

# Long Noncoding-RNA Component of Mitochondrial RNA Processing Endoribonuclease Promotes Gastric Cancer Cell Multiplication, Migration, and Invasion

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## Abstract

Since RMRP was found to target microRNA-766-5p (miR-766-5p) in TNBC cells, it enhanced cell viability and decreased apoptosis by silencing miR-766-5p. Additionally, RMRP has been proven to be highly involved in either pathological or physiological processes. Therefore, the study aims to determine whether RMRP promotes the multiplication, migration, and invasion of gastric cancer cells. The study will overexpress RMRP in GC1401 gastric cancer cells and measure cell growth by MTT assay, migration by wound healing assay, and Boyden chamber assay. Positive control is another treatment previously shown to increase cancer cell growth and migration, such as IQGAP3, which, beginning with the early stages of tumor growth, is markedly up-regulated in human stomach cancer. Furthermore, the negative control is DMSO. The study measures miR206 by qRT-PCR. If the solution gets dark through MTT assay, RMRP promotes gastric cancer cell multiplication. If the migration rate is getting quicker according to the wound healing assay, and the number of migration cells increases by Boyden chamber assay, RMRP promotes gastric cancer cell migration. If miR-206 may affect the expression of HIF-1 to control cell growth and ECM buildup, RMRP promotes gastric cancer cell invasion by acting as a miR-206 sponge for gastric cancer. The RNA component of miRNA processing endoribonuclease, the novel biomarker for gastric cancer, promotes gastric cancer cell multiplication, migration, and invasion by acting as a miR-206 loss of function in cell lines for gastric cancer.

**Keywords-Long** Noncoding-RNA, Mitochondrial RNA Processing Endoribonuclease, Gastric Cancer, Cell Multiplication, Cell Migration, Cell Invasion

## 1. Introduction

The second-leading factor in cancer-related fatalities globally is still gastric cancer, even though the incidence of gastric cancer has declined globally over the past three decades, and Asian nations have seen a similar trend. Meanwhile, gastric cancer mortality has significantly decreased. Nevertheless, it still imposes a sizable burden [1]. In recent years, there are three new gastric cancer screening methods—upper gastrointestinal endoscopy, serological testing, and the “screen and treat” method—were all thoroughly examined for their efficacy. Additionally, using the road map for the development of biomarkers, the stages of development for cancer screening were examined. In Chisato Hamashima’s study, the detail of Phase 3 of Biomarker development process is that “Biomarker detects disease early before it becomes clinical and a ‘screen positive’ rule is defined” [1]. Furthermore, progress in improving disease-free survival in gastric cancer patients is severely limited by the lack of ideal tumor markers for early diagnosis, individualized treatment, prognostic assessment, and prediction of postoperative recurrence risk [2,3]. Therefore, current

gastric cancer research primarily focuses on should be on identifying and searching for new biomarkers [4].

RMRP, a part of the mitochondrial RNA processing endoribonuclease, was initially described as a substance that cleaved miRNA at a priming location of miDNA replication despite being a long non-coding RNA (lncRNA) [5]. It is known to act as a tumor-propeller in some cancers. However, its exact mechanism in gastric cancer is still unclear, even though it has been confirmed to exhibit the role of carcinogen in gastric cancer [4,6]. Furthermore, the lncRNAs have been proven to be highly involved in either pathological or physiological processes [7,8]. In a number of human tissues, it is strongly expressed and plays a crucial role in early embryogenesis development [9].

Three years ago, the miRNA “sponge” technique was introduced as a way to continuously deactivate miRNA in cell lines and transgenic organisms. Sponge RNAs, which are made in cells from transgenes, have complementary binding sites to a miRNA of interest. In this experiment, the miR-206 sponge is considered to be used to target HIF-1 $\alpha$  to modulate cell proliferation and ECM accumulation.

Thus, RMRP can promote gastric cancer cell invasion. Endogenous single-stranded short RNA molecules, miRNAs play no role in coding [10]. Cell death, metastasis, invasion, growth, differentiation, and angiogenesis are just a few of the several tumor-related processes where miRNAs play a crucial regulatory role in biological processes and act as either oncogenes or anti-oncogenes [11]. miRNAs are important in many cancers [12,13]. For instance, miR-206 regulates the expression of KIF2A negatively. Also, it's linked to patients with ovarian carcinoma who had a worse prognosis [12]. Additionally, miR-206 may prevent angiogenesis and cell epithelial-mesenchymal transition induced by hepatocyte growth factor in non-small-cell lung cancer [13]. According to a recent study, miR-206 levels were found to be decreased in oral cancers, and miR-206 may be a useful biomarker for early-stage diagnosis [14]. It has been demonstrated that MiR-206 regulates the behavior of cancer stem cells in hepatocellular carcinoma [15]. A more significant finding was that miR-206 was related to the development of the laryngeal squamous cell carcinoma [16].

The phenomena of RMRP downregulation implies, in accordance with a prior work by Shao's team, that lncRNAs have a significant association with the development of gastric cancer [4]. Recently, as the number of dysregulated lncRNAs in gastric cancer rises, Shao's team found that RMRP was the first to exhibit dysregulated expression in gastric cancer [17–20].

Then, it was looked into how RMRP contributes to gastric tumorigenesis molecularly. The release of cyclin D2 transcripts from RMRP was discovered to accelerate the cell cycle of gastric cancer and expand the tumor [5]. It indicated that RMRP has a major impact on the development and spread of gastric cancer and might serve as a cutting-edge biomarker for early detection and prognosis prediction.

## 1.1. Hypothesis

Predict that the RNA component of mitochondrial RNA processing endoribonuclease, RMRP promotes gastric cancer cell multiplication, migration, and invasion by acting as a miR-206 sponge for gastric cancer.

## 2. Materials and Methods

### 2.1. Overexpress RMRP in GC1401 Gastric Cancer Cells

Pritoneal effusions from patients with gastric adenocarcinoma, mice, and 5% fetal bovine serum need to be prepared.

The cancer cell line, designated as gastric cancer (GC)1401 was derived from peritoneal effusions from

patients with gastric adenocarcinoma. The cell line was created using tissue culture and NOD/SCID (non-obese diabetic, immunodeficient, and severe combined immunodeficiency) mice. The cell line was cultured in Dulbecco's modified Eagle's medium with 5% fetal bovine serum added. The doubling time of the cell line, which was cultured as an adherent monolayer with a doubling time of between 25 and 34 hours (GC1436 cell line), was (GC1401 and GC1415, respectively).

### 2.2. Measure Cell Growth by MTT Assay

Formazan crystals and yellow tetrazolium salt need to be made.

The MTT test measures cell viability, proliferation, and cytotoxicity by measuring cellular metabolic activity. A yellow tetrazolium salt (MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is transformed into purple formazan crystals by metabolically active cells in this colorimetric experiment [21,22,23]. NAD(P) H-dependent oxidoreductase enzymes are present in the living cells and convert the MTT to formazan [24]. A solubilization solution is used to dissolve the insoluble formazan crystals, and a multi-well spectrophotometer is used to measure the colored solution's absorbance at 500–600 nanometers. The more the fluid is opaque, the more living, metabolically active cells there are.

Measure the cell growth with MTT assay through the process of cultivation of gastric cancer (GC)1401.

### 2.3. Migration by Wound Healing Assay

To measure the migration rate of the gastric cells, the basic stages entail making a "wound" in a cell monolayer, taking pictures at various points throughout cell movement to close the wound, and comparing the pictures.

### 2.4. Boyden Chamber Assay

DNA dyes need to be prepared.

Gastric cells are seeded onto a semi-permeable membrane cell culture insert with an addition of chemoattractants below the membrane, and are then allowed to move across a cell monolayer or ECM protein combination. The number of migrating cells can then be determined by staining cells with DNA dyes as CyQUANT GR Dyes or Calcein-AM.

It is ideally suited for the quantitative analysis of different migratory responses of gastric cells and assessing gastric cell motility and invasion.

### 2.5. Positive Control

The IQGAP3 gene, a member of the IQGAP gene family, was discovered to be considerably up-regulated in human gastric cancer in Jinawath's study, beginning with the initial stages of tumor progression. In both 293T and

NIH3T3 cells, which don't express endogenous IQGAP3, overexpression of IQGAP3 caused morphological changes with many protrusions that resembled dendrites and increased migration. Additionally, IQGAP3 overexpression reduced cell-cell adhesion in 293T cells, most likely as a result of interactions with the proteins e-cadherin or -catenin. Additionally, IQGAP3 accumulated along the leading edge of migrating cells as well as close to the cleavage furrow of dividing cells. TMK-1 and MKN1 gastric cancer cells had much less invasion and anchorage-independent growth when IQGAP3 was suppressed by short-interfering RNA (siRNA). They went on to confirm that IQGAP3 did interact with GTPases from the Rho family and had a significant role in cytokinesis. Also, they showed that IQGAP3 affects cytoskeletal reorganization, cell migration, and adhesion and plays a crucial role in the migration and invasion of human gastric cancer cells.

**2.6. Negative Control**

For the purpose of gathering useful data, negative controls are just as crucial as positive controls. Always compare the outcomes of the target RNA or siRNA-treated and control-treated cells by transfecting a batch of cells with an equimolar amount of at least one negative control. Data from these significant controls act as a benchmark for assessing experimental target knockdown.

Negative controls that aren't transfected or consist simply of cells are particularly helpful in the experiment. The impact of transfection on gastric cell viability can be determined by comparing the expression of

a housekeeping gene between cultures that weren't transfected and cultures that were transfected with a non-targeting negative control.

**2.7. Measures miR206 by qRTPCR**

The findings of Cao's study indicate that HG-treated hMCs had downregulated miR-206 levels. In HG-induced hMCs, cell proliferation was stimulated; however, miR-206 mimics greatly inhibited this behavior. MiR-206 mimics significantly elevated p21 expression and decreased cyclin D1 and CDK2 expression, the opposite of what was seen in HG-induced hMCs. Additionally, hMCs treated with HG had noticeably higher levels of ECM proteins, which miR-206 mimics greatly reduced. The effects of a miR-206 inhibitor were the opposite. Additionally, it was discovered that miR-206 directly targets HIF-1 and that miR-206 adversely regulates HIF-1 in hMCs. HIF-1 can be targeted by miR-206 to influence cell growth and ECM buildup [25].

**3. Statistical analysis**

The standard error of the mean and itself was used to present all data (S.E.M.). The 2 analysis, Pearson's coefficient correlation assay and Fisher exact probability analysis were used to evaluating gene expression and examine the association between genes. The log-rank test and Kaplan-Meier method were used to evaluate survival rates and variations. To compare tumor cells, the t-test or analysis of variance (ANOVA) was performed. [26].

**3.1. Possible Results (as shown in Table 1)**

**Table 1. Combination of Possible Results (CR)**

Possible Observation	CR1	CR2	CR3	CR4	CR5	CR6	CR7	CR8
Cellular multiplication increases by MTT?	+	+	+	+	-	-	-	-
Migration increases by wound healing assay?	+	-	+	-	+	+	-	-
Migration increases by boyden chamber assay?	+	+	-	-	+	-	+	-
Supporting hypothesis	YES	PARTIALLY	PARTIALLY	PARTIALLY	PARTIALLY	PARTIALLY	PARTIALLY	NO

Note. "+" represents a positive result or is close to the positive control.  
 "-" represents a negative result or is close to the negative control.

**Possible result 1 (as shown in Table 2):** The gastric cells grow and cellular multiplication increases according to MTT, migration increases based on wound healing assay. At the same time, overexpression of RMRP in GC1401 gastric cancer cells leads to increasing migration based on boyden chamber assay. This demonstrates that RMRP promotes gastric cancer cell proliferation, migration, and invasion by functioning as a miR-206 sponge for gastric cancer.

**Possible result 2 (as shown in Table 2):** The gastric cells grow and cellular multiplication increases according to MTT, migration decreases based on wound healing assay. At the same time, overexpression of RMRP in GC1401 gastric cancer cells leads to increasing migration based on boyden chamber assay. This demonstrates that RMRP can probably promote gastric cancer cell proliferation, migration, and invasion by functioning as a miR-206 sponge for gastric cancer.

**Table 2. Possible Observation 1&2**

Possible Observation	CR1	CR2
Cellular Multiplication increases by MTT?	+	+
Migration increases by Wound Healing Assay?	+	-
Migration increases by Boyden Chamber Assay?	+	+
Supporting hypothesis?	YES	PARTIALLY

**Possible result 3 (as shown in Table 3):** The gastric cells grow and cellular multiplication increases according to MTT, migration increases based on wound healing assay. At the same time, overexpression of RMRP in GC1401 gastric cancer cells leads to decreasing migration based on boyden chamber assay. This indicates that RMRP partially promotes gastric cancer cell proliferation, migration, and invasion by functioning as a miR-206 sponge for gastric cancer.

**Possible result 4 (as shown in Table 3):** The gastric cells grow and cellular multiplication increases according to MTT, migration decreases based on wound healing assay. At the same time, overexpression of RMRP in GC1401 gastric cancer cells leads to the decreasing migration on the basis of boyden chamber assay. This indicates that RMRP might not promote gastric cancer cell proliferation, migration, and invasion by functioning as a miR-206 sponge for gastric cancer.

**Table 3. Possible observation 3&4**

Possible Observation	CR3	CR4
Cellular Multiplication increases by MTT?	+	+
Migration increases by Wound Healing Assay?	+	-
Migration increases by Boyden Chamber Assay?	-	-
Supporting hypothesis?	PARTIALLY	PARTIALLY

**Possible result 5 (as shown in Table 4):** The gastric cells grow and cellular multiplication decreases according to MTT, migration increases based on wound healing assay. At the same time, overexpression of RMRP in GC1401 gastric cancer cells leads to increased migration on the basis of boyden chamber assay. This demonstrates that RMRP can probably promote gastric cancer cell proliferation, migration, and invasion by functioning as a miR-206 sponge for gastric cancer.

**Possible result 6 (as shown in Table 4):** The gastric cells grow and cellular multiplication decreases according to MTT, migration increases based on wound healing assay. At the same time, overexpression of RMRP in GC1401 gastric cancer cells leads to decreasing migration based on boyden chamber assay. This demonstrates that RMRP can probably promote gastric cancer cell proliferation, migration, and invasion by functioning as a miR-206 sponge for gastric cancer.

**Table 4. Possible result 5&6**

Possible Observation	CR5	CR6
Cellular Multiplication increases by MTT?	-	-

Possible Observation	CR5	CR6
Migration increases by Wound Healing Assay?	+	+
Migration increases by Boyden Chamber Assay?	+	-
Supporting hypothesis?	PARTIALLY	PARTIALLY

**Possible result 7 (as shown in Table 5):** The gastric cells grow and cellular multiplication decreases according to MTT, migration decreases based on wound healing assay. At the same time, overexpression of RMRP in GC1401 gastric cancer cells leads to increasing migration based on boyden chamber assay. This indicates that RMRP might not promote gastric cancer cell proliferation, migration, and invasion by functioning as a miR-206 sponge for gastric cancer.

**Possible result 8 (as shown in Table 5):** The gastric cells grow and cellular multiplication decreases according to MTT, migration decreases based on wound healing assay. At the same time, overexpression of RMRP in GC1401 gastric cancer cells leads to decreasing migration based on boyden chamber assay. This indicates that RMRP can't promote gastric cancer cell proliferation, migration, and invasion by functioning as a miR-206 sponge for gastric cancer.

**Table 5. Possible result 7&8**

Possible Observation	CR3	CR4
Cellular Multiplication increases by MTT?	-	-
Migration increases by Wound Healing Assay?	-	-
Migration increases by Boyden Chamber Assay?	+	-
Supporting hypothesis?	PARTIALLY	NO

#### 4. Discussion

The role of lncRNAs in the development and spread of stomach cancer is critical. In prior studies, one of the dysregulated lncRNAs in the overall expression profile of gastric cancer was discovered to be RMRP. The goal of the current investigation was to assess the diagnostic value of RMRP and the molecular mechanisms behind gastric carcinogenesis.

Tumorigenesis has several stages. Phenotypic multistep progression cascades are used to describe the stages of gastric carcinogenesis. Dysplasia is a precancerous stomach lesion that is an important step in the progression of gastric cancer. First, qRT-PCR was used to compare the levels of RMRP expression between gastric cancer tissues and the matching non-tumorous tissues. We discovered that gastric cancer tissues had downregulated RMRP levels. The expression of RMRP in tissues from gastric cancer, gastric dysplasia, erosive gastritis, gastric ulcers, and healthy gastric mucosa was then investigated. The findings demonstrated that tissues with gastric dysplasia and gastric cancer had considerably lower levels of RMRP expression. The phenomena of tissue-specific downregulation revealed a significant association between RMRP and gastric cancer [4]

Thus, we concentrate on the overexpression of RMRP and the association between RMRP and gastric cancer.

From possible observation 2 to possible observation 7, at least one factor contradict with the standard one that leads to the positive result. In possible observation 1, the hypothesis is totally supported. In contrast, possible observation 8 entirely opposes the hypothesis. Therefore, it demonstrates that RMRP promotes gastric cancer cell multiplication, migration, and invasion by functioning as a miR-206 loss of function in cell lines for gastric cancer.

#### 5. Conclusion

RNA component of mitochondrial RNA processing endoribonuclease, RMRP, the novel biomarker for gastric cancer promotes gastric cancer cell multiplication, migration, and invasion by acting as a miR-206 loss of function in cell lines for gastric cancer.

#### Reference

- [1] Hamashima, Chisato. "Current issues and future perspectives of gastric cancer screening." *World Journal of Gastroenterology: WJG* 20.38 (2014): 13767.
- [2] Wu, Hua-Hsi, Wen-chang Lin, and Kuo-Wang Tsai. "Advances in molecular biomarkers for gastric cancer: miRNAs as emerging novel cancer markers." *Expert reviews in molecular medicine* 16 (2014).
- [3] Durães, Cecília, et al. «Biomarkers for gastric cancer: prognostic, predictive or targets of therapy?.» *Virchows Archiv* 464.3 (2014): 367-378.

- [4] Shao, Yongfu, et al. "LncRNA-RMRP promotes carcinogenesis by acting as a miR-206 sponge and is used as a novel biomarker for gastric cancer." *Oncotarget* 7.25 (2016): 37812.
- [5] Hao, Qian, and Xiang Zhou. "The emerging role of long noncoding RNA RMRP in cancer development and targeted therapy." *Cancer Biology & Medicine* 19.2 (2022): 140.
- [6] Qi, Liqiang, et al. "Long Noncoding-RNA Component of Mitochondrial RNA Processing Endoribonuclease Promotes Carcinogenesis in Triple-Negative Breast Cancer Cells via the Competing Endogenous RNA Mechanism." *Journal of Breast Cancer* 24.5 (2021): 428.
- [7] Li, Jing, Zhenyu Xuan, and Changning Liu. "Long non-coding RNAs and complex human diseases." *International journal of molecular sciences* 14.9 (2013): 18790-18808.
- [8] Fatica, Alessandro, and Irene Bozzoni. "Long non-coding RNAs: new players in cell differentiation and development." *Nature Reviews Genetics* 15.1 (2014): 7-21.
- [9] Rosenbluh, Joseph, et al. "RMRP is a non-coding RNA essential for early murine development." *PLoS one* 6.10 (2011): e26270.
- [10] Ghafouri-Fard, Soudeh, et al. "An update on the role of miR-379 in human disorders." *Biomedicine & Pharmacotherapy* 139 (2021): 111553.
- [11] Liu, W. H., et al. "LINC00641 regulates prostate cancer cell growth and apoptosis via the miR-365a-3p/VGLL4 axis." *Eur Rev Med Pharmacol Sci* 25.1 (2021): 108-15.
- [12] Bai, Hua, and Suhui Wu. "miR-451: a novel biomarker and potential therapeutic target for cancer." *OncoTargets and therapy* 12 (2019): 11069.
- [13] Zhou, Zhong-xin, et al. "miR-632 promotes laryngeal carcinoma cell proliferation, migration, and invasion through negative regulation of GSK3 $\beta$ ." *Oncology research* 28.1 (2020): 21.
- [14] Wang, Lili, Hongguang Song, and Shiming Yang. "MicroRNA-206 has a bright application prospect in the diagnosis of cases with oral cancer." *Journal of Cellular and Molecular Medicine* 25.17 (2021): 8169-8173.
- [15] Ghafouri-Fard, Soudeh, et al. "The impact of lncRNAs and miRNAs in regulation of function of cancer stem cells and progression of cancer." *Frontiers in Cell and Developmental Biology* (2021): 2036.
- [16] Wang, Xin, et al. "Regulation of laryngeal squamous cell cancer progression by the lncRNA RP11-159K7. 2/miR-206/DNMT3A axis." *Journal of Cellular and Molecular Medicine* 24.12 (2020): 6781-6795.
- [17] Fang, Xin-yu, et al. "Long noncoding RNAs: novel insights into gastric cancer." *Cancer letters* 356.2 (2015): 357-366.
- [18] Song, Haojun, et al. "Long non-coding RNA expression profile in human gastric cancer and its clinical significances." *Journal of translational medicine* 11.1 (2013): 1-10.
- [19] Xia, Tian, et al. "Long noncoding RNA FER1L4 suppresses cancer cell growth by acting as a competing endogenous RNA and regulating PTEN expression." *Scientific reports* 5.1 (2015): 1-9.
- [20] Xie, Min, et al. "Long noncoding RNA HOXA-AS2 promotes gastric cancer proliferation by epigenetically silencing P21/PLK3/DDIT3 expression." *Oncotarget* 6.32 (2015): 33587.
- [21] Vistica VT, Skehan P, Scudiero D, Monks A, Pittman A, Boyd MR. 1991. Tetrazolium-based assays for cellular viability: a critical examination of selected parameters affecting formazan production. *Cancer Res.* 51(10):2515-20.
- [22] Maehara Y, Anai H, Tamada R, Sugimachi K. 1987. The ATP assay is more sensitive than the succinate dehydrogenase inhibition test for predicting cell viability. *European Journal of Cancer and Clinical Oncology.* 23(3):273-276.
- [23] Slater T, Sawyer B, Sträuli U. 1963. Studies on succinate-tetrazolium reductase systems. *Biochimica et Biophysica Acta.* 77383-393.
- [24] Berridge M, Tan A. 1993. Characterization of the Cellular Reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular Localization, Substrate Dependence, and Involvement of Mitochondrial Electron Transport in MTT Reduction. *Archives of Biochemistry and Biophysics.* 303(2):474-482.
- [25] Cao, Yanchao, et al. "miR-206 inhibits cell proliferation and extracellular matrix accumulation by targeting hypoxia-inducible factor 1-alpha (HIF-1 $\alpha$ ) in mesangial cells treated with high glucose." *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research* 25 (2019): 10036.
- [26] Zheng, Liduan, et al. "miRNA-558 promotes gastric cancer progression through attenuating Smad4-mediated repression of heparanase expression." *Cell death & disease* 7.9 (2016): e2382-e2382.