoring of EMT-mediated therapy adaptation.<sup>474</sup>

In addition to tissue biopsy, liquid biopsies are another promising method for clinical diagnosis and can also be used to analyze EMT characteristics of tumors, mainly CTCs from the blood of cancer patients. For example, a follow-up study comprising 226 blood samples of 39 patients with metastatic breast cancer showed that the expression rates of EMT-related proteins and the stemness marker ALDH1 were 62 and 44%, respectively, in CTCs of patients with no response to therapy. In contrast, the expression rates in responders are 10 and 5%, respectively.<sup>479</sup> Likewise, through investigating related protein expression in CTCs isolated from blood, Ning et al. demonstrated that the coexpression pattern of EMT markers (PI3K $\alpha$  and/or Akt-2) and stemness markers (ALDH1) predicts significantly shortened progression-free survival and overall survival (OS) in metastatic colorectal cancer patients.<sup>480</sup> To date, the clinical trials of CTCs with EMT features are ongoing or have already been activated for diagnosing or treating patients with breast, prostate, pancreatic, and colorectal cancers (NCT04265274, NCT04021394, NCT04323917, and NCT04323813, respectively).

## 8.2 | Therapeutic interventions targeting the EMT program

With the increasing in-depth knowledge of detailed mechanisms modulating the EMT program, the identification of novel agents targeting this program has been an irreversible trend. Thus far, three reasonable strategies aimed at preventing the initiation of EMT activation, selectively eliminating mesenchymal-like cancer cells, and reversing the EMT process by inducing the MET program all seem to be promising for controlling EMT-mediated tumorigenesis and therapeutic tolerance. However, which approach is the best one with long-term efficacy in the clinic remains controversial. In this section, we present various EMT-targeted drugs based on the approaches mentioned above and list the related clinical trials (Table 2).

### 8.2.1 | Strategies to inhibit EMT induction

Intervention with TGF $\beta$  signaling, the confirmed pathways involved in EMT induction by several preclinical studies, is currently the most recognized strategy. Galunisertib (LY2157299) is a small molecule inhibitor targeting TGF $\beta$ R1 and has been investigated in various advanced tumors.<sup>481–483</sup> Importantly, the anticancer effect of galunisertib is at least partially attributed to its capability of inhibiting EMT. For example, galunisertib was revealed to show a marked sensibilization to enzalutamide treatment in prostate cancer due to that galunisertib-mediated TGF $\beta$  signaling inhibition prevented EMT process.<sup>481</sup> To date, more than 10 clinical trials of galunisertib have been completed for treating metastatic or recurrent cancer (<https://clinicaltrials.gov/>). Among them, a clinical trial for advanced or metastatic unresectable pancreatic cancer suggests that galunisertib combined with gemcitabine has a promising anticancer effect on patients, as evidenced by the prolonged OS (NCT01373164).<sup>484</sup> Similarly, the

TABLE 2 Cancer therapeutic strategies based on targeting EMT

Drugs or combination	Targets or pathways	Cancer types	Intervene strategies	Clinical trials
Galunisertib, gemcitabine	TGF $\beta$ signaling	Pancreatic cancer	Inhibiting EMT induction	NCT01373164
Galunisertib, sorafenib	TGF $\beta$ signaling	Hepatocellular carcinoma	Inhibiting EMT induction	NCT01246986
Antisense	TGF $\beta$ 2 mRNA	Gliomas	Inhibiting EMT induction	NCT00431561
Tepotinib, cetuximab	HGFR (Met)	Colorectal cancer	Inhibiting EMT induction	NCT04515394
Tepotinib	HGFR (Met)	NSCLC, hepatocellular carcinoma	Inhibiting EMT induction	NCT01982955, NCT01988493
Capmatinib	c-Met	Glioblastoma multiforme, colorectal cancer, and so on	Inhibiting EMT induction	NCT02386826
Cabozantinib	AXL	Several solid tumors	Inhibiting EMT induction	NCT03170960
ADH-1	N-cadherin	Several solid tumors	Killing cells undergoing EMT	NCT01825603, NCT00421811
Pritumumab	Vimentin	brain cancer	Killing cells undergoing EMT	NCT04396717
Mocetinostat, nivolumab	HDAC-mediated ZEB1	NSCLC	Killing cells undergoing EMT	NCT02954991
All-trans retinoic acid	Activation of MET	Breast cancer, head and neck cancer, neuroblastoma	Reversing the EMT process	NCT05016349, NCT03370367, NCT03042429
Tazemetostat	EZH2	Prostate cancer, lymphomas, and so on	Reversing the EMT process	NCT04179864, NCT01897571

therapeutic effect of galunisertib combined with sorafenib was confirmed in a phase II study regarding advanced hepatocellular carcinoma, showing acceptable safety and a pronounced improvement in OS (NCT01246986).<sup>485</sup> Notably, there also exist many similar small molecule inhibitors depending on TGF $\beta$ R1 inhibition, such as EW-7195, LY580276, and SD-208, displaying potential anticancer effects by blocking TGF $\beta$  pathways.<sup>486</sup> In addition to small molecular inhibitors, antisense therapy targeting TGF $\beta$ 2 mRNA has been revealed to have comparable effects, and related clinical trials have been ongoing (NCT00431561).<sup>487</sup> Nonetheless, it will be counterproductive to perform a blind targeting of TGF $\beta$  signaling, which has multifaceted biological functions in tumorigenesis and development.<sup>488</sup> Indeed, in the early stages of cancer, induction of TGF $\beta$  signaling actually limits the proliferation of tumor cells.<sup>489</sup> Thus, rational patient stratification and optimized drug administration will be key to the effective use of these therapeutic regimens.

The HGF–HGF receptor (also known as Met) axis is another well-known mediator of EMT induction.<sup>490</sup> Excessive activation of the signaling axis is often attributed to point mutation or amplification of the HGFR gene, therefore triggering EMT-induced cell motility and conferring resistance to a series of anticancer agents in cancer.<sup>491</sup> As such, substantial efforts have been made to develop HGF–HGFR signaling inhibitors; furthermore, an increasing number of clinical studies are evaluating the anticancer effect of c-Met inhibitors in different types of cancer.<sup>492</sup>

For example, scientists from the EMD Serono Research & Development Institute recently combined the selective c-Met inhibitor tepotinib with cetuximab for treating metastatic colorectal cancer in a Phase II clinical trial (NCT04515394). Moreover, the effects of tepotinib were also identified in patients with EGFR-mutant non-small-cell lung cancer (NCT01982955)<sup>493</sup> or with advanced hepatocellular carcinoma (NCT01988493),<sup>494</sup> as observed by improved anticancer activity and time to progression, respectively. Capmatinib, another selective c-Met inhibitor, has also been approved for treating Met exon 14-altered non-small-cell lung cancer.<sup>491</sup> Furthermore, a phase I clinical trial of capmatinib is ongoing for the treatment of glioblastoma multiforme, gliosarcoma, colorectal cancer, and renal cell carcinoma (NCT02386826).

In addition, the potential therapeutic agents for blocking EMT induction include other target inhibitors, such as COX-2 inhibitors and AXL inhibitors. The COX-2 selective antagonist celecoxib was revealed to prevent EMT-mediated malignant transformation by modulating  $\beta$ -catenin nuclear localization, the vimentin/E-cadherin proportion and EMT-TFs (Slug, Snail and ZEB1) in colorectal cancer cells and oral squamous cell carcinoma.<sup>495,496</sup> Additionally, inhibition of AXL by the multikinase inhibitor cabozantinib reverses EMT-associated drug resistance in renal cell carcinoma and non-small-cell lung cancer.<sup>497,498</sup> Since 2017, the City of Hope Comprehensive Cancer Center has been conducting a phase I clinical trial of combination therapy of cabozantinib and atezolizumab, treating more than 10 different types of advanced or

metastatic solid tumors (NCT03170960). Recently, their study reported that cabozantinib combined with atezolizumab showed encouraging efficacy and acceptable tolerability in advanced clear cell and nonclear cell renal cell carcinoma.<sup>499</sup>

### 8.2.2 | Strategies to kill cells undergoing EMT

Compared with prevention of EMT induction, a promising alternative strategy is to selectively target EMT-induced mesenchymal-like cancer cells by therapeutically inhibiting the functions of EMT-specific markers. For example, the novel pentapeptide ADH-1 was identified as an N-cadherin antagonist, which breaks extracellular N-cadherin adhesion, therefore disturbing the interaction with FGFR-1 and accelerating the degradation of FGFR-1. ADH-1 treatment markedly enhances the cytotoxicity of chemotherapy.<sup>500</sup> Notably, the clinical applications of cisplatin, gemcitabine hydrochloride, or melphalan combined with ADH-1 have been completed and display considerable promise (NCT01825603 and NCT00421811). Furthermore, vimentin, another classic EMT marker, has been revealed as the direct target of withaferin A, which reduces EMT-associated cancer metastasis by triggering the degradation of vimentin intermediate filaments.<sup>501,502</sup> Pritumumab, a natural IgG1 $\kappa$  antibody derived from cervical carcinoma patients, is also an antagonist of vimentin and was originally evaluated in a clinical trial of patients with brain cancer in Japan.<sup>503</sup> Recently, a clinical trial of primumab in brain cancer therapy has been conducted again, implying promising clinical therapeutic potential (NCT04396717). Beyond the direct suppression of these EMT effectors, targeting EMT-TFs is an alternative approach against EMT-induced mesenchymal-like carcinoma. Indeed, inhibition of the EMT activator ZEB1 by the HDAC inhibitor mocetinostat has been shown to impede drug resistance to oncotherapy in lung and pancreatic cancer.<sup>504,505</sup> Moreover, a phase II clinical trial in which mocetinostat was combined with nivolumab for treating advanced or metastatic non-small-cell lung cancer is ongoing (NCT02954991). Similarly, pharmacologic inhibition of another EMT-TF, Snail, can also prevent EMT-derived malignancy. Lee and colleagues provided a small chemical inhibitor named GN25 and GN29, which showed significant anticancer effects by specifically breaking p53-Snail binding in KRAS-driven human cancer.<sup>506</sup>

Importantly, EMT-driven mesenchymal phenotype of cancer cells seems to hold unique therapeutic vulnerabilities. For example, the aforementioned study reports that mutation-induced FAT1 function defects result in Hippo signaling inhibition and ZEB1 expression by acti-

vating the CAMK2-CD44-SRC axis, thus conferring mesenchymal state-related stemness and metastasis. Interestingly, compared to FAT1 wild-type cancer cells, the loss of FAT1 dramatically enhances the drug sensitivity of cancer cells to dasatinib, saracatinib (SRC inhibitors), and KN93 (CAMK2 inhibitor).<sup>114</sup> Similarly, Kosuke et al. indicated that treatment with the EGFR inhibitor osimertinib causes acquired resistance by promoting the EMT process in lung cancer. These cells with a mesenchymal-like phenotype activate the ATR-CHK1-AURKB axis, simultaneously showing obvious therapeutic vulnerability. AURKB inhibitors and ATR/CHK1 inhibitors.<sup>507</sup> Additionally, the sustainment of endoplasmic reticulum (ER) homeostasis and redox balance is also confirmed as an indispensable requirement for EMT subpopulation survival in various types of cancer, suggesting that ER or oxidative stress inducers can selectively kill these clusters.<sup>394,508</sup> Recently, a novel strategy based on cell plasticity, aiming to force trans-differentiation of EMT-related breast cancer cells into postmitotic adipocytes, seems to work. Ronen and colleagues combined rosiglitazone (antidiabetic drug) with trametinib (MEK inhibitor) to suppress the metastasis of breast cancer.<sup>509</sup> With the in-depth understanding of EMT-driven characteristics, high-throughput screening approaches have been used to identify therapeutic vulnerabilities in carcinoma cells with EMT-induced phenotypes.<sup>510-512</sup> Nevertheless, the clinical application of a similar strategy for treating EMT-mediated metastasis and drug resistance still requires further exploration and validation.

### 8.2.3 | Strategies to reverse the EMT process

Since EMT induction leads to elevated metastatic potential and stemness, activation of MET, a reverse process of EMT, would be a reasonably alternative therapeutic option. This strategy, by forcing differentiation of mesenchymal-like cancer cells into regain epithelial features, resembles “differentiation therapy” treated for AML, whereby all-trans retinoic acid treatment breaks the poorly differentiated state of AML with potential EMT traits, eventually resulting in differentiation-mediated apoptosis.<sup>217,513,514</sup> Notably, all-trans retinoic acid treatment for solid cancers has been universally used in preclinical and clinical studies. In breast cancer cells and paclitaxel-resistant colorectal cancer cells, all-trans retinoic acid has been shown to reverse the EMT process, thus decreasing the motility of cancer cells both in vitro and in vivo.<sup>515,516</sup> Furthermore, all-trans retinoic acid also displays dramatic anticancer potential in phase II and phase III clinical trials of various cancer patients (NCT05016349, NCT03370367, and NCT03042429).



In addition to retinoic acid, which removes EMT-modulated malignant phenotypes by directly inducing differentiation, several epigenetic-associated drugs have been identified as potential inducers of the MET program in preclinical models by preventing EMT activation-mediated metastasis or drug resistance.<sup>517</sup> Pattabiraman et al. indicated that the intracellular second messenger adenosine 3',5'-monophosphate induces epigenetic reprogramming by triggering the activation of protein kinase A and the subsequent phosphorylation of histone demethylase PHF2. Thus, PHF2 decreases histone methylation in mesenchymal cancer cells, in turn inducing a MET process and compromising tumor initiation, implying promising clinical therapeutic potential of inhibitors of histone methyltransferases.<sup>518</sup> Indeed, tazemetostat, a kind of EZH2 histone methyltransferase inhibitor, has rapidly progressed into Phase II or Phase III clinical trials (NCT04179864 and NCT01897571). Additionally, epigenetic regulation-mediated reprogramming of metabolic patterns in cancer cells can control cell state transitions. For example, Loo and colleagues reported that rechanneling fatty acid  $\beta$ -oxidation toward lipid storage by retinoids reverses the mesenchymal-like phenotype to an epithelial-like phenotype in breast cancer, accompanied by a loss of EMT-driven metastasis ability. Mechanistically, fatty acid  $\beta$ -oxidation in the mesenchymal cell state exhibits epigenetic controls of EMT genes via upregulation of acetyl-CoA-dependent histone acetylation.<sup>519</sup> In particular, morphological screening based on an organoid platform from a recent study has identified multiple class I HDAC inhibitors and bromodomain inhibitors as potential inducers of the MET program.<sup>520</sup> However, MET program plays a key role in metastatic colony formation.<sup>48</sup> Thus, the opportune moment of MET induction treatment is undoubtedly the most important. Nonetheless, such an "EMT reverse strategy" still holds a very attractive perspective for clinical application.

## 9 | CONCLUSIONS AND FUTURE PERSPECTIVES

EMT underlies various physiological and pathological processes, including embryonic development, wound healing, tissue fibrosis, and cancer progression. This event is largely driven by a class of EMT-TFs, whose functions are context dependent, therefore EMT status cannot be evaluated based on a single or a very few marks.<sup>1</sup> To this end, EMT status is encouraged to be assessed by certain "EMT signatures" containing dozens of related genes including epithelial marks, mesenchymal marks, EMT-TFs, and upstream or downstream factors.<sup>521–523</sup> In addition to the reliability, EMT signatures can also reveal the hybrid EMT state

(also known as partial EMT, incomplete EMT, or intermediate EMT), which is a common event, whereas absolute epithelial or mesenchymal state are rarely observed during cancer progression. Moreover, it will be more convincing to evaluate EMT status using gene signature in conjunction with morphological changes, such as the presence of a spindle-like shape or the formation of membrane protrusions.

Classical EMT-TFs including Snail, Twist, and ZEB are potent inducers of EMT, which has been extensively investigated. Nevertheless, as a rapid evolving field, novel EMT regulators are gradually identified, notably noncoding RNAs (e.g., microRNAs and lncRNAs).<sup>524,525</sup> These EMT-regulating microRNAs include miR-1, miR-22, miR-29, miR-30, miR-34, miR-125, miR-130, miR-182, miR-192, miR-200, miR-203, miR-205, miR-216, miR-217, miR-331, miR-365, miR-506, miR-517, miR-1199, and many others.<sup>105,453,475,526–540</sup> However, most these microRNAs regulate EMT through targeting classical EMT-TFs, and lncRNAs affect EMT via serving as molecular sponge targeting those microRNAs. For example, miR-205 has been shown to prevent EMT via repressing of ZEB in the metastasis of breast cancer.<sup>105</sup> Meanwhile, lncRNA PNUTS competitively binds with miR-205, leading to the upregulation of ZEB and consequent EMT during tumor progression.<sup>541</sup> Therefore, regulation of EMT by ncRNAs is probably better to be a part of classical EMT-TF pathways, instead of a novel mechanism. Nevertheless, several microRNAs have been found to regulate EMT independent of EMT-TFs. For instance, miR-9 directly targets E-cadherin mRNA for degradation, leading to a EMT phenotype and consequent tumor metastasis in breast cancer.<sup>106</sup> Besides, miR-22 can also directly target E-cadherin, enhancing the invasiveness of prostate cancer cells.<sup>542</sup> Moreover, lncRNA NEAT1 facilitates the formation of FOXN3–NEAT1–SIN3A complex, which directly represses the transcription of GATA3 and ZO-1, leading to EMT.<sup>543</sup> These observations suggest a direct regulatory mechanism of EMT by ncRNAs, but more evidence is still required for ascertain it. Recently, a large number of transcription factors and miRNAs were identified as potential novel regulators of EMT, but as well, the possibility that these novel regulators act through classical EMT-TFs was not precluded.<sup>544</sup> More intriguingly, E-cadherin has been shown to be degraded in response to oxidative stress. TNF- $\alpha$ -induced oxidative stress can modulate the phosphorylation of E-cadherin/ $\beta$ -catenin complex, thus facilitating the degradation of E-cadherin without altering the mRNA level of E-cadherin.<sup>408</sup> Administration of NAC restores the E-cadherin protein expression.<sup>545</sup> Furthermore, E-cadherin has been shown to be degraded through autophagy.<sup>546,547</sup> Hence, the regulation of EMT at the protein level might be at least partially different from the well-known epigenetic

mechanisms induced by EMT-TFs, which deserves further investigation.

In terms of cancer therapy, EMT is an attractive target due to the fact that EMT is closely linked with the hallmarks of cancer such as metastasis, metabolism, stemness, drug sensitivity and immune microenvironment. Generally, EMT gives rise to a more aggressive phenotype in a majority of cancer, thus inhibiting EMT can be a potential therapeutic strategy. However, the reversed process of EMT, namely MET, is also required for distant colonization of metastatic cancer cells. Moreover, incomplete inhibition of EMT program might result in the hybrid EMT state, which is characterized with high plasticity, stemness, invasiveness, and drug resistant property, leading to a more refractory malignancy. In contrast, EMT sometimes provides opportunity for cancer treatment, as evidenced by the fact that tumor cells in complete mesenchymal state are highly susceptible to ferroptosis. This evidence suggests that strategies targeting EMT hold therapeutic potential but still need in-depth understanding of the mechanisms of EMT.

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#### CONFLICT OF INTEREST

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#### ETHICS STATEMENT

Not applicable.

#### DATA AVAILABILITY STATEMENT

Not applicable.

#### AUTHOR CONTRIBUTIONS

C. H., L. L., and C. Z. conceived the manuscript; C. H., Z. H., and Z. Z. wrote and edited the manuscript. All authors read and approved the final manuscript. Z. H. and Z. Z. contributed equally to this work.

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