MIR600HG suppresses metastasis and enhances oxaliplatin chemosensitivity by targeting ALDH1A3 in colorectal cancer

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Running Head: MIR600HG suppress chemoresistance and metastasis in colorectal cancer

Abstract

BACKGROUND: Metastasis and chemoresistance indicate a poor prognosis in colorectal cancer (CRC) patients. However, the mechanisms that lead to the development of chemoresistance and metastasis in CRC remain unclear.

MATERIAL AND METHODS: We combined clinical and experimental studies to determine the role of MIR600HG in CRC metastasis and chemoresistance. The statistical analysis was performed using GraphPad Prism software, version 8.0.

RESULTS: We detected downregulated expression of lncRNA MIR600HG in CRC specimens and cell lines compared to normal controls, and the expression level of MIR600HG was inversely correlated with the overall survival of CRC patients. The inhibition of MIR600HG stimulated CRC cell metastasis and chemoresistance. In addition, our data showed that the inhibition of MIR600HG stimulated CRC stemness, while the overexpression of MIR600HG suppressed stemness. Importantly, our
animal experiments showed that MIR600HG inhibited tumour formation and that the combination of MIR600HG inhibition and oxaliplatin treatment significantly inhibited tumour growth compared to that with either intervention alone. Furthermore, we demonstrated that MIR600HG exerts its anticancer role by targeting ALDH1A3 in CRC.

**CONCLUSIONS:** Our data suggest that MIR600HG functions as a tumour suppressor and that the overexpression of MIR600HG inhibits tumour invasion and enhances chemosensitivity, providing a new strategy for CRC treatment.

**Keywords:** MIR600HG; ALDH1A3; Chemosensitivity; Metastasis; Colorectal cancer

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**Introduction**

Colorectal cancer is one of the most common tumours. Approximately 1.2 million patients worldwide are diagnosed with colorectal cancer each year, and more than 600,000 patients die directly or indirectly from colorectal cancer[1]. However, neoadjuvant chemoradiotherapy and surgery greatly improve patient survival time. Early tumour metastasis and chemoresistance are still the main challenges in the treatment of CRC patients[2-4]. However, the mechanisms that lead to the development of chemoresistance and metastasis in CRC remain unclear.

Cancer stem cells are a small subset of cells within a tumour, and studies show that cancer stem cells are implicated in chemotherapy resistance and metastasis in cancer[5]. Cancer stem cells confer high resistance to chemotherapy drugs that are commonly used in the treatment of CRC, including oxaliplatin and 5-fu [6,7]. In addition, studies
show that cancer stem cells are able to regenerate all of the cell types in the tumour due to their stem cell-like behaviour, resulting in metastatic relapse \cite{7, 8}. Therefore, cancer stem cells are important therapeutic targets in cancer. However, the mechanism of CSC regulation in CRC remains unclear.

IncRNAs are non-coding RNA molecules of more than 200 nucleotides in length\cite{9}. They do not encode proteins, but they do regulate gene expression at the transcriptional, posttranscriptional and epigenetic levels and participate in tumour cell proliferation, apoptosis and invasion, metastasis\cite{10}. Additionally, dysregulated expression of IncRNA was demonstrated in cancer stem cells, and such aberrantly regulated IncRNAs are involved in the development of cancer stem cells and the maintenance of stemness\cite{11}. MIR600HG is an RNA gene and is affiliated with the miRNA class. Diseases associated with MIR600HG include pancreatic ductal adenocarcinoma. MIR600HG was then validated to be an independent prognostic predictor for patients with PDAC\cite{12}. Our previous data showed that the decreased expression of MIR600HG was significantly correlated with the overall survival of CRC patients; however, its function and mechanism in CRC remain unclear. Here, we describe a functional role of MIR600HG as a tumour suppressor lncRNA that regulates metastasis, chemoresistance and cancer stemness. Additionally, we identified ALDH1A3 as a target of MIR600HG in CRC.

Materials and methods

Cell culture and human specimens

All colorectal cancer cells were purchased from the American Type Culture Collection (Manassas, VA) and cultured in DMEM (Sigma-Aldrich, St Louis, MO) supplemented with 10% foetal bovine serum (HyClone, Logan, UT). Human specimens were obtained from diagnostic biopsies. We selected CRC patients from February 2018 to August 2018. A total of 60 patient specimens and 60 adjacent tissues were used in this study (Table 1), and informed consent was obtained from
each patient who participated in this study. This research was approved by the Research Ethics Board of the Eighth Medical Centre of PLA General Hospital.

**RNA extraction and qRT-PCR**

RNA was extracted from cells or tissues using TRIzol reagent (Invitrogen, Carlsbad, CA) and subjected to qRT-PCR. MIR600HG and RNU6 expression was tested by qRT-PCR using a primer set from RiboBio (Guangzhou, China) as a reference\(^{[13]}\). The primer sequence information is defined as follows: MIR600HG forward 5'-'TGAGCAGAGTCAAGTGGCAG-3', reverse 5'-'AAAGCCCCATTTCTAGCCC -3'; U6 forward 5'-'GCTTCG GCAGCATAATACTAAAAT-3'; U6 reverse 5'-'CGCTTCACGAA TTTGCGTGTCAT-3'. The primer sequences for other genes were defined as follows: ALDH1A3 forward 5'-'TGAGTGATTAGCAGGCTGCA-3', reverse 5'-'TGGCCACATACACCAATAGGTTC-3'; and GAPDH forward 5'-'GCAGGGGGGAGCCAAAAGGGT-3', reverse 5'-'TGGGTGGCAGTGATGGCATGG-3'. The MIR600HG expression was normalized against RNU6 expression, and ALDH1A3 mRNA expression was normalized against GAPDH. The 2-ΔΔCT method was used to analyse the relative fold changes.

**Transwell and osteosphere assays**

The Transwell and osteosphere assay protocols were performed as described by Chen et al\(^{[14]}\) and Roscigno et al\(^{[15]}\). Briefly, 1 × 10^5 cells in serum-free growth medium were seeded in the upper wells of chambers (12-well plate). The lower wells contained the same medium with 10% serum. After 24 hours, the cells that had migrated to the lower side of the chamber were fixed with 2.5% glutaraldehyde, stained with 0.1% crystal violet and counted. For the osteosphere assay, 1000 cells were plated in 24-well ultra-low attachment plates in N2B27-defined serum-free medium and cultured for 9 days. Spheres were counted in each plate using a Leica MZ12 inverted microscope.

**Cell viability and apoptosis analysis**
After transfection, evaluation of cell proliferation using cell viability was determined using a cell counting kit-8 (CCK-8, MedChem Express, Monmouth Junction, New Jersey, USA). Apoptosis assays were performed using the FITC Annexin V Apoptosis Detection Kit I (BD Biosciences) according to the manufacturer’s protocol. Stained cells were analysed by a FACSCalibur flow cytometer (BD Biosciences) [9].

**Luciferase reporter assay**

Experiments were performed in triplicate. Luciferase activity was measured using the dual-luciferase assay system (Promega, Madison, WI). The 293T cells were co-transfected with the indicated reporter plasmids and either the pre-MIR600HG or control oligonucleotides (NC) after 48 hours of incubation [16]. The 3’-UTR segments of ALDH1A3 that were predicted to interact with MIR600HG were amplified by PCR from human genomic DNA and inserted into the Hind III and SacI sites of the lncRNA Expression Reporter Vector. The primer sequences for amplifying the 3’ UTR of ALDH1A3 are as follows: forward, 5’-AAAGATCTTTATTAAAGCTTTAATAAAAATGAGGGCCCCTAAGAACCAGTG-3’; and reverse, 5’-GCGCAGGAGGAGCTCTTTGTGGATGCGATCTGCAGCTAGGA-3’.

For the luciferase reporter experiments, the indicated cells were seeded into 24-well cell culture plates and transfected with the indicated reporter plasmids and either the MIR600HG or negative control oligonucleotides (NC). Following 48 hours of incubation, cells were subjected to a luciferase reporter assay.

**Library construction, RNA sequencing and data analysis**

First, rRNAs in samples from the control and PM2.5 groups were removed. Then, the libraries for next-generation sequencing were prepared using the TruSeq RNA Sample Prep Kit (Illumina, USA) according to the manufacturer’s instructions. After enrichment and purification, the libraries were processed for sequencing by Shanghai Origingene Bio-pharm Technology Co., Ltd. (Shanghai, China) according to available protocols. After quality control of the original data, the high-quality sequencing data were compared with the designated reference genome. The expression values were
calculated by the StringTie tool, and the tDESeq algorithm was applied to filter the differentially expressed genes.

**Western blot analysis**

Cells were lysed, and proteins were isolated. Protein (30 µg) was subjected to Western blotting with anti-ALDH1A3 (Abcam, USA; Cat. No.: ab129815; 1:800), anti-E-cadherin (Abcam, USA; 1:4000), anti-vimentin (Abcam, USA; 1:5000), anti-PARP (Abcam, USA; 1:4000), anti-SOX2 (Abcam, USA; 1:4000), anti-CD44 (Abcam, USA; 1:4000), and anti-β-actin (Invitrogen, USA; 1:10000) and subsequent incubation with secondary antibodies conjugated to horseradish peroxidase (HRP) for 2 hours at RT.

**Animal experiments**

Animal experiments were conducted using six-week-old female athymic (nu/nu) mice, and each group included eight mice. A total of 5x10^6 Caco2 cells (stably transfected with a series of lentiviruses were constructed in our laboratory) in 100 µl serum-free medium were injected subcutaneously (s.c.) into per mouse (right back). When the tumours reached ~50 mm^3, mice were intraperitoneally (i.p.) injected with oxaliplatin (6 mg/kg body weight). After 3 weeks of drug treatment, mice were sacrificed, and tumour weights were measured. Euthanasia was carried out by CO₂ in mice when the tumour size reached 1500 mm^3 (no anaesthetics used in these experiments). All animal experiments were performed in the Laboratory of PLA General Hospital and were approved by the Research Ethics Board of the Eighth Medical Center of PLA General Hospital (NO. 0034/18).

**Statistical analysis**

Statistical significance was analysed by unpaired Student’s t tests or one-way ANOVA and Duncan’s multiple range tests using the GraphPad Prism software package version 8.0. P values less than 0.05 were considered statistically significant.
Results

Downregulated expression of MIR600HG was associated with poor clinical outcome

Previous studies have shown that some lncRNAs are inversely correlated with CRC progression; MIR600HG expression was significantly decreased in CRC specimens compared to paracancerous specimens in the analysis of data from the TCGA (Fig. 1A) and our hospital (Fig. 1B). In addition, our clinical data showed that decreased expression of MIR600HG was significantly correlated with the overall survival of CRC patients (Fig. 1C). MIR600HG was downregulated in CRC cell lines compared to the HcoEpiC cell line (Fig. 1D). Taken together, these data suggest that MIR600HG may act as a tumour suppressor lincRNA in CRC.

MIR600HG inhibits metastasis and chemoresistance in CRC

Then, we investigated the effects of MIR600HG on CRC metastasis and chemoresistance using a CRC cell line transfected with MIR600HG or inhibitor (Fig. 2A). We investigated the effects of MIR600HG on the chemotherapy sensitivity of CRC cells. Cell viability and apoptosis analysis results showed that ectopic expression of MIR600HG attenuated CRC cell proliferation and stimulated apoptosis (Fig. 2B,D). The combination of MIR600HG and oxaliplatin more significantly accelerated proliferation and stimulated apoptosis compared to single treatment (Fig. 2B,D). In contrast, the inhibition of MIR600HG significantly attenuated oxaliplatin-induced CRC cell growth inhibition (Fig. 2C). Consistent with cell viability and apoptosis analysis, MIR600HG overexpression significantly enhanced oxaliplatin-induced expression of the pro-apoptotic protein cleaved PARP (Fig. 2E). In addition, Transwell experiments showed that the ectopic expression of MIR600HG significantly inhibited CRC cell metastasis, while the inhibition of MIR600HG stimulated CRC cell invasion (Fig. 2F). In addition, we examined the effects of MIR600HG on CRC cell EMT because EMT plays an important role in cancer metastasis. Our data show that MIR600HG positively regulates E-cadherin expression in CRC cells but negatively regulates vimentin expression (Fig. 2G), suggesting that
MIR600HG inhibits EMT in CRC cells. Together, our data suggest that MIR600HG suppresses CRC progression by inhibiting CRC cell invasion and inducing chemoresistance.

RNA transcriptome sequencing analysis of the MIR600HG downstream regulatory pathway

We constructed MIR600HG-overexpressing Caco2 cell lines for sequencing, with normal controls included. PCA shows that the two groups of samples are almost completely different (Fig. 3A), and the heat map also shows that there is a large difference between the groups (Fig. 3B). A volcano map (Fig. 3C) was generated for the two groups of samples (tools, R language limma package). The volcano map showed that compared with the low miR600HG expression group, the high miR600HG expression group had a large number of downregulated proteins. Enrichment analysis revealed the top 30 signalling pathways related to MIR600HG expression (Fig. 3D), which suggested that MIR600HG may affect the process of colon cancer by affecting the pluripotency of colon cancer cells.

ALDH1A3 is a target of MIR600HG

To investigate how MIR600HG regulates metastasis and chemoresistance in CRC, we searched for candidate target genes of MIR600HG (mirdb.org) and identified ALDH1A3 as a candidate of MIR600HG (Fig. 4A). Thus, we chose to further study ALDH1A3. To investigate whether MIR600HG is involved in ALDH1A3 regulation, Caco2 cells were transfected with MIR600HG or inhibitor. After 72 hours of transfection, ALDH1A3 expression was measured using qRT-PCR and Western blot. Our experimental results showed that ALDH1A3 expression was significantly upregulated or downregulated by the inhibition or ectopic expression of MIR600HG, respectively, in CRC cells at both the mRNA and protein levels (Fig. 4B and 4C). Furthermore, we verified that MIR600HG directly targeted the 3’ UTR of ALDH1A3 by using a luciferase reporter assay (Fig. 4D). Consistent with the in vitro results, the
clinical sample analysis results also showed an inverse association between ALDH1A3 and MIR600HG in CRC specimens (Fig. 4E). These findings indicated that MIR600HG inhibits ALDH1A3 mRNA and protein expression by directly targeting its 3’ UTR.

**MIR600HG inhibits cancer stemness via ALDH1A3 in CRC**

Previous studies have shown that stem cells cause metastasis and chemoresistance in cancers; ALDH1A3 plays a key role in cancer stem cell maintenance and is closely associated with cancer metastasis and chemoresistance \[^{17-19}\]. We investigated whether MIR600HG is involved in cancer stem cell regulation in CRC. As expected, the osteosphere assay showed that the inhibition of MIR600HG significantly increased osteosphere numbers, while the overexpression of MIR600HG reduced osteosphere numbers compared to those in the control (Fig. 5A). Consistent with sphere formation results, Western blot results also showed that the overexpression of MIR600HG significantly inhibited the expression of cancer stem cell marker proteins, including SOX2 and CD44 (Fig. 5B). However, increasing ALDH1A3 expression reversed the inhibition of cancer stem cell marker protein expression by MIR600HG (Fig. 5B). These data suggest that MIR600HG exerts its anticancer effects partially due to the inhibition of cancer stemness in CRC.

**ALDH1A3 is a functional target of MIR600HG that modulates CRC metastasis and chemoresistance**

Finally, we investigated whether ALDH1A3 is involved in the MIR600HG-mediated regulation of metastasis and chemoresistance. The cell viability analysis results showed that the overexpression of ALDH1A3 restored the cell proliferation inhibited by MIR600HG (Fig. 6A); in contrast, silencing of ALDH1A3 suppressed MIR600HG inhibition-induced cell growth (Fig. 6B). Consistent with these results, the apoptosis analysis showed that the overexpression of ALDH1A3 attenuated
MIR600HG-induced cell apoptosis (Fig. 6C). In addition, Transwell experiments showed that the overexpression of ALDH1A3 attenuated the MIR600HG-induced metastasis effect (Fig. 6D). Together, our data suggest that MIR600HG regulates metastasis and chemoresistance via ALDH1A3 in CRC.

**MIR600HG significantly inhibits tumorigenesis and chemoresistance in vivo**

As shown in the animal experiment, tumour volume and weight (Fig. 7A and B) was significantly decreased in the MIR600HG overexpression group compared to the control group. Additionally, we detected the cancer stem cell marker CD133 in tumour tissues, and the data showed that the groups treated with MIR600HG had a significantly reduced CD133 mRNA level compared to that in the groups that were not treated with MIR600HG (Fig. 7C). Consistent with these results, Ki-67 IHC assay (Fig. 7D) results clearly showed that combined oxaliplatin treatment and MIR600HG inhibition suppressed cell proliferation more potently and significantly than the single treatments. Taken together, these data suggest that MIR600HG is essential to blocking tumour formation and improving chemosensitivity (Fig. 7E).

**Discussion**

The occurrence of chemoresistance and metastasis indicates poor survival in CRC patients¹⁰. In this study, we provide insight into the biological effects of MIR600HG in CRC metastasis and chemoresistance by using a series of experiments. Here, we found that increased expression of MIR600HG significantly correlated with good clinical outcomes in CRC patients. This result is consistent with Song’s reports that IncRNA MIR600HG is associated with poor prognosis in patients with PDAC¹². In addition, our in vitro study showed that the overexpression of MIR600HG enhanced the chemosensitivity of CRC cells to anticancer drugs and inhibited CRC cell invasion. In contrast, the inhibition of MIR600HG promoted CRC cell invasion and decreased chemosensitivity. These findings showed that MIR600HG functions as a tumour suppressor and that targeting MIR600HG may be a novel strategy for suppressing CRC metastasis and enhancing chemosensitivity.

We further clarified the anti-CRC mechanism of MIR600HG. We used a series of
experiments to identify ALDH1A3 as a target gene of MIR600HG in CRC. Our data showed that ALDH1A3 expression was upregulated or downregulated in CRC cells by the inhibition or ectopic expression of MIR600HG, respectively. Previous studies have shown that ALDH1A3 is a CSC marker and plays an important role in CSC regulation\textsuperscript{[20, 21]}. Accumulated evidence has shown that increased cancer stemness can stimulate cancer metastasis and induce chemoresistance \textsuperscript{[22-24]}. MIR600HG may regulate CSCs by targeting ALDH1A3, so we investigated the CSC regulation mechanism of MIR600HG in CRC. Our data showed that the inhibition of MIR600HG stimulated CRC stemness. More importantly, our experiments showed that the overexpression of MIR600HG significantly suppressed cancer stemness and reduced CSC populations in tumour tissues, suggesting that MIR600HG plays an anticancer role partially by inhibiting CRC stemness. In addition, our data showed that the restoration of ALDH1A3 blocked the MIR600HG overexpression-induced inhibition of cancer stemness. In general, these data suggest that MIR600HG inhibits CRC metastasis and chemoresistance through the inhibition of cancer stemness by targeting ALDH1A3.

In summary, we combined clinical and experimental studies to establish a role for MIR600HG in CRC metastasis and chemoresistance. The overexpression of MIR600HG dramatically enhances the sensitivity of CRC cells to chemotherapy and inhibits CRC metastasis through suppressing CRC stemness by targeting ALDH1A3. Our findings may also help develop potential therapeutics for the treatment of CRC metastasis and chemoresistance.

**Abbreviations list**

CRC: colorectal cancer  
CSC: Cancer stem cells  
LncRNAs: long noncoding RNA  
Oxa: Oxaliplatin

**Acknowledgement**

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**Author contributions**
Nan Li conceived and designed this study. Yi Yao was responsible for doing the main experimental. Nan Li and Yi Yao were jointly involving in extracting data and writing the manuscript.

**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Declaration of Interest Statement**

The authors declare that they have no conflicts of interest

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**Figure legends**

**Fig. 1.** Decreased expression of MIR600HG correlates with poor survival of CRC patients. **A.** MIR600HG expression was significantly downregulated in CRC patient’s analysis from TCGA database. **B.** MIR22HG expression was significantly downregulated in CRC specimens. The expression levels of MIR600HG were measured using qRT-PCR in normal tissue (n=50) and tumor tissues (n=50). **C.** Survival rates for CRC patients with low (n=20) and high (n=20) MIR600HG expression. **D.** CRC cell lines showed lower expression level of MIR600HG compared to Human normal colon epithelial (HcoEpiC) cells. ***, p<0.01; ***, p<0.001.

**Fig. 2.** MIR600HG negatively regulates CRC cells metastasis and chemosensitivity. **A.** Caco2 cells were transfected with negative control oligonucleotide, MIR600HGmimics or MIR600HG inhibitor. After 72 hours of transfection, isolated mRNAs were subjected to qRT-PCR. **B.** MIR600HG enhanced the sensitivity of Caco2 cells to Oxaliplatin treatment. Caco2 cells were transfected with the MIR600HG and treated with or without 0.2 µM Oxaliplatin for 48 hours and then subjected to an CCK-8 assay. **C.** Knockdown MIR600HG expression inhibited the sensitivity of Caco2 cells to Oxaliplatin treatment. Caco2 cells were transfected with the siMIR600HG and treated with or without 0.2 µM Oxaliplatin for 48 hours and then subjected to an CCK-8 assay. **D.** The MIR600HG overexpression enhanced Oxaliplatin-induced apoptosis in Caco2 cells. MIR600HG overexpression Caco2 cells were treated with DMSO or 0.2 µM Oxaliplatin for 24 hours and analyzed with flow cytometry. **E.** MIR600HG stimulates Oxaliplatin-induced expression of cleaved- and total-PARP in CRC cells. Caco2 cells were transfected with the MIR600HG or treated with 0.2 µM Oxaliplatin for 48 hours, and then subjected to Western blotting. **F.** MIR600HG negatively regulates Caco2 cell metastasis. After 24 hours of transfection, cells were subjected to an invasion assay. **G.** MIR600HG inhibited EMT in CRC cells. Caco2 cells were transfected with negative control oligonucleotides, MIR600HG mimics or MIR600HG inhibitor. After 72 hours of transfection, cells were subjected
to Western blot analysis for the detection of the expression of the indicated proteins. NC, negative control oligonucleotides; mimics, MIR600HG mimics; inhibitor, MIR600HG inhibitor; Oxa, Oxaliplatin; *, p<0.05; **, p<0.01.

Fig. 3 Analysis of MIR600HG downstream regulatory pathway

We constructed MIR600HG over-expressing Caco2 cell lines for RNA sequencing with normal controls. A. PCA analysis can find that the two groups of samples are almost completely different. B. the heat map also shows the sample There is a large difference between them. C. the volcano map was performed on the two groups of samples (tools, R language limma package). The volcano map showed that compared with the miR600HG low expression group, the miR600HG high expression group had a large number of proteins with low expression. D. KEGG Enrichment analysis, taking the first 30 signal pathways as a map, suggesting that MIR600HG may affect the process of colon cancer by affecting the pluripotency of colon cancer cells.

Fig. 4. MIR600HG targets ALDH1A3.

A. The MIR600HG seed sequence is complementary to the 3’ UTR of ALDH1A3. B and C. MIR600HG inhibited ALDH1A3 mRNA and protein expression. After 72 hours of transfection of MIR600HG and siMIR600HG, using qRT-PCR and Western blot measure. D. Activity of the luciferase gene linked to the 3’ UTR of ALDH1A3. The luciferase reporter plasmids of wildtype (WT) or mutated 3’ UTR sequences of ALDH1A3 (MT) were transfected into HEK-293 cells with or without the MIR600HG. E. The expression levels of ALDH1A3 and MIR600HG showed a negative correlation in CRC patients. Tumor samples were obtained from 10 patients with CRC, and the expression of ALDH1A3 and MIR600HG were measured by RT-qPCR. NC, negative control inhibitor, MIR600HG inhibitor; ns, no significance; **, p<0.01.

Fig. 5. MIR600HG negatively regulates CRC stemness.
A. The inhibition of MIR600HG stimulated osteosphere formation in Caco2 cells, whereas the overexpression of MIR600HG inhibited Osteosphere formation. B. MIR600HG negatively regulated CSC marker protein expression. Caco2 cells were transfected with the MIR600HG and siMIR600HG. After 72 hours of transfection, cells were subjected to Western blot analysis. NC, negative control oligonucleotides; inhibitor, MIR600HG inhibitor; *, p<0.05; **, p<0.01.

Fig. 6. ALDH1A3 is a functional target of MIR600HG that modulates CRC metastasis and chemosensitivity of Oxaliplatin

A. The cell viability analysis results show that the overexpression of ALDH1A3 restored the cells viability inhibited by MIR600HG and decreased chemosensitivity of Oxaliplatin. B: Silencing of ALDH1A3 suppressed MIR600HG inhibition-induced cells viability and increased chemosensitivity of Oxaliplatin. C: The apoptosis analysis ALDH1A3 overexpression attenuated the MIR600HG induced cell apoptosis, decreased chemosensitivity of Oxaliplatin. D: transwell experiments showed that the overexpression of ALDH1A3 attenuated the MIR600HG-induced metastasis effect. NC, negative control oligonucleotides; inhibitor, MIR600HG inhibitor; ALDH1A3: ALDH1A3 plasmid; siALDH1A3, siRNA of ALDH1A3; *, p<0.05; **, p<0.01; ***, p<0.001.

Fig. 7. MIR600HG overexpression in combination with Oxaliplatin inhibits tumor relapse. A and B. Tumor volume and weight in xenografts treated with Oxaliplatin, MIR600HG or both at days 29, 32 and 36. Caco2 cells and injected s.c. into nude mice (n = 9/group, 1 × 10⁴ cells/mouse). C. The level of CD133 Mrna derived from xenograft model tumors analysis by RT-PCR. D. Ki-67 immunohistochemistry assay show that combination of MIR600HG inhibition and Oxaliplatin treatment more significantly inhibits cell proliferation in xenograft tumor. E. A schematic model of MIR600HG target ALDH1A3 inhibited CRC metastasis and chemosensitivity. NC, negative control oligonucleotides; inhibitor, MIR600HG inhibitor; Oxa, Oxaliplatin. *, p<0.05; **, p<0.01.
Figure 1
Figure 3
Figure 5
Figure 7
| Characteristics          | Variable                     | Number (%) | P value |
|--------------------------|------------------------------|------------|---------|
| Age (years)              | Range, (means±SD)            | 39-76(60±10) | 0.189   |
| Gender                   | Male                         | 26 (59.1)  | 0.952   |
|                          | Female                       | 18 (40.9)  |         |
| Family history           | No                           | 31 (70.5)  | 0.432   |
|                          | Yes                          | 13 (29.5)  |         |
| Clinic stage             | I                            | 5 (11.4)   | 0.445   |
|                          | II                           | 15 (34.1)  |         |
|                          | III                          | 14 (31.8)  |         |
|                          | IV                           | 10 (22.7)  |         |
| Pathological type        | Adenocarcinoma               | 30 (68.2)  | 0.637   |
|                          | Mucinous carcinoma           | 14 (31.8)  |         |