Histol Histopathol (2022) 37: 301-309

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE **Open Access**

MiR-194-3p modulates the progression of colorectal cancer by targeting KLK10

Tao Liu and Yuejun Fang

Department of Gastrointestinal Surgery, Zhejiang Jinhua Guangfu Tumor Hospital, Jinhua, China

Summary. Background. A rich history of studies have manifested the importance of miRNAs to cancer progression, while miR-194-3p has been seldom explored.

Objective. The purpose of this study is to unearth the way the miR-194-3p/KLK10 axis modulates colorectal cancer (CRC).

Methods. Differentially expressed genes of CRC in TCGA database were analyzed. Western blot and qRT-PCR were employed to test protein and mRNA expressions of two researched genes. Their targeting was confirmed using dual-luciferase. Biological behaviors of cells were tested by a series of cellular functional assays.

Result. Remarkably low miR-194-3p expression and high KLK10 expression were observed in cancer cells. Overexpressing miR-194-3p hindered the progression of CRC cells. Overexpression of miR-194-3p significantly weakened the promoting effect of upregulated KLK10 on cell migration, invasion and proliferation. Their targeting was verified by dual -luciferase assay. Therefore, miR-194-3p hindered cell behaviors of CRC through KLK10.

Conclusion. This investigation casts new light on the treatment of CRC through the miR-194-3p/KLK10 axis.

Key words: miR-194-3p, KLK10, Colorectal cancer, Cell functions

Introduction

Colorectal cancer (CRC) triggers a massive number of deaths every year. Despite the great progress in medical approaches, most CRC patients are still diagnosed in late stages and miss the optimal time for surgery. Therefore, CRC still features a relatively high rate of incidence and mortality (Connell et al., 2017; Thanikachalam and Khan, 2019). Moreover, recurrence, metastasis and drug resistance are also important reasons for the poor prognosis of CRC patients (Brenner and Chen, 2018). Hence, it is necessary to deeply comprehend the underlying mechanisms of CRC pathogenesis and metastasis, so as to develop new therapies and improve the survival rate of CRC patients.

Varying cellular biological processes were reported to be mediated by miRNAs (Zhang et al., 2007; Lee and Dutta, 2009). Therefore, identifying CRC-related miRNAs is of great significance for cancer therapy. Some research showed that miR-194, is pivotal in carcinogenesis, and was found to be down-regulated in liver cancer (Meng et al., 2010), multiple myeloma (Pichiorri et al., 2016), gastric cancer (Song et al., 2012), and CRC (Zhao et al., 2014). MiR-194 also conspicuously reduces colony formative ability and tumor growth as a suppressor gene in CRC (Chang et al., 2017). As a complementary sequence of mature miRNA, miRNA* is generally degraded after the formation of mature miRNA. MiR-194-3p, the mature body of miR-194, has been rarely studied in cancer. A study conducted by Yi and other experts revealed the functions of lncRNA PTPRG-AS1/PRC1/miR-194-3p in nasopharyngeal carcinoma (NPC), and it was found that the corresponding mechanism has clinical significance in the treatment of NPC (Yi et al., 2019). Bioinformatics analysis so far has proved down-regulated miR-194-3p in CRC (Chiang et al., 2012), yet whether the dysregulation of miR-194-3p is related to CRC progression needs further study.

KLK10 belongs to the human kallikrein related peptidase family (Borgono and Diamandis, 2004). Studies exhibited that the expression patterns of KLK10 in different tumors are different. For example, KLK10 is lowly expressed in prostate cancer (Sidiropoulos et al., 2005), breast cancer (Liu et al., 1996), and NSCLC (Zhang et al., 2010). On the contrary, KLK10 is up-regulated in oral squamous cell carcinoma and ovarian cancer as a tumor promoter (Zhang et al., 2010). Although studies showed that KLK10 is pertinent to CRC patient's dismal prognosis (Petraki et al., 2012; Talieri et al., 2011), the upstream regulatory mechanism

Corresponding Author: Yuejun Fang, Department of Gastrointestinal Surgery, Zhejiang Jinhua Guangfu Tumor Hospital, No. 1296 Huancheng North Road, Xinshi Street, WuCheng District, Jinhua, 321000, China. e-mail: fangyuejun393@163.com

DOI: 10.14670/HH-18-413

of KLK10 in in this cancer has rarely been investigated.

We decided to clarify the expression and targeting relationship of the two genes. The effect of their interaction on the development and progression of CRC was also investigated. We posited that miR-194-3p targeted KLK10 to mediate progression and performed the subsequent experiments to prove the speculation. The results may cast light on CRC therapy.

Materials and methods

Microarray analysis

First, miRNA (tumor: 521; normal: 46) and mRNA (tumor: 535; normal: 59) data were accessed from TCGA database. The "edgeR" package was utilized for examining the differential miRNA expressions (|logFC|>2, padj<0.05), and the survival significance of the differentially expressed miRNAs (DEmiRNAs) was examined by "survival" package. Overlap of miR-194-3p-targeted mRNAs (calculated by miRTarBase, TargetScan and miRDB) and differentially expressed mRNAs (DEmRNAs) was deemed as the target mRNA.

Cell culture

BeNa Culture Collection (BNCC) (Shanghai, China) was the provider of the cells used in this study. The cell lines and their mediums used in this study were as follows: normal cell line NCM-460 (BNCC339288) in McCoy's medium with 10% FBS, colon carcer cell lines HCT116 (BNCC337692) in RPMI-1640 medium containing 10% FBS, and SW1116 (BNCC341365) in high glucose-DMEM with 1% streptomycin/penicillin and 10% FBS. All of them were kept in an incubator under routine conditions.

Cell transfection

RiboBio (Guangzhou, China) offered all plasmids used in this investigation. The mimics, plasmids and vectors were respectively transfected or co-transfected with colon cancer cells HCT116 and SW1116 using Lipofectamine 2000 Kit (Invitrogen, Carlsbad, CA, USA). Upon 24 h, these cells could be employed to the follow-up assays.

qRT-PCR

Isolation of total RNA was performed by using Trizol (15596026, Invitrogen, Carlsbad, CA, USA). Complimentary DNA (cDNA) for mRNA and miRNA was synthesized respectively with the PrimeScript RT Master Mix (Takara, Dalian, China) and MiScript II RT kit (Qiagen, USA). Real-time PCR apparatus (ABI7500, ABI, Foster City, CA, USA) was employed with the assistance of SYBR Premix EX Taq kit (RR420A, Takara Bio Inc., Otsu, Shiga, Japan). Data analysis was conducted using $2^{-\Delta\Delta Ct}$ method. Internal references were as follows: miRNA: U6; mRNA: KLK10. The primers were: miR-194-3p: F, 5'-TTCCCGCCCCCTGTAAC-3', R, 5'-GGCCCTCGCCCCAGATAA-3'; U6: F, 5'-CTCGCTTCGGCAGCACA-3', R, 5'-AACGCTTCACGAATTTGCGT□3'; KLK10: F, 5'-GCCCGGAGAGTGAAGTACAA-3', R, 5'-GTAAACACCCCACGAGAGGA-3'; GAPDH: F, 5'-GGACCTGACCTGCCGTCTAG-3', R, 5'GTAGCCCAGGATGCCCTTGA-3'.

Western blot (WB)

Extraction of total proteins was performed through RIPA buffer (Beyotime, China). Concentration measurement was carried out with BCA Kit (Beyotime, China). Proteins were isolated on SDS-PAGE. The proteins were subsequentially mounted on a PVDF (Millipore) membrane under constant current. The membrane was sealed using 5% skimmed milk powder (2 h), followed by a culture overnight with primary rabbit anti-KLK10 antibody (Abcam, China) or rabbit anti-GAPDH antibody (Abcam, China) at 4°C. Afterwards, secondary antibody goat anti-rabbit IgG (Abcam, China) was utilized for an extra 2 h of incubation. Protein signal examination was implemented using ECL kit (GE Healthcare, Chicago, IL, USA).

CCK-8

Cells from each transfected group were plated (2.5×10^3 cells/well). Then, 10 μL CCK-8 reagent (CK04; Dojindo Laboratories, Kumamoto, Japan) was dropped at indicated times (0/24/48/72/96 h). Thereafter, 24 h of routine culture was implemented. A microplate reader (Multiskan MK3; Thermo Fisher Scientific Inc., Waltham, MA) was utilized to read absorbance (490 nm).

Wound healing assay

Cells were plated first. When the coverage of cells in each well reached 80%, a straight line on the surface of the monolayer cells was drawn by pipette tips to form a clear cell-free area. After 24 h of cultivation in medium free of serum, wound closure was calculated (0/24 h).

Transwell method

Matrigel-coated Transwell room was incubated at 37°C for solidification. The upper chamber was then added with about 2×10^4 cells. The lower room was filled by DMEM with 10% FBS (Thermo Fisher, USA). Forty-eight h later, the non-invading cells were wiped with a cotton swab, while invading cells were stained with 0.1% crystal violet.

Dual-Luciferase reporter gene assay

Mutant (mut)/wild type (wt) KLK10 3' untranslated

region (3'UTR) reporter carriers included binding sites with miR-194-3p and mutant binding sites (General Biosystems; Anhui, China). Co-transfection was performed on NC mimic or miR-194-3p mimic and the carriers described with HCT116 and SW1116 cells. In this process, the kit used was Lipofectamine 2000 (Invitrogen). Luciferase activity examination was performed 48 h later. The kit used in this procedure was Dual-Lucy Assay Kit (Solarbio).

Data analysis

GraphPad Prism 6.0 Software (GraphPad Prism 6.0, San Diego, CA, USA) was employed to process statistics obtained. Our experiment processes included three biological and technical replicates. The form of mean ± standard deviation denoted results. Significant differences were compared using methods like two-tail student's t-test and one way of variance, for two group and multiple groups respectively. Afterwards, Turkey HSD test was applied. Statistical significance was defined when p value was less than 0.05.

Results

Down-regulated miR-194-3p in colon cancer cells correlates with patient's prognosis

MiRNA expression in TCGA-COADREAD dataset was analyzed through bioinformatics analysis (patient's clinical information is shown in Supplementary Table 1). As suggested by results, miR-194-3p was conspicuously poorly-expressed in CRC tissue (p=1.7e-07, Fig. 1A). Survival analysis illuminated patients with lower miR-194-3p expression having shorter survival time in CRC patients (p=0.012, Fig. 1B). qRT-PCR discovered conspicuously down-regulated miR-194-3p in colon cancer (p=9.06e-06, 2.96 e-05, Fig. 1C).

MiR-194-3p overexpression inhibits the progression of colon cancer cells

To unearth the functions of the researched miRNA in SW116 and HCT116, it was overexpressed in the two cell lines. After detection by qRT-PCR, conspicuously up-regulated miR-194-3p was recorded (p=2.12e-05,

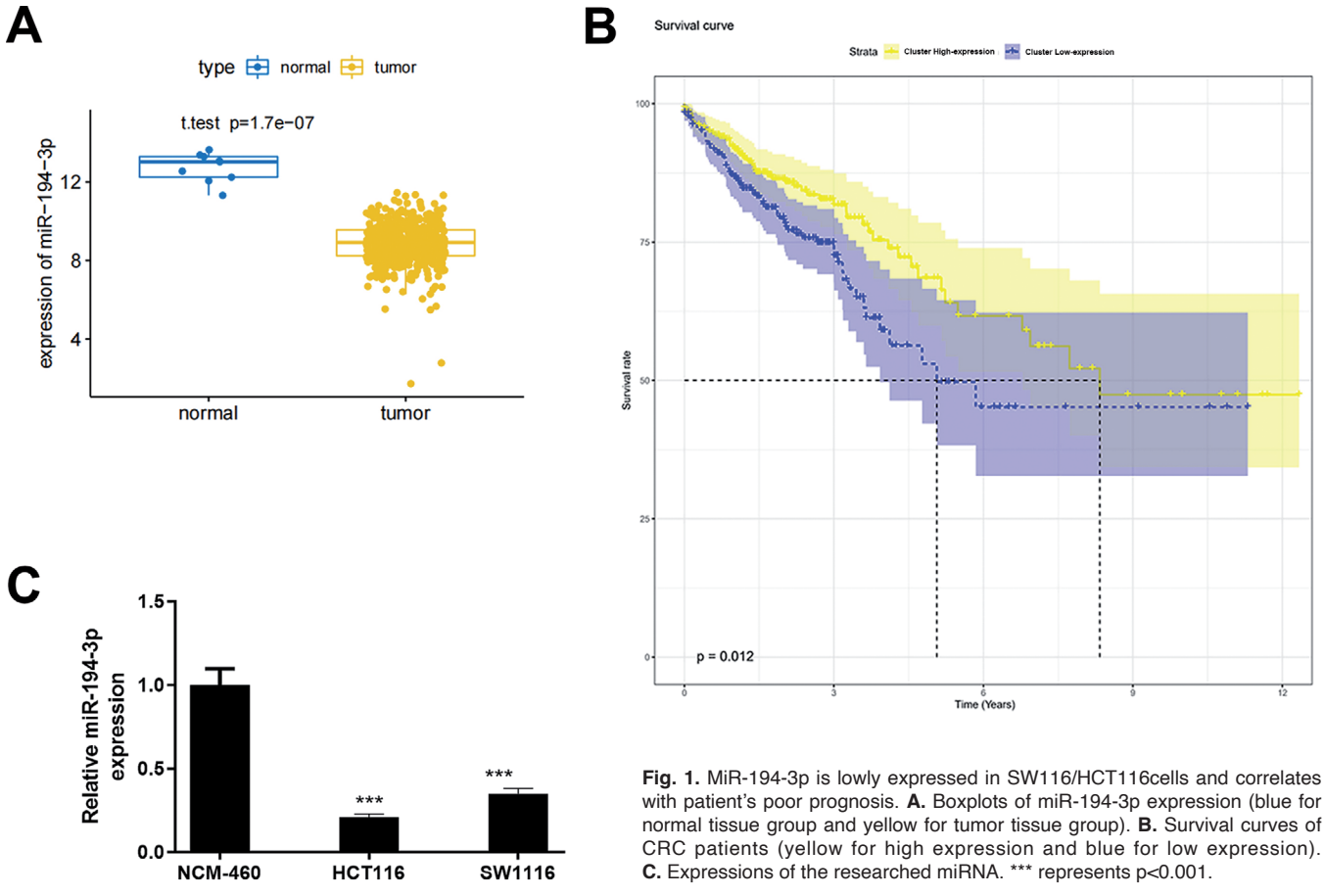

Fig. 1. MiR-194-3p is lowly expressed in SW116/HCT116cells and correlates with patient's poor prognosis. **A.** Boxplots of miR-194-3p expression (blue for normal tissue group and yellow for tumor tissue group). **B.** Survival curves of CRC patients (yellow for high expression and blue for low expression). **C.** Expressions of the researched miRNA. *** represents p<0.001.

p=2.53e-06, Fig. 2A). Thus, the cell lines successfully overexpressing miR-194-3p were eligible for the follow-up assays. Next, CCK-8 demonstrated conspicuously lower proliferative potential in miR-194-3p-mimic (p=2.31e-13, p=6e-15, Fig. 2B). Wound healing and Transwell methods also demonstrated the conspicuous reduction of migratory and invasive potentials in miR-194-3p-mimic (p=3.75e-06, p=7.83e-06, p=1.45e-07, p=2.86e-07, Fig. 2C,D). Altogether, the potentials of SW1116 and HCT116 cells to proliferate, migrate and invade were hindered by miR-194-3p overexpression.

High expression of KLK10 in SW1116 and HCT116cells

MiR-194-3p-targeted mRNAs were found by multiple databases. Besides, these genes were overlapped with the 1,141 up-regulated DEmRNAs screened from TCGA to obtain the differentially expressed gene KLK10 (p<2.2e-16, Fig. 3A) which was with binding sites of miR-194-3p. TCGA data revealed that KLK10 was highly expressed in tumor tissue (p=7.496e-03, Fig. 3B). Correlation analysis based on TCGA data revealed negative correlation between these two genes (Fig. 3C). KLK10 expression was further tested and found to be conspicuously high in two colon cancer cell lines (p=6.72e-03, p=2.7e-03, Fig. 3D). Similarly, WB result showed observably up-regulated KLK10 protein expressions in two cell lines (Fig. 3E). Considering the above results, we speculated the target between KLK10 and the studied miRNA in CRC.

KLK10 is targeted by miR-194-3p

We first predicted the target locations of miR-194-3p on KLK10 3'UTR through bioinformatics analysis. They proved to have target locations (Fig. 4A). Dual-

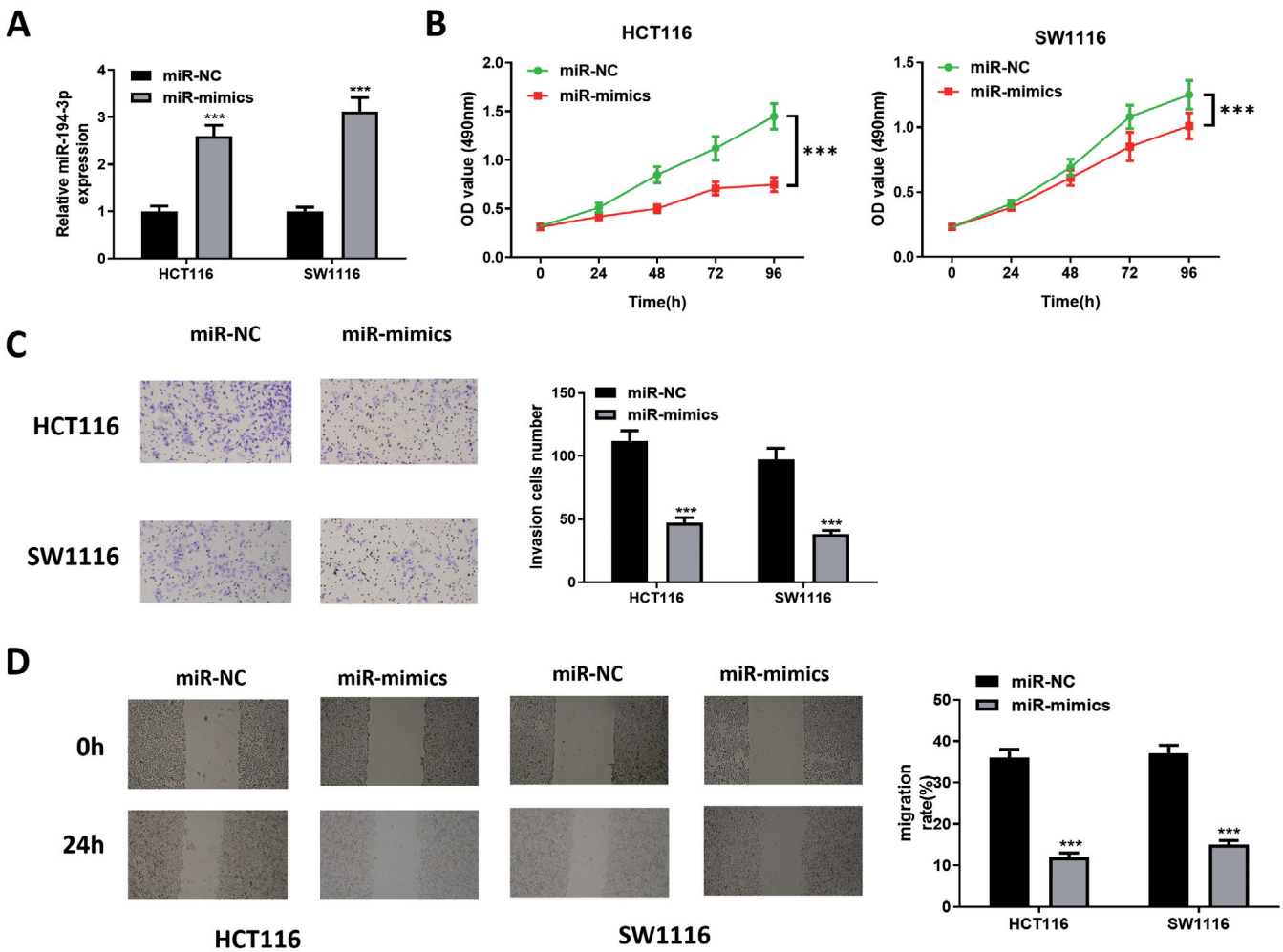

Fig. 2. MiR-194-3p overexpression hinders biological behaviors of SW1116/HCT116 cells. **A.** Expressions of miR-194-3p. **B.** The proliferative potentials of cells. **C.** The invasive potential of cells. **D.** The migratory potential of cells. * p<0.05, ** p<0.01, *** p<0.001.

luciferase assay was used to confirm their targeting. Then, we found inhibited luciferase activity of KLK10-WT by miR-194-3p-mimic, but there was no such effect on KLK10-MUT (p=1.68e-04, p=0.95, p=1.01e-03, p=0.37, Fig. 4B). KLK10 mRNA expression in transfecting groups was tested by qRT-PCR. KLK10 mRNA expression was conspicuously down-regulated in miR-194-3p-mimic, whereas the expression was the opposite in miR-194-3p-inhibitor (p=1.01e-05, p=1.26e-04, p=1.19e-04, p=1.78e-04, Fig. 4C). WB illuminated similar results in mRNA protein expressions (Fig. 4D). Altogether, KLK10 was hindered by miR-194-3p in CRC cells.

MiR-194-3p hinders cellular functions of colon cancer via modulating KLK10

NC mimic+vector, miR-NC+oe-KLK10, miR-194-3p mimic+oe-KLK10 groups were established in rescue assay. The expression of KLK10 in miR-NC+oe-KLK10 was found to be significantly up-regulated, while the expression was rescued in miR-194-3p mimic+oe-KLK10 (p=0.0003, p=5.93e-04, p=3.91e-04, p=6.01e-04, Fig. 5A,B). As shown in CCK-8 results, KLK10 overexpression conspicuously strengthened the potentials for SW1116/HCT116 cells to proliferate, whereas overexpressing miR-194-3p counteracted such an impact (p=7e-15, p=4-e15, p=1.58e-12, p=6.3e-13, Fig. 5C). Wound healing and Transwell methods also displayed the similar trends (p=1.5e-03, p=1.05e-03, p=7.45e-04, p=3.45e-03, p=1.41e-03, p=7.63e-04, p=1.99e-03, p=1.7e-03, Fig. 5D,E). Altogether, miR-194-3p hindered the cellular functions of CRC via KLK10.

Discussion

In previous studies, it was found that during the development of CRC, expression of many miRNAs alters, which is related to the basic cellular function, such as cell differentiation and progression (de Krijger et al., 2011). Here, conspicuously down-regulated miR-194-3p was discovered to hinder the cellular functions of SW1116/HCT116. For miR-194, it was reported that miR-194 displays an anti-tumor effect in assertive cancers (Basati et al., 2016; Yi et al., 2019; Zhou et al., 2020). Sun et al. (2019) covered that miR-194 hampers CRC stem cells to proliferate. MiR-194 modulates epithelial-mesenchymal transition of tumor to cast new lights on CRC onset and metastasis (Cai et al., 2017).

Nonetheless, little data are available about miR-194-3p in CRC. Bioinformatics analysis and cell function

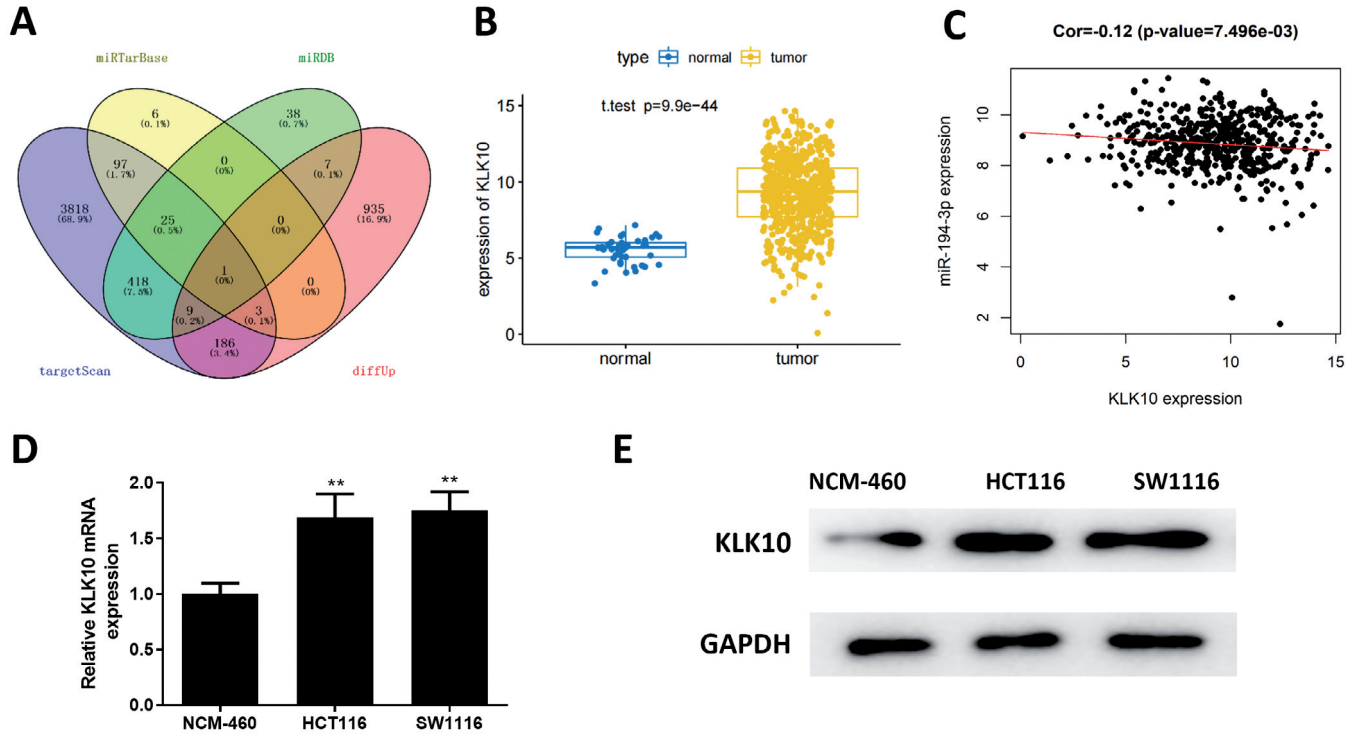

Fig. 3. High expression KLK10 and its negative correlation with miR-194-3p in SW1116/HCT116. **A.** Venn diagram of target mRNAs. **B.** Boxplot of KLK10 expression (yellow for tumor group and blue for normal group). **C.** Pearson analysis of the two studied genes. **D.** The expression of KLK10 mRNA. **E.** The expression of KLK10 proteins. ** p<0.01.

experiments proved that miR-194-3p functions as a tumor suppressor in the processes of CRC cell progression.

Alteration of KLK10 expression in prostate cancer, testicular cancer and ovarian cancer is considered to be an effective prognostic indicator of disease progression (Luo et al., 2001; Shvartsman et al., 2003; Yousef et al., 2003, 2004). In cervical cancer, miR-199b-5p downregulates KLK10 to facilitate tumor growth (Xu et al., 2018). According to the research of Feng and other experts, up-regulated KLK10 in CRC and gastric cancer correlates with the malignant progression of the diseases and poor prognosis (Feng et al., 2006). Constantina Petraki and other researchers proposed that KLK10 is pertinent to CRC patient's survival outcomes (Petraki et al., 2012). The study conducted by Maroulio and other researchers showed that KLK10 may exert as a biomarker for CRC treatment (Petraki et al., 2012). Previous studies only were limited to the clinical aspect, and there has not yet been any study on the mechanism and biological functions of KLK10 in CRC. Here, dual-luciferase assay further confirmed the targeting of these two genes. Moreover, KLK10 overexpression enhanced the cellular functions of SW1116/HCT116, suggestive of dismal prognosis of patients with high KLK10 expression. The results are consistent with those of Feng et al., and the regulatory mechanism of KLK10 was further verified on this basis. In addition, according to the rescue experiments, In CRC, miR-194-3p hindered cancer cellular functions by modulating KLK10.

On the whole, we unearthed the functions of miR-194-3p/KLK10. Specifically, overexpression of miR-194-3p conspicuously hindered cancer cell functions. MiR-194-3p could mediate the KLK10 in a targeting manner, thus exerting its anti-cancer effect on CRC. These findings provide a breakthrough point for finding a new targeted therapy for CRC. Nonetheless, limitations still existed in our study. We did not verify

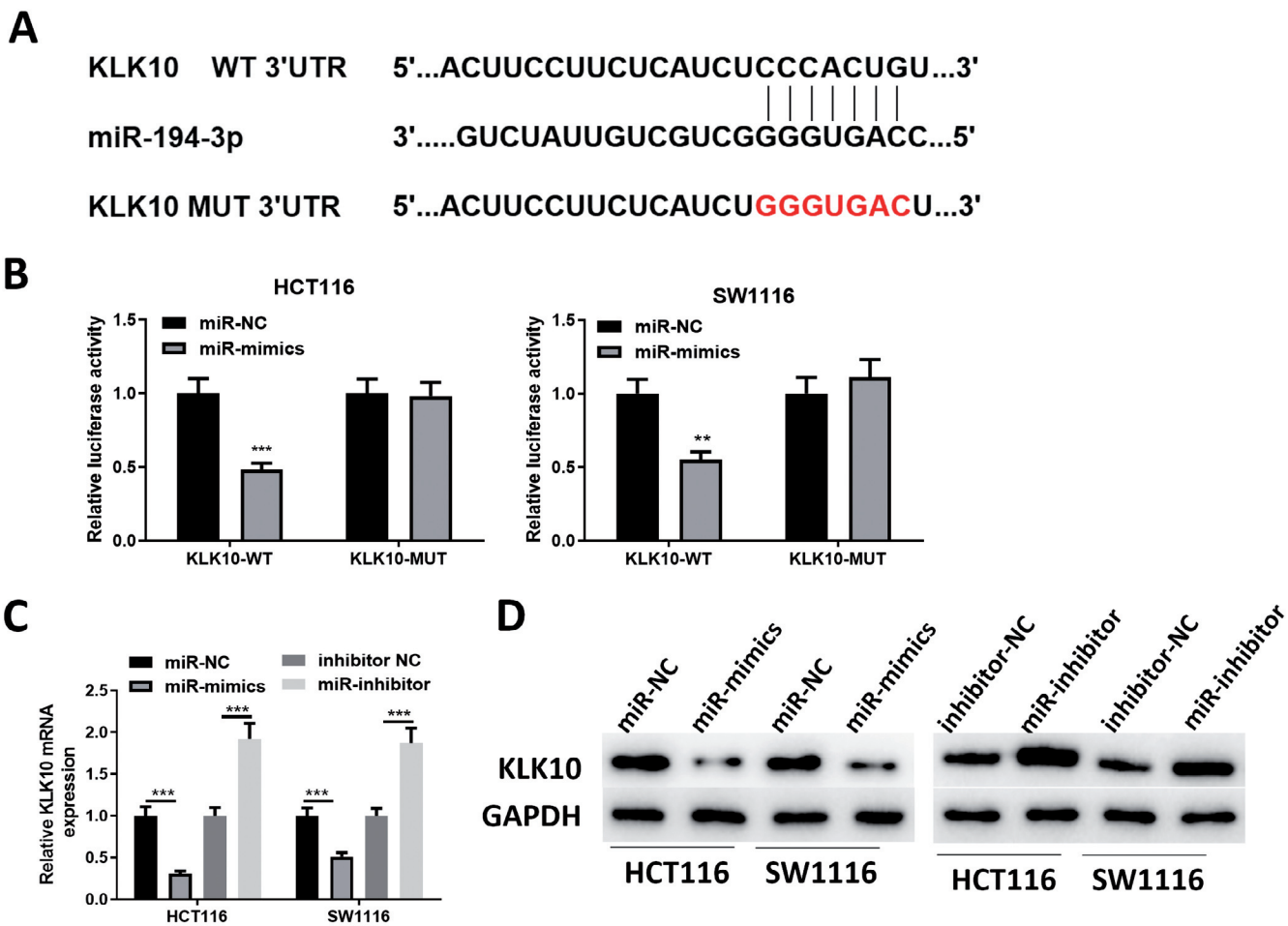

Fig. 4. MiR-194-3p inhibits KLK10. **A.** The target locations of the two studied genes. **B.** Images of dual-luciferase assay. **C.** The expression of KLK10 mRNA. **D.** KLK10 protein expression. ** p<0.01, *** p<0.001.

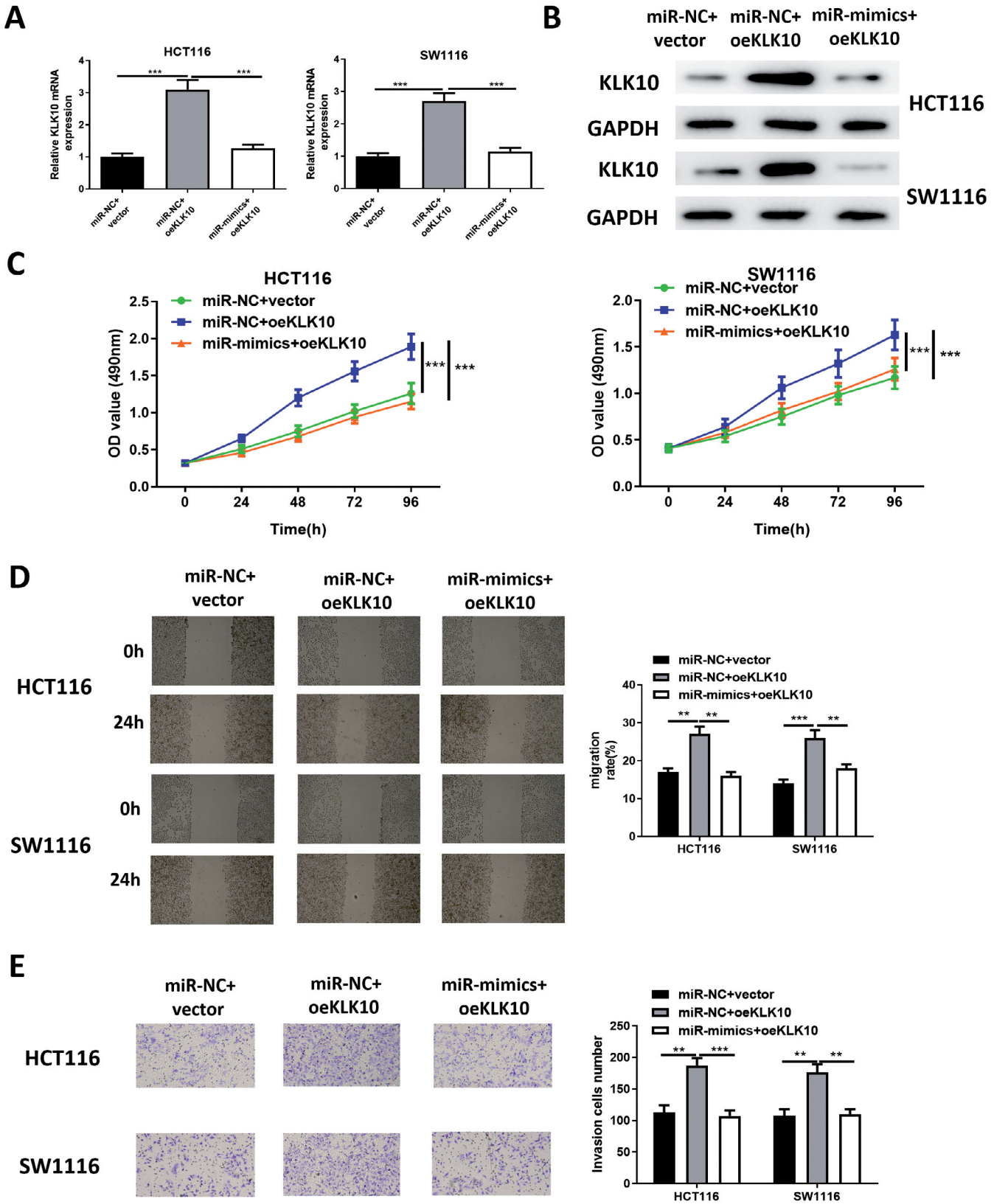

Fig. 5. MiR-194-3p hinders cellular functions of SW1116/HCT116 via modulating KLK10. **A, B.** Expression of KLK10 mRNA and protein. **C.** The proliferative potential of cells. **D.** The migratory potential of cancer cells. **E.** The invasive potential of cancer cells. * p<0.05, ** p<0.01, *** p<0.001.

the expression of two genes in CRC patient's tissue. We did not apply in vivo approaches to probe their impacts on tumor growth. In the future, we will enrich these parts.

Acknowledgements. Not applicable.

Ethics approval and consent to participate. Not applicable.

Consent for publication. All authors consent to submit the manuscript for publication.

Availability of data and materials. The data used to support the findings of this study are included within the article. The data and materials in the current study are available from the corresponding author on reasonable request.

Conflict of Interest Statement. The authors declare that they have no potential conflicts of interest.

Funding. Not applicable.

Authors' contributions. T wrote the main manuscript text and YJ prepared figures 1-5. All authors reviewed the manuscript.

References

Basati G., Razavi A.E., Pakzad I. and Malayeri F.A. (2016). Circulating levels of the miRNAs, miR-194, and miR-29b, as clinically useful biomarkers for colorectal cancer. Tumour Biol. 37, 1781-1788.

Brenner H. and Chen C. (2018). The colorectal cancer epidemic: challenges and opportunities for primary, secondary and tertiary prevention. Br. J. Cancer 119, 785-792.

Borgono C.A. and Diamandis E.P. (2004). The emerging roles of human tissue kallikreins in cancer. Nat. Rev. Cancer 4, 876-890.

Cai H.K., Chen X., Tang Y.H. and Deng Y.C. (2017). MicroRNA-194 modulates epithelial-mesenchymal transition in human colorectal cancer metastasis. Onco. Targets Ther. 10, 1269-1278.

Chang H.Y., Ye S.P., Pan S.L., Kuo T.T., Liu B.C., Chen Y.L. and Huang T.C. (2017). Overexpression of miR-194 Reverses HMGA2-driven Signatures in Colorectal Cancer. Theranostics 7, 3889-3900.

Chiang Y., Song Y., Wang Z., Liu Z., Gao P., Liang J., Zhu J., Xing C. and Xu H. (2012). microRNA-192, -194 and -215 are frequently downregulated in colorectal cancer. Exp. Ther. Med. 3, 560-566.

Connell L.C., Mota J.M., Braghiroli M.I. and Hoff P.M. (2017). The rising incidence of younger patients with colorectal cancer: Questions about screening, biology, and treatment. Curr. Treat. Options Oncol. 18, 23.

Feng B., Xu W.B., Zheng M.H., Ma J.J., Cai Q., Zhang Y., Ji J., Lu A.G., Qu Y., Li J.W., Wang M.L., Hu W.G., Liu B.Y. and Zhu Z.G. (2006). Clinical significance of human kallikrein 10 gene expression in colorectal cancer and gastric cancer. J. Gastroenterol. Hepatol. 21, 1596-603.

de Krijger I., Mekenkamp L.J., Punt C.J. and Nagtegaal I.D. (2011). MicroRNAs in colorectal cancer metastasis. J. Pathol. 224, 438-447.

Lee Y.S. and Dutta A. (2009). MicroRNAs in cancer. Annu. Rev. Pathol. 4, 199-227.

Liu X.L., Wazer D.E., Watanabe K. and Band V. (1996). Identification of a novel serine protease-like gene, the expression of which is down-regulated during breast cancer progression. Cancer Res. 56, 3371-3379.

Luo L.Y., Rajpert-De Meyts E.R., Jung K. and Diamandis E.P. (2001). Expression of the normal epithelial cell-specific 1 (NES1; KLK10) candidate tumour suppressor gene in normal and malignant testicular tissue. Br. J. Cancer 85, 220-224.

Meng Z., Fu X., Chen X., Zeng S., Tian Y., Jove R., Xu R. and Huang W. (2010). miR-194 is a marker of hepatic epithelial cells and suppresses metastasis of liver cancer cells in mice. Hepatology 52, 2148-2157.

Petraki C., Youssef Y.M., Dubinski W., Lichner Z., Scorilas A., Pasic M.D., Komborozos V., Khalil B., Streutker C., Diamandis E.P. and Yousef G.M. (2012). Evaluation and prognostic significance of human tissue kallikrein-related peptidase 10 (KLK10) in colorectal cancer. Tumour Biol. 33, 1209-1214.

Pichiorri F., Suh S.S., Rocci A., De Luca L., Taccioli C., Santhanam R., Zhou W., Benson D.M., Jr., Hofmainster C., Alder H., Garofalo M., Di Leva G., Volinia S., Lin H.J., Perrotti D., Kuehl M., Aqeilan R.I., Palumbo A. and Croce C.M. (2016). Downregulation of p53-inducible microRNAs 192, 194, and 215 Impairs the p53/MDM2 Autoregulatory Loop in Multiple Myeloma Development. Cancer Cell 30, 349-351.

Shvartsman H.S., Lu K.H., Lee J., Lillie J., Deavers M.T., Clifford S., Wolf J.K., Mills G.B., Bast R.C. Jr, Gershenson D.M. and Schmandt R. (2003). Overexpression of kallikrein 10 in epithelial ovarian carcinomas. Gynecol. Oncol. 90, 44-50.

Sidiropoulos M., Pampalakis G., Sotiropoulou G., Katsaros D. and Diamandis E.P. (2005). Downregulation of human kallikrein 10 (KLK10/NES1) by CpG island hypermethylation in breast, ovarian and prostate cancers. Tumour Biol. 26, 324-336.

Song Y., Zhao F., Wang Z., Liu Z., Chiang Y., Xu Y., Gao P. and Xu H. (2012). Inverse association between miR-194 expression and tumor invasion in gastric cancer. Ann. Surg. Oncol. 19 (Suppl 3), S509-517.

Sun B., Fang Y.T., Jin D.J., Chen Z.Y., Li Z.Y., Gu X.D. and Xiang J.B. (2019). miR-194 inhibits the proliferation of SW620 colon cancer stem cells through downregulation of SSH2 expression. Cancer Manag. Res. 11, 10229-10238.

Talieri M., Alexopoulou D.K., Scorilas A., Kypraios D., Arnogiannaki N., Devetzi M., Patsavela M. and Xynopoulos D. (2011). Expression analysis and clinical evaluation of kallikrein-related peptidase 10 (KLK10) in colorectal cancer. Tumour Biol. 32, 737-744.

Thanikachalam K. and Khan G. (2019). Colorectal cancer and nutrition. Nutrients 11, 164.

Xu L.J., Duan Y., Wang P. and Yin H.Q. (2018). MiR-199b-5p promotes tumor growth and metastasis in cervical cancer by down-regulating KLK10. Biochem. Biophys. Res. Commun. 503, 556-563.

Yi L., Ouyang L., Wang S., Li S.S. and Yang X.M. (2019). Long noncoding RNA PTPRG-AS1 acts as a microRNA-194-3p sponge to regulate radiosensitivity and metastasis of nasopharyngeal carcinoma cells via PRC1. J. Cell. Physiol. 234, 19088-19102.

Yousef G.M., Polymeris M.E., Yacoub G.M., Scorilas A., Soosaipillai A., Popalis C., Fracchioli S., Katsaros D. and Diamandis E.P. (2003). Parallel overexpression of seven kallikrein genes in ovarian cancer. Cancer Res. 63, 2223-2227.

Yousef G.M., Yacoub G.M., Polymeris M.E., Popalis C., Soosaipillai A. and Diamandis E.P. (2004). Kallikrein gene downregulation in breast cancer. Br. J. Cancer 90, 167-172.

Zhang B., Pan X., Cobb G.P. and Anderson T.A. (2007). microRNAs as oncogenes and tumor suppressors. Dev. Biol. 302, 1-12.

Zhang Y., Song H., Miao Y., Wang R. and Chen L. (2010). Frequent transcriptional inactivation of Kallikrein 10 gene by CpG island

hypermethylation in non-small cell lung cancer. Cancer Sci. 101, 934-940.

Zhao H.J., Ren L.L., Wang Z.H., Sun T.T., Yu Y.N., Wang Y.C., Yan T.T., Zou W., He J., Zhang Y., Hong J. and Fang J.Y. (2014). MiR-194 deregulation contributes to colorectal carcinogenesis via targeting AKT2 pathway. Theranostics 4, 1193-1208.

Zhou Q., Guo J., Huang W., Yu X., Xu C. and Long X. (2020). Linc-ROR promotes the progression of breast cancer and decreases the sensitivity to rapamycin through miR-194-3p targeting MECP2. Mol. Oncol. 14, 2231-22350.

Accepted December 22, 2021