REVIEW

Epithelial-mesenchymal transition-related circular RNAs in lung carcinoma

Meina Jiang[1,2], Shuai Fang[1,2], Xiaodong Zhao[1,3], Chengwei Zhou[1,3], Zhaohui Gong[1,2]

[1]Department of Biochemistry and Molecular Biology, Ningbo University School of Medicine, Ningbo 315211, China; [2]Zhejiang Province Key Laboratory of Pathophysiology, Ningbo University School of Medicine, Ningbo 315211, China; [3]Department of Thoracic Surgery, The Affiliated Hospital of Ningbo University School of Medicine, Ningbo 315020, China

ABSTRACT   The epithelial-mesenchymal transition (EMT) is a highly complex phenotypic conversion during embryogenesis, and is important for metastasis, which contributes to tumor deterioration and poor prognoses of cancer patients. Lung carcinoma has a high tendency to develop the EMT. Circular RNAs (circRNAs) are involved in EMT-related cell invasion and metastasis in various types of cancers. Moreover, circRNAs have been found to be a link to EMT-related transcription factors and EMT-associated signaling pathways. This review mainly focuses on the influence of EMT-related circRNAs on lung carcinomas. More specifically, the roles of EMT-inducing- and EMT-suppressive circRNAs in lung carcinomas are discussed. With circRNAs potentially becoming promising biomarkers and therapeutic targets for cancer managements, they will hopefully stimulate the interest of medical workers in the early diagnosis, personalized treatment, and positive prognoses in the era of precision oncology.

KEYWORDS   Epithelial-mesenchymal transition; circular RNA; transcription factor; metastasis; lung carcinoma

Introduction

The epithelial-mesenchymal transition (EMT) is a highly complicated phenotypic conversion during embryogenesis, and is a potential process in adults by which epithelial cells gradually downregulate the expressions of cytokeratins and E-cadherin, and upregulate the expressions of mesenchymal genes such as vimentin and fibronectin[1]. Generally, the EMT can be divided into three subtypes: type I EMT in embryonic development, type II EMT in fibrosis, and type III EMT in premalignant and malignant stroma[2]. It is worth noting that EMT is not a purely bipolar state with two well-defined cell populations of mesenchymal cells and epithelial cells. There is also an intermediate state, called partial, involving incomplete hybrid EMT states expressing various levels of epithelial and mesenchymal characteristics and preserving intermediate morphologies[3-5].

Correspondences to: Chengwei Zhou and Zhaohui Gong
E-mail: nbzhouchengwei@163.com and zhaohui@ncri.org.cn
ORCID ID: https://orcid.org/0000-0001-5256-2820 and
https://orcid.org/0000-0002-5662-2752

The EMT is involved in multiple tumor processes including tumor initiation, stemness, migration, cancerous progression, intravasation into the blood, malignant metastasis, and resistance to therapy[6]. However, EMT-inducing transcription factors (EMT-TFs), EMT-related signaling pathways, epigenetic controls, and post-transcriptional regulators have also been found to regulate the EMT[7]. Nonetheless, the roles of circular RNAs (circRNAs) as post-transcriptional regulators in modulating the EMT remain unclear.

With the rapid development of high-throughput RNA sequencing (RNA-seq), a variety of circRNAs have been characterized in humans. In the last few years, it has been reported that circRNAs possess EMT-associated functions and may have an effect on epithelial and mesenchymal cell characteristics including metastasis, migration, and invasion[8]. It is known that circRNAs are a type of covalent single chain closed-loop molecule lacking the 5′end cap and 3′end poly (A) tails *via* a form of alternative splicing, resulting in more stability than linear RNAs in the presence of RNase R[9]. The circRNAs can be divided into three classifications: exonic circRNAs (ecircRNAs), circular intronic RNAs (ciRNAs), and exon-intronic circRNAs (EIciRNAs). Based on their translational capabilities, circRNAs can be classified into noncoding circRNAs and protein-coding circRNAs[10]. In future studies, EMT-related

circRNAs will therefore be extensively studied in the field of oncology.

With a 5-year survival rate of 16.6%, lung cancer is the most common cancer with two major categories of non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), and is the leading cause of cancer-related deaths[11,12]. Despite great progress in experimental research and clinical treatments, lung carcinoma still has a poor diagnosis and prognosis. Thus, there is an urgent need to improve our understanding of the mechanisms of tumorigenesis in the lung[13]. Recently, studies have started to decipher the significance of EMT-related circRNAs in various carcinomas. Lung carcinoma tends to develop as a result of the EMT and metastasis; however, the function of EMT-related circRNAs is still unclear. This review focuses on EMT-related circRNAs in lung carcinoma. It is hoped that it will stimulate interest in the early diagnosis, personalized treatment, and positive prognoses in the field of precision oncology.

Biosynthesis, characteristics, and functions of circRNAs

In the past, numerous circRNAs have been found at lower levels of expression by using outdated detection technologies.

As a result, they have been recognized as nonspecific byproducts[14]. However, it is now known that circRNAs play more critical roles with their unique biosynthesis and characteristics, when compared to cognate linear RNAs. Most circRNAs are created by back-splicing in pre-mRNA, of which 3′ splice donors covalently link to 5′ splice acceptors in the opposite direction (**Figure 1A**). The back-splicing includes four paradigms such as exon-skipping, lariat-driven, intron-pairing-driven circularization, and RNA binding protein-driven circulation[15]. Furthermore, circRNAs have four distinct peculiarities. First, they are conservative despite the length of their evolution[16]. Second, they are specifically expressed spatiotemporally in altered cell types and tissues[17]. Third, they are abundant in all species containing plants, archaea, mice, and humans[9,18-20]. Finally, they have a high stability because of their closed-loop construction and short free terminals. Although circRNAs are stable, the understanding of circRNA degeneration is still unclear. Some hypotheses suggest that endonucleases or N6-methyladenosine (m6A) may initiate degradation of circRNAs. Alternatively, circRNAs may be degraded when combined with miRNAs during Ago2-facilitated cleavage[21,22].

Regarding biological functions, circRNAs play different roles in different subcellular locations. In the cytoplasm, circRNA segregates miRNA as a "sponge" to modulate the
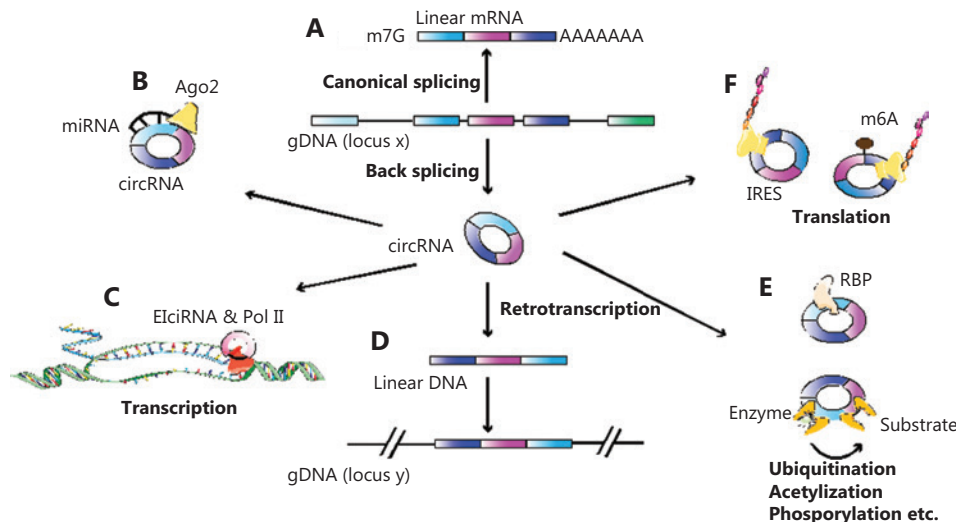
Figure 1 Biosynthesis and functions of circular RNAs (circRNAs). (A) Pre-mRNAs generate mRNAs through canonical splicing while circRNAs are generated through back-splicing. (B) CircRNAs bind to miRNAs. (C) Exon-intronic circRNAs bind to pol II to regulate transcription. (D) The circRNA-derived pseudogenes insert into the genome and switch the genomic DNA configuration through circRNA-retrotranspostion. (E) CircRNAs control protein-protein interactions in a similar manner to reservoirs or scaffoldings. (F) CircRNAs initiate translation through internal ribosome entry sites or *via* N6-methyladenosine.

activity of miRNA and the miRNA-targeted gene (**Figure 1B**). For example, Zhang et al.[23] reported that circFOXO3 (hsa_circ_0006404) promoted the expression of nuclear factor of activated T cells 5 (NFAT5) as a sponge of both miR-138-5p and miR-432-5p in glioblastoma tissues. In the nucleus, circRNA regulates the splicing process or gene transcription (**Figure 1C**). Huang et al.[24] showed that circERBB2, a circRNA generated from the *ERBB2* cognate gene, accumulated in the nucleus to bind with PA2G4, a nucleolus-associated protein, to regulate ribosomal DNA transcription and cell proliferation *via* the circERBB2-PA2G4-TIFIA axis in gallbladder cancer. Like linear mRNAs, circRNAs can also generate pseudogenes. The circRNA-derived pseudogenes are capable of inserting into the genome and switching the genomic DNA configuration during circRNA retrotransposition[25] (**Figure 1D**). Moreover, circRNAs, in a similar manner to reservoirs or scaffoldings, can bind proteins in exact subcellular positions to control protein-protein interactions[26] (**Figure 1E**). Presently, it is well-known that circRNAs involve one of the spliced production coding peptides. For example, Ye et al.[27] showed that circFBXW7 (hsa_circ_0001451) encoded a 185 amino acid peptide (FBXW7-185aa) inhibiting migration and proliferation in triple-negative breast cancer cells. Yet unlike canonic cap-dependent translations, circRNAs commence translation *via* the internal ribosome entry site (IRES) or through m6A[27,28] (**Figure 1F**).

The circRNA link to the EMT in multiple cancers

The EMT comprises fine-tuned phenotypic shifts, in which cells imperceptibly decline expressing E-cadherin and lose cellular adhesion and apical-basal polarity, while showing increased expressions of N-cadherin, extracellular matrix degradation, and cytoskeletal reorganization. Such shifts are initiated by EMT-TFs, which are regulated by EMT-related signaling pathways. EMT-inducing signs are spatiotemporally specific when interacting with numerous regulators and signaling pathways. Although the contributions of the EMT to drug resistance and chemotherapy tolerance have been well-studied, the EMT promotion of tumor metastasis is still controversial[29]. However, increasing evidence has suggested that circRNA-mediated EMT is an essential part of tumor occurrence and development (**Table 1**).

The circRNA link to EMT-TFs

Vimentin is a type of EMT protein marker, which is present in mesenchymal cells and is involved in cancer metastasis and poor prognoses. Cullin2 (Cul2), as a tumor suppressor protein, is a principal part of the multiple cullin-RING-based ECS (Elongin B/C-Cul2/5-SOCS-box protein) E3 ubiquitin-protein ligase complexes engaging in cell cycle control and vasculogenesis[30]. Twist, a vital EMT-TF, combines with the promoter of Cul2 to increase the expression of circCul2 (hsa_circ_10720), which binds to a set of miRNAs to increase vimentin expression in hepatocellular carcinoma (HCC)[31] (**Figure 2A**). In addition to Twist, the EMT is also initiated by Snail, which is a conservative protein of the zinc finger transcription factor family, containing zinc finger domains to bind DNA at the C-terminus, and containing the SNAG domain to interact with epigenetic remodeling complexes at the N-terminus[32,33]. In urothelial carcinoma of the bladder (UCB), circPRMT5 (has_circRNA_101320) generated from *PRMT5* on chromosome 14q11.2, promotes the EMT and aggressiveness through the circPRMT5/miR-30c/SNAIL1/E-cadherin pathway[34] (**Figure 2B**). In melanomas, circRNA_0084043 contributes to cell metastasis and growth *via* the miR-153-3p/snail axis[35] (**Figure 2C**). In cervical cancer, circRNA_000284 induces cell invasion and proliferation *via* the circRNA-000284/miR-506/Snail-2 axis[36]. Apart from Twist and Snail, ZEB2 has been associated with epithelial polarities and diverse malignancies. Li et al.[37] found that circNUP214 (hsa_circ_0089153), as an oncogenic molecule, sponged miR-145 to upregulate the expression of ZEB2 and thereby promote cell proliferation, migration, and invasion in papillary thyroid cancer (PTC). Taken together, the results have shown that circRNAs can bind the classic EMT-TFs to modulate the EMT process and cancer development.

The circRNA link to EMT-related signaling pathways

In addition to the EMT-TFs, EMT-related signaling pathways had been reported to be linked to cancer-associated circRNAs. In triple-negative breast cancer (TNBC), circANKS1B (hsa_circ_0007294), derived from exons 5–8 of the *ANKS1B* gene, is an independent risk factor of the overall survival of patients with TNBC, because of the promotion of tumor metastasis and invasion. Mechanistically, circANKS1B segregates miR-152-3p and miR-148a-3p, and upregulates the expression of transcription factor, USF1, which increases the expression

Table 1 CircRNAs modulating the epithelial-mesenchymal transition (EMT) in various carcinomas

Cancer type	CircRNA	Function	Mechanism	Reference
HCC	circCul2 (hsa_circ_10720)	Promoted EMT	Twist1 promoted vimentin expression and EMT by increasing levels of circCul2, which can absorb miRNAs that target vimentin	[31]
UCB	circPRMT5 (circRNA_101320)	Promoted EMT and aggressiveness	By circPRMT5/miR-30c/SNAIL1/E-cadherin pathway	[34]
Melanoma	circRNA_0084043	Promoted growth and metastasis	By circRNA_0084043/miR-153-3p/Snail axis	[35]
Cervical cancer	circRNA_000284	Promoted proliferation and cell invasion	By circRNA_000284/miR-506/Snail 2 axis	[36]
PTC	circNUP214 (hsa_ circ_0089153)	Promoted proliferation, migration and invasion	By circNUP214/miR-145/ZEB2 axis	[37]
TNBC	circANKS1B (hsa_circ_0007294)	Promoted EMT and metastasis	By circANKS1B/miR-152-3p, miR-148a-3p/USF1/TGF-β1/Smad signaling and a positive feedback loop of USF1/circANKS1B	[38]
OSCC	circUHRF1 (hsa_circ_0002185)	Promoted proliferation, metastasis, invasion and EMT	By a positive feedback loop of circUHRF1/miR-526b-5p/c-Myc/TGF-β1/ESRP1/circUHRF1	[39]
CRC	circRNA_100290	Promoted metastasis and EMT	By circRNA_100290/miR-516b/FZD4/Wnt/β-catenin signaling pathway	[40]
ccRCC	circAKT3	Inhibited metastasis and EMT	By circAKT3/miR-296-3p/E-cadherin pathway	[41]
DLBCL	circAPC (hsa_circ_0127621)	Hindered cell proliferation	By circAPC/APC/TET1/Wnt/β-catenin signaling pathway	[42]
CRC	hsa_circ_0026344	Functioned as anti-tumor molecule	By hsa_circ_0026344/miR-183/Wnt/β-catenin signaling pathway	[43]
Bladder cancer	circPTK2 (hsa_circ_0003221)	Enhanced migration	Unknown	[59]
CRC	circPTK2 (hsa_circ_0005273)	Stimulated EMT	Attaching to vimentin protein at sites Ser38, Ser55, Ser82	[60]

HCC, hepatocellular carcinoma; UCB, urothelial carcinoma of the bladder; PTC, papillary thyroid cancer; TNBC, triple-negative breast cancer; OSCC, oral squamous cell carcinoma; CRC, colorectal cancer; ccRCC, clear cell renal cell carcinoma; DLBCL, diffuse large B-cell lymphoma.

of TGF-β1 by binding with the TGF-β1 promoter, and then initiating TGF-β1/Smad signaling to stimulate the EMT. In addition, there is a positive feedback loop where USF1 upregulates circANKS1B expression by regulating the splicing factor ESRP1[38] (**Figure 2D**). In the oral squamous cell carcinoma (OSCC), circUHRF1 (hsa_circ_0002185) is in excess and promotes migration, invasion, proliferation, and the EMT *in vitro* and *in vivo via* a positive feedback loop pathway of circUHRF1/miR-526b-5p/c-Myc/TGF-β1/ESRP1/circUHRF1[39] (**Figure 2E**). In colorectal cancer (CRC), circRNA_100290 acts as an oncogene to increase metastasis and the EMT by sponging miR-516b, upregulating FZD4 expression, and

then initiating the FZD4-induced Wnt/β-catenin signaling pathway[40] (**Figure 2F**). Other than pro-oncogenic circRNAs, investigators have also discovered some EMT suppressive circRNAs. For example, in clear cell renal cell carcinoma (ccRCC), overexpression of circAKT3 decreases the EMT and metastasis *via* the circAKT3/miR-296-3p/E-cadherin pathway[41] (**Figure 2G**). In diffuse large B-cell lymphoma (DLBCL), circAPC (hsa_circ_0127621), derived from the *APC* exon 7 to exon 14, decreases cell proliferation. CircAPC binds the DNA demethylase, TET1, and binds to the APC promoter to enhance the expression of APC, which decreases the Wnt/β-catenin signaling pathway in the nucleus. In addition, circAPC
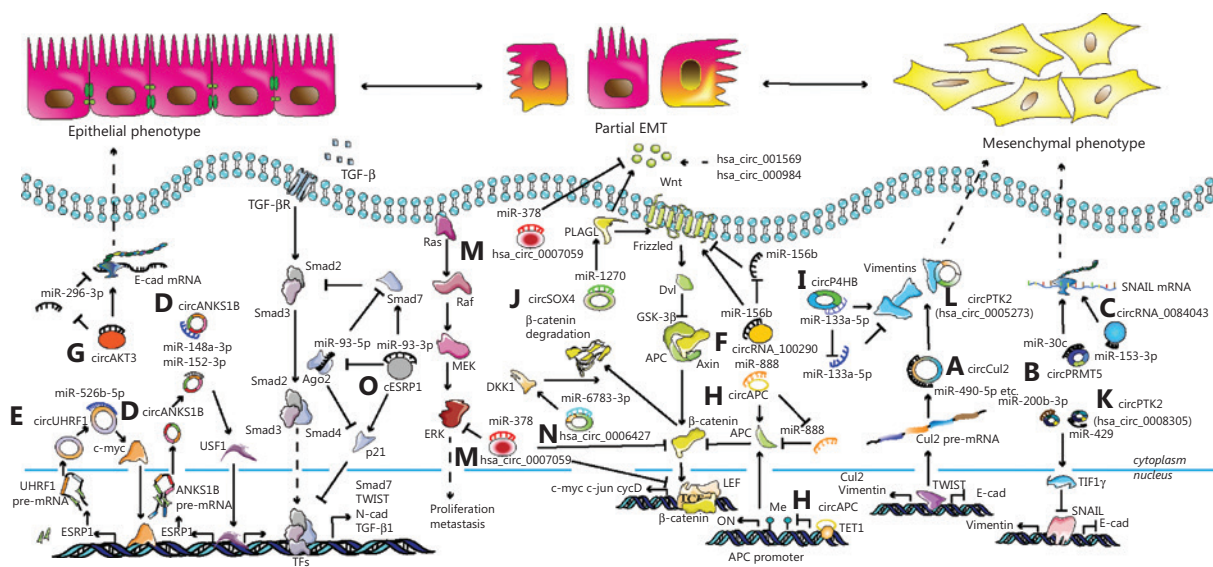
Figure 2   Mechanisms of the circRNA-mediated epithelial-mesenchymal transition (EMT) and metastasis in various carcinomas. (A) Twist promotes circCul2 expression by sponging miR-490-5p, and results in the upregulation of vimentin in hepatocellular carcinoma. (B) CircPRMT5 mediates the EMT in urothelial carcinoma of the bladder through the circPRMT5/miR-30c/SNAIL1 axis. (C) CicrRNA_0084043 increases metastasis in melanomas by regulating the miR-153-3p/Snail axis. (D) CircANKS1B facilitates tumor metastasis and invasion in triple-negative breast cancer through the circANKS1B/miR-148a-3p/miR-152-3p/USF1/TGF-β1 axis and a positive feedback loop of USF1/ESRP1/circANKS1B. (E) CircUHRF1 promotes migration, invasion, and the EMT in oral squamous cell carcinoma through a positive feedback loop of circUHRF1/miR-526b-5p/ c-Myc/TGF-β1/ESRP1/circUHRF1. (F) CircRNA_100290 induces metastasis and the EMT in colorectal cancer (CRC) through the miR-516b/FZD4/ Wnt/β-catenin signaling pathway. (G) CircAKT3 accelerates metastasis and the EMT in clear cell renal carcinoma by the circAKT3/miR-296-3p/ E-cadherin axis. (H) CircAPC decreases the canonical Wnt/β-catenin signaling pathway in diffuse large B-cell lymphoma by the miR-888/APC axis in the cytoplasm and circAPC/TET1/APC in the nucleus. (I) CircP4HB promotes the EMT in non-small cell lung cancer (NSCLC) by the circP4HB/ miR-133a-5p/vimentin axis. (J) CircSOX4 facilitates the EMT in lung adenocarcinoma (LUAD) by the circSOX4/miR-1270/ PLAGL2 axis. (K) CircPTK2 (hsa_circ_0008305) enhances the EMT in NSCLC by the circPTK2/miR-429/miR-200b-3p/TIF1γ/snail axis. (L) CircPTK2 (hsa_circ_005273) stimulates the EMT in CRC *via* directly combining with vimentin. (M) Hsa_circ_0007059 inhibits the EMT, decreases Wnt/β-catenin, and ERK1/2 signaling pathways in A549 and H 1975 cells by suppressing miR-378. (N) Hsa_circ_0006427 represses the Wnt/β-catenin signaling pathway in LUAD *via* the miR-6783-3p/DKK1 axis. (O) cESRP1 suppresses the EMT in NSCLC *via* the cESRP1/miR-93-5p/Smad7/p21 axis.

acts as a sponge of miR-888 to upregulate APC expression in the cytoplasm[42] (**Figure 2H**). Notably, the EMT and metastasis induced by CCL20 and CXCL8 treatment are decreased by overexpression of anti-tumor hsa_circ_0026344, which binds to miR-183 and inhibits the Wnt/-catenin pathway[43]. In general, circRNAs modulate EMT and migration mainly through two signaling pathways: the TGF-β1 signaling and Wnt/ β-catenin signaling pathways.

The influence of EMT-related circRNAs on lung carcinoma

The EMT phenotype is commonly expressed in primary squamous cell carcinomas (SCCs) and lung adenocarcinomas (LUADs), and occurs early in the pathogenesis of SCC,

suggesting a potential target for lung cancer chemoprevention and treatment[44]. In addition, the overexpression of forkhead box Q1 (FoxQ1) influences the poor prognosis in NSCLC and is associated with the EMT[45]. Further studies showed that the cancer stemness marker was associated with the EMT and predicted poor prognoses in patients with LUAD[46]. Thus, lung carcinoma has been shown to be linked to the EMT and metastasis[47], and the EMT-related circRNAs have an effect on lung carcinomas (**Table 2**). Overall, the EMT-inducing and EMT-suppressive circRNAs play very important roles in the occurrence and development of lung carcinomas.

The EMT-inducing circRNAs in NSCLC

Among the most common histological subtypes of lung carcinoma, LUAD is responsible for a majority of cancer-related

Table 2 The epithelial-mesenchymal transition (EMT)-related circRNAs in lung carcinoma

Type	CircRNA	Target/pathway
EMT-inducing circRNAs in NSCLC	circ-ENO1	circ-ENO1/miR-223p/ENO1 axis
	circAGFG1	circAGFG1/miR-203/ZNF281 axis
	circP4HB	circP4HB/miR-133a-5p/vimentin pathway
	hsa_circ_0007534	Unknown
	hsa_circ_0079530	Unknown
	circ_0067934	Unknown
	circRNA CCDC66	HGF and c-Met upregulate circRNA CCDC66, nAchRα7 downregulates circRNA CCDDC 66
	circ_0012673	circ_0012673/miR-320A/LIMK1 axis
	hsa_circ_000984	Wnt/β-catenin pathway
	circ_001569	transcription factor 4 and Wnt/β-catenin pathway
	circ-SOX4 (hsa_circ_0131457)	circ-SOX4/miR-1270/PLAGL2/Wnt signaling pathway
EMT-suppressive circRNAs in NSCLC	circPTK2 (hsa_circ_0008305)	circPTK2/miR-429/miR-200b-3p/Snail axis
	circPTPRA (hsa_circ_102984)	circPTPRA/miR-96-5p/RASSF8/E-cadherin
	hsa_circ_0007059	hsa_circ_0007059/miR-378/Wnt/β-catenin pathway and ERK1/2 pathway
	circ_0006427	circ_0006427/miR-6783e3p/DKK1/Wnt/β-catenin pathway
EMT-suppressive circRNAs in SCLC	cESRP1	cESRP1/miR-93-5p/Smad7/p21(CDKN1A) axis

ENO1, enolase 1; AGFG1, ArfGAP with FG repeats 1; ZNF281, zinc finger protein 281; P4HB, prolyl 4-hydroxylase subunit beta; CCDC66, coiled-coil domain containing 66; HGF, hepatocyte growth factor; nAchRα7, nicotinic acetylcholine receptor Alpha 7; LIMK1, LIM domain kinase 1; SOX4, SRY-box transcription factor 4; PTK2, protein tyrosine kinase 2; PTPRA, protein tyrosine phosphatase receptor type A; RASSF8, Ras association domain family member 8; DKK1, Dickkopf WNT signaling pathway inhibitor 1; EMT, epithelial mesenchymal transition; NSCLC, non-small cell lung cancer.

mortalities worldwide. Zhou et al.[12] reported that circENO1 and its host gene, *ENO1* were both amplified in LUAD cells to augment glycolysis and tumor growth, and silenced circENO1 impeded glycolysis, migration and the EMT, and induced cell apoptosis *via* the circENO1/miR-223p/ENO1 axis. Similarly, circAGFG1 increased the EMT of NSCLC cells *via* invasion and migration by acting as a sponge for miR-203, which targeted ZNF281[48]. In addition, Wang et al.[49] reported that circP4HB was higher in NSCLC tissues than in healthy paired samples, and that circP4HB promoted higher vimentin expression and the EMT *in vivo* and *in vitro via* the circP4HB/miR-133a-5p/vimentin axis (**Figure 2I**). Qi et al.[50] found that EMT-inducing circDDX42 (hsa_circ_0007534), a transcription product of DEAD-box helicase 42, decreased E-cadherin and increased the levels of Snail, N-cadherin, and vimentin.

CircTwist1 (hsa_circ_0079530), which is 664 nt in length, was found to be upregulated in NSCLC. Knockdown of CircTwist1 resulted in the downregulation of mesenchymal marker proteins and the upregulation of epithelial marker proteins in A549 and H1299 cells[51]. Similarly, hsa_circ_0067934 led to an identical trend of changes of N-cadherin and vimentin, and an opposite effect for E-cadherin[52]. In LUAD, it was found that circCCDC66 and SUMO-activating enzyme subunit 2 (SAE2) were both highly expressed and associated with the EMT, lung cancer metastasis, and EGFR drug resistance. Hepatocyte growth factor (HGF) and c-Met upregulate SAE2 and circCCDC66 to enhance EMT and drug resistance; however, nicotinic acetylcholine receptor alpha 7 (nAChRα7) negatively modulates circCCDC66 expression[53]. LIM domain kinase 1 (LIMK1) is a serine-threonine protein kinase, which affects

the actin cytoskeleton and participates in the EMT. Qin et al.[54] showed that hsa_circ_0012673 upregulated LIMK1 *via* binding to miR-320a to manipulate migration, invasion, proliferation, apoptosis, and the EMT in lung cancer cells. Because the EMT is closely correlated with the Wnt-associated pathways, many studies have focused on the effects of circRNAs on the expression levels of cyclin D1, β-catenin, and c-myc in the Wnt/β-catenin signaling pathway. Li et al.[55] reported that hsa_circ_000984 was highly expressed in NSCLC tissues and cells, and correlated with shorter disease-free survival and overall survival. Functionally, hsa_circ_000984 promoted EMT to a pro-oncogenic role *via* upregulating the expression of cyclin D1, β-catenin, and c-myc in the Wnt/β-catenin pathway. It was also reported that hsa_circ_001569 predicted a poor prognosis and enhanced the expressions of crucial members of the Wnt/β-catenin pathway, including Wnt1, β-catenin, and transcription factor 4 (TF4)[56]. In the same manner, upregulated circSOX4 (hsa_circ_0131457) activates the Wnt signaling pathway and promotes the EMT *via* the circSOX4/miR-1270/ PLAGL2 axis in LUAD[57] (**Figure 2J**). Taken together, it is clear that the EMT-inducing circRNAs promote EMT-mediated metastasis, mainly by affecting the major members of the EMT-related signaling pathways in NSCLC.

The EMT-suppressive circRNAs in NSCLC

In contrast to EMT-inducing circRNAs, EMT-suppressive circRNAs usually negatively modulate EMT and inhibit certain cell programs such as migration, metastasis, and invasion. As mentioned above, Snail is one of the classical EMT-TFs. In NSCLC, circPTK2 (hsa_circ_0008305) binds to miR-429/miR-200b-3p to enhance transcriptional intermediary factor 1γ (TIF1γ), which suppresses the function of Snail in the nucleus[58] (**Figure 2K**). Notably, circPTK2 (hsa_circ_0003221), derived from the *PTK2*, enhances migration in bladder cancer cells[59]. Recently, Yang et al.[60] reported that circPTK2 (hsa_circ_0005273), unlike hsa_circ_0008305 in NSCLC, stimulated the EMT *via* binding to vimentin protein at Ser38, Ser55, and Ser82 in CRC (**Figure 2L**). Ras association domain-containing protein 8 (RASSF8) is an acknowledged tumor suppressor of lung cancer[61]. Wei et al.[62] showed that circPTPRA (hsa_circ_102984) derived from protein tyrosine phosphatase receptor type A gene (*PTPRA*) inhibited tumor metastasis and the EMT by sponging miR-96-5p and releasing RASSF8 and E-cadherin. In A549 and H1975 cells, overexpression of hsa_circ_0007059 restrains cell proliferation, decreases

the EMT, and hinders the Wnt/β-catenin and ERK1/2 signaling pathways *via* suppressing miR-378[63] (**Figure 2M**). In addition, Yao et al.[64] have shown that the cytoplasmic-located has_circ_0006427 sponges miR-6783-3p to release Dickkopf WNT Signaling Pathway Inhibitor 1 (DKK1) and inhibit the Wnt/β-catenin pathway, and thereby repress cell proliferation, migration, invasion and EMT in LUAD cells (**Figure 2N**). In summary, the EMT-suppressive circRNAs play important roles in suppressing EMT-mediated metastasis in NSCLC *via* acting as sponges of miRNAs to influence EMT-TFs, EMT-related signaling, and EMT markers.

The EMT-suppressive circRNAs in SCLC

The effects of EMT-related circRNAs on SCLC presently remain largely unknown. Huang et al.[65] showed that cESRP1 (circular RNA epithelial splicing regulatory protein 1) located in the cytoplasm sequestered miR-93-5p to free Smad7/p21(CDKN1A) and further regulate the TGF-β-induced EMT (**Figure 2O**). Moreover, the inhibition of the TGF-β pathway and cESRP1 overexpression improved the responsiveness to chemotherapy in a patient-derived xenograft model suffering acquired chemoresistance.

Conclusions

Although numerous studies have verified that circRNAs are more stable than their cognate mRNAs, the effectiveness of back splicing is lower than canonical splicing from certain expression vectors[66]. Recent advances have shown that circRNAs can act as minimally-invasive or noninvasive biomarkers for various diseases including cancers, although the detection techniques in blood and other body fluids still need to be improved[15,67]. More importantly, circRNAs may work together as a group, with complicated crosstalk with other regulators[68]. The EMT is a chief element of the metastatic cascade, which is important in tumor deterioration. Additionally, the EMT is responsible for chemoresistance and immune resistance[69,70]. Although the correlations between miRNAs and EMT-TFs have been well-established[71], as sponges for miRNAs, the roles of circRNAs in the EMT remain to be discovered. The levels of certain circRNAs could be distinctly changed during the EMT due to splicing programs stimulated by a set of splicing factors such as RBFOX2, SRSF2, and QKI[8]. CircRNAs function as parts of a noncoding RNA adjustment net by separating proteins and miRNAs during the EMT. A quantitative EMT scoring system based on

418

Jiang et al. EMT-related circRNAs in lung carcinoma

gene expression profiles has recently been established to grade the status of the EMT[47]. This system has been used to emphasize the developmental lineage of each cancer subtype from microscopic and macroscopic EMT gradients, and each cancer subtype has a unique tendency to exhibit different EMT states. Lung carcinoma displays higher EMT scores, which always forecast a poor prognosis[47]. Investigators of EMT-related circRNAs can therefore provide novel therapeutic approaches and tactics for patients with lung carcinoma. Specifically, a large range of small molecule agents for targeting characteristics with EMT in lung carcinoma have been developed in preclinical phases[72]. For example, bufalin, a Chinese medicine, impedes the migratory activity of A549 human lung cancer cells and the TGF-β-induced EMT[73]. However, there is controversy concerning the oligonucleotide chemistry that targets circRNAs, but further studies may bring new hope to patients with drug resistance.

Grant support

Conflict of interest statement

No potential conflicts of interest are disclosed.

References

1. Battula VL, Evans KW, Hollier BG, Shi Y, Marini FC, Ayyanan A, et al. Epithelial-mesenchymal transition-derived cells exhibit multilineage differentiation potential similar to mesenchymal stem cells. Stem Cells. 2010; 28: 1435-45.

2. Bronte G, Bravaccini S, Bronte E, Burgio MA, Rolfo C, Delmonte A, et al. Epithelial-to-mesenchymal transition in the context of epidermal growth factor receptor inhibition in non-small-cell lung cancer. Biol Rev Camb Philos Soc. 2018; 93: 1735-46.

3. Nieto MA, Huang Ruby Y-J, Jackson Rebecca A, Thiery Jean P. EMT: 2016. Cell. 2016; 166: 21-45.

4. Blanco MJ, Barrallo-Gimeno A, Acloque H, Reyes AE, Tada M, Allende ML, et al. Snail1a and Snail1b cooperate in the anterior migration of the axial mesendoderm in the zebrafish embryo. Development. 2007; 134: 4073-81.

5. Grigore AD, Jolly MK, Jia D, Farach-Carson MC, Levine H. Tumor budding: the name is EMT. Partial EMT. J Clin Med. 2016; 5: 51.

6. Pastushenko I, Blanpain C. EMT transition states during tumor progression and metastasis. Trends Cell Biol. 2019; 29: 212-26.

7. Shang BQ, Li ML, Quan HY, Hou PF, Li ZW, Chu SF, et al. Functional roles of circular RNAs during epithelial-to-mesenchymal transition. Mol Cancer. 2019; 18: 138.

8. Conn SJ, Pillman KA, Toubia J, Conn VM, Salmanidis M, Phillips CA, et al. The RNA binding protein quaking regulates formation of circRNAs. Cell. 2015; 160: 1125-34.

9. Zhang Q, Wang W, Zhou Q, Chen C, Yuan W, Liu J, et al. Roles of circRNAs in the tumour microenvironment. Mol Cancer. 2020; 19: 14.

10. Li Z, Ruan Y, Zhang H, Shen Y, Li T, Xiao B. Tumor-suppressive circular RNAs: mechanisms underlying their suppression of tumor occurrence and use as therapeutic targets. Cancer Sci. 2019; 110: 3630-8.

11. Di X, Jin X, Li R, Zhao M, Wang K. CircRNAs and lung cancer: biomarkers and master regulators. Life Sci. 2019; 220: 177-85.

12. Zhou J, Zhang S, Chen Z, He Z, Xu Y, Li Z. CircRNA-ENO1 promoted glycolysis and tumor progression in lung adenocarcinoma through upregulating its host gene ENO1. Cell Death Dis. 2019; 10: 885.

13. Wang C, Tan S, Liu W-R, Lei Q, Qiao W, Wu Y, et al. RNA-seq profiling of circular RNA in human lung adenocarcinoma and squamous cell carcinoma. Mol Cancer. 2019; 18: 134.

14. Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. PLoS One. 2012; 7: e30733.

15. Meng S, Zhou H, Feng Z, Xu Z, Tang Y, Li P, et al. CircRNA: functions and properties of a novel potential biomarker for cancer. Mol Cancer. 2017; 16: 94.

16. Dong R, Ma XK, Chen LL, Yang L. Increased complexity of circRNA expression during species evolution. RNA Biol. 2017; 14: 1064-74.

17. Salzman J, Chen RE, Olsen MN, Wang PL, Brown PO. Cell-type specific features of circular RNA expression. PLoS Genet. 2013; 9: e1003777.

18. Lu T, Cui L, Zhou Y, Zhu C, Fan D, Gong H, et al. Transcriptome-wide investigation of circular RNAs in rice. RNA. 2015; 21: 2076-87.

19. Danan M, Schwartz S, Edelheit S, Sorek R. Transcriptome-wide discovery of circular RNAs in Archaea. Nucleic Acids Res. 2012; 40: 3131-42.

20. Fan X, Zhang X, Wu X, Guo H, Hu Y, Tang F, et al. Single-cell RNA-seq transcriptome analysis of linear and circular RNAs in mouse preimplantation embryos. Genome Biol. 2015; 16: 148.

21. Li X, Yang L, Chen LL. The biogenesis, functions, and challenges of circular RNAs. Mol Cell. 2018; 71: 428-42.

22. Zhou C, Molinie B, Daneshvar K, Pondick JV, Wang J, Van Wittenberghe N, et al. Genome-wide maps of m6A circRNAs identify widespread and cell-type-specific methylation patterns that are distinct from mRNAs. Cell Rep. 2017; 20: 2262-76.

23. Zhang S, Liao K, Miao Z, Wang Q, Miao Y, Guo Z, et al. CircFOXO3 promotes glioblastoma progression by acting as a competing endogenous RNA for NFAT5. Neuro Oncol. 2019; 21: 1284-96.

24. Huang X, He M, Huang S, Lin R, Zhan M, Yang D, et al. Circular RNA circERBB2 promotes gallbladder cancer progression by regulating PA2G4-dependent rDNA transcription. Mol Cancer. 2019; 18: 166.

25. Dong R, Zhang XO, Zhang Y, Ma XK, Chen LL, Yang L. CircRNA-derived pseudogenes. Cell Res. 2016; 26: 747-50.

26. Du WW, Zhang C, Yang W, Yong T, Awan FM, Yang BB. Identifying and characterizing circRNA-protein interaction. Theranostics. 2017; 7: 4183-91.

27. Ye F, Gao G, Zou Y, Zheng S, Zhang L, Ou X, et al. circFBXW7 Inhibits malignant progression by sponging miR-197-3p and encoding a 185-aa protein in triple-negative breast cancer. Mol Ther Nucleic Acids. 2019; 18: 88-98.

28. Lei M, Zheng G, Ning Q, Zheng J, Dong D. Translation and functional roles of circular RNAs in human cancer. Mol Cancer. 2020; 19: 30.

29. Grelet S, McShane A, Geslain R, Howe PH. Pleiotropic roles of non-coding RNAs in TGF-β-mediated epithelial-mesenchymal transition and their functions in tumor progression. Cancers (Basel). 2017; 9: 75.

30. Cai W, Yang H. The structure and regulation of Cullin 2 based E3 ubiquitin ligases and their biological functions. Cell Div. 2016; 11: 7.

31. Meng J, Chen S, Han JX, Qian B, Wang XR, Zhong WL, et al. Twist1 regulates vimentin through Cul2 circular RNA to promote EMT in hepatocellular carcinoma. Cancer Res. 2018; 78: 4150-62.

32. Wang Y, Shi J, Chai K, Ying X, Zhou B. The role of snail in EMT and tumorigenesis. Curr Cancer Drug Targets. 2013; 13: 963-72.

33. Stemmler MP, Eccles RL, Brabletz S, Brabletz T. Non-redundant functions of EMT transcription factors. Nat Cell Biol. 2019; 21: 102-12.

34. Chen X, Chen RX, Wei WS, Li YH, Feng ZH, Tan L, et al. PRMT5 circular RNA promotes metastasis of urothelial carcinoma of the bladder through sponging miR-30c to Induce epithelial-mesenchymal transition. Clin Cancer Res. 2018; 24: 6319-30.

35. Luan W, Shi Y, Zhou Z, Xia Y, Wang J. circRNA_0084043 promote malignant melanoma progression via miR-153-3p/Snail axis. Biochem Biophys Res Commun. 2018; 502: 22-9.

36. Ma HB, Yao YN, Yu JJ, Chen XX, Li HF. Extensive profiling of circular RNAs and the potential regulatory role of circRNA-000284 in cell proliferation and invasion of cervical cancer via sponging miR-506. Am J Transl Res. 2018; 10: 592-604.

37. Li X, Tian Y, Hu Y, Yang Z, Zhang L, Luo J. CircNUP214 sponges miR-145 to promote the expression of ZEB2 in thyroid cancer cells. Biochem Biophys Res Commun. 2018; 507: 168-72.

38. Zeng K, He B, Yang BB, Xu T, Chen X, Xu M, et al. The pro-metastasis effect of circANKS1B in breast cancer. Mol Cancer. 2018; 17: 160.

39. Zhao W, Cui Y, Liu L, Qi X, Liu J, Ma S, et al. Splicing factor derived circular RNA circUHRF1 accelerates oral squamous cell carcinoma tumorigenesis via feedback loop. Cell Death Differ. 2019; 27: 919-33.

40. Fang G, Ye BL, Hu BR, Ruan XJ, Shi YX. CircRNA_100290 promotes colorectal cancer progression through miR-516b-induced downregulation of FZD4 expression and Wnt/β-catenin signaling. Biochem Biophys Res Commun. 2018; 504: 184-9.

41. Xue D, Wang H, Chen Y, Shen D, Lu J, Wang M, et al. Circ-AKT3 inhibits clear cell renal cell carcinoma metastasis via altering miR-296-3p/E-cadherin signals. Mol Cancer. 2019; 18: 151.

42. Hu Y, Zhao Y, Shi C, Ren P, Wei B, Guo Y, et al. A circular RNA from APC inhibits the proliferation of diffuse large B-cell lymphoma by inactivating Wnt/β-catenin signaling via interacting with TET1 and miR-888. Aging. 2019; 11: 8068-84.

43. Shen T, Cheng X, Liu X, Xia C, Zhang H, Pan D, et al. Circ_0026344 restrains metastasis of human colorectal cancer cells via miR-183. Artif Cells Nanomed Biotechnol. 2019; 47: 4038-45.

44. Prudkin L, Liu DD, Ozburn NC, Sun M, Behrens C, Tang X, et al. Epithelial-to-mesenchymal transition in the development and progression of adenocarcinoma and squamous cell carcinoma of the lung. Mod Pathol. 2009; 22: 668-78.

45. Feng J, Zhang X, Zhu H, Wang X, Ni S, Huang J. FoxQ1 overexpression influences poor prognosis in non-small cell lung cancer, associates with the phenomenon of EMT. PLoS One. 2012; 7: e39937.

46. Sowa T, Menju T, Sonobe M, Nakanishi T, Shikuma K, Imamura N, et al. Association between epithelial-mesenchymal transition and cancer stemness and their effect on the prognosis of lung adenocarcinoma. Cancer Med. 2015; 4: 1853-62.

47. Tan TZ, Miow QH, Miki Y, Noda T, Mori S, Huang RY, et al. Epithelial-mesenchymal transition spectrum quantification and its efficacy in deciphering survival and drug responses of cancer patients. EMBO Mol Med. 2014; 6: 1279-93.

48. Xue YB, Ding MQ, Xue L, Luo JH. CircAGFG1 sponges miR-203 to promote EMT and metastasis of non-small-cell lung cancer by upregulating ZNF281 expression. Thorac Cancer. 2019; 10: 1692-701.

49. Wang T, Wang X, Du Q, Wu N, Liu X, Chen Y, et al. The circRNA circP4HB promotes NSCLC aggressiveness and metastasis by sponging miR-133a-5p. Biochem Bioph Res Commun. 2019; 513: 904-11.

50. Qi Y, Zhang B, Wang J, Yao M. Upregulation of circular RNA hsa_circ_0007534 predicts unfavorable prognosis for NSCLC and exerts oncogenic properties in vitro and in vivo. Gene. 2018; 676: 79-85.

51. Li J, Wang J, Chen Z, Chen Y, Jin M. Hsa_circ_0079530 promotes cell proliferation and invasion in non-small cell lung cancer. Gene. 2018; 665: 1-5.

52. Wang J, Li H. CircRNA circ_0067934 silencing inhibits the proliferation, migration and invasion of NSCLC cells and correlates with unfavorable prognosis in NSCLC. Eur Rev Med Pharmacol Sci. 2018; 22: 3053-60.

53. Joseph NA, Chiou SH, Lung Z, Yang CL, Lin TY, Chang HW, et al. The role of HGF-MET pathway and CCDC66 cirRNA expression in EGFR resistance and epithelial-to-mesenchymal transition of lung adenocarcinoma cells. J Hematol Oncol. 2018; 11: 74.

420

Jiang et al. EMT-related circRNAs in lung carcinoma

54. Qin H, Liu J, Du ZH, HU R, Yu YK, Wang QA. Circular RNA hsa_circ_0012673 facilitates lung cancer cell proliferation and invasion via miR-320a/LIMK18521 axis. Eur Rev Med Pharmacol Sci. 2020; 24: 1841-52.

55. Li XY, Liu YR, Zhou JH, Li W, Guo HH, Ma HP. Enhanced expression of circular RNA hsa_circ_000984 promotes cells proliferation and metastasis in non-small cell lung cancer by modulating Wnt/β-catenin pathway. Eur Rev Med Pharmacol Sci. 2019; 23: 3366-74.

56. Ding L, Yao W, Lu J, Gong J, Zhang X. Upregulation of circ_001569 predicts poor prognosis and promotes cell proliferation in non-small cell lung cancer by regulating the Wnt/β-catenin pathway. Oncol Lett. 2018; 16: 453-8.

57. Gao N, Ye B. Circ-SOX4 drives the tumorigenesis and development of lung adenocarcinoma via sponging miR-1270 and modulating PLAGL2 to activate WNT signaling pathway. Cancer Cell Int. 2020; 20: 2.

58. Wang L, Tong X, Zhou Z, Wang S, Lei Z, Zhang T, et al. Circular RNA hsa_circ_0008305 (circPTK2) inhibits TGF-β-induced epithelial-mesenchymal transition and metastasis by controlling TIF1γ in non-small cell lung cancer. Mol Cancer. 2018; 17: 140.

59. Xu ZQ, Yang MG, Liu HJ, Su CQ. Circular RNA hsa_circ_0003221 (circPTK2) promotes the proliferation and migration of bladder cancer cells. J Cell Biochem. 2018; 119: 3317-25.

60. Yang H, Li X, Meng Q, Sun H, Wu S, Hu W, et al. CircPTK2 (hsa_circ_0005273) as a novel therapeutic target for metastatic colorectal cancer. Mol Cancer. 2020; 19: 13.

61. Lock FE, Underhill-Day N, Dunwell T, Matallanas D, Cooper W, Hesson L, et al. The RASSF8 candidate tumor suppressor inhibits cell growth and regulates the Wnt and NF-κB signaling pathways. Oncogene. 2010; 29: 4307-16.

62. Wei S, Zheng Y, Jiang Y, Li X, Geng J, Shen Y, et al. The circRNA circPTPRA suppresses epithelial-mesenchymal transitioning and metastasis of NSCLC cells by sponging miR-96-5p. EBioMedicine. 2019; 44: 182-93.

63. Gao S, Yu Y, Liu L, Meng J, Li G. Circular RNA hsa_circ_0007059 restrains proliferation and epithelial-mesenchymal transition in lung cancer cells via inhibiting microRNA-378. Life Sci. 2019; 233: 116692.

64. Yao Y, Hua Q, Zhou Y. CircRNA has_circ_0006427 suppresses the progression of lung adenocarcinoma by regulating miR-6783–3p/DKK1 axis and inactivating Wnt/β-catenin signaling pathway. Biochem Bioph Res Commun. 2019; 508: 37-45.

65. Huang W, Yang Y, Wu J, Niu Y, Yao Y, Zhang J, et al. Circular RNA cESRP1 sensitises small cell lung cancer cells to chemotherapy by sponging miR-93-5p to inhibit TGF-β signalling. Cell Death Differ. 2020; 27: 1709-27.

66. Zhang Y, Xue W, Li X, Zhang J, Chen S, Zhang JL, et al. The biogenesis of nascent circular RNAs. Cell Rep. 2016; 15: 611-24.

67. Memczak S, Papavasileiou P, Peters O, Rajewsky N. Identification and characterization of circular RNAs as a new class of putative biomarkers in human blood. PLoS One. 2015; 10: e0141214.

68. Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. Nature. 2014; 505: 344-52.

69. Chen X, Lingala S, Khoobyari S, Nolta J, Zern MA, Wu J. Epithelial mesenchymal transition and hedgehog signaling activation are associated with chemoresistance and invasion of hepatoma subpopulations. J Hepatol. 2011; 55: 838-45.

70. Wang L, Saci A, Szabo PM, Chasalow SD, Castillo-Martin M, Domingo-Domenech J, et al. EMT- and stroma-related gene expression and resistance to PD-1 blockade in urothelial cancer. Nat Commun. 2018; 9: 3503.

71. Puisieux A, Brabletz T, Caramel J. Oncogenic roles of EMT-inducing transcription factors. Nat Cell Biol. 2014; 16: 488-94.

72. Otsuki Y, Saya H, Arima Y. Prospects for new lung cancer treatments that target EMT signaling. Dev Dyn. 2018; 247: 462-72.

73. Zhao L, Liu S, Che X, Hou K, Ma Y, Li C, et al. Bufalin inhibits TGF-β-induced epithelial-to-mesenchymal transition and migration in human lung cancer A549 cells by downregulating TGF-β receptors. Int J Mol Med. 2015; 36: 645-52.