MINI-REVIEW

Role of Wnt/β-catenin signaling regulatory microRNAs in the pathogenesis of colorectal cancer†

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Abstract

Colorectal cancer (CRC) is one of the leading causes of cancer death worldwide. In more than 90% of all CRC patients, the master oncogenic Ras-Wnt signaling axis is over-activated. MicroRNAs (miRNAs) are potential novel diagnostic and prognostic biomarkers as well as therapeutic targets for several cancers including lung, breast, gastric and colorectal cancers. Oncogenic or tumor suppressor miRNAs modulate tumor cells proliferation, cell cycle progression, angiogenesis, invasion and metastasis through regulating oncogenic pathways including Wnt/β-catenin signaling. This review summarizes the current knowledge about the role of Wnt/β-catenin signaling regulatory miRNAs in the pathogenesis of colorectal cancer for a better understanding and hence a better management of this disease. This article is protected by copyright. All rights reserved.

Keywords: Wnt/β-catenin signaling, MicroRNA, Colorectal cancer
Introduction

Colorectal cancer (CRC) is one of the most common malignancies in the world (Rodrigues et al., 2016). The progression of the CRC from normal colonic epithelial cells to the malignant phenotype is accompanied by the accumulation of mutations in oncogenes and tumor-suppressor genes including adenomatous polyposis coli (APC) (Pandurangan, 2013; Rodrigues et al., 2016; Zhang et al., 2013). The APC is a multi-functional tumor suppressor protein with inhibitory functions on Wnt/β-catenin signaling pathway (Guo et al., 2016a). APC mutations induce over-activation of Wnt/β-catenin signaling and over-expression of Wnt downstream effectors including c-myc, cyclin D1, E-cadherin and etc. leading to cell proliferation, migration, invasion and metastasis of CRC cells (Stanczak et al., 2011). Mutations in APC have been found in almost 90% of CRC as well as in all familial adenomatous polyposis patients (Coppede et al., 2014).

MicroRNAs (miRNAs) are novel promising biomarker candidates for CRC that regulate various oncogenic pathways, including Wnt/β-catenin signaling (Zhang et al., 2013). MiRNA-induced Wnt signaling regulation modulates tumorigenesis in brain (Saydam et al., 2009), colorectal (Kim et al., 2013a; Kim et al., 2011), breast (Cai et al., 2013a; Cai et al., 2013b) and liver cancers (Wang et al., 2012). In this review role of Wnt/β-catenin signaling regulatory miRNAs in the pathogenesis of CRC is summarized.

Wnt signaling pathway

Wnt signaling is classified into canonical (β-catenin-dependent) and non-canonical (β-catenin-independent) pathways (Fortress and Frick, 2015; Niehrs and Acebron, 2012). The canonical Wnt/β-catenin pathway plays essential roles in different stages of tumor development including cancer cell proliferation, migration, invasion, tumorigenesis and metastasis (Chua et al., 2014; Jiang et al., 2016; Song et al., 2015). In the absence of a Wnt ligand, cytoplasmic β-
catenin protein is phosphorylated at Ser33, Ser37, Thr41 (Yost et al., 1996) and Ser45 (Liu et al., 2002) by glycogen synthase kinase 3β (GSK3β) and casein kinase 1 (CK1) respectively, leading to ubiquitination and subsequent degradation of the protein by the proteasomal system (Huang et al., 2010; Song et al., 2015; Wheelock et al., 2001).

However, in the presence of a Wnt ligand, the receptor/co-receptor complex phosphorylates and activates the scaffolding protein Disheveled (Dsh) leading to the inhibition of the GSK-3β. Following deactivation of GSK-3β, β-catenin is not targeted for degradation by the ubiquitin/proteasome system and as a consequence, the β-catenin accumulates in the cytoplasm and travel to the nucleus to form complexes with co-regulators of transcription factors including T cell factor/lymphocyte enhancer factor (TCF/LEF). This complex regulates transcription of multiple downstream factor genes involved in cellular proliferation, differentiation, survival and apoptosis (Anastas and Moon, 2013; Clevers, 2006; Clevers and Nusse, 2012; Kim et al., 2013b; Moon et al., 2004).

Non-canonical Wnt signal transduction is independent of β-catenin stabilization and is classified into the Wnt/Ca^{2+} and planar cell polarity (PCP) pathways (Kühl et al., 2000; Pandur et al., 2002). Wnt/Ca^{2+} pathway activates transcription factor nuclear factor associated with T cells (NFAT) to regulate cytoskeletal rearrangements, cell adhesion, migration, and tissue separation (Kohn and Moon, 2005). In the PCP pathway, the activated Dvl triggers Rho (Habas et al., 2001; Tanegashima et al., 2008) and Rac branch of signaling (Marlow et al., 2002) which regulate myosin activation (Weiser et al., 2007) and actin polymerization in stimulated cells (Gordon and Nusse, 2006). These complicated signaling are integrated for cytoskeletal changes, cell polarization and motility during gastrulation (Gordon and Nusse, 2006; Seifert and Mlodzik, 2007).

Aberrant Wnt/β-catenin signaling has been linked to the pathogenesis of various diseases, including cancer. Over-expression of Wnt or gene mutations in β-catenin, GSK3β,
Axin or APC leads to over-activation of Wnt/β-catenin pathway. Wnt/β-catenin pathway regulates E-cadherin via promoting the expression of repressors of this adhesion molecule including transcriptional factors SNAI1 and SNAI2, and zinc finger E-box binding homeobox 1 (ZEB1) leading to metastasis and invasiveness (Schmalhofer et al., 2009; Yost et al., 1996).

MicroRNAs regulate Wnt/β-catenin signaling pathway

MicroRNAs are short non-coding 18-25 nucleotide RNAs (Zhong et al., 2012). MicroRNAs are transcribed by polymerase II as large RNA precursors of variable length (1 kb-3kb), called pri-miRNA. These precursor molecules are processed within the nucleus by RNase III enzyme Drosha into pre-miRNA with 60–70 nucleotide stem loop structures. Pre-miRNAs are transported to the cytoplasm through nuclear pore complexes by a shuttle protein, exportin 5. Once in the cytoplasm, the Pre-miRNAs are processed by Dicer, a RNase III enzyme, to generate the single stranded mature miRNA (Fabian and Sonenberg, 2012). Mature microRNAs assemble into the RNA-induced silencing complex (RISC), which following binding to the complementary sequence of the target mRNA induces cleavage of the mRNA (Bonfrate et al., 2013; He and Hannon, 2004).

MicroRNAs could regulate the expression of about 30% of the human genome (Schee et al., 2010) including oncogenes or tumor suppressors (Hosseini et al., 2016). MicroRNAs are involved in almost every type of cancers including lung, breast, gastric carcinoma and colorectal cancer (Kayani et al., 2011; Kong et al., 2012). Given their tendency to regulate numerous cellular processes, it is no surprising that aberrant expression of microRNAs be associated with dysregulation of oncogenic signaling pathways such as Wnt/β-catenin in cancers (Kong et al., 2014). Here we discuss about the role of Wnt/β-catenin signaling regulatory microRNAs in the CRC pathogenesis (Fig. 1).
Role of Wnt/β-catenin signaling regulatory oncogenic miRNAs in CRC pathology

APC is a key component of the Wnt/β-catenin pathway which together with other components of the destruction complex suppresses the Wnt/β-catenin signaling through targeting β-catenin for ubiquitination and degradation (Coppede et al., 2014). Mutation in APC is associated with the up-regulation of several miRNAs in CRC patients (Diosdado et al., 2009; Grillari et al., 2010; Lanza et al., 2007; Mogilyansky and Rigoutsos, 2013; Zhou et al., 2014). For instance, β-catenin binds and activates the promoter of miR-17-92, oncogenic polycistronic miRNA cluster, but APC suppresses expression of these miRNAs via inducing degradation of β-catenin. Similarly, up-regulation of β-catenin is positively associated with miR-19a expression in CRC patients (Li et al., 2016b). Consistently, enforced expression of miR-19a attenuates inhibitory effects of APC on cellular migration, invasion and growth by targeting tumor suppressor PTEN (Li et al., 2016b). Consistent with these findings, it has been shown that expression of miR135b, a key downstream effector of oncogenic pathways, is negatively correlated with the APC gene expression. Further studies showed that APC is a target gene of miR135a/b in human CRC cells. Over-expression of miR135b is associated with advanced tumor grade and poor clinical outcomes (Nagel et al., 2008; Valeri et al., 2014).

Furthermore, oncogenic miR-574-5p activates Wnt/β-catenin signaling through down-regulation of Quaking 6/7 (Qki6/7) protein (Ji et al., 2013). Qki 6/7 is a RNA binding protein regulating several cellular processes including cell cycle progression, differentiation and angiogenesis (Lobbardi et al., 2011; van Mil et al., 2012). Consistently, inhibition of miR-574-5p suppresses growth of CRC cells in the nude mice (Ji et al., 2013). To further support the stimulatory effects of oncogenic miRNAs on Wnt/β-catenin pathway, Yu Y et al. showed that oncogenic miR-21 significantly activates Wnt/β-catenin pathway, through down-regulation of transforming growth factor beta receptor 2 (TGFβR2), a negative regulator of the Wnt/β-catenin pathway, in colon cancer cells (Yu et al., 2012). Ectopic expression of miRNA-21 stimulates β-
catenin-induced TCF/LEF activity leading to up-regulation of c-Myc and cyclin D in colon cancer cells (Yu et al., 2013). Consistent with these findings, Farooqi et al. demonstrated that oncogenic miR-155 up-regulates expression of oncogenes including c-Myc, cyclin D1, TCF-1 and LEF-1 through suppressing tumor suppressor HMG-box transcription factor 1 (HBP1) (Farooqi et al., 2014; YC Wan, 2016). Similarly, miR-15b induces proliferation and metastasis through up-regulating β-catenin and suppressing Axin2, a component of the destruction complex, in colon cancer cells. MiR-15b is elevated in CRC individuals which is associated with poor patient prognosis (Guo et al., 2016a). Moreover, Guo et al. demonstrated that miR-150 is the most up-regulated microRNA in response to over-activation of Wnt/β-catenin pathway in human colon cancer cells, HCT116 (Guo et al., 2016b). Interestingly, they showed that TCF/LEF1 transcription factors bind to the promoter of miR-150 leading to trans-activation of the miRNA in Wnt/β-catenin deregulated colon cancer cells. Ectopic expression of miR-150 potently increases cancerous properties including migration and invasion activities and enhances epithelial-mesenchymal transition (EMT) by targeting CAMP responsive element binding protein 1, CREB1, and EP300, a histone acetyl transferase, in colon cancer cells (Guo et al., 2016b).

To further support the role of stimulatory Wnt/β-catenin oncogenic miRNAs in CRC pathogenesis Li et al. showed that miR-224 is an oncogenic miRNA that directly targets Wnt/β-catenin signaling suppressors including GSK3β and secreted Frizzled Related Protein-2 (sFRP-2), resulting in cytoplasmic accumulation and nuclear translocation of β-catenin thereby up-regulating the pathway target genes c-Myc and cyclinD1 (Li et al., 2016a). Similarly, miR-29a activates Wnt/β-catenin signaling via inhibiting Wnt/β-catenin antagonists including Dickkopf Wnt/β-catenin signaling pathway inhibitor 1 (Dkk1) and sFRP-2 proteins. In contrast to miR-29a, miR-29b down-regulates Wnt/β-catenin pathway and inhibits cell growth, tumor angiogenesis and EMT in human colorectal cancer cells. The inhibitory effects of miR-29b on Wnt/β-catenin pathway is mediated through targeting β-catenin transcription cofactor, B-cell CLL/lymphoma 9-
like (BCL9L), and transcription factors including transcription factor 7-like 2 (TCF7L2) and SNAI1 in colon cancer cells (Jiang et al., 2016; Kapinas et al., 2010; Subramanian et al., 2014).

**Wnt/β-catenin signaling regulatory tumor suppressor miRNAs in CRC pathology**

In contrast to oncogenic miRNAs, tumor suppressor counterparts inhibit Wnt/β-catenin pathway in CRC patients. Tumor suppressor miR-23b decreases CRC progression through down-regulation of frizzled 7 (Fzd-7), a Wnt/β-catenin signaling receptor, in CRC individuals (Zhang et al., 2011). Similarly, miR-7 targets the oncogenic transcription factor Ying Yang 1 (YY1) (Zhang et al., 2013). YY1 protein activates Wnt/β-catenin signaling pathway, thereby promoting cell proliferation (Zhang et al., 2013). Moreover, miR-34a is another tumor suppressor miRNA with an inhibitory effect on Wnt/β-catenin signaling pathway (Kim et al., 2011). It has been shown that loss of miR-34 contributes to neoplastic progression in colorectal cancer cells and the epigenetic silencing of this miRNA through CpG methylation contributes to the formation of distant metastases in primary tumors (Siemens et al., 2013). In support of the regulatory role of tumor suppressor miRNAs on Wnt/β-catenin signaling, Zhang et al. showed that compared to colon cancer cells, levels of miR-26b is significantly higher in normal colon cells (Zhang et al., 2014). Further studies showed that miR-26b down-regulates LEF-1 expression by inhibiting Wnt/β-catenin signaling pathway in colon cancer cells (Zhang et al., 2010).

Furthermore, miR-93 level is decreased in CRC tissues and colorectal carcinoma cell lines compared with normal colon mucosa (Xiao et al., 2013). Expression of miR-93 potently decreases expression of Wnt/β-catenin components and effectors. Further studies showed that miR-93-induced Wnt/β-catenin inhibition is partially mediated through targeting of smad-7, a protein essential for nuclear accumulation of β-catenin (Tang et al., 2015). Consistently, miR-101 is another inhibitory Wnt/β-catenin miRNA that its expression is impaired in CRC specimens.
Enforced expression of this miRNA decreases CRC pro-malignant features including cell growth, hypoxic survival and invasion, suggesting that MiR-101 functions as a potent tumor suppressor in CRC patients (Strillacci et al., 2013). Moreover, tumor suppressor miR-145 expression is significantly decreased in colon cancer cells (Akao et al., 2006). Recent studies showed that this miRNA inhibits Wnt/β-catenin pathway by disturbing the intracellular translocation of β-catenin into the nucleus (Yamada et al., 2013). Further studies showed that miR-145 targets catenin δ-1, a regulator of cytoskeletal reorganization that binds to p21-activated kinase 4 (PAK4). The latter one is a modulator of intracellular translocation of β-catenin to the nucleolus which is a key step for the signaling pathway activation. Similarly, miR-185 and miR-320a expressions are significantly down-regulated in colon cancer cells compared with normal colon cells (Dong-Xu et al., 2015; Schepeler et al., 2008). This miRNAs suppress cell proliferation in colon cancer cells through inhibition of Wnt/β-catenin pathway and down-regulation of signaling effector genes including c-Myc and cyclin D1 (Dong-Xu et al., 2015; Sun et al., 2012; Zhang et al., 2015). These studies clearly demonstrate that miRNAs can modulate the pathogenesis of CRC through regulating Wnt/β-catenin signaling pathway and should be pursued as potential novel diagnostic, prognostic biomarkers as well as a therapeutic target for development of novel drugs for CRC patients.

Conclusion

This review summarizes the recent findings on the Role of Wnt/β-catenin signaling regulatory miRNAs in the pathogenesis of colorectal cancer (Table 1). Through regulating Wnt/β-catenin signaling pathway, oncogenic or tumor suppressor miRNAs modulate colon cancer cells proliferation, invasion and metastasis (Fig. 2). Study results clearly support the hypothesis that these regulatory miRNAs could be novel diagnostic factor as well as a clinically invaluable post-treatment marker for CRC patients.
From this point of view, pharmacological inhibition or restoration of miRNAs might attenuate the aggressive behavior of CRC that either alone or in combination with standard clinically chemotherapy agents can be used in patients with advanced CRC cancer. Noting that a role for miRNAs in regulation of Wnt/β-catenin pathway has been reported, several studies investigated the interplay between Wnt/β-catenin signaling and miRNA expression by determining if inhibition of one pathway by specific inhibitors is mirrored in the other. Results demonstrated that not only Wnt/β-catenin signaling components including APC, β-catenin and Axin are targets for miRNAs but also many miRNAs promoters are downstream effectors of Wnt/β-catenin pathway. These findings support the hypothesis that modulation of miRNA profile is a key mechanism in anti-tumor properties of Wnt/β-catenin pharmacological inhibitors in cellular or clinical models. Understanding of the effect of specific Wnt/β-catenin pharmacological inhibitors on miRNA profile could therefore help to design of agent with more specificity and less side effects than those currently in use.
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Figure legends

**Figure 1.** Schematic representation of the role of Wnt/β-catenin signaling regulatory microRNAs in the pathogenesis of colorectal cancer.

**Figure 2.** Regulatory functions of oncogenic and tumor suppressor miRNAs on Wnt/β-catenin signaling pathway in CRC.
Table 1.

Oncogenic and tumor suppressor miRNAs regulate the pathogenesis of colorectal cancer through targeting the specific components of the Wnt/β-catenin signaling pathway.

<table>
<thead>
<tr>
<th>microRNA</th>
<th>Target</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR135b</td>
<td>APC</td>
<td>Oncogene</td>
<td>(Nagel et al., 2008)</td>
</tr>
<tr>
<td>miR-15b</td>
<td>Axin2</td>
<td>Oncogene</td>
<td>(Guo et al., 2016a)</td>
</tr>
<tr>
<td>miR-574-5p</td>
<td>β-catenin, Qki6/7, p27</td>
<td>Oncogene</td>
<td>(Ji et al., 2013)</td>
</tr>
<tr>
<td>miR-21</td>
<td>TCF/LEF, TGFβR2</td>
<td>Oncogene</td>
<td>(Yu et al., 2013)</td>
</tr>
<tr>
<td>miR-224</td>
<td>GSK3β, sFRP-2</td>
<td>Oncogene</td>
<td>(Li et al., 2016a)</td>
</tr>
<tr>
<td>miR-29a</td>
<td>sFRP-2, DKK1</td>
<td>Oncogene</td>
<td>(Kapinas et al., 2010)</td>
</tr>
<tr>
<td>miR-19a</td>
<td>PTEN</td>
<td>Oncogene</td>
<td>(Li et al., 2016b)</td>
</tr>
<tr>
<td>mir-150</td>
<td>CREB1, EP300</td>
<td>Oncogene</td>
<td>(Guo et al., 2016b)</td>
</tr>
<tr>
<td>miR-320a</td>
<td>β-catenin</td>
<td>Tumor suppressor</td>
<td>(Sun et al., 2012)</td>
</tr>
<tr>
<td>miR-29b</td>
<td>TCF7L2,BCL9L,SNAI1</td>
<td>Tumor suppressor</td>
<td>(Subramanian et al., 2014)</td>
</tr>
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<td>miR-23b</td>
<td>Fzd-7</td>
<td>Tumor suppressor</td>
<td>(Zhang et al., 2011)</td>
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<td>Wnt 1</td>
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<td>(Tang et al., 2015)</td>
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<td>miR-145</td>
<td>catenin δ-1, PAK4</td>
<td>Tumor suppressor</td>
<td>(Yamada et al., 2013)</td>
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<tr>
<td>miR-7</td>
<td>YY1</td>
<td>Tumor suppressor</td>
<td>(Zhang et al., 2013)</td>
</tr>
<tr>
<td>mir-155</td>
<td>HBP1</td>
<td>Tumor suppressor</td>
<td>(YC Wan, 2016)</td>
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Figure 1