MicroRNA-125a-5p Is an Independent Prognostic Factor in Gastric Cancer and Inhibits the Proliferation of Human Gastric Cancer Cells in Combination with Trastuzumab

Naohiro Nishida1,2, Koshi Mimori1, Muller Fabbri3, Takehiko Yokobori1, Tomoya Sudo1, Fumiaki Tanaka1, Kohei Shibata1, Hideshi Ishii1,2, Yuichiro Doki2, and Masaki Mori1,2

Abstract

**Purpose:** MicroRNA 125a-5p (miR-125a-5p) has been reported to be a tumor suppressor in malignancies of the breast, ovary, lung, and central nervous system. However, the clinical significance of miR-125a-5p in human gastrointestinal cancer has not been explored. We investigated a tumor inhibitory effect of miR-125a-5p in gastric cancer, focusing in particular on the miR-125a–ERBB2 (HER2, HER-2/neu) pathway.

**Experimental Design:** Quantitative RT-PCR was used to evaluate miR-125a-5p expression in 87 gastric cancer cases to determine the clinicopathologic significance of miR-125a-5p expression. The regulation of ERBB2 by miR-125a-5p was examined with precursor miR-125a–transfected cells. Furthermore, we investigated whether miR-125a-5p suppresses proliferation of gastric cancer cells in combination with trastuzumab, a monoclonal antibody against ERBB2.

**Results:** Low expression levels of miR-125a-5p were associated with enhanced malignant potential such as tumor size (P = 0.0068), tumor invasion (P = 0.031), liver metastasis (P = 0.029), and poor prognosis (P = 0.0069). Multivariate analysis indicated that low miR-125a-5p expression was an independent prognostic factor for survival. *In vitro* assays showed that ERBB2 is a direct target of miR-125a-5p, which potently suppressed the proliferation of gastric cancer cells, and, interestingly, the growth inhibitory effect was enhanced in combination with trastuzumab.

**Conclusions:** miR-125a-5p is a meaningful prognostic marker. Furthermore, miR-125a-5p mimic alone or in combination with trastuzumab could be a novel therapeutic approach against gastric cancer. *Clin Cancer Res;* 17(9); 2725-33. ©2011 AACR.

Introduction

MicroRNAs (miRNA) constitute a class of small (19–25 nucleotides) noncoding RNAs that function as posttranscriptional gene regulators. miRNAs regulate gene expression by binding to their mRNAs (1). Alterations in miRNA expression are involved in the initiation, progression, and metastasis of human cancer, and it is believed that miRNAs function both as tumor suppressors and oncogenes in cancer development (2, 3).

Recent studies have shown that the expression of miR125a-5p is downregulated in several human cancers such as breast cancer (4–6), ovarian cancer (7), lung cancer (8), and medulloblastoma (9). Li and colleagues reported that a germline mutation in mature miRNA 125a-5p (miR-125a-5p) is closely associated with breast cancer tumorigenesis (5). Other reports showed that epidermal growth factor (EGF) receptor signaling suppresses miR-125a-5p expression and leads to cancer metastasis in lung cancer (8) and ovarian cancer (10). Furthermore, in squamous cell carcinoma of the oral cavity, the levels of miR-125a-5p were significantly downregulated in the saliva of patients (11). These findings strongly suggest that the function of miR-125a-5p as a tumor suppressor is not organ specific.

Scott and colleagues revealed that miR-125a-5p and its homologue, miR-125b, regulate ERBB2 and ERBB3 in human breast cancer cells (12). In gastric cancer, ERBB2 overexpression has been increasingly recognized as a frequent molecular abnormality and as an important therapeutic target similar to breast cancer (13, 14). Preclinical and clinical data have revealed significant efficacy of anti-ERBB2 therapies, especially trastuzumab (Herceptin), a monoclonal antibody directed at ERBB2 in gastric cancer (15, 16).
In this study, we showed that miR-125a-5p functions as a crucial tumor suppressor in human gastric cancer. Low miR-125a-5p expression was correlated with more aggressive disease and poorer prognosis and was an independent prognostic factor. Of the numerous target genes of miR-125a-5p, we focused on ERBB2 and discovered that miR-125a-5p regulates ERBB2 in human gastric cancer cells. miR-125a-5p potently suppressed the proliferation of gastric cancer cells. Moreover, the growth inhibitory effect was enhanced in combination with trastuzumab, a monoclonal antibody against ERBB2. miR-125a-5p is a meaningful prognostic indicator. Furthermore, miR-125a-5p mimic alone or in combination with trastuzumab could be a novel therapeutic approach against gastric cancer.

Materials and Methods

Clinical cases

Patients and sample collection. Eighty-seven gastric cancer samples were obtained during surgery and used after obtaining informed consent. All patients underwent resection of the primary tumor at Kyushu University Hospital at Beppu and affiliated hospitals between 1992 and 2000. Written informed consent was obtained from all patients. All patients had a clear histologic diagnosis of gastric cancer, based on the clinicopathologic criteria described by the Japanese Gastric Cancer Association (17). All patients were closely followed every 3 months. The follow-up periods ranged from 0.2 months to 12.3 years, with a mean of 2.6 years. Resected cancer samples were obtained during surgery and used after obtaining informed consent. All patients underwent resection of the primary tumor at Kyushu University Hospital after obtaining informed consent. All patients underwent resection of the primary tumor at Kyushu University Hospital after obtaining informed consent.

Evaluation of miR-125a-5p expression in clinical samples

For miR-125a-5p quantitative real-time reverse transcriptase PCR (RT-PCR), cDNA was synthesized from 10 ng of total RNA using TaqMan MicroRNA hsa-miR-125a-5p specific primers (Applied Biosystems) and a TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems). RT-PCR protocols are described in Supplementary Data.

Evaluation of ERBB2, DACH1, and PDCD6 mRNA expression in gastric cancer cells

For RNA analysis, each cell line was seeded at $2 \times 10^5$ cells per well in a volume of 2 mL in 6-well flat-bottomed microtiter plates. Total RNA from cell lines was isolated using the mirVana miRNA Isolation Kit (Ambion) after 48-hour incubation. Quantitative RT-PCR was carried out to measure ERBB2, dachshund homolog 1 (DACH1), and programmed cell death 6 (PDCD6) mRNA expression with the Universal Probe Library Probe (UPL; Roche Diagnostics). Primer sequences corresponding to UPL and RT-PCR protocols are described in Supplementary Data.

Immunohistochemistry

Immunohistochemical studies of ERBB2 were conducted on formalin-fixed, paraffin-embedded (FFPE) surgical sections obtained from patients with gastric cancer. Tissue sections were deparaffinized, soaked in 0.01 mol/L sodium citrate buffer, and boiled in a microwave oven for 5 minutes at 500 W to retrieve cell antigens. Mouse monoclonal antibody against ERBB2 (Epitomics, Inc.) diluted 1:400 was used as the primary antibody. All tissue sections were immunohistochemically stained with the avidin–biotin-peroxidase method (LSAB+ System HRP; Dako, Inc.) and counterstained with hematoxylin.

Evaluation of ERBB2 immunohistochemical staining

The slides were examined and scored independently by 2 experienced pathologists. Evaluation of the results was done according to the criteria recommended by Hofmann and colleagues and other groups (19, 20), by assigning a score from 0 to 3+. Scores were defined as follows: 0, no reactivity or membranous reactivity in less than 10% of cells; 1+, faintly perceptible membranous reactivity in 10% or more of cells; cells are reactive only in part of their membrane; 2+, weak to moderate complete or basolateral membranous reactivity in more than 10% or more of tumor cells; 3+, moderate to strong complete or basolateral membranous reactivity in more than 10% or more of tumor cells.

Quantitative real-time reverse transcriptase PCR analysis of microRNA 125a-5p (miR-125a-5p) in 87 cases of gastric cancer revealed that low expression levels of miR-125a-5p were associated with enhanced malignant potential such as tumor size, tumor invasion, liver metastasis, and poor prognosis. To evaluate the function of miR-125a-5p, we focused on the miR-125a–ERBB2 (HER2, HER-2/neu) pathway. In gastric cancer, ERBB2 overexpression has been increasingly recognized as an important therapeutic target similar to that in breast cancer. Our data suggested that miR-125a-5p directly targets ERBB2. miR-125a-5p reportedly suppressed the proliferation of gastric cancer cells and, moreover, the growth inhibitory effect was enhanced in combination with trastuzumab, a monoclonal antibody against ERBB2. miR-125a-5p is a meaningful prognostic indicator. Furthermore, miR-125a-5p mimic alone or in combination with trastuzumab could be a novel therapeutic approach against gastric cancer.

Translational Relevance

Quantitative real-time reverse transcriptase PCR analysis of microRNA 125a-5p (miR-125a-5p) in 87 cases of gastric cancer revealed that low expression levels of miR-125a-5p were associated with enhanced malignant potential such as tumor size, tumor invasion, liver metastasis, and poor prognosis. To evaluate the function of miR-125a-5p, we focused on the miR-125a–ERBB2 (HER2, HER-2/neu) pathway. In gastric cancer, ERBB2 overexpression has been increasingly recognized as an important therapeutic target similar to that in breast cancer. Our data suggested that miR-125a-5p directly targets ERBB2. miR-125a-5p reportedly suppressed the proliferation of gastric cancer cells and, moreover, the growth inhibitory effect was enhanced in combination with trastuzumab, a monoclonal antibody against ERBB2. miR-125a-5p is a meaningful prognostic indicator. Furthermore, miR-125a-5p mimic alone or in combination with trastuzumab could be a novel therapeutic approach against gastric cancer.

In this study, we showed that miR-125a-5p functions as a crucial tumor suppressor in human gastric cancer. Low miR-125a-5p expression was correlated with more aggressive disease and poorer prognosis and was an independent prognostic factor. Of the numerous target genes of miR-125a-5p, we focused on ERBB2 and discovered that miR-125a-5p regulates ERBB2 in human gastric cancer cells. miR-125a-5p potently suppressed the proliferation of gastric cancer cells. Moreover, the growth inhibitory effect was enhanced in combination with trastuzumab. This is the first report describing the clinical significance of miR-125a-5p and its growth inhibitory effect in human gastric cancer.

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membranous reactivity in ≥10% or more of tumor cells. Specimens with scores of 0 and 1+ were regarded as being negative for ERBB2 expression, whereas scores of 2+ and 3+ indicated positive expression of ERBB2.

**Experimental studies**

**Cell lines and cell culture.** The human gastric cancer cell lines AZ521, KATO, MKN1, MKN45, MKN74, NUGC3, and NUGC4 were provided by the Cell Resource Center of Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University. These cell lines were maintained in RPMI 1640 containing 10% FBS with 100 units/mL penicillin and 100 units/mL streptomycin sulfates and cultured in a humidified 5% CO2 incubator at 37°C.

**Transfection of miRNA-125a precursor (Pre-miR-125a)**

Using NUGC4, a gastric cancer cell line that expresses a high level of ERBB2 mRNA, either Pre-miR-125a or Pre-miR negative control (Ambion Pre-miR miRNA Precursors Applied Biosystems), was transfected at 30 nmol/L (final concentration) by using Lipofectamine RNAiMAX (Invitrogen Life Technologies) according to the manufacturer’s instruction.

**In vitro assays**

The MTT assay for gastric cancer cell growth after transfection with Pre-miR-125a with/without trastuzumab treatment. Logarithmically growing NUGC4 cells were transfected with Pre-miR-125a or Pre-miR negative control with or

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**Table 1. miR-125a-5p expression and clinicopathologic factors**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Low expression group (n = 55)</th>
<th>High expression group (n = 32)</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>n %</td>
<td>n %</td>
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<tr>
<td>Age (mean ± SD)</td>
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<td>18 32.7</td>
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<td>37 67.3</td>
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<td>Depth of tumor invasionb</td>
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<tr>
<td>ss, se, si</td>
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<td>21 65.6</td>
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<tr>
<td>III–IV</td>
<td>33 60</td>
<td>11 34.4</td>
<td></td>
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</table>

*P < 0.05.

bTumor invasion of mucosa (m), submucosa (sm), muscularis propria (mp), subserosa (ss), penetration of serosa (se), and invasion of adjacent structures (si).
without addition of trastuzumab (0.1 or 1 μg/ml) and were seeded at 8.0 × 10^3 cells per well in 96-well flat-bottomed microtiter plates in a final volume of 100 μL of culture medium per well. Cells were incubated in a humidified atmosphere (37°C and 5% CO_2) for 24, 48, 72, and 96 hours after initiation of transfection. MTT assays were used to measure cell proliferation at each period, as described in Supplementary Data. The assay was carried out with 6 replicates.

**Plasmid construction**

The 3’ untranslated region (3’-UTR) and open reading frame (ORF) of ERBB2 were amplified by RT-PCR. The amplified product was subcloned and ligated into the pmirGLO Dual-Luciferase miRNA Target Expression Vector (Promega). The resultant reporter vector position was confirmed by sequencing and termed Luc-ERBB2 WT. To make miR-125a-5p binding site mutants, positions 37 to 43 of ERBB2 3’-UTR (the sequence; CTCAAGG) were mutated to the sequence CACCGGG (mutated nucleotides are underlined), using the QuickChange Lightning Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer’s protocol. The resultant reporter vector position was confirmed by sequencing and termed Luc-ERBB2 mutant.

**Luciferase assay**

Luciferase assays were conducted using 1 × 10^4 NUGC4 cells plated in a 96-well plate. Transfections were done with Lipofectamine 2000 (Invitrogen) in OptiMEM reduced serum media (GIBCO). Cells were transfected with 30 ng of Luc-ERBB2 WT vector or Luc-ERBB2 mutant vector and 100 nmol/L of either Pre-miR negative control or Pre-miR-125a. Twenty-four hours following transfection, cells were assayed for both firefly and Renilla luciferase, using Dual-Glo Luciferase Assay System (Promega). All transfection experiments were conducted in triplicate.

**ERBB2 and miR-125a-5p expression in the NCI60 panel**

For analysis of the correlation between ERBB2 and miR-125a-5p expression in the NCI60 panel (21), the normalized expression levels of the cDNA array and the miRNA expression in the NCI60 panel (21) was used to measure cell proliferation at each period, as described in Supplementary Data. The assay was carried out with 6 replicates.

**Protein expression analysis**

Western blotting was used to confirm the expression of ERBB2 and phosphorylated AKT, BAK1, and p53 in Pre-miR-125a-transfected cells. Primary antibodies and dilutions were as follows: ERBB2 rabbit monoclonal antibody (Epitomics, Inc.) at a 1:500 dilution; AKT rabbit monoclonal antibody (Cell Signaling Technology, Inc.) at a 1:1,000 dilution; phosphorylated AKT (p-AKT) rabbit monoclonal antibody (Cell Signaling Technology, Inc.) at a 1:2,000 dilution; BAK1 (Cell Signaling Technology, Inc.) at a 1:1,000 dilution; p53 (Dako, Inc.) at a 1:1,000 dilution. Detailed protocols are described in Supplementary Data.

**Statistical analysis**

Data from RT-PCR analysis and in vitro transfected cell assays were analyzed with JMP 5. Overall survival rates were calculated actuarially according to the Kaplan–Meier method and were measured from the day of surgery. Differences between groups were estimated using the χ² test, Student’s t test, repeated-measures ANOVA test, and the log-rank test. Variables with a value of P < 0.05 in univariate analysis were used in a subsequent multivariate analysis based on the Cox proportional hazards model. A probability level of 0.05 was chosen for statistical significance.

**Results**

**The clinicopathologic significance of miR-125a-5p mRNA expression in gastric cancer**

In this study, patients with values less than the average expression level of miR-125a-5p (8.66, normalized to RNU6B) were assigned to a low expression group (n = 32) whereas those with expression values above average were assigned to a high expression group (n = 55). Patients in the low miR-125a-5p expression group had a significantly poorer prognosis than those in the high miR-125a-5p expression group (P = 0.0069; Fig. 1). Clinicopathologic factors were significantly different in the low miR-125a-5p expression group. There were greater tumor size (P = 0.0068), tumor invasion (P = 0.031), liver metastasis...
indicated that the high expression level of overall survival are shown in Table 2. Multivariate analysis indicated that the high expression level of miR-125a-5p was an independent and significant prognostic factor for survival (OR, 2.44; CI, 1.04–6.73; P = 0.041; Table 2). Expression of ERBB2, which is a putative miR-125a-5p target, is shown to be an indicator of patient prognosis by univariate analysis (P = 0.048), although it is not an independent prognostic factor. For the comparison, using the same RNA samples, we also investigated DACH1 and PDCD6 mRNA expression, which was previously reported as prognostic factors for gastric cancer patients (22). However, in univariate analysis for overall survival, the expression levels of those 2 molecules were not superior to miR-125a-5p expression as prognostic factors, at least in the group we investigated (Supplementary Table S1).

**ERBB2 mRNA expression in gastric cancer cell lines and the effect of trastuzumab**

ERBB2 mRNA expression was examined in 7 gastric cancer cell lines by using RT-PCR. NUGC4, a human cell line derived from a signet ring cell carcinoma of the stomach, showed a remarkably high level of ERBB2 mRNA compared with other cell lines, (P < 0.0001; Supplementary Fig. S1A) and was chosen for experiments on validation of ERBB2 suppression by miR-125a-5p. MTT assays were carried out to evaluate the growth inhibitory effect of trastuzumab in gastric cancer cell lines, NUGC4, AZ521, and NUGC3. AZ521 and NUGC3 were chosen as representative low ERBB2 expression cell lines. The results indicated that trastuzumab exerted its activity selectively on NUGC4, the high expression gastric cancer cell line. At the maximum concentration of 100 μg/mL, the cell viability of NUGC4 was reduced by 28.3% ± 3.98% whereas the viability of NUGC3 and AZ521 remained above 90% (Supplementary Fig. S1B).

**miR-125a-5p regulates ERBB2 in gastric cancer cells**

Using *in silico* miRNA target prediction tools, such as miRanda (23), PicTar (24), and TargetScan (25), we identified the sequence of the miR-125a-5p binding sites in the 3’-UTRs of transcripts encoding ERBB2 (Fig. 2A). To investigate binding and repression, a luciferase reporter assay was carried out with a vector which included the ORF sequence and 3’-UTR of ERBB2 downstream from the luciferase reporter gene (Luc-ERBB2 WT). Transient cotransfection of NUGC4 cells with the reporter plasmid and Pre-miR-125a significantly reduced luciferase activity in comparison with the negative control (P < 0.05). However, the activity of the reporter construct with mutant sequence (Luc-ERBB2 mutant) was unaffected by simultaneous transfection with Pre-miR-125a (Fig. 2B). These data suggest that ERBB2 mRNA is a direct functional target of miR-125a-5p.

**miR-125a-5p expression and ERBB2 protein expression in clinical samples**

Of the 87 gastric cancer patients we examined for the expression of miR-125a-5p, FFPE surgical sections were available in 52 cases. To explore the association between miR-125a-5p expression and ERBB2 protein expression status, we carried out immunohistochemical analysis with these samples. The results showed that in the low ERBB2 expression group (ERBB2 staining 0 or 1+; n = 45), the expression of miR-125a-5p was significantly increased compared with that in the high ERBB2 expression group (ERBB2 staining 2+ or 3+; n = 7; mean ± SEM; 4.97 ± 0.88 vs. 2.34

| Table 2. Univariate and multivariate analysis for overall survival (Cox proportional regression model) |
|-----------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| **Factors**                       | **Univariate analysis** | **Multivariate analysis** |
| Age (65/>66)          | RR    | 95% CI    | P       | RR    | 95% CI    | P       |
| Sex (male/female)    | 0.928 | 0.648–1.314 | 0.673   | –     | –     | –     |
| Histology grade (well, moderately/poorly and others) | 1.309 | 0.913–1.946 | 0.146   | –     | –     | –     |
| Depth of tumor invasion (<m, sm, mp/ss, se, si) | 8.387 | 2.531–51.86 | 0.0001b | 2.415 | 0.650–15.87 | 0.208 |
| Lymph node metastasis (negative/positive) | 4.675 | 2.162–19.74 | 0.0001b | 2.892 | 1.219–12.88 | 0.011b |
| Lymphatic invasion (negative/positive) | 2.684 | 1.474–6.676 | 0.0003b | 1.777 | 0.481–5.237 | 0.761 |
| Venous invasion (negative/positive) | 3.324 | 1.627–6.080 | 0.0012b | 1.316 | 0.883–1.971 | 0.176 |
| ERBB2 mRNA expression (low/high) | 1.466 | 1.003–2.263 | 0.0482b | 1.010 | 0.991–1.025 | 0.277 |
| miR-125a-5p expression (high/low) | 3.018 | 1.372–7.588 | 0.0051b | 2.438 | 1.035–6.727 | 0.041b |

Abbreviation: RR, Relative risk.

aTumor invasion of mucosa (m), submucosa (sm), muscularis propria (mp), subserosa (ss), penetration of serosa (se), and invasion of adjacent structures (si).

bP < 0.05.
tumor cell panel carcinoma cells of different origin from the NCI60 miR-125a-5p and are shown in Supplementary Figure S2. correlated with the expression of in Material and Methods. array and the miRNA array data set of NCI60, as described in 23 cell lines including colon, lung, prostate, and renal cancer (\(n = 45\)), the expression of miR-125a-5p was significantly increased compared with that in the high ERBB2 expression group (ERBB2 staining 2+; n = 7; \(P < 0.05\)). Dots represent the miR-125a -5p expression of each sample. Horizontal lines indicate mean value of each group. D, miR-125a-5p and ERBB2 expression levels of carcinoma cells of different origin from the NCI60 tumor cell panel. ERBB2 expression is inversely correlated with expression of miR-125a-5p in 23 cell lines including colon, lung, prostate, and renal cancer.

\[ \pm 2.24; P < 0.05, \text{Fig. 2C}. \] Images of immunohistochemistry are shown in Supplementary Figure S2.

**miR-125a-5p and ERBB2 expression levels in carcinoma cells of different origin from the NCI60 tumor cell panel**

To evaluate whether the miR-125a–ERBB2 pathway functions in cells of different origin, we exploited the cDNA array and the miRNA array data set of NCI60, as described in Material and Methods. ERBB2 expression was inversely correlated with the expression of miR-125a-5p in 23 cell lines including colon, lung, prostate, and renal cancer (\(r = -0.3348, P = 0.0086\); Fig. 2D).

**Both ERBB2 and its primary downstream signal through AKT were suppressed by miR-125a-5p**

Using RT-PCR, we confirmed that miR-125a-5p expression in Pre-miR-125a-treated cells was significantly higher than that in untreated cells (parent) and in Pre-miR negative control–treated cells (\(P < 0.05\), Fig. 3A). To determine whether miR-125a-5p suppresses ERBB2 and its downstream signaling in the gastric cancer cell line NUGC4, cell lysates of transfected cells were analyzed by Western blotting. Remarkable suppression of ERBB2 and phosphorylated AKT (p-AKT) was observed in Pre-miR-125a–treated cells, in comparison with untreated cells (parent) or Pre-miR negative control–treated cells (Fig. 3B). However, AKT itself did not show significant reduction. We also investigated the inhibition of previously reported miR-125a-5p targets, including apoptosis-related gene BAK1 (26) and tumor suppressor gene p53 (27). Western blot analysis showed that protein expression of BAK1 and p53 was moderately suppressed in Pre-miR-125a–treated cells; however, the reduction was not as significant as that of ERBB2 (Supplementary Fig. S3).
miR-125a-5p inhibited the proliferation of gastric cancer cells in combination with trastuzumab

To explain the antitumor efficacy of miR-125a-5p in gastric cancer cells, a proliferation assay was carried out with Pre-miR-125a–treated cells or negative control cells by using NUGC4. Furthermore, we investigated whether the additional administration of trastuzumab, the ERBB2-targeting antibody, enhanced the antitumor efficacy of miR-125a-5p. These experiments were carried out at 2 different concentrations of trastuzumab [0.1 µg/mL (Fig. 4A) and 1 µg/mL (Fig. 4B)]. Interestingly, miR-125a not only potently suppressed the proliferation of gastric cancer cells by itself but also inhibited growth more potently when combined with trastuzumab ($P = 0.0032$, Fig. 4A; $P = 0.0033$, Fig. 4B). The combined growth inhibitory effect was more robust at the higher concentration of trastuzumab.

Discussion

Recent evidence has shown that altered patterns of miRNA expression correlate with various human cancers. The behavior of miRNAs is complex because they regulate hundreds of targets, resulting in the downregulation of numerous target genes including oncogenes and tumor suppressors. Therefore, exploring their clinical potential is especially worthwhile.

In the current study, we show that altered miR-125a-5p expression significantly affected cancer progression and prognosis in human gastric cancer. Multivariate analysis revealed that miR-125a-5p is an independent prognostic factor for survival. Clinicopathologic analysis revealed that low miR-125a-5p expression contributes to more advanced tumor size and tumor invasion (Table 1).
suggests that this miRNA primarily achieves its antiproliferative effect through downregulation of proliferation-related genes, including ERBB2, a member of the EGF receptor family of receptor tyrosine kinases, which regulate a key initiator of phosphoinositide-3 kinase (PI3K)-AKT and RAS/RAF/mitogen-activated protein kinase signaling (28). miR-125a-5p is shown to be a superior biomarker to previously reported gastric cancer biomarkers such as DACH1 and PDCD6 (ref. 22; Supplementary Table S1). However, because of the differences in patient backgrounds such as clinical stage and the presence or absence of chemotherapy, further investigation is required for adequate use of these biomarkers.

We confirmed miR-125a-ERBB2 interaction in the human gastric cancer cell line NUGC4. MiR-125a-5p significantly repressed ERBB2 expression and the phosphorylation of its downstream molecule, AKT (Fig. 3B). In addition, ERBB2 expression was shown to be inversely correlated with expression of miR-125a-5p both in vitro and in clinical samples. Overexpression of Pre-miR-125a also led to the inhibition of previously reported miR-125a-5p targets, such as apoptosis-related gene BAK1 (26) and tumor suppressor gene p53 (ref. 27; Supplementary Fig. S3). However, the inhibition of these tumor suppressor genes was modest compared with that of ERBB2, suggesting ERBB2 is a crucial target of miR-125a-5p, at least in the gastric cancer cell line NUGC4.

It is noteworthy that the growth inhibitory effect of miR-125a-5p was enhanced when combined with trastuzumab (Fig. 4A and B). This could be partly due to the fact that miR-125a-5p and trastuzumab share the same target, ERBB2. miR-125a-5p and trastuzumab silence the ERBB2 pathway through 2 different mechanisms. miR-125a-5p suppresses the molecule at the posttranscriptional level before protein synthesis, whereas trastuzumab is a monoclonal antibody targeted against completed ERBB2 protein. In other words, miR-125a-5p blocks the synthesis of the oncoprotein at an earlier phase than does trastuzumab. These considerations suggest that miR-125a-5p mimic and trastuzumab have the potential to be highly effective against ERBB2 when used together.

ERBB2-positive gastric cancer patients constitute about 19.0% (8.2%–53.4%) of all gastric cancer patients (19, 29, 30). A recent phase III study (the ToGA trial) combining treatment of trastuzumab and conventional chemotherapy against ERBB2-positive gastric cancer showed a statistically significant advantage in overall survival for patients who received combined therapy compared with chemotherapy alone. These reliable large-scale clinical data indicate that ERBB2 is a crucial therapeutic target in gastric cancer (31).

In conclusion, our data suggest that miR-125a-5p functions as a powerful tumor suppressor and could be a bona fide prognostic marker for gastric cancer patients. Furthermore, miR-125a-5p mimic alone or in combination with trastuzumab could be a novel therapeutic approach against gastric cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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