Original Article
Functions of the Wnt/β-catenin pathway in esophageal cancer

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Abstract: Esophageal cancer is a common cancer worldwide. The Wnt/β-catenin signaling pathway plays important roles in cell differentiation, proliferation, and death. This pathway has been extensively studied because its abnormal activation is related to the occurrence of various cancers. This review focuses on the relationship of the Wnt/β-catenin signaling pathway to esophageal cancer. It highlights key aspects in Wnt/β-catenin signaling and its downstream effectors that regulate processes involved in tumor initiation, tumor growth, metastasis, and therapy resistance.

Keywords: Esophageal cancer, Wnt/β-catenin, biomarker, cancer biology

Introduction

Esophageal cancer is the sixth leading cause of cancer death and the eighth most common cancer worldwide [1]. The incidence rate of this disease is expected to increase over the next 10 years. Adenocarcinoma and squamous cell carcinoma (SCC), the major histological types of esophageal carcinoma, significantly differ in etiology and epidemiology. SCC is the most common type of esophageal carcinoma worldwide, and its major risk factors include alcohol consumption and tobacco use; esophageal adenocarcinoma is linked to gastroesophageal reflux disease and obesity [2]. Esophageal SCC (ESCC) is the most common subtype of esophageal cancer in the Chinese population; its incidence is much higher than that of esophageal adenocarcinomas [3].

Current treatment options for esophageal cancer include surgery, radiation, and chemotherapy. Surgery remains the first-choice treatment for resectable esophageal carcinoma [4]. Resection of early esophageal carcinoma (T1-2) usually results in long-term survival, but the prognosis is poor with esophagectomy alone for advanced stages (T3-4) [5]. Radiotherapy is usually used to treat advanced esophageal cancer, but the prognosis remains poor and the five-year survival rate is approximately 10% because of local control and distant metastasis [6, 7]. The existence of radioresistant tumor cells is another cause of local recurrence. The Wnt signaling pathway may be involved in the radioresistance of esophageal cells [8].

A relationship exists between epithelial-mesenchymal transition (EMT) and tumor invasion, distant metastasis, and treatment resistance, which are the most important causes of tumor death [9]. Recent studies have indicated that EMT is essential for cancer radioresistance development [10] and that the Wnt/β-catenin pathway is involved in EMT [11].

Wnt/β-catenin signaling pathway

The components of Wnt/β-catenin signaling include Wnt ligands, Wnt receptors (Frizzled and LRP5/6), Wnt antagonists and agonists (sFRPs and WIF), β-catenin, and β-catenin...
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destruction complex (composed of APC, Axin, GSK3b, and CK1a) [12]. Wnt ligands are secreted glycoproteins that have 19 isoforms in humans [13]. Wnt/β-catenin signaling is initiated upon the binding of Wnt to the Frizzled family and LRPS or LRPS6 [14]. In the absence of Wnt signals, β-catenin in the cytoplasm is phosphorylated by two multidomain scaffolding proteins, namely, Axin and APC, via kinases GSK3 and CKIa. The E3 ubiquitin ligase β-Trcp recognizes the phosphorylated β-catenin, which is then ubiquitinated and degraded by the proteasome. Finally, free β-catenin is maintained at low levels in the cytoplasm and nucleus [12]. Meanwhile, the binding of Wnt ligands to Fz receptors and LRPS6 coreceptors induces the phosphorylation of Disheveled and prevents the GSK3 β-dependent phosphorylation of β-catenin. Unphosphorylated β-catenin accumulates in the cytoplasm and eventually translocates to the nucleus [16]. The accumulation of β-catenin in the cytoplasm and the nucleus promotes β-catenin and transcription factor T-cell factor (TCF)/lymphoid enhancer factor (LEF) transcription factors, which simultaneously activate Wnt-response gene expression [15].

The Wnt/β-catenin signaling pathway is crucial in cell differentiation, proliferation, and death. Consequently, alterations in this pathway are involved in abnormal development, growth, and homeostasis in animal organisms. Wnt proteins include a numerous family of secretion glycoproteins that bind to Frizzled receptors and low-density lipoprotein receptor-related proteins to stabilize β-catenin and initiate an intricate signaling cascade related to multiple nucleocytoplasmatic processes [17].

The abnormal activation of Wnt/β-catenin signaling is associated with various human cancers. Genetic mutations of Wnt signaling pathway components are primarily responsible for this aberrant activation. Genetic mutations of the components of the β-catenin destruction complex are common in human cancers [18]. Mutations, epigenetic inactivation, or deregulated expression of components of the Wnt pathway can induce human birth defects, cancer, and other diseases [19]. Mutations cause the aberrant expression of Wnt ligand-proteins, such as Wnt1, Wnt2, Wnt5A, Wnt7A, SFRPs, and downstream components of the Wnt pathway, such as β-catenin, axin-2, and APC, which are involved in various types of human cancer [20-22]. The current review focuses on alterations of the association of the Wnt/β-catenin pathway or its defects with esophageal cancer.

Wnt/β-catenin pathway and esophageal cancer

The Wnt/β-catenin pathway is indispensable in cell differentiation, proliferation, and death [23]. Hence, the abnormal activation of this pathway contributes to carcinogenesis and malignant behaviors [18]. Constitutive activation of the Wnt/β-catenin signaling pathway can be observed in many human cancers, including esophageal cancer [16]. Many studies found that the aberrant activation of this pathway is associated with the occurrence and development of esophageal cancer. Liu K et al. [24] investigated the relationship between LKB1 and WNT signaling in esophageal cancer and found that LKB1 overexpression in TE10 cells inhibits TOPFlash luciferase reporter activity and WNT target gene expression even in the presence of WNT3A. By contrast, LKB1 knockdown enhances WNT signaling activity in esophageal cancer cells. They also found that LKB1 antagonizes the WNT signaling pathway by interacting with GSK3β to downregulate β-catenin expression. Lastly, they suggested that LKB1 interaction with GSK3β increases GSK3β activity and inhibits WNT-induced cell proliferation in esophageal cancer cells. Loss of LKB1 expression may deregulate the WNT/β-catenin pathway and promote the malignant progression of esophageal cancer.

HOTAIR is a long noncoding RNA that can directly decrease WIF-1 expression and subsequently activate the Wnt/β-catenin signaling pathway. Xiao-Song Ge et al. [25] found that the level of HOTAIR is much higher in esophageal squamous carcinoma than in adjacent normal esophageal tissues. In addition, patients with high HOTAIR expression have a poor prognosis. Chan, S L et al. [26] found that WIF1 acts as a tumor suppressor gene in esophageal cancer by inhibiting the Wnt signaling pathway; their study showed that inactivating WIF1 expression can lead to the abnormal activation of the signaling pathway and the development of esophageal carcinoma. Therefore, they suggested that WIF1 methylation can also serve as a specific marker for these tumors. Clement, G et al.
[27] found that WIF-1 hypermethylation is more frequent in patients with esophageal adenocarcinoma than in those with Barrett’s esophageal cancer that did not progress to esophageal adenocarcinoma. They showed that the activity of the Wnt/β-catenin signaling pathway decreases when WIF-1 is refectious in EAC cell lines that lack WIF-1 expression. This phenomenon suppresses the growth of esophageal adenocarcinoma cells.

Lee, E J et al. [28] studied the CpG island hypermethylation of the CDH1, sFRP1, integrin alpha4, p16, DAP kinase, Wif-1, and RARbeta2 genes in 251 ESCCs. They suggested that the lack of constitutive activation of β-catenin induces CDH1 methylation, which can be used as a recurrence-associated prognostic indicator in stage I and stage II ESCCs.

Methylation-specific polymerase chain reaction with the DNA in the plasma of esophageal cancer patients can reveal the promoter methylation of WIF-1, DKK-3, RUNX3, and sFRP-1. Liu J B et al. [29] analyzed the relationship between the Wnt signaling pathway regulation gene promoter methylation and the two-year recurrence in patients. They found that the hypermethylation of SFRP-1, DKK-3, and RUNX-3 increases the risk of esophageal cancer recurrence.

Saito T et al. [30] suggested that sFRP-2, which is a target gene of hypermethylation in esophageal basaloid SCC (BSCC), is related to BSCC tumorigenesis through the Wnt/β-catenin signaling pathway. Fu L et al. [31] reported that TF-secreted Wnt2 is involved in the growth and invasion of esophageal cancer by activating the canonical Wnt/β-catenin signaling pathway.

The Wnt pathway proteins include β-catenin, Axin, β-TrCP, and APC. Axin negatively regulates the Wnt signaling pathway, and genetic alterations of AXIN1 influence the occurrence of certain tumors. Li AF et al. [32] found that Axin expression decreases in 46% of tumor specimens, which results in the poor prognosis of ESCC. Kudo J et al. [33] reported that β-catenin accumulates at abnormal levels in the nucleus of some patients with ESCC. These results suggest that the nuclear localization of β-catenin is associated with esophageal cancer. Moreover, the abnormal localization of β-catenin is apparently not caused by the genetic alterations of either the Axin or β-catenin gene. Nakajima M et al. [34] found that AXIN expression negatively correlates with lymph node metastasis, invasion depth, and lymphatic invasion. They believed that reduced Axin expression may lead to the tumor progression of esophageal SCC.

Various studies indicated that Wnt/β-catenin signaling pathway is involved in ESCC occurrence and development. Peng H et al. found that the expression of E-cadherin, APC, and cyclin D1 in ESCC gradually decreases while that of β-catenin increases from high-grade ESCC to poorly differentiated ESCC. β-Catenin expression negatively correlates with E-cadherin and positively correlates with cyclin D1 but shows no correlation with APC. APC, β-catenin, cyclin D1, and E-cadherin may participate in the mechanism of ESCC. Thus, detection of cyclin D1, β-catenin, and E-cadherin proteins may contribute to the diagnosis of early esophageal cancer [35]. Salahshor S et al. [36] analyzed the expression and subcellular localization of key Wnt signaling components (Axin2, alpha-catenin, kinase-3 alpha/beta, E-cadherin, cyclin D1, β-catenin, and MYC) in 30 cases of ESCCs and found that Wnt signaling is deregulated in ESCC. Wang Y et al. reported that EB1 overexpression in the esophageal squamous carcinoma line EC9706 significantly promotes cell growth and that EB1 protein suppression through RNA interference significantly inhibits ESCC growth. They also found that EB1 overexpression can cause β-catenin accumulation in the cell nucleus and promote the transcriptional activity of β-catenin/TCF. Furthermore, the interaction between β-catenin and APC can be affected by EB1. Overall, EB1 overexpression participates in ESCC development by affecting APC function and activating the β-catenin/TCF pathway [37]. Tanaka S et al. [38] found that Fz3 expression may reduce APC function and enhance β-catenin-mediated signals in poorly differentiated esophageal cancers.

Targeting the Wnt/β-catenin pathway in esophageal cancer treatment

β-Catenin in the nucleus interacts with TCF and/or LEF, which promotes the expression of specific genes. Hill TP et al. [39] reported that β-catenin translocation is a key molecular event that leads to EMT. EMT is an important cellular mechanism in embryonic development,
Evidence suggests that cancer metastasis is driven by EMT [41]. TGF-β1, which is involved in the progression and metastasis of various cancer types, is a potent EMT inducer that is present in the tumor microenvironment [42]. TGF-β1 can engage the Wnt/β-catenin pathway [43]. Current data show that reducing β-catenin expression in cancer cells decreases the expression of vimentin, MMP-2, snail1, MMP-9, VEGF-C, and VEGF-A; thus, EMT and metastasis may be regulated by the Wnt/β-catenin signaling pathway [44].

Fangfang Bu et al. [45] showed that TIP30 silencing can induce the nuclear translocation and transcriptional activation of β-catenin in an AKT-dependent manner, initiate EMT, intensify the migration and invasion of esophageal carcinoma cells, and thus facilitate tumor metastasis in nude mice. By contrast, TIP30 overexpression inhibits the EMT and metastatic abilities of esophageal cancer cells. These data suggest that TIP30 can predict ESCC prognosis. Su Huafang et al. [46] reported that the acquisition of radioresistance and EMT in esophageal cancer cells is associated with the activation of the Wnt/β-catenin pathway. EMT phenotypes can be reduced, and the radiosensitivity of esophageal cancer cells can be enhanced by inhibiting the Wnt/β-catenin pathway with FH535 treatment. Chen D et al. [44] found that IL-23 is markedly expressed in esophageal carcinoma patients with distant metastasis, which can inhibit β-catenin expression. This finding indicates that IL-23 plays an important role in the development of esophageal cancer via EMT.

Xin Tong et al. [47] found that SOX10 is expressed in normal adult and fetal tissues; however, SOX10 is often silenced or less expressed in digestive system tumors, such as gastric cancer, esophageal cancer, and colorectal cancer, because of promoter methylation. They found that abnormal SOX10 expression can inhibit the proliferation and promote the apoptosis of tumor cells in vitro and in nude mice. In addition, they found that SOX10 can inhibit the migration and invasion of tumor stem cells and EMT by resisting the Wnt/β-catenin signaling pathway.

Wang JS et al. [48] investigated the effect of the β-catenin gene on tumor growth by silencing its expression through RNA interference. Their research showed that inhibiting β-catenin expression can reduce cellular proliferation and tumor growth in nude mice. They concluded that β-catenin is crucial in the regulation of cell proliferation and growth in esophageal cancer and that β-catenin expression is a potential treatment for esophageal cancer.

Geneviève Clément et al. [49] showed that WIF-1 expression sensitizes esophageal adenocarcinoma cells that lack WIF-1 expression to the chemotherapeutic agent cisplatin. Their findings suggest that the restoration of WIF-1 alone or in combination with chemotherapeutic drugs provides a novel therapeutic approach for patients with esophageal adenocarcinoma.

Ren HZ et al. [50] found that the low expression of nm23-H1 and 14-3-3sigma or the high expression of Prohibition is significantly associated with the proliferation, invasion, and lymph node metastasis of esophageal cancer. Moreover, these three proteins are related to β-catenin expression. They suggested that the result provides a potential protein marker to reveal the mechanism of the biological characteristics of the esophageal carcinoma by β-catenin.

Li HZ et al. [51] found that LEF1 and CTNNB1 are critical genes in the Wnt signaling pathway. Microarray and RT-PCR showed that the expression of LEF1 and CTNNB1 is significantly higher in radioresistant cells than in their parental cells. LEF1 and CTNNB1 are both upregulated in radioresistant cells, suggesting that these two genes directly interact to induce radioresistance.

PITX2 is a downstream effector in Wnt/β-catenin signaling and plays a key role during normal embryonic development. Zhang JX et al. [52] found that PITX2 expression is markedly higher in ESCCs than in normal esophageal mucosa and positively correlates with ESCC invasion. These data suggest that PITX2 expression is associated with poor prognosis in ESCC. They also showed that ESCC cell sensitivity to ionizing radiation or cisplatin significantly increases in vitro through PITX2 reduction. Che SM et al. [53] reported that NS398 can improve the sensitivity of CSC-like Eca109R50Gy cells to radiation by downregulating β-catenin expression. Su H et al. hypothesized that the Wnt/β-catenin pathway...
signaling pathway plays an important role in radioresistance. They found that hsa-miR-301a downregulation through Wnt1 upregulation promotes radioresistance in KYSE-150R [54].

Ge C et al. [55] found that miR-942 overexpression promotes stem cell-like traits and tumorigenesis in ESCC by directly suppressing sFRP4, GSK3β, and TLE1, which are multiple negative regulators of Wnt/β-catenin signaling. Their findings reveal a novel molecular mechanism by which the constitutive activation of the Wnt/β-catenin pathway is maintained in cancers and suggest that miR-942 is a potential therapeutic target for ESCC. Zhang H et al. [56] found that WISP1, which is a downstream target gene of the Wnt/β-catenin pathway, is re-expressed in 67.3% of ESCC patients as an oncofetal gene. They also revealed that WISP1 is a potential target in overcoming radioresistance in ESCC. Considering that calmodium kinase II and protein kinase C can be activated by Wnt ligands in esophageal adenocarcinoma, Keld R.R. et al. [57] reported that the components of the Wnt signaling pathway are potential targets for drug inhibition. Xu J et al. [58] found that celastrol can inhibit the cell metastasis of esophageal cancer by inhibiting the Wnt pathway and suppressing the expression of integrins. Further high-quality clinical trials of the Wnt/β-catenin pathway in esophageal cancer treatment are needed in the future.

Conclusions

The Wnt/β-catenin signaling pathway is crucial in cell regulation, differentiation, proliferation, and death. Alteration in this signaling pathway contributes to esophageal cancer development. Targeting the Wnt/β-catenin pathway might be an innovative approach to treat esophageal cancer. The Wnt signaling pathway is difficult to target because many components of this pathway are also involved in other cellular processes. Understanding the mechanism of the Wnt signaling pathway and searching for drugs for esophageal cancer treatment form a solid foundation for developing innovative approaches to treat esophageal cancer.

Disclosure of conflict of interest

None.

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