Roles of Wnt/β-catenin signaling pathway related microRNAs in esophageal cancer

Chao-Yang Chu, Rui Wang, Xian-Li Liu

Abstract

MicroRNAs (miRNAs) are endogenous, noncoding, single-stranded small RNAs that regulate expression of tumor suppressor genes and oncogenes and are involved in almost all tumor-related processes. MiRNA dysregulation plays an important role in the occurrence and development of esophageal cancer through specific signal pathways, including the Wnt/β-catenin signaling pathway, and is closely related to the malignant characteristics of esophageal cancer. The interaction between miRNAs and the Wnt/β-catenin signaling pathway, which is specifically expressed in esophageal cancer tissues, shows potential as a new biomarker and therapeutic target. This article reviews the role of miRNAs related to the Wnt pathway in the carcinogenesis of esophageal carcinoma and its role in Wnt signal transduction. The content of this review can be used as the basis for formulating or improving the treatment strategy of esophageal cancer.

Key Words: Wnt signal pathway; MicroRNA; Esophageal cancer; Esophageal cancer tissues

©The Author(s) 2022. Published by Baishideng Publishing Group Inc. All rights reserved.
MicroRNAs (miRNAs) related to the Wnt pathway are regulated in varying degrees in esophageal cancer. They regulate the proliferation, invasion and metastasis, radiosensitivity, autophagy, phenotype and chemotherapy resistance of esophageal cancer cells and promote stem-cell-like characteristics of esophageal cancer cells. The interaction between the Wnt signaling pathway and miRNA plays an important role in the occurrence and development of tumors. These results suggest that the miRNA-Wnt signaling pathway can be used as a potential target for tumor therapy and a diagnostic index for predicting treatment response.

**Core tip:** MicroRNAs (miRNAs) related to the Wnt pathway are regulated in varying degrees in esophageal cancer. They regulate the proliferation, invasion and metastasis, radiosensitivity, autophagy, phenotype and chemotherapy resistance of esophageal cancer cells and promote stem-cell-like characteristics of esophageal cancer cells. The interaction between the Wnt signaling pathway and miRNA plays an important role in the occurrence and development of tumors. These results suggest that the miRNA-Wnt signaling pathway can be used as a potential target for tumor therapy and a diagnostic index for predicting treatment response.

**Citation:** Chu CY, Wang R, Liu XL. Roles of Wnt/β-catenin signaling pathway related microRNAs in esophageal cancer. *World J Clin Cases* 2022; 10(9): 2678-2686

**URL:** https://www.wjgnet.com/2307-8960/full/v10/i9/2678.htm

**DOI:** https://dx.doi.org/10.12998/wjcc.v10.i9.2678

**INTRODUCTION**

Esophageal squamous cell carcinoma (ESCC) accounts for more than half of esophageal cancer patients in China. ESCC and esophageal adenocarcinoma (EAC) differ at the genomic level and have contrasting molecular characteristics[1]. China is one of the countries with the highest incidence of esophageal cancer. In recent years, although the incidence of esophageal cancer in China has declined, the absolute incidence of esophageal cancer is still high due to the large population[2]. At present, the 5-year survival rate of esophageal cancer is 15%-25%, but if esophageal cancer is diagnosed in the early stage, the survival rate can be as high as 80%[3]. In fact, most patients with esophageal cancer are diagnosed and treated at an advanced stage, which is the main reason for the poor prognosis[4]. Although endoscopy has proven to be an effective method for detecting early esophageal cancer and can reduce mortality[5], its high cost and invasiveness limit its use as a tool for extensive screening of early esophageal cancer [6]. Therefore, developing a noninvasive method for the early detection of esophageal cancer is undoubtedly an effective way to improve the early diagnosis and prognosis of esophageal cancer patients.

MicroRNAs (miRNAs) are a class of small, conservative, noncoding RNAs that play an important role in regulating mRNA translation. miRNAs are involved in a plethora of biological and pathological processes, including cell differentiation, apoptosis, proliferation and metabolism[7]. Since their discovery, miRNAs have been shown to play a potential role in cancer pathogenesis through their function as oncogenes or tumor suppressors. Since a large number of miRNAs are differentially expressed in esophageal cancer tissues, the potential of miRNA use for diagnosis has been extensively studied. In addition, more attention has been paid to characterization of the downstream targets and action mechanisms of miRNAs and evaluate their usefulness as prognostic markers, as well as to assess their potential use for monitoring therapeutic responses.

The Wnt signaling pathway is an important extracellular signaling pathway discovered together with proto-oncogene Int1 (also known as Wnt1) in 1982[8]. This complex and conserved pathway is involved in various developmental processes, such as cell growth, differentiation, ontogeny, migration, genetic stability, apoptosis, self-renewal of stem cells, maintenance of homeostasis in adult tissue, tissue regeneration and tumorigenesis[9,10].

The Wnt signaling pathway consists of two different intracellular signaling pathways, including the typical (initiated by Wnt proteins such as Wnt1, Wnt2, Wnt3, Wnt3a, Wnt7 and Wnt8) and the atypical (activated by Wnt proteins such as Wnt4, Wnt5A, Wnt5B and Wnt11) pathways. Typical Wnts activate the transcription of β-catenin/Tcf/LEF target genes through β-catenin transduction signal[11,12]. Atypical Wnt pathways can be further divided into the Wnt/Ca²⁺ pathway and the Wnt/planer cell polarity pathway. Atypical Wnts are related to the activation of the Wnt plane cell polarity pathway, Wnt/NK signaling pathway, Wnt/Ror receptor pathway, Wnt/GSK3MT pathway, Wnt/aPKC pathway, Wnt/Ryk pathway and Wnt/mTOR pathway[13].

The Wnt signaling pathway should be maintained at a normal level to perform normal physiological functions. However, extensive research has shown that the Wnt pathway is structurally activated in many cancers[14]. Therefore, the determination of genetic factors and biomarkers is important in predicting the efficacy of Wnt pathway modulator therapy, diagnosing and judging the prognosis of tumors, and developing new treatment methods. In order to explore the different roles of miRNAs in the progression of esophageal cancer through the Wnt pathway, we enumerated all the reported miRNAs related to the Wnt pathway.
MIRNAS REGULATE PROLIFERATION, INVASION AND METASTASIS OF ESOPHAGEAL CANCER CELLS THROUGH THE WNT SIGNALING PATHWAY

Ma et al [15] collected 58 cases of ESCC and adjacent normal tissue samples for comparative analysis. Expression of long noncoding RNA MEG3 is downregulated in ESCC, which is associated with tumor progression and poor prognosis. The expression level of miR-4261 in ESCC tissues is significantly higher than that in normal tissues, and negatively correlated with expression of MEG3 in ESCC tissues. In addition, in vivo and in vitro experiments have shown that miR-4261 is the target of MEG3, and MEG3 can directly regulate the expression of miR-4261, upregulate Dickkopf-related protein 2 (DKK2) and block the Wnt/β-catenin signaling pathway to inhibit cell proliferation, migration and invasion [15].

Qiao et al [16] used real-time quantitative polymerase chain reaction (RT-qPCR) to detect expression of miR-106b-3p in 50 pairs of ESCC and paracancer tissues in surgical specimens. It was confirmed that the expression of miR-106b-3p was upregulated in ESCC tissues, while zinc and ring finger 3 (ZNRF3) was downregulated in ESCC. ZNRF3 is a negative regulator of Wnt/β-catenin signal transduction and is considered to be a direct target of miR-106b-3p. Clinical research has shown that miR-106b-3p promotes the proliferation and invasion of ESCC cells by downregulating ZNRF3 and inducing epithelial–mesenchymal transition (EMT) of ESCC cells through the Wnt/β-catenin signal pathway [16].

Some laboratory studies have found that expression of miR-30a is upregulated in esophageal cancer cells. According to the results of luciferase reporter analysis, it has been confirmed that Wnt1 is the target gene of miR-30a1, indicating that miR-30a1 inhibits the activity of ESCC cells by targeting Wnt1. MiR-30a1 blocked the Wnt/β-catenin signal pathway and reversed EMT by targeting Wnt1, which inhibits the proliferation and migration of esophageal cancer cells and enhances radiosensitivity [17].

MiRNA let-7 is one of the most prominent miRNAs associated with human malignant tumors [18]. A study has previously shown that, compared with clinically collected paracancer tissues, the inhibition of let-7a in tumors is closely related to the invasion, metastasis and poor prognosis of ESCC. LIN28 is an RNA-binding protein that plays a key role in signal transduction and regulates the expression of genes downstream of LET-7a and Wnt signaling pathways [19,20]. Wnt/β-catenin/LIN28 signal transduction induces EMT and promotes the invasion, metastasis and poor prognosis of ESCC by eliminating let-7a [21].

According to the analysis of public microarray data and the verification of ESCC biopsy, miR-30a-3p/5p is downregulated in ESCC tissues compared with the paired adjacent normal tissues. The published microarray analysis showed that the downregulation of miR-30a-3p/5p is related to the activation of the Wnt signal in ESCC. Fzd2 is one of the receptors of Wnt2 that contributes to increasing the invasiveness of ESCC cells [22]. MiR-30a-3p/5p can directly target the 3′-UTR of Wnt2 and Fzd2, inhibiting their expression and resulting in the inhibition of the Wnt signaling pathway. This might be the main mechanism by which miR-30a-3p/5p regulates the proliferation of ESCC cells [23].

MiR-455-3p is upregulated in ESCC and many different types of cancer, and miR-455-3p can promote or inhibit tumors according to different tumor types [24]. Liu et al [25] used the PDX model to prove that miR-455-3p is significantly upregulated in ESCC and correlated with the overall survival time and short disease survival time of ESCC patients. At the same time, it has been confirmed that silencing of miR-455-3p decreases luciferase reporter activity and expression of genes downstream of β-catenin and transforming growth factor (TGF)-β/Smad signaling pathways. This suggests that miR-455-3p contributes to the activation of Wnt/β-catenin and β-Smad pathways in ESCC and promotes the metastasis and invasion of esophageal cancer cells.

Previous basic studies have shown that miR-200b is an invasive inhibitor of ESCC [26]. Zhang et al [27] have verified that miR-200b mainly induces G2 phase arrest, apoptosis, inhibition of cell growth and clone formation potential of ESCC lines, thereby mediating its tumor inhibition in ESCC. Cyclin-dependent kinase 2 and platelet-activating factor (an effective oncoprotein) have been identified as miR-200b targets and inhibit cell growth by reducing Wnt/β-catenin signal transduction. These studies have suggested that miR-200b is a promising therapeutic target in ESCC.

Xu et al [28] established 30 ESCC samples and adjacent normal tissue models, which showed that the level of β-catenin in ESCC specimens was significantly increased, while the level of miR-214 was significantly decreased. There was a negative correlation between the levels of β-catenin and miR-214 in ESCC specimens. The binding of miR-214 to the 3′-UTR of β-catenin mRNA inhibits the protein translation of β-catenin mRNA and directly promotes tumor growth and metastasis. It has been shown that the downregulation of miR-214 promotes the growth and invasion of ESCC cells by activating the Wnt/β-catenin pathway, highlighting that miR-214 is an effective inhibitor of ESCC.

Several clinical studies have shown that human papilloma virus-16 (HPV-16) infection may be an important risk factor for ESCC [29]. Zang et al [30] found that HPV-16 E6 activated the Wnt/β-catenin pathway at different levels by directly downregulating several regulatory factors, including transducer-like enhancer of split 1 (TLE1), glycogen synthase kinase-3β (GSK-3β) and secreted frizzled-related proteins (SFRPs). The overexpression of miR-125b restored the expression level of these proteins. The expression of miR-125b was low in HPV-16 E6 positive esophageal carcinoma, and it was negatively correlated with the level of HPV-16 E6 mRNA. These clinical results indicate that HPV-16 E6 promotes the tumorigenesis of esophageal cancer by downregulating miR-125b, and this potential mechanism is involved in the activation of the Wnt/β-catenin signal pathway.
MiRNAs REGULATE RADIOSENSITIVITY OF ESOPHAGEAL CANCER THROUGH THE WNT PATHWAY

Radiotherapy is one of the main treatments for advanced esophageal cancer. A randomized study showed that radical radiotherapy and chemotherapy for locally advanced esophageal cancer have greater survival advantages than radiotherapy alone, but the local recurrence rate and distant metastasis rate are still high[31]. Radiotherapy resistance has long been considered to be the most important cause of local tumor recurrence or metastasis. Studies have shown that miR-301a is a candidate for abnormal distribution of radiosensitive miRNAs and is related to the radiosensitivity of ESCC. MiRNA hsa-miR-301a is downregulated in esophageal cancer cell lines, while its target gene Wnt is upregulated, suggesting that the Wnt/β-catenin signal pathway plays an important role in the radiation resistance of esophageal cancer cells[32]. Su et al[17] and others have shown that the proliferation rate of radiosensitive ESCC cell line KYSE-150R transfected with miR-301a is decreased, while radiosensitivity and mobility are increased. Dual-luciferase report analysis has shown that Wnt1 is the target gene of miR-301a, suggesting that miR-301a may be a new type of radiosensitivity-related miRNA and could be a potential target for radioresistant ESCC therapy.

Xie et al[33] have demonstrated that miR-1275 inhibition increases the radioresistance of KYSE-150 cells by promoting EMT, while the enhanced expression of miR-1275 increases the radiosensitivity of KYSE-150R cells by inhibiting EMT. It has been shown that the direct targeting of miR-1275 to Wnt1 inactivates the Wnt/β-catenin signaling pathway in esophageal cancer cells. In addition, Wnt1 deletion counteracts the effect of miR-1275 inhibition on radiation resistance of KYSE-150 cells by inhibiting EMT, while overexpression of Wnt1 rescues miR-1275 upregulation-mediated damage of EMT, reducing the radiation sensitivity of KYSE-150R cells. These results suggest that miR-1275 inhibits the radiosensitivity of esophageal cancer cells by targeting the Wnt/β-catenin signaling pathway activated by Wnt1, which provides a new therapeutic approach for overcoming radioresistance in patients with esophageal cancer.

MiRNAs REGULATE AUTOPHAGY AND PHENOTYPE OF ESOPHAGEAL CANCER CELLS THROUGH THE WNT PATHWAY

Autophagy is an evolutionarily conservative process from yeast to mammals, mainly through the degradation of nonessential proteins and damaged organelles to maintain intracellular metabolic homeostasis[34,35]. Limited energy in the form of ATP can activate AMP kinase and drive autophagy. Similarly, autophagy can occur by inhibiting mammalian target of rapamycin, using rapamycin or the deprivation of growth factors and amino acids. Autophagy can be induced by some anticancer treatments, such as chemotherapy, radiotherapy and targeted therapy, and can promote the survival of cancer cells from stress-induced damage. Increasing evidence has shown the important role of autophagy in cancer due to its function in inducing the survival or death of cancer cells[36]. In addition, anticancer therapy can also induce autophagy to cause the death of cancer cells. Therefore, the contribution of autophagy to cancer development is still controversial.

MiR-638 is a potential oncogene that promotes tumorigenicity, including cell proliferation, migration and invasion, and overexpression of miR-638 promotes hunger and rapamycin-induced autophagy[37]. Disheveled-associated antagonist of β-catenin 3 (DACT3), a member of the DACT gene family, is a negative regulator of Wnt/β-catenin signal transduction and is transcriptionally suppressed in a variety of malignant tumors[38]. Ren et al[39] have shown that miR-638 regulates transport of DACT3 and is involved in autophagy. Downregulation of DACT3 leads to strong induction of Disheveled (Dvl) expression and Dvl-mediated Wnt/β-catenin signaling pathway. This clinical research has shown that the expression of miR-638 is increased while DACT3 expression is decreased in human clinical ESCC specimens. The autophagy-related miR-638/DACT3 axis may be an attractive target for cancer therapy intervention.

MiRNAs REGULATE CHEMOTHERAPY RESISTANCE OF ESOPHAGEAL CANCER THROUGH THE WNT PATHWAY

In the past 20 years, more evidence has shown that a minority cell group in tumors, called tumor stem cells or tumor-initiation cells, are related to cancer recurrence, metastasis and drug resistance to conventional treatment, and they are the key determinants of human cancer prognosis[40,41]. Liu et al[25] successfully enriched chemotherapy-resistant ESCC cells by using a xenograft model derived from chemotherapy-resistant human ESCC patients. MiRNA analysis was carried out in chemotherapy-resistant and normal esophageal cancer cells. Expression of miR-455-3p in chemotherapy-resistant esophageal cancer cells was significantly higher than that in normal esophageal cancer cells. Inhibition
Table 1 MicroRNA associated with Wnt pathway in esophageal carcinoma

| MicroRNAs | Target genes | Molecular functions | Expression pattern | Interaction with Wnt signaling | Cancer phenotype | Ref. |
|-----------|--------------|---------------------|-------------------|------------------------------|-----------------|------|
| MiR-1275  | Wnt1         | Receptor targeting Wnt protein | Up            | Repress                      | Inhibition of radiosensitivity of ESCC cells | [32] |
| MiR-4261  | DKK2         | The proliferation, migration and invasion of esophageal cancer cells can be inhibited | Up            | Repress                      | Involvement in tumor progression and poor prognosis of ESCC | [15] |
| MiR-106b-3p | ZNRF3       | Promote epithelial-mesenchymal transition | Up            | Activate                     | Promote cell proliferation and invasion | [16] |
| MiR-301a  | Wnt1         | Increased resistance to autophagy and radiation | Up            | Repress                      | Inhibition of proliferation and migration of ESCC cells and enhancement of radiosensitivity | [17] |
| MiR-let-7a | LIN28        | Transformation, matrix decomposition and angiogenesis | Down          | Activate                     | Promoting invasion, metastasis and poor prognosis of ESCC | [21] |
| MiR-30a-3p/5p | Wnt2 and Fzd2 | Gene transcription | Down          | Activate                     | Promote the proliferation of ESCC cells | [22] |
| MiR-455-3p | DKK1/GSK3β/TCF7L1 | Stem cell development | Up            | Activate                     | Promoting chemotherapy resistance and invasiveness of ESCC | [24] |
| MiRNA-638 | DACT3        | Autophagy            | Up            | Activate                     | Promote autophagy and malignant phenotype of cancer cells | [38] |
| MiR-221   | DKK2         | Imbalance of signal transduction and chemoresistance target genes | Up            | Activate                     | Mediating chemotherapy resistance of esophageal adenocarcinoma | [41] |
| MiR-200b  | CDK2 and PAF | Apoptosis and cell cycle progression | Down          | Repress                      | Induction of cell cycle arrest and inhibition of cell growth in ESCC | [26] |
| MiR-214   | β-catenin mRNA3'-UTR | Inhibit protein translation | Down          | Repress                      | Inhibit cell growth and invasion | [27] |
| MiR-125b  | SFRP4        | The cause is unknown | Down          | Activate                     | Promote the growth of ESCC cells | [29] |
| MiR-942   | SFRP4/GSK3β/TLE1 | Promote stem-cell-like characteristics | Up            | Activate                     | Promoting tumor stem cell-like characteristics of ESCC | [43] |

ESCC: Esophageal squamous cell carcinoma; DKK2: Dickkopf-related protein 2; ZNRF3: Zinc and ring finger 3; PAF: Platelet-activating factor; CDK2: Cyclin-dependent kinase 2; TLE1: Transducer-like enhancer of split 1; GSK3β: Glycogen synthase kinase-3β; SFRP: Secreted frizzled-related protein; DACT3: Dishevelled-associated antagonist of β-catenin.

of miR-455-3p increased the sensitivity of ESCC cells. MiR-455-3p, as a negative regulatory factor, simultaneously activates the Wnt/β-catenin signaling pathways involved in chemoresistance of esophageal cancer.

Wang et al.[42] established a series of EAC cell lines resistant to 5-fluorouracil (5-FU) and analyzed their miRNAs differential expression by RT-qPCR. In a group of 5-FU-resistant esophageal cancer cells (OE19, OE33, PT1590 and LN1590), miR-221 was overexpressed in all drug-resistant variants. Increased expression of miR-221 led to a decrease in the expression of DKK2, resulting in the release of Wnt/β-catenin signaling pathway blockade mediated by DKK2, indicating that miR-221-induced chemical resistance was mediated by the Wnt/β-catenin signaling pathway.

**MIRNAs PROMOTE TUMOR STEM-CELL-LIKE CHARACTERISTICS THROUGH THE WNT PATHWAY**

The Wnt/β-catenin signaling pathway is a key molecular pathway that can control stem cell function and promote tumor progression and has been demonstrated to play an important role in tumor stem cells[43]. Ge et al.[44] analyzed the microarray data set of the cancer genome map composed of 177 cases of primary esophageal cancer and 13 cases of normal esophageal tissue. Compared with normal tissues, miR-942 was significantly upregulated in tumor tissues. It was also shown that the overexpression of miR-942 upregulated Wnt/β-catenin signal transduction activity and promoted stem-cell-like characteristics and tumorigenesis in ESCC by directly inhibiting SFRP4, GSK3β and TLE1, which are negative
regulators of the Wnt/β-catenin signal pathway. This revealed a new molecular mechanism revealing how the constitutive activation of the Wnt/β-catenin pathway is maintained in cancer and suggests that miR-942 is a potential therapeutic target for esophageal cancer.

CONCLUSION

Recent clinical data indicate that the incidence of esophageal cancer and the rate of recurrence and metastasis are still high, and the overall therapeutic effect is not promising. miRNAs regulate gene expression through different mechanisms. Importantly, miRNAs can activate or inhibit Wnt signaling by interacting with other cellular macromolecules, thereby providing signals for the malignant transformation of esophageal cancer. Besides, miRNAs related to the Wnt pathway are regulated to varying degrees in esophageal cancer, regulating the proliferation, invasion and metastasis, radiosensitivity, autophagy, phenotype and chemotherapy resistance of esophageal cancer cells, as well as promoting stem-cell-like characteristics of esophageal cancer cells (Table 1). The interaction between the Wnt signaling pathway and miRNA plays an important role in the occurrence and development of tumors (Figure 1). These results suggest that the miRNA/Wnt signaling pathway can be used as a potential target for tumor therapy and a diagnostic index for predicting treatment response. Although considerable effort has been made to develop new treatments, there is still some controversy about the molecular mechanism of the Wnt pathway targeting in esophageal cancer. Further studies are warranted to improve the efficacy and develop new potent combination therapies.

FOOTNOTES

Author contributions: All authors contributed equally to this work.

Conflict-of-interest statement: There are no conflict of interest.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-
Chu CY et al. Wnt/β-catenin signaling pathway related miRNAs

NC 4.0 license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China
ORCID number: Chao-Yang Chu 0000-0002-8602-8949; Rui Wang 0000-0002-3998-4604; Xian-Li Liu 0000-0003-1114-5291.
S-Editor: Wang JJ
L-Editor: Kerr C
P-Editor: Wang JJ

REFERENCES

1. Cancer Genome Atlas Research Network; Analysis Working Group: Asan University; BC Cancer Agency; Brigham and Women’s Hospital; Broad Institute; Brown University; Case Western Reserve University; Dana-Farber Cancer Institute; Duke University; Greater Poland Cancer Centre; Harvard Medical School; Institute for Systems Biology; KU Leuven; Mayo Clinic; Memorial Sloan Kettering Cancer Center; National Cancer Institute; Nationwide Children’s Hospital; Stanford University; University of Alabama; University of Michigan; University of North Carolina; University of Pittsburgh; University of Rochester; University of Southern California; University of Texas MD Anderson Cancer Center; University of Washington; Van Andel Research Institute; Vanderbilt University; Washington University; Genome Sequencing Center; Broad Institute; Washington University in St. Louis; Genome Characterization Centers: BC Cancer Agency; Broad Institute; Harvard Medical School; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University; University of North Carolina; University of Southern California Epigenome Center; University of Texas MD Anderson Cancer Center; Van Andel Research Institute; Genome Data Analysis Centers: Broad Institute; Brown University; Harvard Medical School; Institute for Systems Biology; Memorial Sloan Kettering Cancer Center; University of California Santa Cruz; University of Texas MD Anderson Cancer Center; Biospecimen Core Resource: International Genomics Consortium; Research Institute at Nationwide Children’s Hospital; Tissue Source Sites: Analytic Biologic Services; Asan Medical Center; Asterand Bioscience; Barretos Cancer Hospital; BioreclamationIVT; Botkin Municipal Clinic; Chonnam National University Medical School; Christiana Care Health System; Cureline; Duke University; Emory University; Erasmus University; Indiana University School of Medicine; Institute of Oncology of Moldova; International Genomics Consortium; Invisumed; Israeliitshes Krankenhaus Hamburg; Keimyung University School of Medicine; Memorial Sloan Kettering Cancer Center; National Cancer Center Goyang; Ontario Tumour Bank; Peter MacCallum Cancer Centre; Pusan National University Medical School; Ribeirão Preto Medical School; St. Joseph’s Hospital & Medical Center; St. Petersburg Academic University; Tayside Tissue Bank; University of Dundee; University of Kansas Medical Center; University of Michigan; University of North Carolina at Chapel Hill; University of Pittsburgh School of Medicine; University of Texas MD Anderson Cancer Center; Disease Working Group: Duke University; Memorial Sloan Kettering Cancer Center; National Cancer Institute; University of Texas MD Anderson Cancer Center; Yonsei University College of Medicine; Data Coordination Center: CSRA Inc; Project Team: National Institutes of Health. Integrated genomic characterization of oesophageal carcinoma. Nature 2017; 541: 169-175 [PMID: 28052061 DOI: 10.1038/nature20805]

2. Li J, Qi Z, Hu YP, Wang XY. Possible biomarkers for predicting lymph node metastasis of esophageal squamous cell carcinoma: a review. J Int Med Res 2019; 47: 544-556 [PMID: 30616477 DOI: 10.1177/0300060518196696]

3. Pennathur A, Gibson MK, Jobe BA, Luketch JD. Oesophageal carcinoma. Lancet 2013; 381: 400-412 [PMID: 23374478 DOI: 10.1016/S0140-6736(12)60643-6]

4. Wang J, Wu N, Zheng QF, Yan S, Lv C, Li SL, Yang Y. Evaluation of the 7th edition of the TNM classification in patients with resected esophageal squamous cell carcinoma. World J Gastroenterol 2014; 20: 18397-18403 [PMID: 25561808 DOI: 10.3748/wjg.v20.i48.18397]

5. Wei WQ, Chen ZF, He YT, Feng H, Hou J, Lin DM, Li XQ, Guo CL, Li SS, Wang GQ, Dong ZW, Abnet CC, Qiao YL. Long-Term Follow-Up of a Community Assignment, One-Time Endoscopic Screening Study of Esophageal Cancer in China. J Clin Oncol 2015; 33: 1951-1957 [PMID: 25940715 DOI: 10.1200/JCO.2014.58.0423]

6. Lao-Sirieix P, Fitzgerald RC. Screening for oesophageal cancer. Nat Rev Clin Oncol 2012; 9: 278-287 [PMID: 22430857 DOI: 10.1038/nrcrine.2012.35]

7. Grady WM, Tewari M. The next thing in prognostic molecular markers: microRNA signatures of cancer. Gut 2010; 59: 706-708 [PMID: 20551450 DOI: 10.1136/gut.2009.200022]

8. Nusse R, Varmus HE. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell 1982; 31: 99-109 [PMID: 6297757 DOI: 10.1016/0092-8674(82)90409-3]

9. Sun X, He Y, Huang C, Ma TT, Li J. Distinctive microRNA signature associated of neoplasms with the Wnt/β-catenin signaling pathway. Cell Signal 2013; 25: 2805-2811 [PMID: 24041653 DOI: 10.1016/j.cellsig.2013.09.006]

10. Nusse R, Clevers H. Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. Cell 2017; 169: 985-999 [PMID: 28575679 DOI: 10.1016/j.cell.2017.05.016]

11. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell 2009; 17: 9-26 [PMID: 19619488 DOI: 10.1016/j.devcel.2009.06.016]

12. Sokol SY. Wnt signaling through T-cell factor phosphorylation. Cell Res 2011; 21: 1002-1012 [PMID: 21606952 DOI: 10.1038/cr.2011.86]

13. De A. Wnt/Ca2+ signaling pathway: a brief overview. Acta Biochim Biophys Sin (Shanghai) 2011; 43: 745-756 [PMID: 21606952 DOI: 10.1038/cr.2011.86]
Lu X, Zhu W, Lu G, Cao L, Tan Z, Zhang S, Jiang L, Wu J, Li M, Song L, Li J. Antagonizing miR-214 inhibits β-catenin-mediated esophageal cancer growth and invasion by miR-214. Am J Transl Res 2018; 10: 2316-2325 [PMID: 28636362 DOI: 10.18632/oncotarget.14307].

Yong Z, Jiang Y, Zhang L, Shi L, Niu H, Sun X, Wang Y, Cao L, Lu S, Jia Q, Chen Y, Sun T, Li J, Zou B. LIN28A-mediated miR-205/302 enhances cell proliferation and migration in esophageal squamous cell carcinoma via targeting of ZNRF3. Front Mol Med 2015; 9: 298-306 [PMID: 26343939 DOI: 10.4103/0973-1482.163830].

Ma J, Li TF, Han XW, Yuan HF. Downregulated MEG3 contributes to tumour progression and poor prognosis in oesophageal squamous cell carcinoma by interacting with miR-4261, downregulating DKK2 and activating the Wnt/β-catenin signaling. Artif Cells Nanomed Biotechnol 2019; 47: 1513-1523 [PMID: 30990378 DOI: 10.1111/acn.13132].

Su H, Wu Y, Fang Y, Shen L, Fei Z, Xie C, Chen M. MicroRNA301a targets WNT1 to suppress cell proliferation and demonstrate radiosensitivity in esophageal cancer cells. Oncol Rep 2019; 41: 599-607 [PMID: 30365079 DOI: 10.3892/orn.2018.6799].

Yan G, Zhan W, Yu C, Ren J, An Z. MicroRNA let-7: Regulation, single nucleotide polymorphism, and therapy in lung cancer. J Cancer Res Ther 2015; 11 Suppl 1: C1-C6 [PMID: 26323902 DOI: 10.4103/0973-1482.163830].

Wang T, Wang G, Hao D, Liu X, Wang D, Ning N, Li X. Aberrant regulation of the LIN28A/LIN28B and let-7 loop in human malignant tumors and its effects on the hallmarks of cancer. Mol Med 2015; 14: 125 [PMID: 26123544 DOI: 10.1186/s12943-015-0402-5].

Hamano R, Miyata H, Yamazaki M, Sugimura K, Tanaka K, Kurokawa Y, Nakajima K, Takiguchi S, Fujiiwa Y, Mori M, Doki Y. High expression of Lin28 is associated with tumour aggressiveness and poor prognosis of patients in oesophageal cancer. Br J Cancer 2012; 106: 1415-1423 [PMID: 22433967 DOI: 10.1038/bjc.2012.90].

Ling R, Zhou Y, Zhou L, Dai D, Wu D, Mi L, Mao C, Chen D. Lin28/microRNA-let-7a promotes metastasis under circumstances of hyperactive Wnt signaling in esophageal squamous cell carcinoma. Mol Med Rep 2018; 17: 5265-5271 [PMID: 29393461 DOI: 10.3892/mmr.2018.8548].

Fu Y, Zheng Q, Mao Y, Jiang X, Chen X, Liu P, Lv B, Huang T, Yang J, Cheng Y, Dai X, Dai C, Wang X, Yin Y, Song T, Jin W, Zou C, Chen T, Fu L, Chen Z. WNT2-Mediated FZD2 Stabilization Regulates Esophageal Cancer Metastasis via STAT3 Signaling. Front Oncol 2020; 10: 1168 [PMID: 32766155 DOI: 10.3389/fonc.2020.01168].

Qi B, Wang Y, Chen ZJ, Li XN, Qi Y, Yang Y, Cui GH, Guo HZ, Li WH, Zhao S. Down-regulation of miR-30a-3p/5p promotes esophageal squamous cell carcinoma cell proliferation by activating the Wnt signaling pathway. World J Gastroenterol 2017; 23: 7965-7977 [PMID: 29259372 DOI: 10.3748/wjg.v23.i45.7965].

Li Z, Meng Q, Pan A, Wu X, Cui J, Wang Y, Li L. MicroRNA-455-3p suppresses invasion and migration in triple negative breast cancer by targeting tumor suppressor EI24. Oncotarget 2017; 8: 19455-19466 [PMID: 28038450 DOI: 10.18632/oncotarget.14307].

Liu A, Zhu J, Wu G, Cao L, Tan Z, Zhang S, Jiang L, Wu J, Li M, Song L, Li J. Antagonizing miR-455-3p inhibits chemoresistance and aggressiveness in esophageal squamous cell carcinoma. Mol Med 2017; 16: 106 [PMID: 28633662 DOI: 10.1186/s12943-017-0669-9].

Zhang HF, Alshareef A, Wu C, Li S, Jiang JW, Wu HL, Cao HH, Li R, Xue LY, Li EM. Loss of miR-200b promotes invasion via activating the Kidlin-2/integrin β1/ALKT pathway in esophageal squamous cell carcinoma. An E-cadherin-independent mechanism. Oncotarget 2015; 6: 28949-28960 [PMID: 26334393 DOI: 10.18632/oncotarget.5027].

Zhang HF, Alshareef A, Wu C, Cao J, Sorensen PH, Lai R, Xu LY, Li EM. miR-200b induces cell cycle arrest and represses cell growth in esophageal squamous cell carcinoma. Carcinogenesis 2016; 37: 858-869 [PMID: 27496804 DOI: 10.1093/carcin/bgw079].

Xu Y, Lu S. Regulation of β-catenin-mediated esophageal cancer growth and invasion by miR-214. Am J Transl Res 2015; 7: 2316-2325 [PMID: 26807179].

Hardefeldt HA, Cox MR, Eslick GD. Association between human papillomavirus (HPV) and oesophageal squamous cell carcinoma: a meta-analysis. Epidemiol Infect 2014; 142: 1119-1137 [PMID: 24721187 DOI: 10.1017/S0950268814000016].

Zang B, Huang G, Wang X, Zheng S. HPV-16 E6 promotes cell growth of esophageal cancer via downregulation of miR-125b and activation of Wnt/β-catenin signaling pathway. Int J Clin Exp Pathol 2015; 8: 13687-13694 [PMID: 26722596].

Tepper JE. Is radiation therapy needed in the treatment of gastroesophageal junction adenocarcinoma? Gastrointest Cancer Res 2008; 2: S2-S5 [PMID: 19343142].

Su H, Jin X, Zhang X, Xue S, Deng X, Shen L, Fang Y, Xie C. Identification of microRNAs involved in the radiosensitivity of esophageal cancer cells. Cell Biol Int 2014; 38: 318-325 [PMID: 24155113 DOI: 10.1002/cbin.10202].

Xie C, Wu Y, Fei Z, Fang Y, Xiao S, Su H. MicroRNA-1275 induces radiosensitization in esophageal cancer by regulating epithelial-to-mesenchymal transition via Wnt/β-catenin pathway. J Cell Mol Med 2020; 24: 747-759 [PMID: 31733026 DOI: 10.1111/jcmm.14784].

Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. Science 2000; 290: 1717-1721 [PMID: 11090406 DOI: 10.1126/science.290.5497.1717].

Levine B, Klionsky DJ. Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev Cell 2004; 6: 463-477 [PMID: 15068787 DOI: 10.1016/s1534-5807(04)00099-1].

Karantza-Wadsworth V, Patel S, Kravchuk O, Chen G, Mathew R, Jin S, White E. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. Genes Dev 2007; 21: 1621-1635 [PMID: 17606641 DOI: 10.1101/gad.156570].

Zhang X, Wei J, Zhou L, Zhou C, Shi J, Yuan Q, Yang M, Lin D. A functional BRCA1 coding sequence genetic variant contributes to risk of esophageal squamous cell carcinoma. Carcinogenesis 2013; 34: 2309-2313 [PMID: 23749772 DOI: 10.1093/carcin/bgt213].

Jiang X, Tan J, Li J, Kivimäe S, Yang X, Zhuang L, Lee PL, Chan MT, Stanton LW, Liu ET, Cheyette BN, Yu Q. DACT3 is an epigenetic regulator of Wnt/β-catenin signaling in colorectal cancer and is a therapeutic target of histone modifications. Cancer Cell 2008; 13: 529-541 [PMID: 18538736 DOI: 10.1016/j.ccr.2008.04.019].
| Page | Reference |
|------|-----------|
| 39   | Ren Y, Chen Y, Liang X, Lu Y, Pan W, Yang M. MiRNA-638 promotes autophagy and malignant phenotypes of cancer cells via directly suppressing DACT3. *Cancer Lett* 2017; **390**: 126-136 [PMID: 28108314 DOI: 10.1016/j.canlet.2017.01.009] |
| 40   | Sampieri K, Fodde R. Cancer stem cells and metastasis. *Semin Cancer Biol* 2012; **22**: 187-193 [PMID: 22774232 DOI: 10.1016/j.semcancer.2012.03.002] |
| 41   | Visvader JE, Lindeman GJ. Cancer stem cells: current status and evolving complexities. *Cell Stem Cell* 2012; **10**: 717-728 [PMID: 22704512 DOI: 10.1016/j.stem.2012.05.007] |
| 42   | Wang Y, Zhao Y, Herbst A, Kalinski T, Qin J, Wang X, Jiang Z, Benedix F, Franke S, Wartman T, Camaj P, Halangk W, Kolligs FT, Jauch KW, Nelson PJ, Bruns CJ. miR-221 Mediates Chemoresistance of Esophageal Adenocarcinoma by Direct Targeting of DKK2 Expression. *Ann Surg* 2016; **264**: 804-814 [PMID: 27501171 DOI: 10.1097/SLA.0000000000001928] |
| 43   | Hoffmeyer K, Rudloff S, Anton R, Hierholzer A, Del Valle I, Hein K, Vogt R, Kemler R. Wnt/β-catenin signaling regulates telomerase in stem cells and cancer cells. *Science* 2012; **336**: 1549-1554 [PMID: 22723415 DOI: 10.1126/science.1218370] |
| 44   | Ge C, Wu S, Wang W, Liu Z, Zhang J, Wang Z, Li R, Zhang Z, Li Z, Dong S, Wang Y, Xue Y, Yang J, Tan Q, Song X. miR-942 promotes cancer stem cell-like traits in esophageal squamous cell carcinoma through activation of Wnt/β-catenin signalling pathway. *Oncotarget* 2015; **6**: 10964-10977 [PMID: 25844602 DOI: 10.18632/oncotarget.3696] |
