Interaction between Wnt/β-catenin pathway and microRNAs regulates epithelial-mesenchymal transition in gastric cancer (Review)

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Abstract. Gastric cancer (GC) is the third primary cause of cancer-related mortality and one of the most common type of malignant diseases worldwide. Despite remarkable progress in multimodality therapy, advanced GC with high aggressiveness always ends in treatment failure. Epithelial-mesenchymal transition (EMT) has been widely recognized to be a key process associating with GC evolution, during which cancer cells go through phenotypic variations and acquire the capability of migration and invasion. Wnt/β-catenin pathway has established itself as an EMT regulative signaling due to its maintenance of epithelial integrity as well as tight adherens junctions while mutations of its components will lead to GC initiation and diffusion. The E-cadherin/β-catenin complex plays an important role in stabilizing β-catenin at cell membrane while disruption of this compound gives rise to nuclear translocation of β-catenin, which accounts for upregulation of EMT biomarkers and unfavorable prognosis. Additionally, several microRNAs positively or negatively modify EMT by reciprocally acting with certain target genes of Wnt/β-catenin pathway in GC. Thus, this review centers on the strong associations between Wnt/β-catenin pathway and microRNAs during alteration of EMT in GC, which may induce advantageous therapeutic strategies for human gastric cancer.

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Contents

1. Introduction

Gastric cancer, one of the most common malignancies and causes almost one million new cases each year in the world (1). To make matters worse, despite decreasing incidence over the recent decades, GC remains the third leading cause of global cancer-related death preceded by lung and liver cancer and maintains the highest morbidity in Eastern Asia (2,3). The main reasons for failure and mortality of gastric cancer are cell infiltration and metastasis. Metastatic cancer cells fractionally retain their epithelial properties and obtain mesenchymal characteristics which give them the ability to invade or distract. E-cadherin is a major protein prominently required for cell-cell junctions and polarity, whose loss has been established to be the principal event in metastatic progression and tumor invasion. Moreover, downregulation of E-cadherin might induce the occurrence of EMT, a multistage process giving rise to the transformation of polar epithelial cells to mesenchymal phenotype and authorizes cell migration as well (4,5).

Two decades ago, Heuberger and Birchmeier made the first ascertainment to expound that the presence of E-cadherin/β-catenin complex allows for steady cell junctions (6). β-catenin, one key protein in Wnt signaling, separates from the degradation complex and then accumulates in the cytoplasm, translocates into nucleus subsequently to sensitize the Wnt pathway during

carcinoma progression. In the presence of downregulation of E-cadherin and Wnt signals activation, both cytoplasmic and nuclear levels of β-catenin increase where it interacts with cell growth and EMT (7,8). MicroRNAs are small non-coding molecules with 19-25 nucleotides that bind to 3'UTR of target mRNAs with complementary base pairing to inhibit their translation or speed up decomposition. Latest experiments investigated either suppressive or promotive function of crosstalk between miRNAs and certain signaling pathways during EMT and metastasis, in spite of this, little attention has been paid to the interplay between Wnt/β-catenin signaling and miRNAs in regulating EMT in gastric cancer (9). Taking efforts to comprehend the mechanism of Wnt adjustment by miRNAs may benefit our perspectives of reversing the course of EMT or potential therapeutic targets in GC. Therefore, this review focuses on crosstalk between microRNAs and Wnt pathway in the course of modulating EMT in gastric cancer.

2. Epithelial-mesenchymal transition and gastric cancer

Epithelial-mesenchymal transition, firstly put forward by Greenburg and Hay in 1982 (10), has been considered to be the feature of embryonic development. However, emerging evidence has indicated that the occurrence of EMT is critical for the initiation and progression of diverse tumors. An EMT process is distinguished by loss of E-cadherin and it creates profound phenotypic alterations through which epithelial cells with apico-basal polarity convert into front-rear polarity to gain mesenchymal characteristics as well as the capacity of migration, invasion and apoptotic resistance (11) (Fig. 1). The epithelial cells going through EMT become invasive and then migrate into distant tissues where they experience a reverting mechanism mesenchymal-epithelial transition (MET), afterward these cells retrieve membrane junctions and form metastatic focus (12). EMT is characterized by the mutative expression of three distinct families of protein consist of cadherins (cell membrane surface protein), Vimentin (cytoskeletal protein) and transcription factors including Snail, Twist, and ZEB (13,14).

E-cadherin, encoded by CDH1, interacts with α, β, γ and P120-catenin to constitute E-cadherin/catenin complex that plays a critical role in adherens junctions between epithelial cells (15). Loss of E-cadherin on account of promoter hypermethylation has been widely considered to correlate with GC aggressiveness and metastasis as well as unfavorable prognosis of patients (16,17). Hansford *et al* (18) reported that HDGC families, particularly over the age of 80, may be best defined by mutations in CDH1 and different clinical ramifications, and that CDH1 mutation might provide more accurate estimations of age-connected risk of GC. Nevertheless, Li *et al* (19) demonstrated that CDH1 promoter hypermethylation probably contributes to E-cad reduction in sporadic instead of hereditary gastric cancer. It is widely acknowledged that in the presence of deviant EMT, E-cadherin switches to N-cadherin which is generally distributed in neural and mesenchymal cells and has ability to expedite the degeneration of stroma as well as cell movement. Jun *et al* suggested that median survival is significantly correlated with N-cadherin expression in cases of gastric cancer with brain metastasis (20). Similarly, Kamikihara *et al* (21) performed a genetic association study in 146 gastric cancer patients to analyze the relationship between N-cadherin expression and clinicopathological features. The result exhibited that N-cadherin expression positively correlates to hematogenous relapse while negatively connects to patients' postoperative outcome independent of E-cadherin expression, even if N-cadherin has no association with tumor histology or lymphatic invasion.

Vimentin, a hallmark of interstitial cells, is known to be tightly associated with the potential of tumor cell invasiveness and migration. Vimentin expresses obviously higher diffuse type than intestinal in patients with gastric adenocarcinoma and it might be a feasible mark of relapse, far metastasis or even decreased survival (22). Shirahata *et al* (23) recommended we use quantitative methylation-specific PCR (qMSP), a sensitive technique that can catch as few as one methylated gene copy out from 1,000 unmethylated ones, to detect promoter methylation in the serum of GC patients. In addition, they recorded a high correlation between Vim methylation and gastric carcinogenesis.

The zinc finger protein Snail consists of three members containing Snail1, Snail2 (Slug) and Snail3 (Smuc), among which Snail1 and Snail2 act synergistically in cases of gastric tumor. Snail is initially recognized to facilitate EMT by way of distinguishing E-box motifs in CDH1 to inhibit E-cadherin, whereas growing indication has inferred the mechanism involves not only downregulation of claudins, occludins, cytokeratins but also upregulation of fibronectin and vitrinectin (24). Besides, the involvement of NF-κB-Snail-E-cadherin axis or CCR7 pathway in induction of EMT through upregulating Snail signaling proved in GC both implied the decisive role of Snail explicitly (25,26). Furthermore, Shin *et al* (27) reported that Snail could be a prognostic predictor of gastric cancer due to its strong interrelationship with tumor progression, lymph node metastasis, lymphovascular or perineural invasion. Twist protein is a member of the basic/helix-loop-helix transcription factor family and binds selectively to the E-box consensus sequence to regulate target genes such as E-cadherin (28). A series of experiments have illustrated the considerable function of Twist as an oncogene *in vivo*, suggestive of its potency to be a target for the treatment of gastric carcinoma. Liu *et al* (29) discovered that Twist is expressed much higher in gastric cancer tissues compared to adjacent normal tissues at mRNA and protein levels by means of RT-PCR and western blotting. Likewise, abnormal Twist expression related strongly to lower 5-year survival rates in patients of GC in the Ru *et al* study, yet, they emphasized that the significance disappears in cases of stage IV (30). Various examinations *in vitro* collaboratively implied the carciogenesis of Twist. GC cells transfected with Twist1 accelerated cell cycle progression by recruiting p300 to enhance FoxM1 gene expression, whereas those treated with Twist siRNA reversely experienced obvious cell cycle arrest at G0/G1 phase, apoptosis induction as well as decreased ability of proliferation and invasion (31,32). Apart from this, Twist was proved to be a pivotal gene of *Helicobacter pylori*-triggered GC cell stemness while decreased after *H. pylori* eradication (33), reminding us to excavate therapeutic targets against gastric cancer via Twist-dependent mechanism. ZEB family members, ZEB1 and ZEB2, are transcriptional factors suppressing E-cadherin, and they play a decisive role in tumor

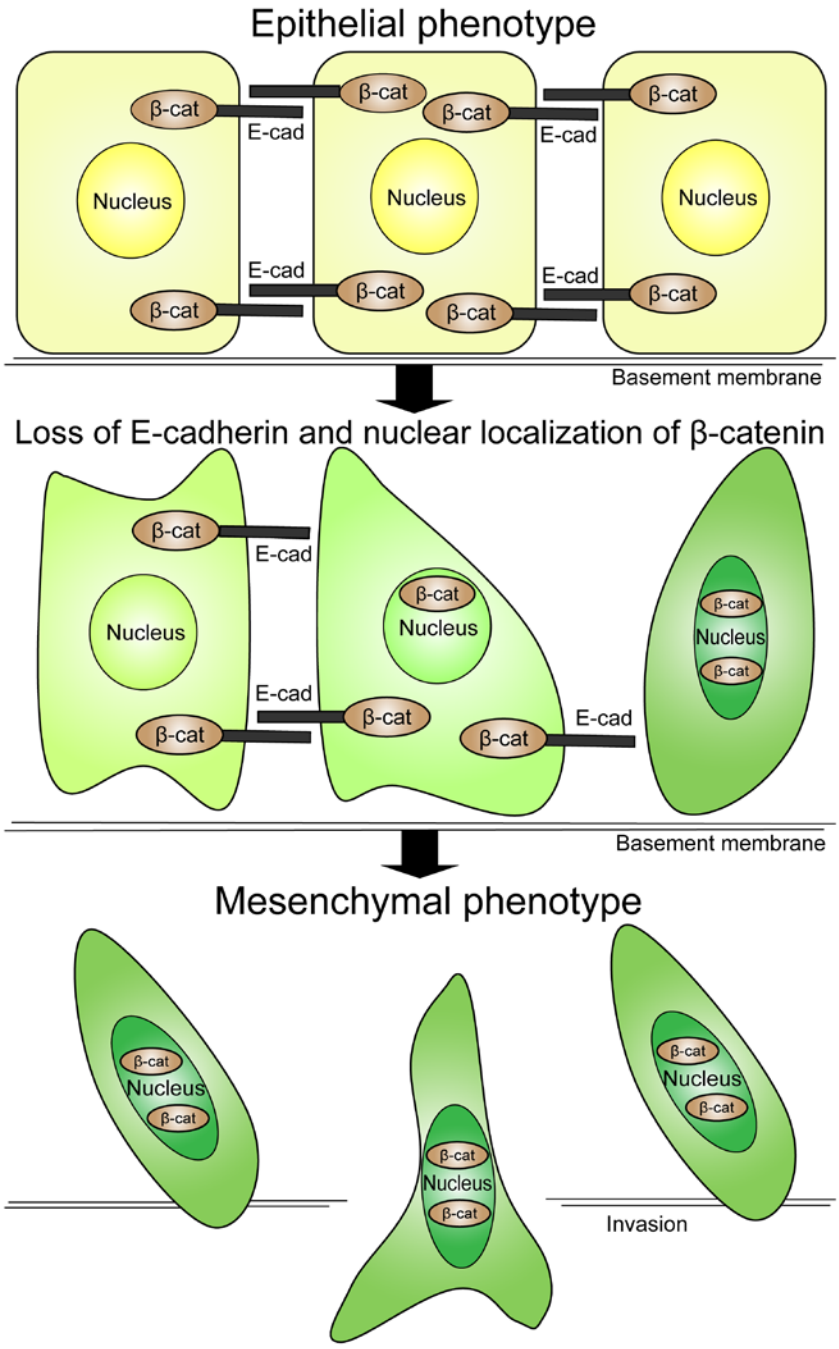

Figure 1. Loss of E-cadherin is generally accepted to be a hallmark event of the EMT process, during which epithelial cells undergo profound phenotypic modification and lack of polarity to turn into mesenchymal cells, reducing contact with surrounding cells or matrix as well as acquiring the ability of migration and invasion.

metastasis. Substantial evidence showed that intense expression of ZEB1 associates with both primary gastric cancer and peritoneal diffusion which predicted earlier recurrence as well as lower disease-free survival rate (34,35). Murai *et al* (36) classified 116 GC patients into epithelial or mesenchymal group through Vimentin/E-cadherin ratio (V/E), as they pointed, the mesenchymal group with high V/E ratio indicated an EMT status and carried anomalous ZEB1 expression, suggesting aggressive clinicopathological characteristics and poorer survival. Moreover, less AGS cells passed through the Transwell chamber which signified reduced capability of invasion in response to transfection with ZEB1 siRNA (37). ZEB2 can also bind to the E-box of E-cadherin gene promoter similarly to the Snail family. In light of the exploration by Dai *et al*, ZEB2 was closely tied to poor clinicopathological parameters such as depth of invasion, lymph node metastasis, TNM stage along with higher expression of matrix metalloprotease (MMP) family (38). A recent study from Korea on brain metastatic gastric adenocarcinoma argued that four of eight primary GC cases represent evident nuclear ZEB2 expression. Nevertheless, 3 cases showed decreased nuclear ZEB2 in brain metastatic gastric cancer samples corresponding to the consequence of brain metastatic adenocarcinoma cell research, which might result from the complex mechanisms

of brain metastasis or small sample size due to low incidence (39). However, this finding might support the theory of MET process that anchors circulating cancer cells to metastatic organs, although the effect of this in metastatic dissemination is still under debate (40).

'Cancer stem cell' was first put forward by Reya *et al* in 2011 and they protested conspicuous parallels between stem cells and cancer cells through the discovery of the origination of tumors from normal stem cells as well as analogical signaling pathways in regulating self-renewal of either stem cells or cancer cells (41). The existence of gastric cancer stem cells (GCSCs) was subsequently demonstrated and investigators isolated GCSCs through employing surface markers involving CD44/CD24 (42), EpCAM/CD44 (43), CD90 (44), CD44/CD54 (45), and CD47 (46). To date, increasing number of research studies strongly suggest that, GC cells acquire cancer stem cell (CSC) properties including self-renew, prevention of apoptosis, chemotherapy or irradiation resistance when undergoing EMT. Yang *et al* (47) obtained viable GCSCs from SGC7901 by use of serum-free and epidermal growth factor (EGF) containing medium. Whereafter, they detected greater empowerment of invasion and metastasis remained with decreased E-cadherin, but increased Vimentin expression of these cells that intensively implied the occurrence of EMT. *H. pylori* has been publicly accepted to be one of the pathogenic factors for gastric cancer while a number of studies have testified that *H. pylori* generates stemness maintenance of GC cells or clinical samples via induction of epithelial-mesenchymal transition simultaneously (33,48). Therefore, investigators advocated to combine CSC with EMT markers, specifically E-cadherin, Vimentin and CD44, as a key predictor of GC recurrence (49,50). Additionally, further exploration ought to be carried out to illuminate the impact of EMT on gastric cancer progression, which could bring about more accurate cancer therapies.

3. Wnt/β-catenin pathway and gastric cancer

The Wnt pathway named from wingless and Int-1, plays a foremost role in cell proliferation, differentiation, adhesion, migration and stem cell self-renewal. This signaling could be divided into two categories through whether it is β-catenin dependent (canonical) or β-catenin independent (non-canonical). Normally, cytoplasmic β-catenin is trussed up by the destruction complex made up of Axin, adenomatous polyposis coli (APC), casein kinase 1α (CK1α) and glycogen synthase kinase 3β (GSK3β), which is distinguished by the E3 ubiquitin ligase β-TrCP and the proteosomal degradation ultimately (51). The steady state is destroyed when secreted Wnt ligands bind to receptors on the surface of cell membrane mainly refer to as Frizzled (Fz)/low-density lipoprotein receptor-related protein 5/6 (LRP5/6) receptor compound. Afterwards, Dishevelled (Dsh) protein gets phosphorylated and forms a complex with Axin, which subsequently binds GSK-3β so as to block its activation (52). This association further results in decomposition of the degradation complex and β-catenin gathers in the cytoplasm (53). Cumulative β-catenin translocates into the nucleus, which has been generally acknowledged to be the principle event of canonical Wnt pathway activation, next interacts with TCF/LEF to compose a complex of activators and accordingly set off the transcription of target genes of Wnt/β-catenin signaling including c-Myc, cyclinD1, and MMP-7 (54) (Fig. 2).

Several mutated components among the members of canonical Wnt signaling, which further result in aberrant activation of the Wnt/β-catenin pathway, play a paramount role in malignant transformation and invasiveness of gastric cancer. For instance, human Wnts has been proved to participate in stomach tumor progression in autocrine or paracrine manner. Upregulation of Wnt-1 ligand was reported to be capable of either maintaining the stemness of GCSC or promoting the advanced stages of gastric cancer (55,56). In addition, augmented Wnt-2 might simultaneously result in cytoplasmic β-catenin accumulation and nuclear localization in both intestinal- and diffuse-type gastric carcinoma, which was positively associated with stomach cancer formation as well as tumor invasion or dissemination (57). Kurayoshi *et al* (58) argued that Wnt-5a gives rise to stimulating cell migration and invasion in gastric cancer cells by means of activating focal adhesion kinase and the small GTP-binding protein Rac. Previous studies stated that upregulation of Wnt10A induced by tumor necrosis factor α (TNFα) and *Helicobacter pylori* (*H. pylori*) infection might shed light on gastric carcinogenesis (59). Saitoh *et al* (60) confirmed the truth of the ability of Wnt10B to trigger the development of gastric cancer. Moreover, both Wnt10A and Wnt10B in GC was tied up with activation of the β-catenin-TCF signaling pathway. CDH1 gene was verified to encode E-cadherin so as to sustain epithelial cell-cell adhesion and suppress tumor invasion while germline gene alterations of CDH1 had a causative role in ~30-50% of hereditary diffuse gastric cancer (HDGC) (61,62). In particular, non-mutated (second) CDH1 allele resulting chiefly from promoter hypermethylation was likely to disintegrate cell adherens junctions and was privy to loss of cell polarity, subsequent to which, β-catenin was activated and HDGC initiated (63,64). Intestine-specific transcription factor CDX2 has been found responding to the generation of gastric adenocarcinoma from intestinal metaplasia, which revealed nuclear staining of β-catenin and APC gene mutations simultaneously (65,66).

Exon 3 of CTNNB1, where almost all the β-catenin mutations reported in human cancers are localized, is widely accepted to encode serine-threonine phosphorylation sequence for GSK3β that regulates destruction of β-catenin through the ubiquitin-proteasome pathway. Multiple experiments provided evidence indicating that mutations in exon 3 of CTNNB1 precipitates not only continuous activation of Wnt pathway but also multistep stomach carcinogenesis (67,68). Galectin-3, whose mutation locates at position 191, has been widely recognized equipped to substitute proline to histidine (gal-3H64), followed by increased nuclear accumulation of β-catenin as well as promotion of TCF transcription during gastric cancer evolvement (69). RNF43, a transmembrane E3 ligase, has been shown to eliminate Wnt receptors on cell surface in order to restrain Wnt signaling (70,71). Of note, according to a study from Korea, RNF43 gene might harbor mutational regional intratumoral heterogeneity (ITH), which could be accountable for tumorigenesis of GC (72). Lu *et al* (73) discussed that enhancer of zeste homolog 2 (EZH2) has ectopic expression in gastric cancer tissues. In a mechanistic manner, repressing CXXC finger protein 4 (CXXC4), a protein

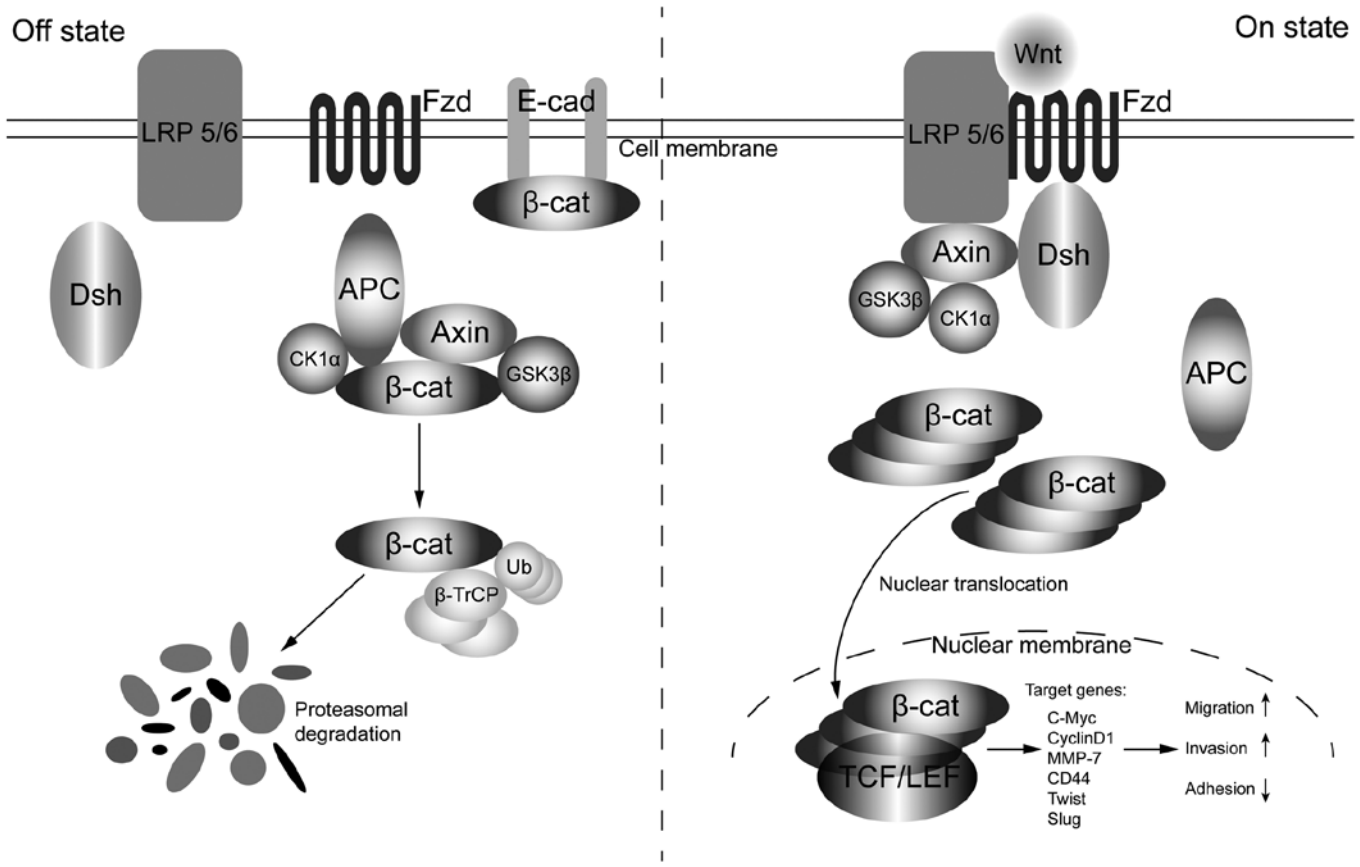

Figure 2. (Off state) In the absence of a Wnt ligand, cytoplasmic β-catenin is restrained by the degradation compound composed of Axin, APC, CK1α and GSK3β, which is discerned by the E3 ubiquitin ligase β-TrCP and sequentially is ubiquitinated. (On state) In the presence of a Wnt ligand, it binds to Fzd/LRP5/6 receptor complex on the surface of the cell membrane. Afterwards, Dsh protein is recruited and constitutes a complex with Axin, which binds GSK-3β and CK1α so as to emancipate β-catenin later on. This sequence of steps further leads to cytoplasmic accumulation of β-catenin subsequent to decomposition of the degradation complex. Stabilized β-catenin translocates into the nucleus and take part in interplay with TCF/LEF to trigger target genes including c-Myc, cyclin D1, and MMP-7.

stabilize the degradation complex of β-catenin, was necessary for the contribution of EZH2 to GC. Many investigations in GC cases identified crucial association between Actin-binding protein anillin (ANLN) expression and clinical features while transcription factor analysis demonstrated the possession of TCF binding sequence in ANLN gene promoter. Interestingly, ANLN manifested higher expression among proliferative type gastric tumors compared to invasive or metabolic types (74). Overexpression of cadherin-17 (CDH17), frizzled (FzE3), Yin Yang 1 (YY1), porcupine (PPN) has all been discovered to account for Wnt motivation, together with cell growth in gastric cancer (75-78).

Besides, loss of Wnt repressor function has been authenticated answerable to gastric cancer conformation. Dickkopf (DKK) family has been shown to bring about Wnt signal inhibition by way of binding to LRP5/6 while epigenetic silence of DKK genes is frequently detected in GC specimens. As an example, DKK-1 was found to be capable of antagonizing Wnt/β-catenin pathway as well as weakening the self-renewing ability of cancer stem-like cells (CSLCs) isolated from MKN-45 cell line (79). Furthermore, DKK-3 is known as negative regulator of Wnt, whose reduced expression primarily is due to promoter methylation involved in poor outcome of gastric carcinoma (80). Than *et al* (81) reported that administration of adenovirus vector carrying REIC/DKK-3 (Ad-REIC/DKK-3) can inhibit scirrhous gastric carcinoma (SGC) both *in vitro* and in nude mice by boosting apoptosis and recruitment of NK cells. On the contrary, despite DKK-1 or DKK-3 being Wnt suppressors, these protein have been detected upregulated in GC as well as significantly correlated to pT-stage and UICC stage (82,83), revealing that DKKs could have potential in oncogenesis of gastric cancer rather than as tumor inactivators, which might be explained either as a negative feedback loop in response to initial Wnt/β-catenin signaling activation or mediation by non-canonical Wnt pathways (84). Shin *et al* (85) sought to assess the role of secreted frizzled-related proteins (SFRPs) as antagonist of Wnt pathway and confirmed that aberrant methylation of SFRPs is the major mechanism by which Wnt signaling is activated in human GC cells. Intestinal-type gastric cancer patients exhibit somatic mutation in germline APC, a pivotal Wnt inhibitor, whose locus at the chromosome sequence 5q21-22 is frequently lost in heterozygosity (86). APC gene deletion was chiefly collected in advanced GCs, suggesting that it might take place in the progression but not initiation of tumors and had profound influence on aggressiveness (87). Axin is the scaffold protein in β-catenin degradation compound while both germline and somatic

alterations in Axin1 or Axin2 genes have been affirmed in gastrointestinal cancers. Pan *et al* (88) set forth five SNPs (334 C>T, 874 C>T, 1,396 G>A, 1,690 C>T and 1,942 T>G) and frameshift mutations in Axin1 involved in GC, in the meantime, a highly mutable G mononucleotide repeat sequence on exon 7 of Axin2 continually had a frameshift mutation (1-bp deletion) in gastric cancer with microsatellite instability (MSI) and nuclear β-catenin stabilization (89). Thus, devoting our efforts to the regulation on Wnt/β-catenin pathway in GC will notably benefit anti-metastatic approaches in the clinic.

4. Interaction between EMT and Wnt/β-catenin signaling in gastric cancer

E-cadherin plays a crucial role in negatively regulating Wnt signaling in addition to the proverbial ingredients of β-catenin degradation complex. β-catenin makes up a direct contact between cadherins and α-catenin that interacts with the actin cytoskeleton to create tight cell-cell junctions. Accumulating testimony of the significance of cadherin in catenin-mediated cell adherence junctions has confirmed that cytoplasmic carboxyl-terminus of E-cadherin could bind to β-catenin so as to suppress its nuclear localization (6). On the contrary, Howard *et al* (90) reported that the capability of β-catenin to combine with cadherins is necessary for its transcriptional activity since that cadherins might stabilize β-catenin at membrane by outcompeting its degradation machinery during EMT. Collectively, it appears that crosstalk between cadherins and β-catenin may be responsible to the process of epithelial-mesenchymal transition. Czyzewska *et al* (91) proved a statistical connection between not only invasion depth and abnormal β-catenin expression but also postoperative survival time and expression of E-cadherin. Additionally, notable significance was observed between E-cadherin along with β-catenin expression in both main mass of tumor and lymph nodes involved. Moreover, Yoshii *et al* (92) detected that combined loss of membranous E-cadherin and β-catenin expression in original mass remarkably correlates to lymph node diversion in patients with intestinal type early gastric cancer. It is noteworthy that, Silva *et al* (93) studied a total of 515 gastric adenocarcinoma patients and separated them into two groups on the basis of their age. In their series, the young group (age ≤40 years) presented with higher percentage of E-cadherin/β-catenin membranous expression compared with the old group (age >40 years), which could be attributed to the distinguish between diffuse-type and intestinal-type tumors or the mutual effect between E-cadherin and other proteins of Wnt signaling such as APC, suggesting that young GC patients might advance carcinomas through diversified genetic pathways.

Various metastasis molecules, such as leucine zipper transcription factor-like 1 (LZTFL1) (94), paired-related homeobox 1 (PRRX1) (95), Vestigial-like 4 (VGLL4) (96), high mobility group protein A2 (HMGA2) (97), erythropoietin-producing hepatocellular A2 (EphA2) (98), FAT4 (a member of the cadherin superfamily) (99), have been showed to regulate EMT via modulating nuclear location of β-catenin or target genes of Wnt signaling in gastric cancer. Secreted frizzled-related protein 1 (SFRP1) is classically considered as a conditioner of Wnt pathway by binding Wnt ligands and its ectopic transcription has eventful clinical relevance in gastric cancer development. Qu *et al* (100) reported that overexpressed sFRP1 is correlated with EMT induction in GC cells as well as lymph node migration and reduced survival time. Han *et al* (101) established an EMT model by treating BGC-823 cells with doxorubicin and elaborated upregulation of β-catenin, LEF1, c-Myc which indicates activated β-catenin signaling. Thereafter, noticeable reversion of EMT biomarkers was found when applying indomethacin or siRNA to stamp down β-catenin. Given that β-catenin is critical to cadherin-mediated cell adhesion as the reciprocity between β-catenin and cadherin could promote EMT in gastric cancer, Zhao *et al* (102) assessed luciferase reporter activity of BGC823 cells where theoretical binding site of β-catenin on the Vimentin promoter was knocked down by mutagenesis, as a result, Vimentin transcription failed to be regulated. Thus, we may safely draw a conclusion that intervention of EMT progression could be achieved only if β-catenin operates steadily. Another member of collagen genes, the collagen type I (COL1) was investigated to enhance GC cell motility by means of prompting disconnection of the E-cadherin/catenin compound. Moreover, researchers declared that the dissociation of β-catenin from E-cadherin and actin cytoskeleton is attributable to its tyrosine phosphorylation, which modifies its chemical attraction to cadherins and induces β-catenin nuclear trans-situation (103).

Besides, complementary affiliation between EMT and Wnt pathway in regulation of gastric cancer can be referred to as microRNA-dependent post-transcriptional modification. Past evidence is suggestive of the concern of certain miRNAs in different phases of EMT through Wnt signaling and elaboration of which might furnish novel diagnostic or therapeutic options for human gastric cancer hereafter.

5. MicroRNA-mediated EMT regulatory network in gastric cancer through Wnt pathway

MicroRNAs have been reported either as anomalously expressed or mutated in various tumors. Specifically, above half of miRNAs locate on tumor associated genomic regions. Analysis of expression patterns of various miRNAs in different gastric cancer tissues has showed the diversity of particular miRNA expression in tumor mass relative to normal mucus. Abundant evidence has verified that a number of miRNAs act as oncogenes or anti-oncogenes while taking part in controlling EMT through targeting at constituents of Wnt pathway (Table I), which indicats the intimate connection between GC evolution and miRNAs.

MicroRNA-200 family comprises five members including miR-200a, miR-200b, miR-200c, miR-141, miR-429 and this family has been extensively acknowledged to be representative of regulatory factors of EMT by means of terminating Wnt/β-catenin pathway in gastric cancer. In an effort to determine the suppressive role of miR-200a in gastric cancer, Su and colleagues (104) employed TOP/FOP flash luciferase assay to elucidate the collaboration beiween miR-200a and Wnt signaling in influencing metastasis potency on GC cells. As a result, they displayed an inverse relationship between miR-200a expression and luciferase activity of CTNNB1, indicating that β-catenin might be a direct target of miR-200a.

Table I. MicroRNAs targeting Wnt/β-catenin pathway in regulating EMT of GC.

MicroRNA	Target(s) of Wnt signaling	Promoter/inhibitor of EMT	Refs.
miR-200a	β-catenin/E-cadherin	Inhibitor	(104)
miR-200b	ZEB1/ZEB2/ E-cadherin /Wnt-1	Inhibitor	(106-108)
miR-27	APC	Promoter	(109)
miR-199a-5p	CDH1/β-catenin	Promoter	(110)
miR-544a	CDH1/AXIN2	Promoter	(111)
miR-145	CTNND1	Inhibitor	(112)

Another mechanism by which miR-200a hinders EMT as Cong *et al* (105) explained was that miR-200a facilitates E-cadherin through upregulating its transcriptional antagonists ZEB1/2 and impacting E-cadherin/β-catenin productive competence. Besides, negative correlation of miR-200a to WHO grades as well as EMT proteins via Wnt/β-catenin pathway was demonstrated in gastric adenocarcinoma. Furthermore, a cohort of 90 gastric cancer cases was investigated by Song *et al* (106) to ascertain two microRNA subtypes in accordance with distinct prognosis and the poor-prognosis subtype was identified to possess deregulation of EMT markers. A further identification of the contribution of three pivotal miRNAs (miR-200a, miR-200b, and miR-125b) to poor survival by targeting EMT gene network was conducted while miR-200b was peculiarly confirmed to be able to confront ZEB1 and strengthen E-cadherin *in vitro*. miR-200b was also validated to act on the 3'UTR of ZEB2 mRNA to antagonize it and localize E-cadherin to the plasma membrane (107). Tang *et al* (108) explored obvious loss of miR-200b expression in five gastric cancer cells (HGC-27, AGS, MGC-803, BGC-823, SGC-7901 and MKN-28) as well as 27 gastric cancer tissues by qRT-PCR, and the 3'UTR of Wnt-1 was confirmed to contain the binding regions of miR-200b and they showed restraining expression of Wnt1, β-catenin and TCF-4 in MGC-803 transfected with miR-200b mimics.

Many other microRNAs have been implied to be significant in modulating EMT through Wnt signaling in GC over and above miR-200 family. Zhang *et al* (109) recently introduced miR-27 as GC promoter on account of its incremental level in gastric cancer specimens. They stated that overexpressing miR-27 brings about increased ZEB1, ZEB2, Slug, and Vimentin as well as decreased E-cadherin expression level. APC was eventually identified as the immediate target of miR-27 that in turn facilitated Wnt/β-catenin pathway and EMT. Serum response factor (SRF) functions as a pro-metastatic factor in the context of stomach cancer formation, current research performed by Zhao *et al* (110) provided proof that miR-199a-5p is transactivated when SRF binds to CArG elements in its promoter. The authors reported that exceptional activation of miR-199a-5p leads to not only inhibition of CDH1 at posttranscriptional level but also import of β-catenin from adherens junctions to the cytoplasm and nucleus, forcefully indicating that the adjustive effect of miR-199a-5p on EMT may be based on Wnt signaling in GC. miR-544a potentially conferred a role during EMT abduction in gastric cancer cell line, Yanaka and colleagues (111) verified it by way of transfecting MKN1 with a cell-based reporter system which comprises a promoter sequence of Vim. Deeper investigation made clear that miR-544a alters expression of Snail and ZEB1 by directly aiming at the 3'UTR in CDH1 and AXIN2 which successively accelerates nuclear shift of β-catenin. Xing *et al* (112) furnished insight into poor clinicopathological parameters arisen from catenin-δ1 (CTNND1) perversion by inspecting 126 human GC samples utilizing immunohistochemistry. Since Wnt signaling assumes one of the downstream effectors of CTNND1, luciferase assay was implemented to identify the complementariness of miR-145 to the 3'UTR element of CTNND1 mRNA, where enables the diversion of CTNND1-E-cadherin complex from cytoplasm to membrane by downregulating N-cadherin. To sum up, microRNAs keep an intimate connection with EMT and Wnt related genes in gastric cancer as the key regulatory factors in post-transcription network. Clarification of the participation of miRNAs in reversing EMT process may exert profound influence on gastric cancer diagnosis, therapy or prognostic evaluation.

6. Promising targeted therapy in gastric cancer

Epithelial-mesenchymal transition has established itself as a foundational process through which epithelial cells turn into mesenchymal accompanied by apparent genetic and phenotypic modifications. Assertive evidence is mounting that Wnt/β-catenin signaling is one of the considerable pathways orchestrating EMT mainly due to the catenin/cadherin complex. Owing to the mutuality among EMT and Wnt/β-catenin pathway in GC pathogenesis, researchers are making efforts to excavate potential molecules as therapeutic targets for more precise treatment.

The Runt-domain transcription factor 3 (Runx3) has a defending effect on gastric epithelium as it mediates cellular proliferation and apoptosis while as many as 80% of GC patients are observed to carry loss of Runx3. Gastric epithelial cells become more plastic and susceptible to spontaneous EMT in absence of Runx3 and perverse Wnt signaling fuels the immortal cells to become carcinogenic, while both could be abolished by endogenous Runx3 inversely, signifying that Runx3 could be deemed as a capable treatment targets (113). Sox10 is a member of the SRY-related-HMG-box family that has been documented to be silenced by promoter methylation in GC while its overexpression remarkably attenuates distinct organ metastasis in nude mice. Mechanistically, competition with TCF4 for β-catenin binding consensus element is required for the EMT restraint of Sox10 (114). Cai *et al* (99)

examined the repressive role of FAT4, a cadherin-associated protein, in gastric cancer and the underlying theory. Knocking out FAT4 boosted pulmonary and hepatic metastatic lesions *in vivo* as well as the upregulation of EMT biomarkers *in vitro*. Notably, this tumorous promotion was abrogated on the condition of siRNA-mediated silencing of β-catenin. Furthermore, many other molecules serve as agitators via motivating Wnt/β-catenin pathway durung GC progress. EphA2 is a member of the RTK family whose upregulated level is usually connected to higher aggressiveness in gastric cancer. Huang *et al* (98) found that overexpression of EphA2 contributes to upregulation of N-cadherin and Snail while knockdown of EphA2 elicits inverted effect. More significantly, the result of EphA2 enhancement was neutralized when applying XAV939 to terminate Wnt signaling, whereas agitation of Wnt pathway by LiCl remedied the influence of EphA2 silencing on EMT in the GC cell lines AGS and SGC7901. Zha *et al* (97) verified the oncogenic character of HMGA2 in nude mice subcutaneously transplanted with MKN-28 and further study conclusively elucidated HMGA2 as a contributor to EMT by preventing β-catenin from phosphorylation degradationin in order to activate Wnt signaling. Analogously, PRRX1 was proven to be positively related to EMT and Wnt/β-catenin signaling downstream the molecule markers in GC specimens, while the reinforced EMT and cell mobility was offset by inhibition of Wnt pathway in BGC823 and SGC7901 lentiviral overexpression of PRRX1 (95). Since a great number of similar molecules are playing a part in EMT through modulating Wnt/β-catenin pathway during GC progression, further insight into directing these potential targets to reverse EMT can be applicable to gastric cancer clinically.

7. Conclusions and perspectives

In brief, numerous potential molecules act the part of inducers or suppressors of EMT by coordinating Wnt/β-catenin pathway in gastric cancer. MicroRNAs at the same time serve as a link between EMT and Wnt signaling in human GC and miRNA-based therapeutics such as antisense oligonucleotides, Locked nucleic acid (LNA) antimiR constructs, miRNAs Sponges and miR-Mask, have been investigated and developed (115). Nonetheless, elaborate pharmacodynamic and pharmacokinetic investigation *in vivo* models as well as rigorous clinical trials in GC patients are urged to eliminate off target side effects and ensure regional effective concentration as far as possible. We firmly believe that reformative understanding of the impact of related molecules on catenin/cadherin compound would contribute to advanced therapeutical applications and better prognosis for human gastric cancer.

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