Role of Long Noncoding RNA Regulator of Reprogramming in Colon Cancer Progression via Epidermal Growth Factor Receptor Signaling

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Abstract
Background: Long intergenic noncoding RNA regulator of reprogramming (linc-ROR) is a novel long noncoding RNA that exhibits significant effects on cancer progression. This research presented that linc-ROR had a crucial part in promoting biological characteristics associated with worse prognosis in colon cancer. Method: Bioinformatics analysis was performed to predict signaling pathways related to linc-ROR. In addition, western blot, quantitative reverse transcription-polymerase chain reaction, RNA-pulldown, cell proliferation assays, colony formation assays, wound healing assays, and transwell assays were applied to detect the role and regulation of particular molecules. Results: Our results showed that the knockdown of linc-ROR reduced cell invasion, proliferative ability, and migration in colon cancer. Further evaluation verified that downregulating linc-ROR inhibited the activation of epidermal growth factor receptor (EGFR) signaling. In addition, cbl-b, a kind of E3 ubiquitin ligase that increases the degradation of EGFR, was found to be a potential linc-ROR target. Conclusions: Based on our findings, it was presented that linc-ROR served a role as a tumor-promoting factor via repressing the ubiquitination and degradation of EGFR signaling, which indicated that it could be a possible prognostic marker and therapeutic target for colon cancer.

Keywords
linc-ROR, EGFR, cbl-b, tumor progression, colon cancer

Abbreviations
AKT, protein kinase B; CCK-8, Cell counting kit-8; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinase; FBS, Fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; KEGG, Kyoto Encyclopedia of Genes and Genomes; linc-ROR, long intergenic noncoding RNA regulator of reprogramming; IncRNA, long noncoding RNA; mRNA, messenger RNA; OS, overall survival; p-AKT, phospho-protein kinase B; p-EGFR, phospho-epidermal growth factor receptor; qRT-PCR, quantitative reverse-transcription-polymerase chain reaction; SDS, sodium dodecyl sulfate; shRNA, short hairpin RNA; siRNA, small interfering RNA

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Introduction

As a malignant disease with high prevalence around the world, colorectal cancer (CRC) is responsible for one-tenth of all cancer-related deaths annually. Although new therapeutic approaches have been developed, the prognosis in colon cancer patients remains unfavorable due to rapid development and metachronous metastasis. Many CRC-related deaths could be avoided if diagnosed early. Therefore, the identification of disease-specific biomarkers is essential for early detection. However, only a few biomarkers are currently used clinically for CRC screening and treatment, such as mutated KRAS and BRAF. Finding potential biomarkers and revealing the associated mechanisms is crucial for developing a CRC cure.

Long noncoding RNAs (lncRNAs) were demonstrated as new and effective markers for early diagnosis and the prognosis of colon cancer. LncRNAs, containing over 200 nucleotides, belong to a group of nonprotein-coding RNAs. P metastasis, invasion, and proliferation are the main biological features that contribute to a poor prognosis for a patient with cancer. An increasing number of trials have indicated that lncRNAs influence these features of cancer cells by mediating gene and protein expression in various malignant cancers, including CRC. Reports have shown that a high bladder cancer-associated transcript 1 (BLACAT1) expression level signifies the tumor with larger size, greater depth, enhanced metastasis in lymph nodes, and advanced TNM stages of CRC. Meanwhile, upregulation of lncRNA Fetal-lethal non-coding developmental regulatory RNA (FENDRR) in patients with CRC have been shown as related to a poor survival prognosis. Unquestionably, lncRNAs are functionally crucial in colon cancer biology and prognoses.

Long intergenic noncoding RNA regulator of reprogramming (linc-ROR) is a lncRNA with a length of 2.6 kbp. It has been sequentially reported to be a prooncogenic factor in certain malignancies, including CRC, lung cancer, and hepatocellular carcinoma. The previous study by our research team presented that linc-ROR served a role as competing endogenous RNA in mediating the hepatocellular carcinoma cell invasion and metastasis via targeting miR-145. Nonetheless, the effects of linc-ROR on CRC development remain to be revealed. The aim of this research was to illustrate the molecular mechanisms related to linc-ROR in CRC progression.

Materials and Methods

Bioinformatics Analysis

Data related to colon cancer were obtained from the TCGA database portal (https://tcga-data.nci.nih.gov/tcga/). The Kaplan–Meier approach was applied to plot the overall survival (OS) curves. The pathway enrichment analysis was performed by using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The correlation network was retrieved from the molecular characteristic database (MSigDB) and analyzed by Pearson correlation analysis.

Clinical Specimens and Cell Lines

Five colon polyps and tumor tissues were obtained from patients after informed consent was provided and approval was granted from the Nantong City No 1 People’s Hospital and Second Affiliated Hospital of Nantong University. Human colon cell lines (HCT116 and SW480) and normal colon cell line (FHC) were obtained from the Typical Culture Preservation Commission Cell Bank, Chinese Academy of Science. Fetal bovine serum (FBS) and RPMI1640 culture medium, penicillin, streptomycin, and trypsin were bought from Gibco. RPMI1640 added with antibiotics (100 U/mL streptomycin and 100 U/mL penicillin) was used for all the experiments.

Figure 1. The clinical importance of linc-ROR in CRC. (A) The TCGA survival data present that the patients with upregulated linc-ROR showed poor overall survival. (B) The qRT-PCR measured linc-ROR expression in colon polyp tissues and CRC tissues. C) The qRT-PCR showed linc-ROR expression in normal colon cell and CRC cell lines. *p<.05, **p<.01, ***p<.001.

Abbreviations: linc-ROR, long intergenic noncoding RNA regulator of reprogramming; CRC, colorectal cancer; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.
penicillin) and FBS (10%) were used for cell culture. Cells were kept in a 37 °C incubator with 5% CO₂ and humidified atmosphere. The cell passage number was less than P10 when the cells were applied in the whole experiment.

**RNA Extraction, Reverse Transcription, and Quantitative Reverse Transcription-Polymerase Chain Reaction**

TRIzol reagent was utilized to extract RNA which was subsequently treated by RQ1 RNase-Free DNase (Promega) for 30 min. Transcriptor First Strand cDNA Synthesis Kit (Roche) was employed in cDNA synthesis following the instructions of the manufacturer. SYBR qPCR Master Mix (Applied Biosystems™) was utilized to perform quantitative polymerase chain reaction. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was set as the internal control. ABI® 7500 Fast Real-Time PCR system (Applied Biosystems) was used to conduct the reactions in duplicate. The conditions for the reaction were set as follows: 95 °C for 30 s, 95 °C for 5 s (40 cycles), 60 °C

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**Figure 2.** Knockdown of linc-ROR negatively regulated CRC cell proliferation, migration, and invasion. (A) qRT-PCR analysis detected the expression of linc-ROR in CRC cell knockdown of linc-ROR. (B) A CCK-8 assay evaluated the influence of linc-ROR knockdown on cell viability. (C) A colony formation assay was carried out to assess the influence of linc-ROR knockdown on the proliferation ability of cells. (D) A transwell migration assay was used to assess the influence of linc-ROR knockdown on the migration ability of cells. (E) The influence of linc-ROR knockdown on the invasion ability of cells according to a transwell assay. (F) Wound healing assay was applied to assess the influence of linc-ROR knockdown on cell invasion. (G) Western blot analysis detected the expression level of EMT markers in CRC cell knockdown of linc-ROR. *p < .05, **p < .01, ***p < .001.

Abbreviations: linc-ROR, long intergenic noncoding RNA regulator of reprogramming; CRC, colorectal cancer; CCK-8, cell counting kit-8; EMT, epithelial-mesenchymal transition.
for 30 s, 95 °C for 15 s, 60 °C for 60 s, and 95 °C for 15 s. The samples were analyzed by the melting curve. The relative content of linc-ROR was calculated by using the average value from each duplicate and the 2$^{-\Delta\Delta Ct}$ method was applied. The sequences of the primers are shown in Table S1.

Small Interfering RNA and Short Hairpin RNA Synthesis, and Transfection

The sequences of short hairpin RNA (shRNA) and small interfering RNA (siRNA) for linc-ROR are shown in Table S1. GenePharma synthesized the specific shRNA and siRNA for linc-ROR. A Lipofectamine 2000 kit (Invitrogen) was utilized for cell transfection following the instructions from the manufacturer.

Western Blotting

RIPA buffer was used for cell lysis. The BCA Protein Assay Kit (Beyotime) was applied in the quantification of total protein. Sodium dodecyl sulfate (SDS)-polyacrylamide gels were used for electrophoresis of denatured protein (30 μg) which was then transferred onto the polyvinylidene fluoride membranes (Millipore). The incubation of primary antibodies with the blots was conducted in a 4 °C environment overnight. The blots were then incubated with secondary antibodies at room temperature for 1 h. SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific) was applied to detect immunocomplexes. Cell Signaling Technology (BST) provided the following primary antibodies: phospho-extracellular signal-regulated kinase 1/2 (p-ERK1/2), phospho-epidermal growth factor receptor (p-EGFR), phospho-protein kinase B (p-AKT), epidermal growth factor receptor (EGFR), extracellular signal-regulated kinase 1/2 (ERK1/2), protein kinase B (AKT), and phosphorylated signal transducer and activator of transcription 3 (p-STAT3). Abcam (Cambridge) provided GAPDH. Image J software was applied to analyze the gray value.

Cell Proliferation Assays

Phosphate-buffered saline or different drugs were used to treat each cell sample of colon cancer for 72 h. Then cell samples at 1 × 10^4 cells/well were inoculated in the 96-well plate added with cell counting kit-8 (CCK-8; 10 μL) (Dojindo Molecular Technologies). The absorbance was set at 450 nm to measure the cell viability.

Colony Formation Assay

Cell samples (800 cells/well) were inoculated in 6-well plates for 14 days in a 37 °C incubator with 5% CO2. A crystal violet solution was added to the cells, and colonies with more than 50 cells were counted.

Wound Healing Assays

Cell samples (1.25 × 10^5 cells/well) were inoculated into the 24-well plate and starved for 24 h. The medium containing 10% FBS was then replaced. The plastic tip was used to create the wounds on the monolayered cells. The wound was photographed at 0 and 48 h under the microscope linked to a digital camera.

Transwell Assay and Transwell Migration Assay

Different samples of 1 × 10^5 cells were inoculated into the upper chamber of a Transwell insert (Corning Incorporated) or transwell insert covered with a Matrigel membrane (BD Biosciences). The chamber below was added with complete culture medium. After 24 h, crystal violet solution was used.
Figure 4. Inhibition of activation of EGFR signaling negatively mediated CRC cell proliferation, migration, and invasion. (A) Western blot analysis was employed to evaluate the influence of Gefitinib on the phosphorylation and baseline protein level of EGFR and the downstream target. (B) A CCK-8 assay evaluated the influence of gefitinib on the viability of cells. (C) A colony formation assay was performed to assess the influence of gefitinib on cell proliferation. (D) A transwell migration assay was used to evaluate the effect of gefitinib on cell migration. (E) The effect of gefitinib on cell invasion according to a transwell assay. (F) A wound-healing assay was used to assess the influence of Gefitinib on cell invasion. *p<.05, **p<.01, ***p<.001.

Abbreviations: EGFR, epidermal growth factor receptor; CRC, colorectal cancer; CCK-8, cell counting kit-8.
to visualize the invaded cells and the cells were counted under microscopy (magnification ×200).

**RNA Pull-Down Assay**

Biotin-RNAs were mixed with streptavidin beads (Thermo Fisher Scientific), and the lysates of cells were added to each binding reaction. The mixture for RNA-protein binding was washed briefly and boiled with SDS buffer. Finally, the retrieved protein was identified with western blot analysis. NICE designed and synthesized the biotinylated linc-ROR probe.

**Statistics Analysis**

GraphPad Prism 5V5.01 software was applied for statistical analysis. Data were shown as the average value ± standard deviation (x ± s). Student’s t-test was carried out to make comparison between two groups or multiple groups, respectively. P-value of <0.05 was regarded as statistically significant.

### Results

**Dysregulation of Linc-ROR was Related to a CRC Poor Prognosis**

Kaplan–Meier survival curves presented that upregulated linc-ROR was related to a poor CRC prognosis (Figure 1A). It was also observed that linc-ROR had increased expression in CRC tissues in comparison with colon polyps via quantitative reverse-transcription-polymerase chain reaction (qRT-PCR; Figure 1B). Meanwhile, linc-ROR manifested higher expression in CRC cell lines relative to normal ones (Figure 1C). Their findings suggested that linc-ROR had a functional part in CRC.

**Linc-ROR Regulates Proliferative Ability and the Invasion Capabilities of CRC Cells**

To assess the influence of linc-ROR on CRC cells, HCT116 and SW480 cells were transfected with si-linc-ROR#1, si-linc-ROR#2, si-linc-ROR#3, or scramble siRNA. The relative linc-ROR expression in linc-ROR-siRNA-transfected cells was demonstrated to be remarkably decreased in comparison with the negative control group, as...
revealed by qRT-PCR (Figure 2A). Moreover, CCK-8 assay and colony formation assay were applied to determine if linc-ROR was related to cell proliferation (Figure 2B and C and Supplemental Figure 1A). The results demonstrated that linc-ROR knockdown attenuated the growth of CRC cell lines. Then, with a wound transwell assay and healing assays, it was discovered that linc-ROR knockdown the invasion and migration abilities of CRC cells in comparison with the control (Figure 2D-F and Supplemental Figure 1B-D). Western blotting results suggested that linc-ROR knockdown remarkably elevated the expression level of epithelial markers (E-cadherin) while reduced that of the mesenchymal markers (N-cadherin and Vimentin) in CRC cells lines (Figure 2G). It was shown that epithelial-mesenchymal transition (EMT) could be induced by linc-ROR, thus promoting invasion and migration in CRC.

Linc-ROR is Related to Activation of EGFR Signaling

The KEGG database was searched to reveal the effect of linc-ROR on CRC cells. ErbB signaling was one of the most enriched pathways (Figure 3A). The EGFR is an important member of the ErbB family. To evaluate whether linc-ROR interacted with EGFR, western blotting was applied to measure the expression level of baseline and phosphorylated protein of EGFR and its downstream target, including ERK and AKT. After the knockdown of linc-ROR, EGFR, p-EGFR, p-ERK, p-AKT, and p-SAT3 protein levels were significantly decreased at 24 h compared to the control group (Figure 3B). Collectively, these data provided evidence suggesting that linc-ROR partly regulated the activation process of the EGFR signaling pathway in CRC cells.

Biological Role of the EGFR Signaling Pathway in CRC

As a common inhibitor of EGFR signaling, gefitinib was used to suppress the activation of EGFR in CRC cells. Western blotting was conducted to demonstrate that gefitinib reduced the expression levels of phosphorylated and baseline proteins of EGFR and its downstream target (Figure 4A). Furthermore, the inhibition of EGFR contributed to a marked reduction in the proliferation ability in comparison with control cells, as indicated by colony formation and CCK-8 assays (Figure 4B and C and Supplemental Figure 2A). Meanwhile, transwell and wound healing assays indicated that the inhibition of EGFR repressed the migration and invasion capabilities of CRC cell in comparison with the control group (Figure 4D-F and Supplemental Figure 2B-D).

Figure 6. Cbl-b was a potential target of linc-ROR. (A) The correlation network between linc-ROR and the EGFR pathway showed potential target genes. (B) The interaction between cbl-b and linc-ROR was evaluated by RNA-pulldown assays. (C) Western blot analysis and (D) qRT-PCR analysis measured the protein and mRNA level of cbl-b in sh-linc-ROR cells. **p < .01, ***p < .001. 
Abbreviations: linc-ROR, long intergenic noncoding RNA regulator of reprogramming; EGFR, epidermal growth factor receptor; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; mRNA, messenger RNA.
Linc-ROR Regulates CRC Cells via the EGFR Signaling Pathway

NSC228155 was used to activate EGFR signaling in CRC to further clarify the association between linc-ROR and EGFR signaling. Through western blotting, the expression of EGFR, p-EGFR, p-ERK, p-AKT, and p-SAT3 were demonstrated to be gradually increased after CRC cell treatment with NSC228155 (Figure 5A). Then, we found that NSC228155 also promoted cell growth after inhibition of linc-ROR expression (Figure 5B and C and Supplemental Figure 3A). It was also observed that the invasion and migration capabilities were increased by NSC228155 in CRC cells without linc-ROR (Figure 5D-F and Supplemental Figure 3B-D). The results indicated that activation of EGFR signaling could ameliorating the influences of downregulating linc-ROR on CRC cell proliferation, migration, and invasion.

Linc-ROR Reduces the Expression of Cbl-b to Activate CRC Cells via the EGFR Signaling Pathway

To reveal the regulatory mechanism between linc-ROR and EGFR pathway, transcriptome data of 521 CRC patients were uploaded from the TCGA and C2 gene set was downloaded from MSigDB, and then analyzed by Pearson correlation analysis (Figure 6A). Previous studies demonstrated that cbl-b facilitates EGFR ubiquitination and degradation. RNA-pulldown assays showed that linc-ROR was not binding to cbl-b directly (Figure 6B). However, our results demonstrated that the expression
level of cbl-b messenger RNA (mRNA) and protein was remarkably increased after downregulating linc-ROR (Figure 6C and D). Based on the result of western blotting, the expression of EGFR and p-EGFR was found to be gradually increased after downregulating cbl-b in CRC cell lines (Figure 7A). Proliferation, migration and invasion abilities of CRC cell lines were also increased while the expression of cbl-b was decreased (Figure 7B-F and Supplemental Figure 4A-D). Meanwhile, inhibition of cbl-b upregulated the expression of mesenchymal markers (Figure 7G). The findings above suggested that upregulated linc-ROR could downregulated cbl-b, ensuring the EGFR stability, then promoting proliferation, migration and invasion of CRC cell.

Discussion

Recently, lncRNAs have received increasing attention in CRC. In our research, via using both bioinformatics and experimental analyses, we documented that upregulated linc-ROR was linked to an increased risk of poor survival in CRC. Additionally, we provided evidence that linc-ROR functioned as an important player in promoting the migration, invasion, and proliferation abilities in CRC cells. Moreover, linc-ROR regulated CRC progression, at least partly, by inhibiting EGFR signaling pathway activation.

Linc-ROR, first found in induced pluripotent stem cells, contains four exons and is expressed both in the cytoplasm and nucleus. Accumulating evidence has proved that linc-ROR exerted significant functions in several malignant tumors. For instance, linc-ROR acts as a tumor-promoting factor and exerts a positive effect on epithelial-to-mesenchymal transition, contributing to the metastasis and tumorigenesis of breast cancer. In hepatocellular carcinoma, linc-ROR serves as a molecular sponge for miR-145 to enhance cancer metastasis and radioresistance. It was found that upregulated linc-ROR was detected in colon cancer tissues and associated with poor OS, which was consistent with previously published reports. Furthermore, linc-ROR could induce the progression of CRC since knockdown of linc-ROR suppressed cell proliferation, migration, invasion and EMT. Hence it was demonstrated that linc-ROR had a similar role as an oncogene in CRC.

We carried out an enrichment analysis to reveal the specific mechanism related to linc-ROR in colon cancer. The data revealed that linc-ROR might interact with EGFR signaling. EGFR, a glycoprotein in the tyrosine kinase receptor family, is associated with the occurrence and development of many cancers, as well as invasion and metastasis of cancer. Anti-EGFR therapy is widely used in CRC patients. It might be helpful to achieve the optimal response to anti-EGFR therapy by exploring the role of linc-ROR and EGFR in CRC progression. In this study, activation of EGFR signaling was found to be inhibited by low expression of linc-ROR. Additionally, we used NSC228155 to activate EGFR signaling in the CRC cell line with a low level of linc-ROR expression to further investigate the regulatory association between linc-ROR and EGFR signaling. Our results revealed that activation of EGFR signaling could reverse the effects described in the linc-ROR knockdown experiments.

Subsequently, we uncovered the molecular mechanisms through which linc-ROR promoted the EGFR signaling activation. In our bioinformatics analysis of TCGA and MSigDB data, cbl-b was found to be significantly associated with linc-ROR. Cbl-b belongs to the cbl protein family, functioning as a ligase for E3 ubiquitin-protein, which has been reported to serve as a negative regulator of many receptor tyrosine kinases. Previous research has reported that the cbl-b/EGFR/ERK/AKT signaling pathway regulates metastasis in gastric cancer cells. Another study showed that lncRNA DUXAP9-206 could directly bind to cbl-b to mediate EGFR signaling. Recently, there was no research investigating whether cbl-b is regulated by linc-ROR. Our research demonstrated that linc-ROR modulated the expression level of cbl-b rather than binding to it directly. After decreasing the expression of cbl-b, phosphorylated EGFR levels increased. Evidence in this study also demonstrated that inhibition of cbl-b promoted the malignant phenotype of colon cancer cells, including proliferation, migration, invasion, and EMT. Consistent with these findings, linc-ROR might decrease the expression of cbl-b to inhibit the ubiquitination mediated by cbl-b and EGFR degradation.

All the findings suggest that the linc-ROR regulates the EGFR signaling activation via cbl-b, finally promoting the cell growth, migration, and metastasis of CRC (Figure 7).

However, several limitations exist in our research. Specific molecular mechanisms of linc-ROR in the regulation of cbl-b remained to be unclear. Meanwhile, our research demonstrated...
that linc-ROR has an effect on the activation EGFR pathway, so the role of linc-ROR in affecting anti-EGFR therapy in colon cancer treatment needs to be further understood (Figure 8).

Conclusions

Our results indicated that upregulated linc-ROR was related to a CRC cell aggressive phenotype. More specifically, linc-ROR exerts its oncogenic activity by activating EGFR signaling via the cbl-b downregulation. Thus, our findings revealed that linc-ROR was a promising biomarker and therapeutic target in CRC. However, specific molecular mechanisms associated with the role of linc-ROR in CRC still require further in-depth study.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

The present study was authorized by the Research Ethics Review Board of The Nantong City No. 1 People’s Hospital and Second Affiliated Hospital of Nantong University (No. 2021KYG013).

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Supplemental Material

Supplemental material for this article is available online.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. https://doi.org/10.3322/caac.21492
2. De Rosa M, Pace UGO, Rega D, et al. Genetics, diagnosis and management of colorectal cancer (Review). Oncol Rep. 2015;34(3):1087-1096. https://doi.org/10.3892/or.2015.4108
3. Taib J, Le Malicot K, Shi Q, et al. Prognostic value of BRAF and KRAS mutations in MSI and MSS stage III colon cancer. J Natl Cancer Inst. 2016;109(5). https://doi.org/10.1093/jnci/djw272.
4. Deng H, Wang JM, Li M, et al. Long non-coding RNAs: new biomarkers for prognosis and diagnosis of colon cancer. Tumor Biol. 2017;39(6):1010428317706332. https://doi.org/10.1177/1010428317706332
5. Kopp F, Mendell JT. Functional classification and experimental dissection of long noncoding RNAs. Cell. 2018;172(3):393-407. https://doi.org/10.1016/j.cell.2018.01.011
6. Peng WX, Koirala P, Mo YY. LncRNA-mediated regulation of cell signaling in cancer. Oncogene. 2017;36(41):5661-5667. https://doi.org/10.1038/onc.2017.184
7. Esmaeili M, Keshani M, Vakilian M, et al. Role of non-coding RNAs as novel biomarkers for detection of colorectal cancer progression through interaction with the cell signaling pathways. Gene. 2020;753:144796. https://doi.org/10.1016/j.gene.2020.144796
8. Galamb O, Barták BK, Kalmár A, et al. Diagnostic and prognostic potential of tissue and circulating long non-coding RNAs in colorectal tumors. World J Gastroenterol. 2019;25(34):5026-5048. https://doi.org/10.3748/wjg.v25.i34.5026
9. Luo K, Geng J, Zhang Q, et al. LncRNA CASC9 interacts with CPSF3 to regulate TGF-β signaling in colorectal cancer. J Exp Clin Cancer Res. 2019;38(1):249. https://doi.org/10.1186/s13046-019-1263-3
10. Poursheikhani A, Abbasazdeegan MR, Kerachian MA. Mechanisms of long non-coding RNA function in colorectal cancer tumorigenesis. Asia Pac J Clin Oncol. 2020;17(1):7-23. https://doi.org/10.1111/jaco.13452
11. Su J, Zhang E, Han L, et al. Long noncoding RNA BLACAT1 indicates a poor prognosis of colorectal cancer and affects cell proliferation by epigenetically silencing of p15. Cell Death Dis. 2017;8(3):e2665-e2665. https://doi.org/10.1038/cddis.2017.83
12. Liu J, Du W. LncRNA FENDRR attenuates colon cancer progression by repression of SOX4 protein. Onco Targets Ther. 2019;12:4287-4295. https://doi.org/10.2147/ott.s195853
13. Chen W, Yang J, Fang H, Li L, Sun J. Relevance function of linc-ROR in the pathogenesis of colorectal cancer. Front Cell Dev Biol. 2020;8:696. https://doi.org/10.3389/fcell.2020.00696
14. Pan Y, Li C, Chen J, et al. The emerging roles of long noncoding RNA ROR (lincRNA-ROR) and its possible mechanisms in human cancers. Cell Physiol Biochem. 2016;40(1-2):219-229. https://doi.org/10.1159/000452539
15. Chen Y, Shen Z, Zhi Y, et al. Long non-coding RNA ROR (lincRNA-ROR) and its possible mechanisms in human cancers. Cell Physiol Biochem. 2016;40(1-2):219-229. https://doi.org/10.1159/000452539
16. Hardy KM, Booth BW, Hendrix MJ, Salomon DS, Strizzi L. ErbB/EGFR signaling and EMT in mammary development and breast cancer. J Mammary Gland Biol Neoplasia. 2010;15(2):191-199. https://doi.org/10.1007/s10911-010-9172-2
17. Singh D, Kumar Attri B, Kaur Gill R, Barivwal J. Review on EGFR inhibitors: critical updates. Mini-Rev Med Chem. 2016;16(14):1134-1166. https://doi.org/10.2174/1389557516666160321114917
18. Deng M, Liu B, Song H, et al. β-Element inhibits the metastasis of multidrug-resistant gastric cancer cells through miR-1323/cbl-b/ EGFR pathway. Phytomedicine. 2020;69:153184. https://doi.org/10.1016/j.phymed.2020.153184
19. Tang X, Qiao X, Chen C, Liu Y, Zhu J, Liu J. Regulation mechanism of long noncoding RNAs in colon cancer development and progression. Oncol Rep. 2019;60(4):319. https://doi.org/10.3349/ymj.2019.60.4.319
20. Wang Y, Xu Z, Jiang J, et al. Endogenous miRNA sponge lincRNA-RoR regulates Oct4, Nanog, and Sox2 in human embryonic stem cell self-renewal. *Dev Cell.* 2013;25(1):69-80. https://doi.org/10.1016/j.devcel.2013.03.002
21. Hou P, Zhao Y, Li Z, et al. LincRNA-ROR induces epithelial-to-mesenchymal transition and contributes to breast cancer tumorigenesis and metastasis. *Cell Death Dis.* 2014;5(6):e1287-e1e87. https://doi.org/10.1038/cddis.2014.249
22. da Cunha Santos G, Shepherd FA, Tsao MS. EGFR Mutations and lung cancer. *Annu Rev Pathol: Mech Dis.* 2011;6(1):49-69. https://doi.org/10.1146/annurev-pathol-011110-130206
23. Sigismund S, Avanzato D, Lanzetti L. Emerging functions of the EGFR in cancer. *Mol Oncol.* 2017;12(1):3-20. https://doi.org/10.1002/1878-0261.12155
24. Xu L, Zhang Y, Qu X, et al. E3 ubiquitin ligase cbl-b prevents tumor metastasis by maintaining the epithelial phenotype in multiple drug-resistant gastric and breast cancer cells. *Neoplasia.* 2017;19(4):374-382. https://doi.org/10.1016/j.neo.2017.01.011
25. Zhu T, An S, Choy M-T, et al. LncRNA DUXAP9-206 directly binds with Cbl-b to augment EGFR signaling and promotes non-small cell lung cancer progression. *J Cell Mol Med.* 2019;23(3):1852-1864. https://doi.org/10.1111/jcmm.14085