Journal of Clinical and Nursing Research, 2023, Volume 7, Issue 3
http://ojs.bbwpublisher.com/index.php/JCNR
ISSN Online: 2208-3693
ISSN Print: 2208-3685

The Role and Mechanism of LncRNA-p21 in Regulating Gastric Cancer Metastasis by Mediating Wnt/β-Catenin Signaling Pathway

Peng Liu[1†], Haizhe Chang[1†], Xinyu Peng[2], Xiu Zhang[1], Jianjun Zhang[1], Jiusong Luan[3*]

[1]Emergency Medicine, Affiliated Hospital of Hebei University, Baoding 071000, China

[2]Gastrointestinal Surgery, Affiliated Hospital of Hebei University, Baoding 071000, China

[3]Department of Respiratory and Critical Care Medicine, Affiliated Hospital of Hebei University, Baoding 071000, China

[†]These authors contributed equally to the work.

***Corresponding author:** Jiusong Luan, luanjiusong183@sina.com

Abstract: *Objective:* To study the mechanism of lncRNA p21 inhibiting the growth and metastasis of human gastric cancer SGC7901/GES-1 cells by mediating the Wnt/β-catenin signaling pathway. *Methods:* Lentiviral overexpression of lncRNA-p21 in human gastric cancer SGC7901/GES-1 cell transfections was observed and analyzed for *in vitro* migration, invasion, cell morphology and proliferation. Besides. Wnt/β-catenin signaling pathway was tested for direct involvement in lncRNA-p21-mediated inhibition of gastric cancer cell growth and proliferation. Wnt/β-catenin signaling pathway was validated using Li-C1. *Results:* Gastric cancer SGC7901/GES-1 cells in the overexpression of lncRNA-p21 showed changes in stellate morphology, low invasion, or spindle-shaped morphology. LncRNA-p21 inhibited the growth and proliferation of gastric cancer SGC7901/GES-1 cells both *in vivo* and *in vitro*, and Wnt/β-catenin signaling pathway mediated the proliferation, invasion, and migration of lncRNA-p21 on gastric cancer SGC7901/GES-1 cells. *Conclusion:* LncRNA-p21 can inhibit the growth and metastasis of gastric cancer SGC7901/GES-1 cells *in vitro* and *in vivo*, and the inhibition of lncRNA p21 is mainly mediated by inhibiting the Wnt/β-catenin signaling pathway.

Keywords: LncRNA-p21; Wnt/beta-catenin signaling pathway; Gastric cancer

Online publication: May 31, 2023

1. Introduction

Stomach cancer is a common malignant tumor, which is the second cause of malignant tumor- and cancer-related death in China. Metastasis occurs in gastric cancer, and metastasis and invasion are important factors for the low overall survival rate and poor prognosis of gastric cancer patients. Therefore, clarifying the molecular mechanism of gastric cancer occurrence and metastasis is of great significance for improving the prognosis of gastric cancer patients and developing new clinical treatments. The current research on gastric cancer metastasis mainly focuses on cytokines, glucose metabolism, and signaling pathways. LncRNA is closely related to tumor development and metastasis, and there have been many studies on the mechanism of lncRNA in tumor metastasis. LncRNA-p21 has a regulatory effect, in which it interacts with the downstream target gene *TCF4* through binding proteins and promotes its accumulation in the cytoplasm to increase the expression of β-catenin protein, thereby mediating the Wnt/β-catenin signaling pathway.

Through *in vivo* and *in vitro* experiments, this study further confirmed that inhibiting lncRNA-p21 can inhibit the invasion and metastasis of gastric cancer cells and increase the expression of β-catenin protein, thereby improving the sensitivity and survival of gastric cancer cells to chemotherapy drugs. About 10% of the DNA in the human genome is RNA, a ratio that may be linked to many diseases. The expression of these molecules is influenced by many factors — genetic mutations, environmental or intrinsic factors and genetic traits, and many more. The expression of these molecules is influenced by many factors — genetic mutations, environmental or personal factors, and genetic characteristics. In addition, in epigenetics, RNA production is regulated by mechanisms such as specific chromatin modifications, demethylation, or changes in RNA splicing resulting from gene expression. Therefore, epigenetics is complex and confusing. There is currently no complete system that comprehensively and systematically describes how various genetic alterations are discovered and characterizes the molecular mechanisms involved in biological processes. Although the human genome contains approximately 3 billion base pairs, they are extremely fragmented, randomly distributed, and difficult to understand and analyze. In fact, DNA methylation is a phenomenon produced by gene encoding proteins such as DNA methyltransferase, demethylase, and histone acetylase through catalyzing a series of biochemical reactions. Many types of epigenetic regulatory functions have been discovered and characterized in the human genome. Epigenetic regulation, as an important and widespread process in life systems, can regulate a series of life activities such as cell cycle, apoptosis, and differentiation and development in tissues and organs by affecting gene expressions. In some tumors, this regulation occurs through changes in DNA methylation. Many tumors, including lung cancer, breast cancer, and colon cancer, are associated with mutations in DNA methylation sites, for example: (1) DNA methylation changes appearing in cancer tissues or cancer patients; (2) the decrease of expression in normal tissues or even absence; (3) tumor growth due to gene mutation or loss; (4) abnormal gene mutation leading to abnormal cell function; (5) disease caused by tumor treatment-related gene mutation and deletion, and many more. RNA-binding protein (RBP) is a molecular RNA complex related to gene post-transcriptional regulation, which can bind to target molecules to regulate gene expression; RBP can regulate gene expression related to cell proliferation and migration. Studies have shown that RBP plays an important role in human diseases and cancers, especially in processes such as DNA methylation, demethylation, and RNA splicing. At the cellular level, RBP regulates the expression of cytokines, receptors, and inflammatory mediators, growth factors, or apoptosis molecules by interacting with proteins. In addition, at the tissue level, RNA-binding proteins can regulate the expression of related signaling pathways and key proteins such as Wnt/ β-catenin signaling pathways in a variety of tumor cells to regulate the complex interactions between various cell types and pathway processes during tumorigenesis and development. In addition, RBPs can also regulate various molecular signaling systems such as endoplasmic reticulum stress and autophagy, mitochondrial inner membrane-dependent pathways, membrane homeostasis and cell cycle, and other biophysical processes such as DNA damage repair. RBP is a common molecular network in tumors, and it plays an important role (including a variety of biological functions such as post-transcriptional expression regulation, protein interaction, chromatin-binding protein, post-translational modification, protein degradation, etc.) and regulates the specific role of various proteins such as NDRG1, RBP2, and PAPE1 in the occurrence and development of gastric cancer. The method and its involvement in the regulation of key signaling pathways and key proteins such as Wnt/β-catenin signaling pathway and the expression regulation mechanism of various downstream cancer factors such as VEGF and PDGF should be further studied. Therefore, the purpose of this paper is to explore the mechanism of gastric cancer development by exploring RBP and its upstream non-coding RNA. Besides the specific role and mechanism of RBP in regulating gastric cancer cell proliferation, migration and invasion are also explored, so as to further understand the role of RBP in gastric cancer. This paper is important for understanding the occurrence and development of tumor cells, and deeply studies and elucidates the molecular mechanism

and the interaction relationship between genes that may be involved, as well as the possible regulatory pathways and molecular basis, and explores the regulatory process involved in tumor cell migration, invasion, etc. [1-6].

2. Materials and methods

2.1. Materials

RPMI1640 was purchased from Invitrogen; nude mice were purchased from Beijing Experimental Animal Co., Ltd.; fetal bovine serum, paraformaldehyde, streptomycin, β-actin antibody, basement membrane, and Transwell chamber were all selected from Corning; coating matrix, TritonX-100 and rabbit anti-β-cate-nin were purchased from CST; CCK-8 reagent and RIPA lysis buffer were purchased from Beijing Suolaibao.

2.2. Method

(1) *In vitro* cell experiment

Human gastric cancer cell lines SGC7901/GES-1 were cultured in an incubator at 37℃ and 5% CO_2. Cell growth was observed under an inverted microscope containing 20% FBS at a cell density of 4×10^6, and cell proliferation was detected by CCK-8.

(2) *In vivo* experiment:

The human gastric cancer cell lines SGC7901/GES-1 were cultured and randomly divided into two groups. The cells in the two groups were subcutaneously inoculated into mice at 4×10^6, and they were sacrificed on the 3rd and 7th day (the body weight decreased by 10%). Then, the tumor tissues were taken for immunohistochemical analysis.

3. Results

3.1. Effect of overexpression of lncRNA-p21 on the morphology of gastric cancer SGC7901/GES-1 cells

Compared to the control group, the star shape or spindle of SGC7901/GES-1 cells in the lncRNAp21-overexpression (lncRNA-p21-OE) group changed to a rounder and less invasive state, as shown in **Figure 1**.

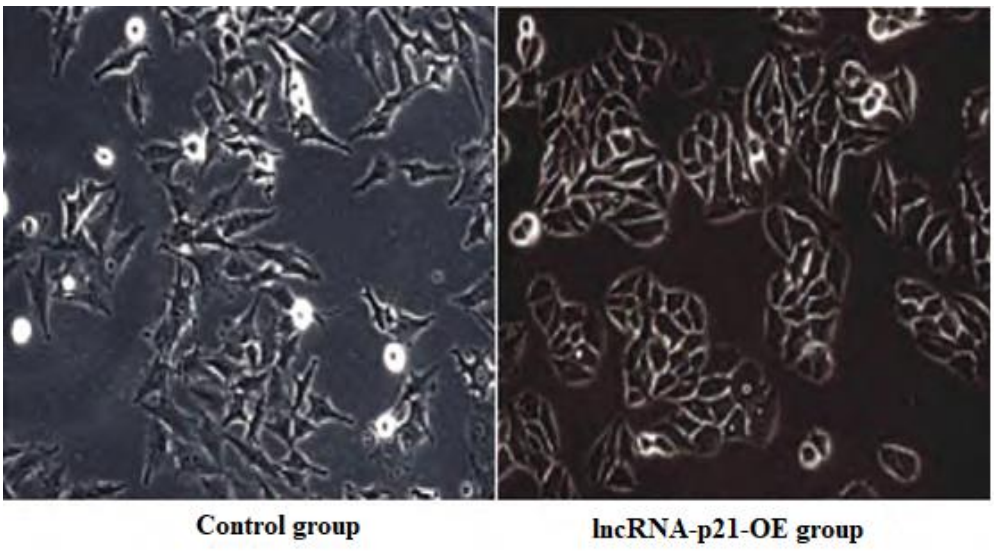

Figure 1. Overexpression of lncRNA-p21 changes the morphological characteristics of SGC7901/GES-1 cells (×400)

3.2. Effect of overexpression of lncRNA-p21 on the invasion and migration of gastric cancer SGC7901/GES-1 cells

Compared to the control group, the migration and invasiveness of SGC7901/GES-1 cells in the lncRNAp21-OE group were significantly reduced ($P < 0.05$), as shown in **Table 1**.

Table 1. The effect of overexpression of lncRNAp21 on the invasion and migration of SGC7901/GES-1 cells (mean ± SD, individual, $n = 3$)

Group	Migration	Invasiveness
Control group	461.62 ± 51.37	367.89 ± 42.52
LncRNAp21-OE group	104.54 ± 27.05	85.9 ± 12.17

3.3. Overexpression of lncRNA p21 inhibits gastric cancer cells *in vitro* and *in vivo*

The differences in cell proliferation between the lncRNAp21-OE group and the control group on the 3rd and 4th day was statistically significant ($P < 0.05$). The lncRNAp21-OE group significantly inhibited the transplanted tumor volume on the 10th day, 20th day, and 30th day ($P < 0.05$) compared to the control group, as shown in **Table 2**.

Table 2. Effects of overexpression of lncRNA p21 on gastric cancer cells *in vivo* and *in vitro*

Group	1d	2d	3d	4d
Control group	0.26 ± 0.02	0.38 ± 0.01	0.63 ± 0.05	0.89 ± 0.04
LncRNAp21-OE group	0.28 ± 0.03	0.30 ± 0.02	0.42 ± 0.01	0.47 ± 0.02

3.4. Inhibition of Wnt/β-catenin signaling pathway activity by overexpression of lncRNA p21

Immunofluorescence staining showed that the lncRNAp21-OE group significantly the accumulation of β-catenin in SGC7901/GES-1 cells was significantly inhibited compared to the control group ($P < 0.05$). Western blotting showed that the expression of β-catenin protein in the lncRNAp21-OE group was significantly reduced compared to the control group, ($P < 0.05$), as shown in **Table 3**.

Table 3. Inhibition of Wnt/β-catenin signaling pathway activity by overexpression of Lnc RNA p21

Group	β-catenin fluorescence	β-catenin protein
Control group	161.62 ± 21.37	97.89 ± 12.52
Lnc RNA p21-OE group	34.54 ± 7.05	25.9 ± 6.17

4. Discussion

Gastric cancer is one of the common malignant tumors in China and it has poor prognosis. Due to the lack of effective diagnostic and treatment methods, most gastric cancer patients are already in the middle and advanced stages when they are diagnosed and have lost the chance of radical surgery and long-term survival, and the prognosis is poor. The mechanism of action of lncRNA-p21 in malignant tumors is still unclear. This study found that lncRNA-p21 is related to the malignant degree of gastric cancer through gene expression profiling analysis; using *in vitro* cell experiments and *in vivo* animal models, it was found that lncRNA-p21 can promote the invasion, migration and clone formation ability of gastric cancer cells and their metastasis *in vivo*. In addition, this study also revealed that lncRNA-p21 can regulate the Wnt/β-catenin signaling pathway by down-regulating the expression of β-catenin protein. Although there is no

drug that directly acts on β-catenin protein, the accumulation of β-catenin protein in cells can activate its downstream target gene transcription factors, thereby promoting the proliferation, migration, and invasion of cancer cells. LncRNA-p21 can inhibit the expression of β-catenin protein in gastric cancer cells by regulating this pathway. On the other hand, there is a certain correlation between lncRNA-p21 and the activation of Wnt/β-catenin signaling pathway in gastric cancer tissues, so this study provides a basis for the clinical diagnosis of gastric cancer. It is a new target and provides a theoretical basis for clinical chemotherapy [7-9].

LncRNA-p21 is highly expressed in gastric cancer tissues and cells, and its expression is correlated with the malignancy of gastric cancer. Through gene expression profiling analysis, it was found that the expression of lncRNA-p21 was closely related to invasion and metastasis and was highly expressed in gastric cancer tissues and cells. LncRNA-p21 plays an important role in tumor development and metastasis. Inhibiting the expression of lncRNA-p21 in gastric cancer cells can promote the invasion and metastasis of cells, and down-regulate the protein expression of β-catenin in cells [10-15]. LncRNA-p21-binding protein is a gene located on chromosome 1q12.2-12.4 discovered by TCGA database analysis. LncRNA-p21 can inhibit β-catenin protein binding enzyme, so that β-catenin accumulates in the cytoplasm to promote the combination of β-catenin and *TCF4* to regulate the increase of β-catenin protein expression. After knocking down lncRNA-p21 with siRNA, we found that downregulation of lncRNA-p21 inhibited the formation of GSK3/GSK4α complex, decreased the functional activity of GSK3/β-Catenin complex, and decreased the expression of transcription factors. Finally, we found that knocking down lncRNA-p21 inhibited the accumulation of *TCF4* in the cytoplasm and promoted β-catenin into the nucleus to increase protein expression. This study shows that lncRNA-p21 can affect the invasion, metastasis, chemosensitivity, and survival of gastric cancer tissues and cells by regulating its downstream target gene *TCF4*, providing a theoretical basis for clinical molecular targeted therapy of gastric cancer.

We found that knocking down lncRNA-p21 can down-regulate the protein expression of β-catenin in cells, while overexpressing lncRNA-p21 can up-regulate the protein expression level of β-catenin in cells. lncRNA-p21 has a unique tissue distribution feature, suggesting that it may function in a variety of tumors. Knockdown of lncRNA-p21 promoted the invasion and metastasis of gastric cancer cells and down-regulated the protein expression level of its downstream target gene *TCF4* in cells after knockdown. Knockdown of lncRNA-p21 can up-regulate the phosphorylation level of β-catenin and down-regulation of cyclinD1 and Rac1.

In conclusion, (1) lncRNA-p21 can affect the invasion and metastasis of gastric cancer by regulating the Wnt/β-catenin signaling pathway, and silencing lncRNA-p21 can down-regulate the expression of *TCF4*, RAC1, etc. in the downstream target genes of Wnt/β-catenin signaling pathway. (2) LncRNA-p21 can inhibit the formation of GSK3/β-catenin complex by binding to its binding protein, thereby down-regulating the accumulation of *TCF4* in the cytoplasm and then regulating the increase of β-catenin protein expression. Knocking down the expression of TCF4 can significantly reduce the expression of β-catenin protein expression. (3) Both lncRNA-p21 and *TCF4* were found to be highly expressed in gastric cancer cell lines and clinical tissue specimens and were correlated with the degree of malignancy of tumors, suggesting that they contribute to the formation of gastric cancer tissue, overall survival of patients, tumor recurrence and metastasis, and chemotherapy sensitivity. (4) LncRNA-p21 regulates the activation of Wnt/β-catenin signaling pathway through binding proteins to promote the development and metastasis of gastric cancer, which may serve as a new target for the diagnosis and treatment of gastric cancer and a potential target for new drug development.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Ruan X, Huang Y, Weng Y, et al, 2022, Exploration on the Preliminary Mechanism of p21 Regulating POLD1 Gene in Gastric Cancer. Journal of Clinical and Experimental Pathology, 38(04): 404–409.

[2] Lu C, Yuan S, Wu Y, 2021, Mechanism of FoxF1 and FoxF2 Transcription Factors Regulating Energy Metabolism in Early Gastric Cancer by Inhibiting p21Cip1CDK. Journal of Clinical and Experimental Medicine, 20(03): 245–248.

[3] Zhao Y, 2019, Research on Annexin A7 Regulating the Proliferation of Gastric Cancer Cells Through the p21-cyclin/CDK-PCNA-E2F Signaling Axis, dissertation, Chengde Medical College.

[4] Lin Z, 2017, The Role of HoxC10 in Gastric Cancer and its Molecular Mechanism of Transcriptional Regulation of p21, dissertation, Zhejiang University.

[5] Wang X, Guo W, 2014, Bmi-1 Regulates the Stem Cell-Like Characteristics of Gastric Cancer Through miR-21. Proceedings of the 9th National Gastric Cancer Academic Conference and the 2nd Sunshine Great Wall Cancer Academic Conference, 56–57.

[6] Luo X, 2012, The Expression of SCF-E3 Ubiquitination Ligase Subunit ROC1 in Gastric Cancer and its Regulation on the Growth of Gastric Cancer Cells, dissertation, Fudan University.

[7] Ruan X, 2012, Expression of P125 in Gastric Cancer Tissue and Regulation of POLD1 Expression by p21 in Gastric Cancer Cells, dissertation, Guangxi Medical University.

[8] Ruan X, Li Y, Wu Q, et al., 2011, p21 Inhibits the Proliferation of Gastric Cancer SGC7901/GES-1 and Regulates the Expression of *POLD1*. World Chinese Journal of Digestion, 19(19): 1990–1995.

[9] Li H, Geng M, 2008, Advances in Research on the Relationship Between Cell Cycle Regulatory Factors p21 and CyclinD1 and Gastric Cancer. Journal of Diagnostic Pathology, 2008(02): 154–156.

[10] Li X, 2001, Effects of Xiaotan Sanjie Recipe on Gastric Cancer Cell Cycle Regulation System and Serum Tumor Markers. Second Military Medical University.

[11] Huang R, Zhang J, 2010, Expression and Clinical Significance of Mouse Doubled Microgenes, P53 and P21 in Gastric Cancer Tissue. Journal of Nantong University, 30(3): 177–180.

[12] Zhang F, Li B, 2010, The Clinical Significance of p21 Expression in Esophageal Squamous Cell Carcinoma and Benign Hyperplastic Lesions of Esophageal Squamous Epithelium. Clinical Medicine, 35(4): 372–376.

[13] Ding HL, Qin Z, Sun KH, et al., 2014, Differential Expression of Notch1 Intracellular Domain and P21proteins, and Their Clinical Significance in Gastric Cancer. Oncology Letters, 7: 471–478.

[14] Gu X, Zhu H, Chen J, et al., 2013, Expression and Significance of p27 Protein and Cyclin D1 in Verrucous Gastritis and Gastric Cancer Tissues. Gastroenterology, 18(10): 591–595.

[15] Hu Z, Wei S, Tan M, et al., 2013, Discussion on the Clinicopathological Relationship of p21, HER2, EGFR Expression in Gastric Adenocarcinoma. International Medicine and Health Herald, 19(21): 3263–3268.