Gastric cancer remains the main cause of cancer death all around the world, and upregulated activation of the nonreceptor tyrosine kinase c-SRC (SRC) is a key player in the development. In this study, we found that expression of Src is also increased in clinical gastric cancer samples, with the protein level increased more significantly than that at the RNA level. Further study revealed that miR34a and miR203, two tumor suppressive miRNAs, inversely correlate with the expression of Src. Restoration of miR34a and miR203 decreased Src expression in gastric cancer cell lines, which in turn inhibited cell growth and cell migration. In summary, our study here revealed that posttranscriptional regulation of Src contributes to the deregulated cell growth and metastasis in gastric cancer, and targeting Src by miR34a or miR203 mimics would be a promising strategy in therapy. [BMB Reports 2013; 46(6): 316-321]

INTRODUCTION

Gastric cancer is one of the most common malignant tumors of the digestive system (1). Due to its genomic instability and the resultant altered gene expression, gastric cancer are of aggressive growth and metastasis ability, and thus remains the leading cause of cancer death all over the world. Elucidating the underlying mechanism, especially the molecular switch for the uncontrolled cell growth and metastasis holds the promise for gastric cancer therapy.

Src-Family Kinases (SFKs) participate in the regulation of proliferation, differentiation, apoptosis, autophagy, adhesion, migration, invasion and angiogenesis in normal and cancer cells. Abnormal expression of SFKs has been documented in cancers that arise in breast, colon, ovary, melanocyte, gastric mucosa, head and neck, pancreas, lung and brain (2). c-Src, the founding member of the SFKs, has been found to be overexpressed or activated in various solid tumors including colon, breast, ovarian, brain cancers and so on. And targeting Src is widely believed to be an optional strategy to cure cancer (3, 4). However, since Src acts both in cancer and normal cells, suggesting that revealing how Src was aberrantly overexpressed and activated is of significant importance for targeting cancer stem cell specifically (5).

Recently, posttranscriptional regulation is believed to play an essential role in development and cancer progress (6). microRNAs (miRNAs) are a class of small non-coding RNAs of 19-25 nt in length, which regulate gene expression at the post-transcriptional level (7, 8). Recent research suggests that miRNAs are involved in tumor invasion and metastasis, in multiple cancers, including gastric cancer (9-11). Among these miRNAs, miR34 and miR203 are characterized as a tumor suppressor in multiple cancers, such as colon cancer and breast cancer by targeting different oncogenes (12-15). However, their roles in gastric cancer is largely unknown.

Here we examined the expression of Src in gastric cancer samples and its relation with miR34a and miR203. Our study here revealed that Src is increased in clinical gastric cancer samples, while miR34a and miR203, two known miRNAs targeting Src, inversely correlate with the expression of Src. Restoration of miR34a and miR203 decreased Src expression in gastric cancer cell lines, which in turn inhibited cell growth and cell migration. In summary, our study here revealed that posttranscriptional regulation of Src contributes to the deregulated cell growth and metastasis in gastric cancer, and targeting Src using miR34a or miR203 mimics would be a promising
miR34a and miR203 contributes to gastric cancer development
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strategy in therapy.

RESULTS

Increased Src expression both at protein and RNA levels in gastric cancer

Although increased Src activation has been revealed in many cancer types, including gastric cancer (16, 17), its basal expression still needs further confirmation. To this end, we compared the expression of Src in both cancer samples and adjacent normal tissues. As shown in Fig. 1A, Src was found to lowly or moderately expressed in most of the adjacent normal tissues, while its expression in cancer samples was found to be significantly higher. To further clarify how Src expression was increased, we further examined the expression of Src in both cancer and normal tissue samples at RNA level. As expected, Src RNA increased in most cancer samples, compared with their normal counterpart (Fig. 1B), however, the fold change was much smaller, suggesting that posttranscriptional regulation might involve in the process.

miR34a and miR203 inversely correlates with Src expression

From the above data, we then analyzed the 3'UTR of Src using targetscan (18) and revealed that Src might be targeted by miR34a and miR203 (Fig. 2A). Further literature searching further confirmed that miR203 are the posttranscriptional regulators of Src (19, 20). In this regard, we test the expression of miR34a and miR203 in the above cancer samples. Strikingly, both miR34a and miR203 were significantly downregulated in cancer samples, which was inversely correlated with the expression of Src in cancer samples (Fig. 2B and C). We next test whether miR34a and miR203 could really target Src in gastric cancer. The available gastric cancer cell lines were screened for miR34a, miR203 and Src expression. Among these cells, MKN45 cell displayed moderate expression of the above molecules (data not shown). In this regard, we transfected either miR34a or miR203 or their antagonisms respectively and tested the expression of both Src and phosphorylated Src. Efficient transfection were confirmed by qRT-PCR (Supplementary Fig. 1A and B). As expected, transfection of miR34a or miR203 repressed Src expression and subsequent its activity (Fig. 2D and E). In contrast, inhibiting endogenous miR34a or miR203 function by transfection of their antagonisms further increased Src expression and activity in the same cell (Fig. 2D and E). Luciferase activity assay revealed that either miR34a or miR203 inhibited the 3'UTR of Src activity, while their antagonisms increased 3'UTR activity of Src (Fig. 2F).

miR34a and miR203 inhibit the cell growth and migration in Src dependent manner

All of the above findings suggest that miR34a and miR203 might be the main cause of deregulated Src, and it might be also responsible for the deregulated Src function in cancer. As expected, knockdown of Src by different RNAi reduced Src expression and its activation in MKN45 cells (Fig. 3A). With the Src expression decreased, migrating cell numbers reduced significantly (Fig. 3B and C). Similar as knockdown of Src, transfection of miR34a decreased the cell growth (Fig. 4A) and reduced the cell migration (Fig. 4B and C). In contrast, inhibiting miR34a had an opposite effect on cell migration and proliferation. While inhibiting miR34a by antago-miR34a nearly has no effects on cell proliferation and migration when cells were together treated with si-Src (Fig. 4A-C), suggesting that Src is the main target of the tumor suppressor role of miR34a. Similar as miR34a, miR203 plays a similar role on cell proliferation and migration (Fig. 4D-F).

DISCUSSION

Gastric cancer remains the main cause of cancer death worldwide, as the conventional strategies based on radical surgery for the treatment of gastric cancer are not yet satisfactory.