INTRODUCTION

Colon cancer ranks the third and fourth highest prevalence and mortality in cancers worldwide, respectively (Zeng, Zhu, Li, & Kang, 2017). Over one million colon cancer cases newly emerge, and approximate 700,000 individuals die of this cancer each year (Haggar & Boushey, 2009). Colon cancer is high in the 40–50 age group (Zhai, Xue, Liu, Guo, & Chen, 2017). The occurrence and development of colon cancer involve multiple factors, including inactivated tumor suppressor
genes and activated oncogenes (Fearon & Vogelstein, 1990). Surgery is the primary therapy for colon cancer and patients exhibit a 5-year survival rate of 50% following surgery (Shi et al., 2014). However, 15%–20% patients experience recurrence following treatment. Tumor recurrence following curative surgery is a major hindrance for the improvement of overall survival (Gerger et al., 2011). Therefore, to improve the clinical outcome of colon cancer, the exploration of its pathogenesis becomes necessary.

As a noncoding RNA with over 200 nucleotides in length, long noncoding RNAs (lncRNA) cannot encode proteins as a result of the absence of open reading frame (Yu, Nangia-Makker, Farhana, & Majumdar, 2017). LncRNA has long been recognized as the noise of genomic transcription free of biological role. However, lncRNA has been shown to both directly regulate gene expression and interact with miRNA as a ceRNA (competing endogenous RNA) from recent studies (Zhang et al., 2016). The potential role of lncRNA in the regulation of malignant manifestations of tumor cells has attracted extensive attentions (Tay, Rinn, & Pandolfi, 2014; Zhou et al., 2016). Multiple tumors (e.g., prostate cancer, esophageal cancer, cervical cancer, cholangiocarcinoma) have showed abnormal expression of lncRNAs (Dong et al., 2017; Liu et al., 2017; Peng, Yuan, Jiang, Tang, & Li, 2016; Wang et al., 2016). In spite of some advances, there is few studies on lncRNA in colon cancer.

Previous studies have shown high expression of lncRNA NEAT1 in hepatocellular carcinoma, which is strongly associated with the recurrence, portal vein tumor thrombus, and tumor size. NEAT1 is considered as an independent prognostic factor for hepatocellular carcinoma from multivariate analysis (Wang, Zou, Song, & Chen, 2017; Zheng et al., 2018). In vitro experiments have demonstrated that long non-coding RNA NEAT1 contributes to laryngeal squamous cell cancer by the regulation of miR-107/CDK6 pathway (Wang et al., 2016). Rare reports exist on the clinical significance of lncRNA NEAT1 in colon cancer. This study investigated the specific expression mode of NEAT1 and described its biological roles in the development of colon cancer.

2  MATERIALS AND METHODS

2.1 Ethical compliance

The study obtained the approval opinion from the Ethics Committee of Shenzhen Second People’s Hospital.

2.2 Tissue samples

Samples were collected according to the agreement of the Medical Ethics Committee of Shenzhen Second People’s Hospital. All patients provided the informed consent. This study enrolled 10 colon cancer patients who underwent surgery in Shenzhen Second People’s Hospital from January 2017 to December 2018. Tissue samples were maintained in liquid nitrogen before RNA extraction. Among all patients enrolled, no antitumor treatment was received before operation, such as surgery, chemotherapy, and radiotherapy.

2.3 Cell culture and transfection

American Type Culture Collection (ATCC) provided colon cancer cell lines (SW620 HT-29, HCT 116, LoVo, and SW480) and normal colon epithelial cells (NCM460). Culture medium was Roswell Park Memorial Institute 1640 (HyClone) containing 10% fetal bovine serum (FBS) (Gibco), 100 μg/ml streptomycin, and 100 μg/ml penicillin. Cells were cultured in a 5% CO2 incubator at 37°C.

Cell transfection was done using Lipofectamine 2000 (Invitrogen) at 70%–80% of confluence. In brief, small interference RNA or pcDNA was subject to dilution in Opti-MEM, and followed by mixing with RNA/DNA-Lipofectamine 2000 after 5 min. Transfection solution was used in each well after 20 min. At 48 hr, transfected cells were harvested for subsequent experiments.

2.4 Quantitative real-time polymerase chain reaction

TRIzol reagent (Invitrogen) was applied for extracting total RNA. MicroRNAs were isolated using the two-column protocol of the High Pure miRNA Isolation Kit (Sigma-Aldrich) resulted enriched miRNA fraction. In brief, Binding Buffer was used to produce supernatant of centrifuge lysate from colon cancer tissues or cell lines. Then, the purified small RNA was isolated according to the two-column protocol of manufacturer’s instructions. Then, Primerscript RT Reagent (TaKaRa) was used for synthesizing their relative complementary deoxyribose nucleic acid. Quantitative real-time polymerase chain reaction (qRT-PCR) was done using SYBR® Premix Ex Taq™ Reagent (TaKaRa) and StepOne Plus Real-Time PCR system (Applied Biosystems) at 95 °C, 95 °C, 58 °C, and 74 °C for 5 min, 15, 30, and for 30 s, respectively, in a total of 40 cycles. GAPDH was used as an endogenous control for lncRNA and mRNA. The expression of miRNA was normalized to small nuclear U6. The 2−ΔΔCt method was applied to quantify gene expression. The following primers were applied for qRT-PCR:

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\text{GAPDH, F: GCACCGTCAAGGCTGAGAAC, R: GGATCGCTTCCTGGAAAGATG} \\
\text{U6, F: CTCGCTTCGAGACAGCACA, R: AACGCTTCAAGTTTGGG} \\
\text{ZHUANG et al.}
\]
2.5 | Western blot

Total proteins were gained after cells were lysed in RIPA buffer (Beyotime) for 30 min and were then centrifuged at 17,000 g at 4°C for 45 min. Protein concentrations were determined by BCA protein assay kit (Beyotime). Protein samples were subjected to SDS-PAGE and electrophoretically transferred to PVDF membranes (Millipore). After blocking in 5% nonfat milk at room temperature for 2 hr, membranes were then incubated with primary antibodies as follows: IGF2 (1:100, ab170304, Abcam), vimentin (1:1000, ab92547, Abcam), cytokeratin 19 (1:1000, ab52625, Abcam), E-cadherin (1:500, ab15148, Abcam), and GAPDH (1:1000, sc-25778). After washed three times with TBST, membranes were incubated with horseradish peroxidase conjugated secondary antibodies at room temperature for 2 hr. Immunoblots were visualized with ECL (enhanced chemiluminescence) Kit (Beyotime) and scanned using ChemImager 5500 V2.03 software (Alpha Innotech).

2.6 | Transwell cell invasion and migration assay

With cells suspended in 1.0 × 10^5/ml serum-free medium, Matrigel precoated Transwell chamber or that without pre-coating was placed in 24-well plates. The apical chamber contained 200 μl suspension and the basolateral chamber had 500 μl medium containing 10% FBS. At 48 hr, chambers were removed and penetrating cells were fixated in 5% paraformaldehyde for 20 min and stained with 0.1% crystal violet for 20 min. Penetrating cells of each sample were collected from four random fields for counting (magnification 20x). Matrigel was used for precoating Transwell chambers for invasion assay.

2.7 | Subcellular fractionation location

The location of NEAT1 in colon cancer cells was determined using a PARIS Kit (Life Technologies) according to the manufacturer's instructions, and fractions of the nucleus and cytoplasm were isolated. RNAs were diluted and tested via qRT-PCR to analyze NEAT1, GAPDH, and U6 expression levels with GAPDH as the cytoplasmic control and U6 as the nuclear control.

2.8 | RNA pull-down assay

Colon cancer cells were transiently transfected with biotinylated IncRNA-NEAT1 WT, IncRNA-NEAT1 MUT, and negative control (JINKAIRUI), respectively, and then harvested. Cell lysates were incubated with Dynabeads/ M280 Streptavidin (Invitrogen) according to the manufacturer's protocol. Similarly, the synthesized biotinylated DNA probe complementary to NEAT1 was incubated with Dynabeads/M280 Streptavidin (Invitrogen) to generate probe-coated beads. Then, cell lysates were incubated with the probe-coated beads at 4°C overnight. The RNA complexes bound to these beads were eluted and detected by qRT-PCR analysis.

2.9 | RNA-binding protein immunoprecipitation assay

RNA-binding protein immunoprecipitation (RIP) assay was performed using Magna RIP™ RNA-binding protein immunoprecipitation kit (Millipore) according to the manufacturer's guidelines with minor modifications. Briefly, the magnetic beads were incubated with 5 μg anti-AGO2 (ab32381, Abcam) or anti-IgG antibodies for 30 min at room temperature to generate antibody-coated beads. Colon cancer cells (1 × 10^7) were lysed in 100 μl RIP lysis buffer and then diluted with 900 μl RIP immunoprecipitation buffer and incubated with the antibody-coated beads overnight at 4°C. After that, beads were washed six times using RIP wash buffer. The immunoprecipitate was treated with Proteinase K at 55°C for 30 min. And the isolated RNA was extracted using TRIzol regent (Invitrogen), followed by qRT-PCR.

2.10 | Dual-luciferase reporter gene assay

We inserted the binding site of NEAT1 and IGF2 into the KpnI and SacI sites of pGL3 promoter vector (Realgene) to construct the following fragments: pGL3-NEAT1-Wild, pGL3-NEAT1-Mut, pGL3-IGF2-Wild, and pGL3-IGF2-Mut. Cells were plated into 24-well plates and cultured to 50%–70% density. Then we cotransfected 80 ng plasmid, 5 ng renilla luciferase vector pRL-SV40, 50 nM miR-185-5p mimics and negative control into cells using lipofectamine 2000 (Invitrogen). After incubation for 48 hr, changes in luciferase activity were analyzed through the dual Glo luciferase assay system (Promega) in accordance with the manufacturer's protocol. All above experiments were done in triplicate.
2.11 | Statistical analyses

All statistical analyses were performed with SAS9.1 Software (SAS Institute Inc.). Data were reported as mean ± SD. Measurement data in homogeneity of variance and normal distribution were analyzed by t test. The potential diagnostic value of NEAT1 in colon cancer was evaluated by the receiver operating characteristic (ROC) curve, whereas the survival of colon cancer patients was analyzed with Kaplan–Meier method. \( p < .05 \) was accepted as statistical difference.

3 | RESULTS

3.1 | Highly expressed NEAT1 in colon cancer

Quantitative real-time polymerase chain reaction was used for examining the expression levels of miR-185-5p, IGF2, and NEAT1 in 10 colon cancer tissues and matched normal tissues, and showed a high expression of NEAT1 and IGF2 and a low expression of miR-185-5p in colon cancer tissues relative to the controls (Figure 1a–d). Colon cancer patients were stratified into low-level and high-level groups by the median NEAT1 expression. Survival analysis indicated that the overall survival of colon cancer patients in high-level group was less than those in low-level group (Figure 1e). It is worth mentioning that, there are two isoforms of NEAT1, named NEAT1_1 and NEAT1_2, respectively. Here, we focused on the long isoform (NEAT1_2) for its higher abundance in colon cancer cell lines (Figure S1a). Moreover, based on ROC curve, NEAT1 was shown to be of diagnostic potential for distinguishing colon cancer tissues from normal ones (AUC = 0.89, Figure 1f). It turned out that an abnormal expression of NEAT1, IGF2, and miR-185-5p may be crucial in the development of colon cancer.

3.2 | Overexpressed NEAT1 promoted invasion and migration of colon cancer cells

Like the expression in colon cancer tissues, a high NEAT1 expression was observed in colon cancer cell lines (SW620, HT-29, HCT 116, LoVo, and SW480) than normal colon epithelial cells (NCM460). Among these two cells that were applied for subsequent experiments, SW480 cells showed a relatively high expression of NEAT1, whereas HT-29 cells showed a low expression (Figure 2a). For the assessment of biological roles of NEAT1, NEAT1 expression in SW480 and HT-29 cells was downregulated and upregulated by

![FIGURE 1](image-url)  Highly expressed NEAT1 in colon cancer. (a) High NEAT1 expression in colon cancer tissues relative to the controls by qRT-PCR. (b) Low miR-185-5p expression in colon cancer tissues relative to the controls by qRT-PCR. (c and d) High IGF2 expression in colon cancer tissues relative to the controls by both qRT-PCR and Western blot. (e) Shorter overall survival of colon cancer patients in high-level group than those in low-level group by survival analysis. (f) Potential diagnostic value of NEAT1 for distinguishing colon cancer tissues from normal ones as confirmed by ROC curve (AUC = 0.89). qRT-PCR, quantitative real-time polymerase chain reaction; ROC, receiver operating characteristic. **p < .01, ***p < .001
transfecting with si-NEAT1 and pcDNA-NEAT1, respectively (Figure 2b,c). Overexpressed NEAT1 in HT-29 cells contributed to the invasion and migration as showed by Transwell migration and invasion assays. On the contrary, NEAT1 knockdown showed inhibiting effect on their invasion and migration in SW480 (Figure 2d). In this study, EMT-related genes in colon cancer cells were determined. Overexpressed NEAT1 in HT-29 cells showed upregulation of vimentin and downregulation of cytokeratin 19 and E-cadherin (Figure 2e). Inversely, downregulation of vimentin and upregulation of cytokeratin 19 and E-cadherin were observed in SW480 cells with NEAT1 knockdown (Figure 2f).

3.3 | NEAT1 upregulated IGF2 expression

In previous studies, ceRNA has been considered important to exert the biological role of lncRNA (Gu, Li, Jin, Liu, & Wei, 2017; Yu, Zhao, et al., 2017). Thus, through bioinformatics, it was predicted that both NEAT1 and IGF2 presented with binding sites for miR-185-5p. Colon cancer cells showed higher IGF2 expression compared with NCM460 cells (Figure 3a). IGF2 expression was considerably upregulated or downregulated at protein and mRNA levels, respectively, through transfection of pcDNA-IGF2 in HT-29 cells or si-IGF2 in SW480 cells (Figure 3b,d).
positively regulated by NEAT1 as demonstrated by subsequent qRT-PCR data (Figure 3c). Western blot analysis also yielded similar association at their protein levels (Figure 3e).

3.4 | NEAT1 could bind to IGF2 and miR-185-5p

Long noncoding RNAs function in a manner depending on their subcellular distribution. Hence, qRT-PCR was adopted to examine NEAT1 expression in the nucleus and cytoplasm of colon cancer cells. NEAT1 was primarily present in the cytoplasm of colon cancer cells (Figure S1b). So, NEAT1 may participate in the development of colon cancer through posttranscriptional regulation. Given that NEAT1 may participate in the development of colon cancer through posttranscriptional regulation, it was assumed to act as a ceRNA in the development of colon cancer. According to bioinformatics prediction (http://starbase.sysu.edu.cn), the binding sites between miR-185-5p and NEAT1 in the binding region (chr11: 65443172–65443195) is similar to the binding sites between miR-185-5p and IGF2. Based on qRT-PCR data, MiR-185-5p was lowly expressed in colon cancer cells relative to NCM460 cells (Figure 4a). Through plasmid transfection, miR-185-5p mimics or inhibitor considerately downregulated and upregulated miR-185-5p expression in SW480 and HT-29 cells, respectively (Figure 4b). The construction of Luciferase reporter plasmids (NEAT1-WT, NEAT1-MUT, IGF2-WT, IGF2-MUT) was performed on the basis of the predicted binding sites to miR-185-5p (Figure 4c). The data indicated that luciferase activity was inhibited in colon cancer cells after cotransfection with miR-185-5p mimics and NEAT1-WT/IGF2-WT, suggesting that NEAT1 and IGF2 bound to miR-185-5p (Figure 4d). In addition, as the outcomes of a RIP-binding assay presented, the level of NEAT1 and miR-185-5p was higher in anti-Ago2 group than that in antinormal IgG group which indicating that NEAT1 and miR-185-5p were in the same RNA induced silencing complex (Figure 4e). The specific interaction between miR-185-5p and NEAT1 was further demonstrated by RNA pull-down assay using biotin-labeled NEAT1. miR-185-5p was pulled down by NEAT1, whereas NEAT1 mutant with disrupting putative-binding sequence resulted in failure of miR-185-5p coprecipitation. These
results validated that the recognition of miR-185-5p to NEAT1 was in a sequence-specific manner (Figure 4f). Furthermore, Western blot revealed negative regulation of miR-185-5p on IGF2 expression (Figure 4g).

3.5 | NEAT1/MiR-185-5p axis was important in migratory and invasive potentials of colon cancer

Finally, the effect of miR-185-5p/NEAT1 on cellular performances of colon cancer was evaluated. HT-29 cells with overexpressed IGF2 contributed to migratory and invasive potentials, whereas SW480 cells with IGF2 knockdown presented with the contrary trends (Figure 5a). It may be concluded the promotive role of IGF2 on migratory and invasive potentials of colon cancer. To identify whether NEAT1/miR-185-5p/IGF2 axis participated in the progression of colon cancer, NEAT1 and miR-185-5p were co-overexpressed in HT-29 cells. Overexpressed miR-185-5p downregulated IGF2 expression in HT-29 cells, but was further upregulated by co-overexpressed NEAT1 and miR-185-5p (Figure 5b). Overexpressed NEAT1 partially reversed the inhibited migration and invasion of HT-29 cells (Figure 5c). Conversely, cotransfection of miR-185-5p inhibitor and si-NEAT1 inhibited upregulated IGF2 expression in SW480 cells with miR-185-5p knockdown (Figure 5d). NEAT1 knockdown partially reversed the enhanced migration and invasion of SW480 cells (Figure 5e). It was concluded that the regulatory role of NEAT1 in colon cancer depended on miR-185-5p/IGF2. In conclusion, this study revealed that the migration and invasion of colon cancer were regulated by NEAT1/miR-185-5p/IGF2 axis (Figure 6).
DISCUSSION

Several lncRNAs have been shown to participate in the progression of colon cancer. For instance, long noncoding RNA HNF1A-AS1 mediated repression of miR-34a/SIRT1/p53 feedback loop acts as a ceRNA to contribute to the metabolism of colon cancer (Fang et al., 2017). LncRNA BCAR4 contributes to the progression of colon cancer through the activation of Wnt/β-catenin signaling (Ouyang et al., 2017). LncRNA MALAT1 induces the development of colon cancer through the regulation of miR-129-5p/HMGB1 axis (Wu, Meng, Jie, & Zhao, 2018). In this study, colon cancer patients presented with a high NEAT1 expression relative to the controls. Moreover, the survival of colon cancer patients at a high NEAT1 expression was shorter than those at low level. Overexpressed NEAT1 considerably promoted migratory and invasive rates of colon cancer cells. Based on a series of functional analyses, NEAT1 could mediate the progression of colon cancer through regulating IGF2 by absorbing miR-185-5p.

![FIGURE 5](image-url) NEAT1/MiR-185-5p axis was important in migratory and invasive potentials of colon cancer. (a) Invasive and migratory potentials were promoted by HT-29 cells transfected with pcDNA-IGF2, whereas the contrary trends were observed in SW480 cells transfected with si-IGF2. (b) Overexpressed miR-185-5p downregulated IGF2 expression in HT-29 cells, but was further upregulated by co-overexpressed NEAT1 and miR-185-5p. (c) Overexpressed NEAT1 partially reversed the migration and invasion of HT-29 cells suppressed by miR-185-5p mimics. (d) Cotransfection of miR-185-5p inhibitor and si-NEAT1 suppressed the upregulated IGF2 expression in SW480 cells with miR-185-5p knockdown. (e) NEAT1 knockdown partially reversed the migration and invasion of SW480 cells promoted by miR-185-5p inhibitor. *p < .05
CeRNA hypothesis was proposed in 2011 for the first time (Giza, Vasilescu, & Calin, 2014). Moreover, lncRNAs shows interaction with target miRNA and further regulation of the expression of the target gene as ceRNAs together with direct gene expression regulation (Peng et al., 2016). CeRNA hypothesis is of crucial significance in regulating malignancies. In theory, all RNAs with miRNA-binding sites could bind to miRNAs, followed by playing their roles as a ceRNA (Karreth & Pandolfi, 2013; Li, Sun, Hicks, & Raikhel, 2015). LncRNA/miRNA/mRNA network under ceRNA regulatory mode complements the miRNA function. This study revealed that the biological role of NEAT1 in colon cancer depended on absorbing miR-185-5p. Bioinformatics prediction identified IGF2 as a potential target gene of miR-185-5p. NEAT1 showed positive regulation on IGF2 expression, whereas miR-185-5p showed negative regulation on it.

The expression of insulin-like growth factor 2 (IGF2) is observed in the liver and many other tissues as a 7.5 kDa mitogenic peptide hormone (Livingstone, 2013). Overexpressed IGF2 is observed in many cancers and is associated with a poor prognosis (Xu et al., 2017). The risk of various cancers (e.g., prostate, breast, ovarian) also increases with higher serum IGF2 (Dong et al., 2015; Schagdarsurengin et al., 2017; Tominaga et al., 2017). This study showed a high expression of IGF2 in colon cancer cells and tissues. Overexpressed IGF2 contributed to invasive and migratory rates of colon cancer cells. It was more important that overexpressed NEAT1 reversed the decrease in IGF2 caused by miR-185-5p mimics, further suggesting that IGF2 was a key target for NEAT1 and miR-185-5p.

This research has several deficiencies. First, a larger sample size is required to further explore the clinical value of NEAT1. Second, which RNA domain of NEAT1 long isoform comes in the contact with miR-185-3p needs further study. Third, more target genes or miRNAs should be applied to interact with NEAT1.

This paper had some noteworthy limitations. This study placed emphasis on the in vitro roles of lncRNA NEAT1/miR-185-5p/IGF2 axis in the progression of colon cancer. More experiments are warranted to confirm the effects of lncRNA NEAT1/miR-185-5p/IGF2 on development and metastasis of colon cancer in vivo.

5 | CONCLUSION

High NEAT1 expression is observed in colon cancer, which contributes to the migration and invasion of colon cancer cells through absorbing miR-185-5p to upregulate IGF2 expression. NEAT1 is identified as an independent risk factor for poor prognosis of colon cancer.
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None.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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REFERENCES

Dong, Y., Li, J., Han, F., Chen, H., Zhao, X., Qin, Q., … Liu, J. (2015). High IGF2 expression is associated with poor clinical outcome in human ovarian cancer. Oncology Reports, 34(2), 936–942. https://doi.org/10.3892/or.2015.4048

Dong, Z., Zhang, A., Liu, S., Lu, F., Guo, Y., Zhang, G., … Guo, W. (2017). Aberrant methylation-mediated silencing of lncRNA MEG3 functions as a ceRNA in esophageal cancer. Molecular Cancer Research, 15(7), 800–810. https://doi.org/10.1158/1541-7786.MCR-16-0385

Fang, C., Qi, S., Sun, F., Li, W., Wang, Z., Yue, B., … Yan, D. (2017). Long non-coding RNA HNFA1-AS1 mediated repression of miR-34a/SIRT1/p53 feedback loop promotes the metastatic progression of colon cancer by functioning as a competing endogenous RNA. Cancer Letters, 410, 50–62. https://doi.org/10.1016/j.canlet.2017.09.012

Fearon, E. R., & Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. Cell, 61(5), 759–767. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2188735. https://doi.org/10.1016/0092-8674(90)90186-1

Gerger, A., Zhang, W., Yang, D., Bohanes, P., Ning, Y., Winder, T., … Lenz, H.-J. (2011). Common cancer stem cell gene variants predict colon cancer recurrence. Clinical Cancer Research, 17(21), 6934–6943. https://doi.org/10.1158/1078-0432.CCR-11-1180

Giza, D. E., Vasilescu, C., & Calin, G. A. (2014). MicroRNAs and ceRNAs: Therapeutic implications of RNA networks. Expert Opin Biol Ther, 14(9), 1285–1293. https://doi.org/10.1517/1472598.2014.920812

Gu, X., Li, M., Jin, Y., Liu, D., & Wei, F. (2017). Identification and integrated analysis of differentially expressed lncRNAs and circRNAs reveal the potential ceRNA networks during PDLSC osteogenic differentiation. BMC Genetics, 18(1), 100. https://doi.org/10.1186/s12863-017-0569-4

Haggar, F. A., & Boussehy, R. P. (2009). Colorectal cancer epidemiology: Incidence, mortality, survival, and risk factors. Clinics in Colon and Rectal Surgery, 22(4), 191–197. https://doi.org/10.1055/s-0029-1242458

Karreth, F. A., & Pandolfi, P. P. (2013). ceRNA cross-talk in cancer: When ce-bling rivalries go awry. Cancer Discovery, 3(10), 1113–1121. https://doi.org/10.1158/2159-8290.CD-13-0202

Li, R., Sun, R., Hicks, G. R., & Raikher, N. V. (2015). Arabidopsis ribosomal proteins control vacuole trafficking and developmental programs through the regulation of lipid metabolism. Proceedings of the National Academy of Sciences of the United States of America, 112(1), E89–E98. https://doi.org/10.1073/pnas.1422656112

Liu, T. E., Chi, H., Chen, J., Chen, C., Huang, Y., Xi, H., … Si, Y. (2017). Curcumin suppresses proliferation and in vitro invasion of human prostate cancer stem cells by ceRNA effect of miR-145 and lncRNA-ROR. Gene, 631, 29–38. https://doi.org/10.1016/j.gene.2017.08.008

Livingstone, C. (2013). IGF2 and cancer. Endocrine-Related Cancer, 20(6), R321–R339. https://doi.org/10.1530/ERC-13-0231

Ouyang, S., Zheng, X., Zhou, X., Chen, Z., Yang, X., & Xie, M. (2017). LncRNA BCAR4 promotes colon cancer progression via activating Wnt/beta-catenin signaling. Oncotarget, 8(54), 92815–92826. https://doi.org/10.18632/oncotarget.21590

Peng, L., Yuan, X., Jiang, B., Tang, Z., & Li, G. C. (2016). LncRNAs: Key players and novel insights into cervical cancer. Tumour Biology, 37(3), 2779–2788. https://doi.org/10.1007/s13277-015-4663-9

Schagdarsurengin, U., Lammert, A., Schunk, N., Sheridan, D., Gattenlohner, S., Steger, K., … Dansranjavin, T. (2017). Impairment of IGF2 gene expression in prostate cancer is triggered by epigenetic dysregulation of IGF2-DMR0 and its interaction with KLF4. Cell Communication and Signaling, 15(1), 40. https://doi.org/10.1186/s12943-017-0197-7

Shi, D., Zheng, H., Zhuo, C., Peng, J., Li, D., Xu, Y. E., … Cai, S. (2014). Low expression of novel lncRNA RP11-462C24.1 suggests a biomarker of poor prognosis in colorectal cancer. Medical Oncology, 31(7), 31. https://doi.org/10.1007/s12323-014-0031-7

Tay, Y., Rinn, J., & Pandolfi, P. P. (2014). The multilayered complexity of ceRNA crosstalk and competition. Nature, 503(7483), 344–352. https://doi.org/10.1038/nature12986

Tominaga, K., Shimamura, T., Kimura, N., Murayama, T., Matsubara, D., Kanauchi, H., … Gotoh, N. (2017). Addiction to the IGF2-1D1-IGF2 circuit for maintenance of the breast cancer stem-like cells. Oncogene, 36(9), 1276–1286. https://doi.org/10.1038/onc.2016.293

Wang, W. T., Ye, H., Wei, P. P., Han, B. W., He, B., Chen, Z. H., & Chen, Y. Q. (2016). LncRNAs H19 and HULC, activated by oxidative stress, promote cell migration and invasion in cholangiocarcinoma through a ceRNA manner. Journal of Hematology & Oncology, 9(1), 117. https://doi.org/10.1186/s13045-016-0348-0

Wang, Z., Zou, Q., Song, M., & Chen, J. (2017). NEAT1 promotes cell proliferation and invasion in hepatocellular carcinoma by negative regulating miR-613 expression. Biomedicine & Pharmacotherapy, 94, 612–618. https://doi.org/10.1016/j.biopha.2017.07.111

Wu, Q., Meng, W. Y., Jie, Y., & Zhao, H. (2018). LncRNA MALAT1 induces colon cancer development by regulating miR-129-5p/ HMGB1 axis. Journal of Cellular Physiology, 233(9), 6750–6757. https://doi.org/10.1002/jcp.26383

Xu, W. W., Li, B., Guan, X. Y., Chung, S. K., Wang, Y., Yip, Y. L., … Cheung, A. L. M. (2017). Cancer cell-secreted IGF2 instigates fibroblasts and bone marrow-derived vascular progenitor cells to promote cancer progression. Nature Communications, 8, 14399. https://doi.org/10.1038/ncomms14399

Yu, S., Zhao, Y., Lai, F., Chu, M., Hao, Y., Peng, Y., … Min, L. (2017). LncRNA as ceRNAs may be involved in lactation process. Oncotarget, 8(58), 98014–98028. https://doi.org/10.18632/oncotarget.20439

Yu, Y., Nangia-Makker, P., Farhana, L., & Majumdar, A. P. N. (2017). A novel mechanism of lncRNA and miRNA interaction: CCAT2 regulates miR-145 expression by suppressing its maturation process in colon cancer cells. Molecular Cancer, 16(1), 155. https://doi.org/10.1186/s12943-017-0725-5

Zeng, M., Zhu, L., Li, L., & Kang, C. (2017). miR-378 suppresses the proliferation, migration and invasion of colon cancer cells by inhibiting SDAD1. Cellular & Molecular Biology Letters, 22, 12. https://doi.org/10.1186/s11658-017-0041-5
Zhai, X., Xue, Q., Liu, Q., Guo, Y., & Chen, Z. (2017). Colon cancer recurrence-associated genes revealed by WGCNA coexpression network analysis. *Molecular Medicine Reports, 16*(5), 6499–6505. https://doi.org/10.3892/mmr.2017.7412

Zhang, Y., Xu, Y., Feng, L. I., Li, F., Sun, Z., Wu, T., … Li, X. (2016). Comprehensive characterization of lncRNA-mRNA related ceRNA network across 12 major cancers. *Oncotarget, 7*(39), 64148–64167. https://doi.org/10.18632/oncotarget.11637

Zheng, X., Zhang, Y., Liu, Y., Fang, L., Li, L., Sun, J., … Huang, P. (2018). HIF-2alpha activated lncRNA NEAT1 promotes hepatocellular carcinoma cell invasion and metastasis by affecting the epithelial-mesenchymal transition. *Journal of Cellular Biochemistry, 119*(4), 3247–3256. https://doi.org/10.1002/jcb.26481

Zhou, M., Diao, Z., Yue, X., Chen, Y., Zhao, H., Cheng, L., & Sun, J. (2016). Construction and analysis of dysregulated lncRNA-associated ceRNA network identified novel lncRNA biomarkers for early diagnosis of human pancreatic cancer. *Oncotarget, 7*(35), 56383–56394. https://doi.org/10.18632/oncotarget.10891

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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