Department of Cardiothoracic Surgery[1], Tianjin Xiqing Hospital; Department of General Surgery[2], Tianjin Fourth Center Hospital, Tianjin; Second Department of Oncology[3], Tengzhou Central People's Hospital, Tengzhou, China

miR-122-5p inhibits tumor cell proliferation and induces apoptosis by targeting MYC in gastric cancer cells

Z. J. Pei[1, #], Z. G. Zhang[2, #], A. X. Hu[3,*], F. Yang[3], Y. Gai[3]

Received December 30, 2016, accepted February 2, 2017

**Corresponding author: Anxiang Hu, Second Department of Oncology, Tengzhou Central People's Hospital, No. 181, Xingtan Road, Tengzhou 277500, China*
huanxiang781@126.com
[#]These authors contributed equally to this work.

Pharmazie 72: 344–347 (2017) *doi: 10.1691/ph.2017.6404*

MicroRNAs (miRNAs) play crucial roles in the development and progression of human cancers, including Gastric cancer (GC). In this study, we investigated the correlation of miR-122-5p expression with cell proliferation, and apoptosis in a GC cell line. GC cells SCG 7901 were transfected with control, miR-122-5p or miR-122-5p inhibitor and MTT assay, western blot, and BrdU staining were respectively used to investigate the effect of miR-122-5p on GC cell cycle. The overexpression of miR-122-5p could reduce cell proliferation in SCG7901 cells, and BrdU staining finally verified miR-122-5p induced cell growth arrest by upregulation p27 expression in SCG7901cells. On the other hand, cells apoptosis research showed that miR-122-5p induced apoptosis by targeting MYC in SCG7901 cells. Finally, in this study, miR-122-5p was confirmed inhibiting tumor GC cells proliferation and inducing cells apoptosis by targeting MYC. All these findings suggest that miR-122-5p may be involved in progression of GC and could be a new therapeutic target for this disease.

1. Introduction

Gastric cancer (GC) is one of the most common malignant tumors accounting for more than 700,000 deaths annually (Mirmajidi et al. 2016; Rui et al. 2016). Current studies suggested that gastric cancer development is a complicated multi-factor and multi-stage process, involving various genetic and epigenetic changes, such as age, gender, level of health and environmental risk factors, and so on (Liu et al. 2014; Lu et al. 2014; Zhang et al. 2016). Although there has been considerable progress in therapies, as well as diagnosis and mechanism research, incidence and mortality in patients with GC are still high (Fan et al. 2015; Parvathi et al. 2013). As a result, it is of great importance to elucidate the molecular mechanisms for cell proliferation, and apoptosis in GC cells.

MicroRNAs (miRNAs) are small noncoding RNAs regulators of oncogenesis that function as oncomiRs or tumor suppressors, potentially playing a critical role in tumorigenesis (Du et al. 2013; Pei-Fei et al. 2014; Venkatadri et al. 2016). Mounting evidence indicates that one specific tumor-suppressive miRNA: miR-122-5p plays important roles in numerous human cancers, such as breast cancer, and hepatocellular carcinoma (Ergün et al. 2015; Indovina et al. 2012; Tan et al. 2014). This suggests that miR-122-5p may play a key role in tumorigenesis.

p27 is once reported as cyclin-dependent kinase inhibitor and the nuclear localization of p27 is crucial for malignant mesothelioma (MM)tumor-suppressive function (Fussbroich et al. 2011). Moreover, it acts as cell-cycle marker in colon cancer, esophageal squamous cell carcinoma, prostate cancer and so on (Huang et al. 2012; Li and Simon 2013; Lin et al. 2012; Lin and Chiang 2011).

MYC is a key cell growth regulator of different cancers, which mediates a transcriptional program spanning cell growth, cell cycle, metabolism, as well as cell survival (Fiorentino et al. 2016). The function of MYC in cancers has been sufficiently reported, in small cell lung cancer (SCLC) patients proteins are potential therapeutic targets (Calcagno et al. 2013). Moreover, a significant function of MYC in GC has also been mentioned (Yan et al. 2013). But, to clearly understand the role of MYC in GC, the approaches now are far from sufficient. The goal of this study was to better clarify the role of miR-122-5p in tumor growth of gastric cancer cells and the underlying molecular mechanism.

2. Investigations and results

In this study, SCG7901 cells were transfected with control, miR-122-5p or miR-122-5p inhibitor and MTT assay was performed to test the effect of miR-122-5p on GC cell proliferation. The MTT results demonstrated that miR-122-5p overexpression strongly inhibited the proliferation of SCG7901 cells. Furthermore, p27 expression was tested to explore the regulation mechanism of miR-122-5p. Next, cell apoptosis assay was performed and affirmed miR-122-5p **i**nduced SCG7901 cells apoptosis by targeting MYC.

2.1. miR-122-5p overexpression reduced cell proliferation in SCG 7901 cells

To test miR-122-5p effect on cell proliferation, SCG7901 cells were transfected with control, miR-122-5p or miR-122-5p inhibitor and performed MTT assay. The MTT results shown in Fig. 1 tell miR-122-5p overexpression strongly inhibited the proliferation of SCG7901 cells, while cells transfected with miR-122-5p inhibitor increased SCG7901 cell viability.

2.2. miR-122-5p overexpression induced cell growth arrest by up-regulation p27 expression in SCG7901cells

To further understand the mechanisms of miR-122-5p induced cell growth inhibition, we performed BrdU staining and analyzed the effect of miR-122-5p on various cell cycle regulators in SCG7901 cells. The BrdU staining results shown in Fig. 2 witnessed strong accumulation of p27 in response to the miR-122-5p expression. The other major CDKN1 family member, p21CIP1, was unaffected by miR-122-5p expression. The results confirmed miR-122-5p inhibits cell growth by up-regulation p27 expression in SCG7901cells.

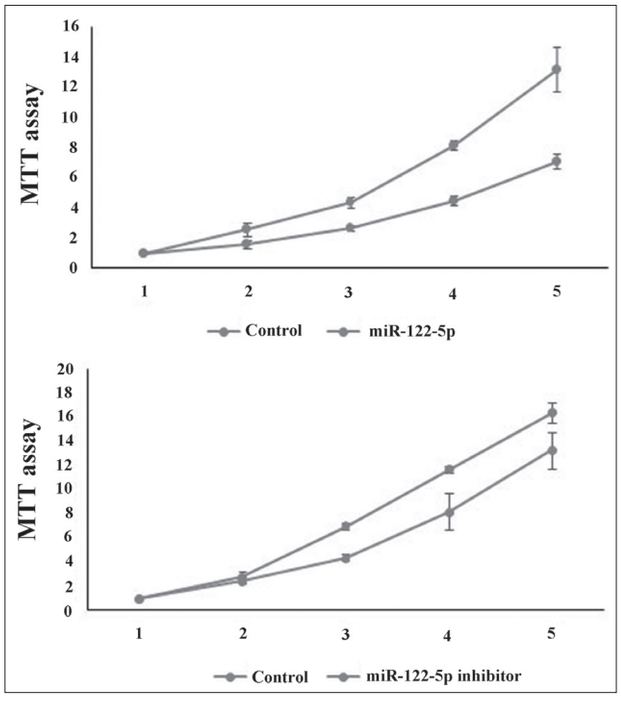

Fig. 1: MTT results showing the effects of miR-122-3p expression on SCG7901cells proliferation. The cell proliferation of SCG7901 cells transfected with miR-122-5p or miR-122-5p inhibitor was tested by MTT assay. MiR-122-5p down-regulates cells proliferation of GC cells.

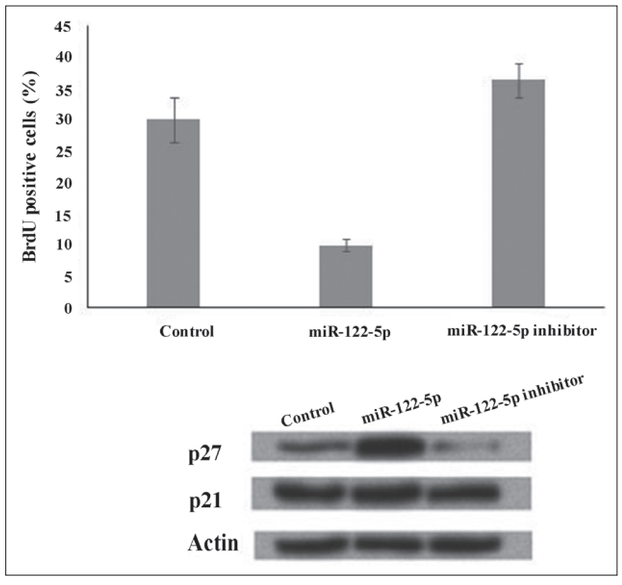

Fig. 2: BrdU staining and western blot results showing effects of miR-122-5p expression on cell cycle regulator p27. GC cells transfected with miR-122-5p show lower percent of BrdU positive cells, while inhibition of miR-122-5p showing high cells viability. Western blot results show high expression of p27. miR-122-5p may upregulate p27 in the cell progression.

2.3. Overexpression of miR-122-5p induced apoptosis in SCG7901 cells

To test miR-122-5p effect on cell apoptosis, SCG7901 cells were transfected with control, miR-122-5p or inhibitor and performed apoptosis assay. The images and cells apoptosis rates shown in Fig. 3 clearly stated that miR-122-3p increases the apoptosis of GC cell.

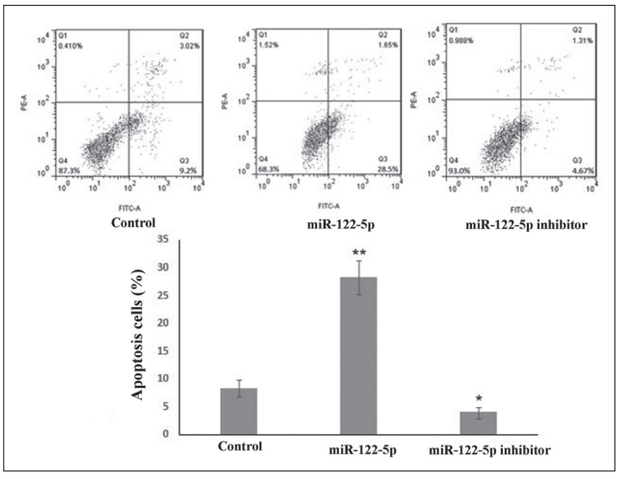

Fig. 3: MTT results showing effects of miR-122-5p expression on cells apoptosis of SCG7901. The percent of GC cell apoptosis transfected with miR-122-5p or miR-122-5p inhibitor was tested by MTT assay. Cells transfected with miR-122-5p show high apoptosis rate, reversely, cells transfected with miR-122-5p inhibitor showing opposite result. MiR-122-5p overexpression promotes cells apoptosis of SCG7901.

2.4. miR-122-5p induced apoptosis by targeting MYC in SCG7901 cells

To further understand the mechanisms of miR-122-5p effect on apoptosis, SCG7901 cells were transfected with control, miR-122-5p or inhibitor and MYC, bax, pro and activated caspase-3 expression. The western blot results shown in Fig. 4 convince us miR-122-5p induced apoptosis by targeting MYC in SCG7901 cells.

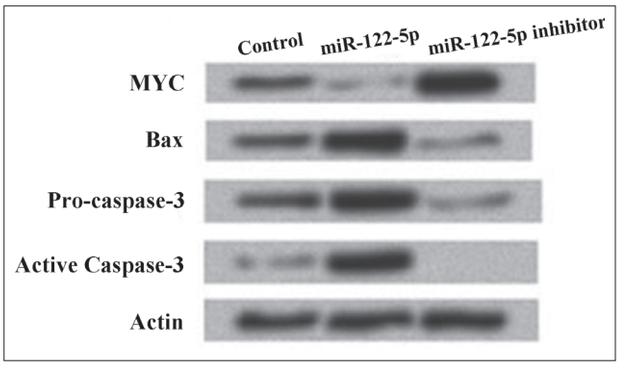

Fig. 4: Western blot results showing pathway of miR-122-5p on cell apoptosis. Expression of MYC, bax, pro and activated caspase-3 was performed by western blot. Overexpression of miR-122-5p promotes MYC expression. MiR-122-5p induced GC cells apoptosis by targeting MYC.

2.5. Conclusion

In summary, our study firstly interpreted the relationship of miR-122-5p expression with GC cell viability. miR-122-5p inhibits cell proliferation by regulating p27 expression, and induces cell apoptosis by targeting MYC in SCG7901 cells. All these findings suggest that miR-122-5p may be a promising biomarker candidate in gastric cancer therapy and could be a new therapeutic target for this disease.

3. Discussion

Gastric cancer (GC) is reported the third primary cause of cancer-related mortality and one of the most common type of malignant diseases (Margue et al. 2015). MiRNAs are increasingly

recognized as biomarkers for the diagnosis of cancers (Tsai et al. 2016), including GC. miR-26b is identified as a significantly downregulated miRNA that could inhibit GC metastasis (Wu et al. 2016). It was also demonstrated that miR-448 works as a tumor suppressor miRNA through targeting ADAM10 expression (Green et al. 2016). Many kinds of miRNAs are related to GC progression, one of them is miR-122-5p. miR-122-5p has a tumor suppressor function through different molecular pathways (Ergün et al. 2015; Indovina et al. 2012; Tan et al. 2014), while the role of miR-122-5p in GC is not sufficiently known so far.

In this study, we [XXX]restigated the role of miR-122-5p in GC cell proliferation. The GC cells were firstly transfected with miR-122-5p or inhibitor, and then we performed MTT and recorded the optical density to measure the GC cells proliferation. The results confirmed that miR-122-5p can inhibit GC cells proliferation.

Furtherly, to clearly understand the pathway of miR-122-5p inhibition in GC cells viability, we constructed BrdU staining assay and monitored the expression changes of cell cycle regulators. As a result, p27 showed strong correlation with the miR-122-5p inhibition function in GC cells. So we came to the conclusion that miR-122-5p may inhibit cell proliferation by upregulating p27 in GC.

MYC is an important cell growth mediator in different cancers (Fiorentino et al. 2016). In a mechanism research of breast cancer, MYC was shown regulating breast cancer cell proliferation and tumor growth (Choi et al. 2016). Also, many studies have focused on the MYC effect in the GC progression (Liu et al. 2011; Schmidt et al. 2015).

In this study, we firstly proved that miR-122-5p can induce cell apoptosis. Next, we researched the MYC expression and the miR-122-5p regulation. We performed western blot assay and tested MYC, Bax, pro and activated caspase-3 expression after GC cells were transfected with control, miR-122-5p or inhibitor. The western blot results clearly proved miR-122-5p induced cell apoptosis by targeting MYC.

miR-122-5p inhibits cell proliferation and induces apoptosis by targeting MYC in SCG7901cells.

Our study found that overexpression of miR-122-5p could inhibit cell proliferation, and induce apoptosis via targeting MYC in GC cells. All these findings suggest that miR-122-5p may be involved in progression of GC and could be a new therapeutic target for this disease.

4. Experimental

4.1. Cell culture

Human Gastric Cancer Cells Line SGC 7901 cells were obtained from the China General Microbiological Culture Collection Center (Beijing, China), and maintained in minimal RPMI (Roswell Parker Memorial Institute)-1640 medium (Gibco BRL, Gaithersburg, MD, USA). The medium containing 10% fetal bovine serum (FBS, Gibco, USA) and 1% antibiotic antimycotic (Gibco,USA), should be restored in 5% CO_2 and 95% air, 37 °C (Liang et al. 2013).

4.2. Cell proliferation and MTT assay

Cells were seeded at 5×10^4 cells/ well on 12-well plates. They were harvested with trypsin, resuspended in 3 mL of culture medium, and counted with a hemocytometer after exposure to 5 μg/mL of Danshen extract at the indicated time intervals. For MTT assays, 5×10^3 cells/well were seeded onto 96-well culture plates. After incubation for 3 h at 37 °C, cell viability was assayed by adding 20 μL of 10 mg/mL 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma). Then the medium was removed, the formazan (in DMSO) was added, and the 590 nm optical density was recorded with a Multiskan EX (Thermo, Finland) (Fernandez et al. 2015).

4.3. BrdU assay

Cells were seeded in 6-well plates at a density of 20000 cells/well on sterilized coverslips. 10 μM of BrdU (Sigma-Aldrich, St. Louis, MO) were added to the medium. After 5 h incubation at 37 °C, the cells were fixed in cold 70% ethanol for 5 min and by adding 1.5M HCl to the cells, DNA was denaturated for 30 min. By using a mouse anti-BrdU antibody (BD Biosciences, San Jose, CA), immunofluorescence to visualize incorporated BrdU was performed as the manufacturer reminded. Primary antibody was followed by 45 min incubation with secondary green-fluorescence dye conjugated antibody (Alexafluor 488, Invitrogen, Carlsbad, CA). VECTASHIELD mounting medium with DAPI (Vector Laboratories, Burlingame, CA) was used to stain the nuclei. Images were visualized using a Leica inverted fully automated microscope (DMI6000B) with digital camera DFC 420 RGB (Leica Microsystems, Wetzlar, Germany) (Zhang et al. 2013).

4.4. Apoptosis assay

24 h after transfection, cells were harvested and suspended in Annexin-binding buffer, and then cells were incubated with Annexin V-FITC and PI (Becton Dickinson, NJ, USA) for 30 min in the dark at 25 °C, Flowjo was used to analyze the apoptotic percentage of cells (Nam et al. 2015).

4.5. Western blot analysis

Cells were washed once with PBS and lysed in a lysis buffer. Protein samples were separated on SDS-PAGE gels and transferred to nitrocellulose membrane (Whatman, Dassel, Germany) after boiling for 10 min in SDS sample buffer. Then, the membranes were incubated with the appropriate primary antibodies overnight after blocking with 5% skim milk in TBS-T for 1 h. Membranes were washed once with TBS-T and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 2 h. Protein bands were visualized with the WEST-ZOL-plus Western Blot Detection System.

Antibodies specific to Actin (C-2) was purchased from Cell Signaling Technology (Beverly, MA, USA). c-Myc (ab32072), active caspase-3 (ab2302), p27 (ab54563), p21 (ab7960) and procaspase 3 (ab32150) from Abcom (Nam et al. 2015).

Acknowledgement: The work was not supported by any funding agency.

Conflicts of interest: The authors declared there was no conflict of interests.

References

Calcagno DQ, Freitas VM, Leal MF, Souza CRD, Demachki S, Montenegro R, Assumpção PP, Khayat AS, Smith MA, Santos AKD (2013) MYC, FBXW7 and TP53 copy number variation and expression in Gastric Cancer. BMC Gastroenterol 13: 141.

Choi JH, Kim JS, Won YW, Uhm J, Park BB, Lee YY (2016) The potential of deferasirox as a novel therapeutic modality in gastric cancer. World J Surg Oncol 14: 1-7.

Du Z, Fei T, Verhaak RG, Su Z, Zhang Y, Brown M, Chen Y, Liu XS (2013) Integrative genomic analyses reveal clinically relevant long noncoding RNAs in human cancer. Nature Struct Mol Biol 20: 908-913.

Ergün S, Ulasli M, Igci YZ, Igci M, Kırkbes S, Borazan E, Balik A, Yumrutaş Ö, Camci C, Cakmak EA (2015) The association of the expression of miR-122-5p and its target ADAM10 with human breast cancer. Mol Biol Rep 42: 497-505.

Fan H, Guo Z, Wang C (2015) Combinations of gene ontology and pathway characterize and predict prognosis genes for recurrence of gastric cancer after surgery. DNA Cell Biol 34: 579-587.

Fernandez S, Risolino M, Mandia N, Talotta F, Soini Y, Incoronato M, Condorelli G, Banfi S, Verde P (2015) miR-340 inhibits tumor cell proliferation and induces apoptosis by targeting multiple negative regulators of p27 in non-small cell lung cancer. Oncogene 34: 3240-3250.

Fiorentino FP, Tokgün E, Solé-Sánchez S, Giampaolo S, Tokgün O, Jauset T, Kohno T, Perucho M, Soucek L, Yokota J (2016) Growth suppression by MYC inhibition in small cell lung cancer cells with TP53 and RB1 inactivation. Oncotarget 7: 31014-31028.

Fussbroich B, Wagener N, Macher-Goeppinger S, Benner A, Fälth M, Sültmann H, Holzer A, Hoppe-Seyler K, Hoppe-Seyler F (2011) EZH2 depletion blocks the proliferation of colon cancer cells. Plos One 6: e21651.

Green AR, Aleskandarany MA, Agarwal D, Elsheikh S, Nolan CC, Diez-Rodriguez M, Macmillan RD, Ball GR, Caldas C, Madhusudan S (2016) MYC functions are specific in biological subtypes of breast cancer and confers resistance to endocrine therapy in luminal tumours. Br J Cancer 114: 917-928.

Huang JX, Chen WC, Lin M, Zhang YL, Li FY, Song ZX, Xiao W, Chen P, Qian RY, Salminen E (2012) Clinicopathological significance of cyclooxygenase-2 and cell cycle-regulatory proteins expression in patients with esophageal squamous cell carcinoma. Dis Esophagus 25: 121-129.

Indovina P, Giorgi F, Rizzo V, Khadang B, Schenone S, Marzo DD, Forte IM, Tomei V, Mattioli E, D'Urso V (2012) New pyrazolo[3,4-d]pyrimidine SRC inhibitors induce apoptosis in mesothelioma cell lines through p27 nuclear stabilization. Oncogene 31: 929-938.

Li B, Simon MC (2013) Molecular pathways: Targeting MYC-induced metabolic reprogramming and oncogenic stress in cancer. Clin Cancer Res 19: 5835-5841.

Liang L, Li C, Li D, Wang Y, Zhou C, Shao W, Peng J, Yan Y, Zhang X, Shen X (2013) Cryptotanshinone inhibits human glioma cell proliferation by suppressing STAT3 signaling. Mol Cell Biochem 381: 273-282.

Lin P, Sun X, Feng T, Zou H, Jiang Y, Liu Z, Zhao D, Yu X (2012) ADAM17 regulates prostate cancer cell proliferation through mediating cell cycle progression by EGFR/PI3K/AKT pathway. Mol Cell Biochem 359: 235-243.

Lin YW, Chiang BH (2011) 4-Acetylantroquinonol B isolated from Antrodia cinnamomea arrests proliferation of human hepatocellular carcinoma HepG2 cell by affecting p53, p21 and p27 levels. J Agricult Food Chem 59: 8625-8631.

Liu H, Zhu L, Liu B, Yang L, Meng X, Zhang W, Ma Y, Xiao H (2011) Genome-wide microRNA profiles identify miR-378 as a serum biomarker for early detection of gastric cancer. Cancer Lett 316: 196-203.

Liu J, Zhu X, Xu X, Dai D (2014) DNA promoter and histone H3 methylation downregulate NGX6 in gastric cancer cells. Med Oncol 31: 138-143.

Lu WD, Zuo Y, Xu Z, Zhang M (2014) MiR-19a promotes epithelial-mesenchymal transitionthrough PI3K/AKT pathway in gastric cancer. Int J Clin Exp Pathol 7: 7286-7296.

Margue C, Reinsbach S, Philippidou D, Beaume N, Walters C, Schneider JG, Nashan D, Behrmann I, Kreis S (2015) Comparison of a healthy miRNome with melanoma patient miRNomes: Are microRNAs suitable serum biomarkers for cancer? Oncotarget 6: 12110-12127.

Mirmajidi SH, Najafi M, Mirmajidi ST, Nasrabadi NN (2016) Study of regulatory promoter polymorphism (-248 G>A) of Bax gene in patients with gastric cancer in the northern provinces of Iran. Gastroenterol Hepatol Bed Bench 9: 36-44.

Nam KS, Oh S, Lee KM, Yoo SA, Shin I (2015) CD44 regulates cell proliferation, migration, and invasion via modulation of c-Src transcription in human breast cancer cells. Cell Signal 27: 1882-1894.

Parvathi MVS, Murthy PB, Vennila M, Suresh BV (2013) Regulation of BMI1 Polycomb gene expression in histological grades of invasive ductal breast carcinomas and its correlation with hormone receptor status. Tumour Biol 34: 3807-3815.

Pei-Fei, Sheng-Can, Chen, Tian, Xiao-Ming, Jiang, Yong-Fu, Shao, Bing-Xiu, Xiao (2014) Non-coding RNAs and gastric cancer. World J Gastroenterol 20: 5411-5419.

Rui H, Wenming L, Xingfeng Q, Zhenghe L, Yan X, Xingya H, Reyila P, Zhongquan Q, Guohong Z, Zhongchen L (2016) Expression of tumor necrosis factor-α-induced protein 8 in stage III gastric cancer and the correlation with DcR3 and ERK1/2. Oncol Lett 11: 1835-1840.

Schmidt K, Moser C, Hellerbrand C, Zieker D, Wagner C, Redekopf J, Schlitt HJ, Geissler EK, Lang SA (2015) Targeting fibroblast growth factor receptor (FGFR) with BGJ398 in a gastric cancer model. Anticancer Res 35: 6655-6665.

Tan Y, Ge G, Pan T, Wen D, Chen L, Yu X, Zhou X, Gan J (2014) A serum microRNA panel as potential biomarkers for hepatocellular carcinoma related with hepatitis B virus. Plos One 9: e107986-e107986.

Tsai MM, Huang HW, Wang CS, Lee KF, Tsai CY, Lu PH, Chi HC, Lin YH, Kuo LM, Lin KH (2016) MicroRNA-26b inhibits tumor metastasis by targeting the KPNA-2/c-jun pathway in human gastric cancer. Oncotarget 7: 39511-39526.

Venkatadri R, Muni T, Iyer AKV, Yakisich JS, Azad N (2016) Role of apoptosis-related miRNAs in resveratrol-induced breast cancer cell death. Cell Death Dis 7: e2104.

Wu X, Tang H, Liu G, Hui W, Jie S, Feng S (2016) miR-448 suppressed gastric cancer proliferation and invasion by regulating ADAM10. Quart J Royal Meteorol Soc 268: 1-7.

Yan JY, Tian FM, Hu WN, Zhang JH, Cai HF, Li N (2013) Apoptosis of human gastric cancer cells line SGC 7901 induced by garlic-derived compound S-allylmercaptocysteine (SAMC). Eur Rev Med Pharmacol Sci 17: 745-751.

Zhang Z, Dou M, Yao X, Tang H, Li Z, Zhao X (2016) Potential biomarkers in diagnosis of human gastric cancer. Cancer Invest 34: 115-122.

Zhang N, Su Y, Xu L (2013) Targeting PKCε by miR-143 regulates cell apoptosis in lung cancer. Febs Lett 587: 3661-3667.