News Release

Title

Amido-bridged Nucleic Acid-modified Antisense Oligonucleotides Targeting SYT13 to Treat Peritoneal Metastasis of Gastric Cancer

Key Points

・Synaptotagmin XIII (SYT13) contributes to peritoneal metastasis of gastric cancer by interfering with FAK-mediated intracellular signals.
・mido-bridged nucleic acid -modified anti-SYT13 antisense oligonucleotides (ASOs) inhibited cellular functions associated with metastatic potential of gastric cancer cell lines in a concentration-dependent manner.
・ntra-abdominal administration of amido-bridged nucleic acid -modified anti-SYT13 ASOs inhibited the formation of peritoneal nodules and significantly increased survival in a mouse xenograft model of metastasis.

Summary

Prof. Yasuhiro Kodera and Dr. Mitsuro Kanda (Department of Gastroenterological Surgery) in Nagoya University Graduate School of Medicine (Dean: Dr. Kenji Kadomatsu), and Prof. Satoshi Obika and Dr. Yuuya Kasahara in National Institutes of Biomedical Innovation, Health and Nutrition (Dean: Dr. Yoshihiro Yoneda) developed an intraperitoneal treatment strategy using an amido-bridged nucleic acid -modified antisense oligonucleotides (ASOs), targeting synaptotagmin XIII (SYT13), and to identify the function of SYT13 in gastric cancer cells. Through a screening of 71 candidate oligonucleotide sequences, the ASOs (designated hSYT13-4378 and hSYT13-4733) with the highest knockdown efficiencies and lowest off-target effects were selected and their abilities to inhibit cellular functions associated with the metastatic potential of gastric cancer cells were determined. SYT13 interfered with FAK-mediated intracellular signals. Intraperitoneal administration of hSYT13-4378 and hSYT13-4733 in a mouse xenograft model of metastasis inhibited the formation of peritoneal nodules and significantly increased survival. Reversible, dose- and sequence-dependent liver damage was induced by ASO treatment without causing abnormal morphological and histological changes in the brain. Intra-abdominal administration of amido-bridged nucleic acid -modified Anti-SYT13 ASOs represents a promising strategy for treating peritoneal metastasis of gastric cancer. This work was published online in Molecular Therapy - Nucleic Acids on October 6, 2020.

Research Background

Peritoneal metastasis represents a devastating form of gastric cancer progression despite intensive efforts to improve the efficacy of systemic chemotherapy. A major impediment to this strategy is that a small fraction of a drug is delivered to peritoneal tumors. Thus,

direct intraperitoneal chemotherapy represents a reasonable alternative.

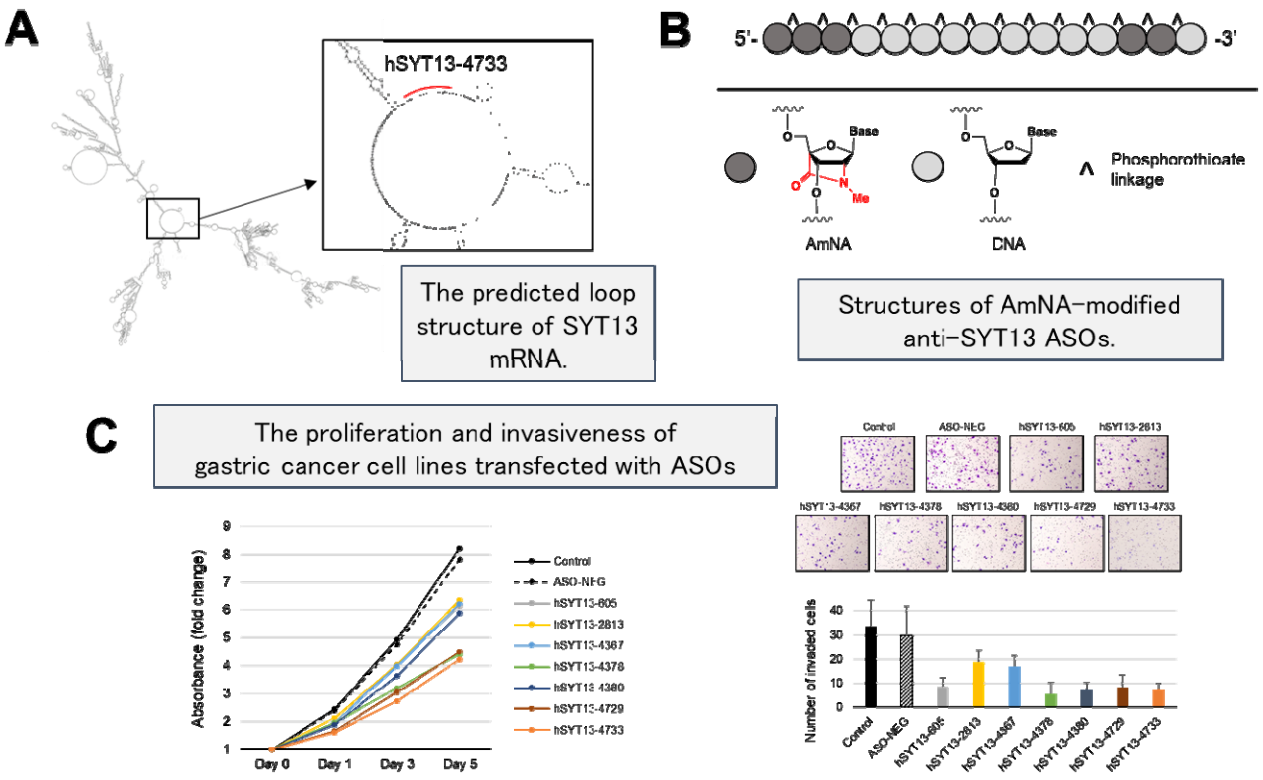
We recently reported that synaptotagmin XIII (SYT13) contributes to peritoneal metastasis of gastric cancer. Thus, SYT13 is specifically expressed in primary cancer tissues from such patients; and in a mouse model of peritoneal metastasis, intraperitoneal administration of an SYT13-specific small interfering RNA (siRNA) significantly inhibits the growth of peritoneal nodules and prolongs survival.6 However, serious problems must be addressed to translate these findings to the clinic, which include limited efficacy and drug delivery using transfection techniques.

The use of antisense oligonucleotides (ASOs) that inhibit the expression of SYT13 may provide an alternative therapeutic approach, although ASOs are vulnerable to endogenous nucleases, and their delivery to target tissues is inefficient. Two key technologies are available to address these obstacles. First, compared with their unmodified precursors, ASOs modified by incorporating amido-bridged nucleic acids (AmNA) with phosphorothioate-linked structures bind to mRNAs with higher affinities, are more resistant to nucleases, and are less toxic. Second, Ca^{2+} enrichment medium (CEM) potentiates the activity of oligonucleotides, independent of net charge and structural modifications, which contributes to enhanced in vivo silencing activity compared with conventional transfection methods.

We reasoned therefore that intraperitoneal administration of AmNA-modified anti-SYT13 ASOs transfected using CEM represents a promising technique for treating peritoneal metastasis of gastric cancer. Here we describe two lines of evidence that identify the function of SYT13 in gastric cancer cells and indicate that AmNA-modified SYT13-specific ASOs show promise for treating peritoneal metastasis of gastric cancer.

Research Results

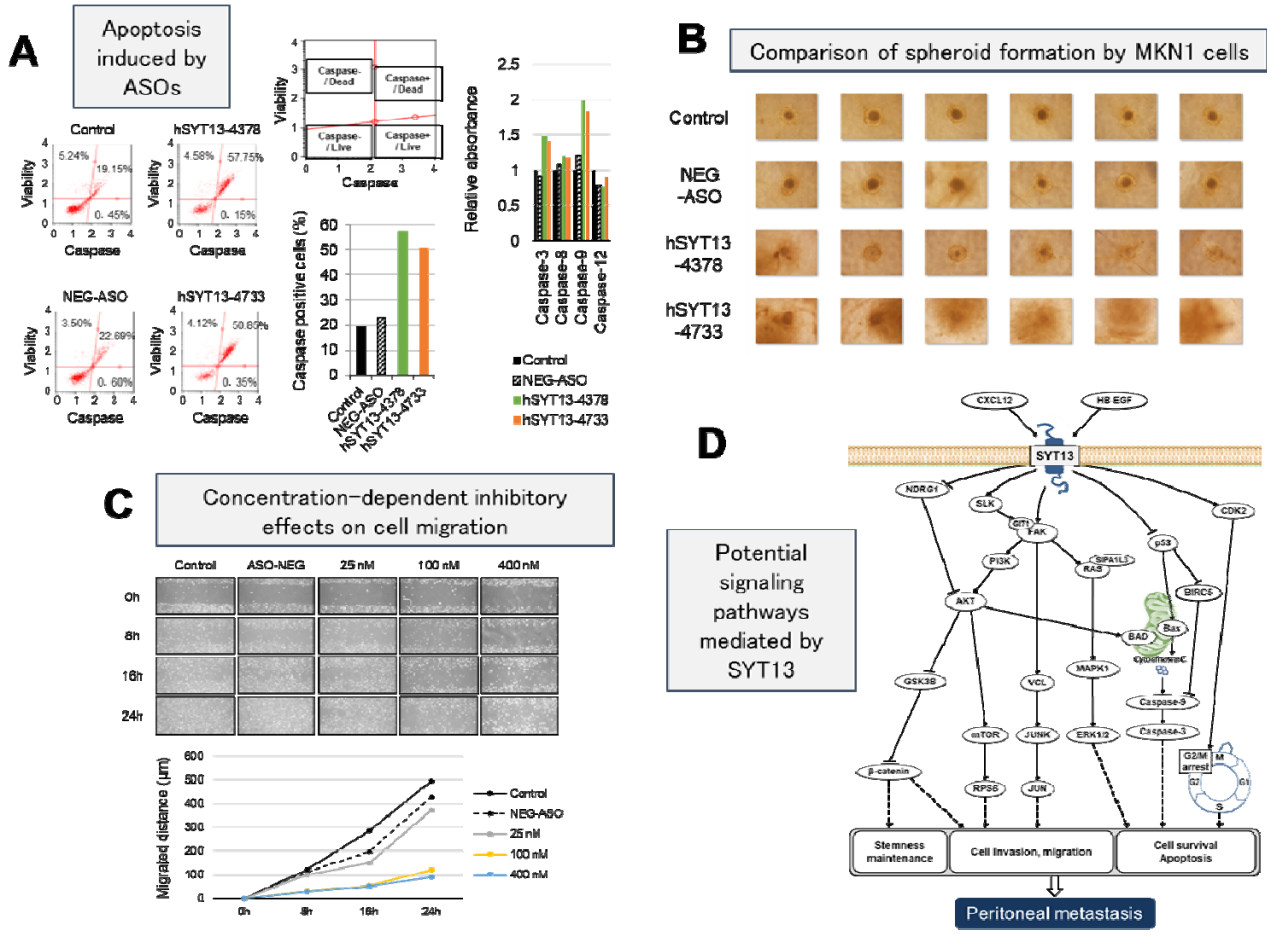
We designed 71 sequences according to the predicted loop structure of SYT13 mRNA (Figure 1A), and to screen for optimal ASOs, we compared their abilities to inhibit SYT13 mRNA expression in gastric cancer cell lines. AmNA-modified Anti-SYT13 ASOs included flanking regions of artificial nucleotides, and all phosphate groups were phosphorothioated (Figure 1B). Alterations of functions associated with the metastatic potential of gastric cancer cells, such as proliferation, migration, invasiveness, and adhesion were determined using the candidate ASO-transfectants. For example, significant inhibition of proliferation was observed in cells transfected with hSYT13-4378, hSYT13-4729, and hSYT13-4733, and invasion abilities of gastric cancer cells were decreased by transfection of hSYT13-605, hSYT13-4378, hSYT13-4380, hSYT13-4729, and hSYT13-4733 (Figure 1C).



We selected hSYT13-4378 (15-mer) and hSYT13-4733 (17-mer) for further analyses. To determine if SYT13-knockdown induced apoptosis associated with caspase activation, caspase activities were assessed. As shown in Figure 2A, ASO-mediated knockdown of SYT13, particularly by hSYT13-4378, increased caspase activities compared with untransfected NUGC4 cells. Further, caspase-3 and -9 activities were preferentially increased by ASO-mediated knockdown of SYT13 expression. To determine whether apoptosis induced by SYT13-knockdown involved the mitochondrial apoptotic pathway, mitochondrial membrane potential was evaluated. The percentages of cells with loss of mitochondrial membrane potential (depolarized/live cells) were increased by ASO-mediated knockdown of SYT13 expression, particularly by hSYT13-4378. The ALDH assay was used to detect the presence of subpopulations with cancer stem cell-like properties vs control cells. The percentage of NUGC4 cells expressing the stemness marker ALDH was decreased by ASO-mediated knockdown of SYT13 expression, particularly by hSYT13-4733, compared with that of the untransfected control and NEG-ASO cells. When we employed a spheroid cell culture assay to assess cancer cell stemness, we found that hSYT13-4733 significantly inhibited spheroid formation, indicative of the stemness phenotype (Figure 2B). To further demonstrate the effects of AmNA-modified Anti-SYT13 ASOs, MKN1 cells were transfected with different concentrations of hSYT13-4378 and hSYT13-4733. Each ASO inhibited SYT13 expression as well as the proliferation, invasiveness, and migration (Figure 2C) of MKN1 cells.

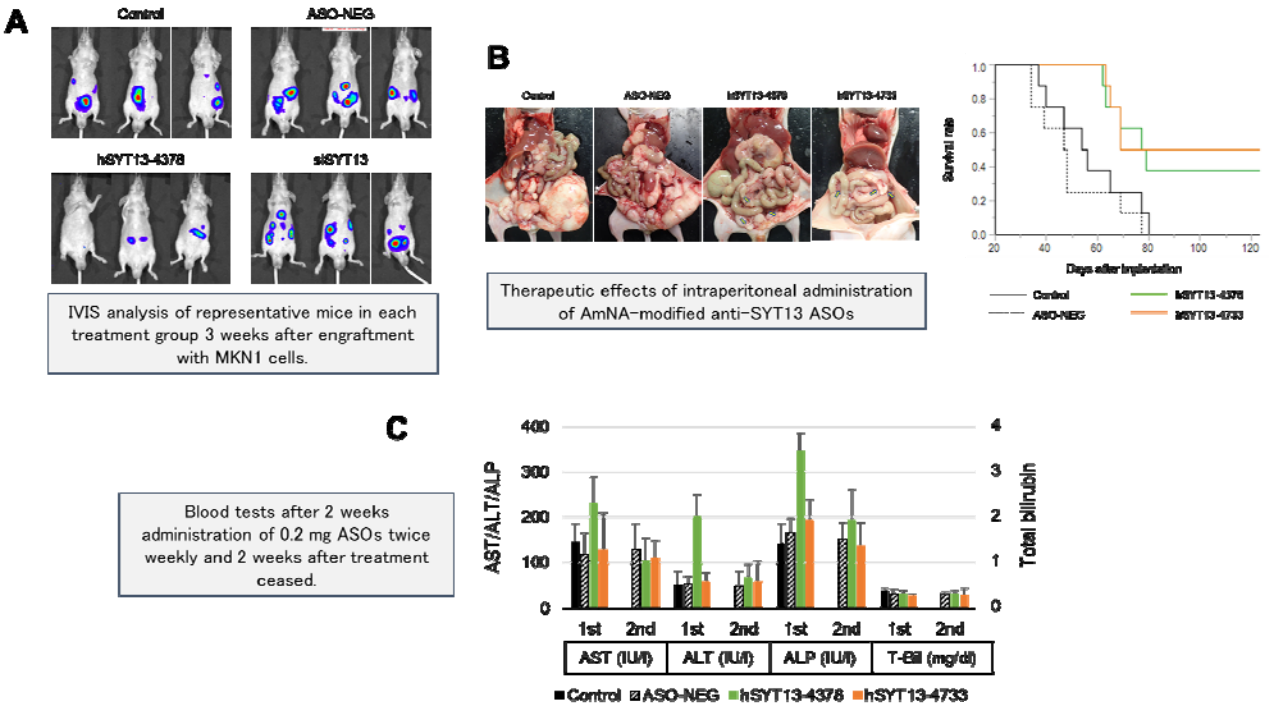
We performed microarray analysis and in silico analysis to detect the effects of the ASOs on candidate off-target genes, and found that hSYT13-4378 lacked an identical match, but had two single mismatches (JRK-like, transcript variant 1 and cell division cycle and

apoptosis regulator 1, transcript variant 4), and 21 sequences with two mismatches. Furthermore, hSYT13-4733 lacked an identical match or one mismatch, and had one sequence with two mismatches (pyruvate dehydrogenase phosphatase regulatory subunit, transcript variant X10). Thus, off-target effects caused by hSYT13-4378 or hSYT13-4733, particularly the latter, appeared unlikely. Pathway analysis indicated that SYT13 inhibits multiple intracellular proliferative signals generated by the activation of FAK (Figure 2D).



MKN1 cells were used to compare the effects of intraperitoneal administration of hSYT13-4378 (0.2 mg) compared with that of mock-transfected cells and cells transfected with ASO-NEG or an SYT13-specific siRNA. The in vivo imaging of mice engrafted with MKN1 cells show the luciferase signal throughout the abdominal areas in mice treated with vehicle, ASO-NEG, or siRNA groups, but markedly fewer spots in mice treated with hSYT13-4378. (Figure 3A). We next evaluated the therapeutic effects on survival of intraperitoneal administration of hSYT13-4378 and hSYT13-4733. Mice were intraperitoneally engrafted with NUGC4 cells and were subsequently abdominally administered vehicle, ASO-NEG, hSYT13-4378, or hSYT13-4733. The macroscopic appearances of satellite mice 8 weeks after cell implantation are shown in Figure 3B. Scattered peritoneal nodules were observed in engrafted mice treated with the hSYT13-4378 or hSYT13-4733. In contrast, we observed gross peritoneal metastasis across the peritoneal cavity in mice treated with vehicle or ASO-NEG. Mice treated with hSYT13-4378 or hSYT13-4733 survived significantly longer compared with those administered vehicle or

ASO-NEG. When we measured the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) to evaluate the toxicities of hSYT13-4378 and hSYT13-4733, we did not detect elevated levels of either enzyme in mice administered hSYT13-4733, while their levels were transiently increased (2 weeks) in mice administered hSYT13-4378 (Figure 3C). These findings indicate that hepatotoxicity induced by AmNA-modified anti-SYT13 ASOs was reversible and sequence-dependent. Renal dysfunction and metabolic abnormalities were undetectable in mice administered either ASO.



Research Summary and Future Perspective

The involvement of SYT13 in the cellular functions associated with metastasis was demonstrated. Intra-abdominal administration of amido-bridged nucleic acid-modified anti-SYT13 ASOs represents a promising strategy for treating the peritoneal metastasis of gastric cancer. Since anti-SYT13 treatment is based on different mechanisms of action from existing molecularly targeted therapies, it can open new frontiers in treatment of gastric cancer and possibly other malignancies causing peritoneal metastasis including pancreatic and ovarian cancer.

Publication

Kanda M, Kasahara Y, Shimizu D, Miwa T, Umeda S, Sawaki K, Nakamura S, Kodera Y, Obika S. Amido-bridged Nucleic Acid-modified Antisense Oligonucleotides Targeting SYT13 to Treat Peritoneal Metastasis of Gastric Cancer. Molecular Therapy - Nucleic Acids. 2020 in press
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Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Mole_The_201006.pdf