News Release

Title

Therapeutic monoclonal antibody targeting of neuronal pentraxin receptor to control metastasis in gastric cancer

Key Points

- We identified neuronal pentraxin receptor (NPTXR) as being specifically overexpressed in gastric cancer tissues with metastatic potential from global expression profiling of 57749 molecules.
- NPTXR silencing promoted caspase-mediated apoptosis and attenuated gastric cancer cell proliferation, cell cycle progression, migration, invasion, adhesion, stem cell-like properties, and resistance to 5-fluorouracil.
- Anti-NPTXR monoclonal antibodies inhibited gastric cancer metastasis in mice.

Summary

Prof. Yasuhiro Kodera and Dr. Mitsuro Kanda (Department of Gastroenterological Surgery) in Nagoya University Graduate School of Medicine (Dean: Dr. Kenji Kadomatsu) identified identified neuronal pentraxin receptor (NPTXR) as being specifically overexpressed in gastric cancer tissues with metastatic potential from global expression profiling of 57749 molecules. NPTXR mRNA expression in clinical specimens was associated with disease progression and was significantly higher in tissues from GC patients with distant metastasis compared with those without. NPTXR regulated expression of genes involved in metastatic behaviors as well as activation of the PI3K-AKT-mTOR, FAK-JNK, and YAP signaling pathways. NPTXR silencing promoted caspase-mediated apoptosis and attenuated GC cell proliferation, cell cycle progression, migration, invasion, adhesion, stem cell-like properties, and resistance to 5-fluorouracil in vitro, and also inhibited the tumorigenicity of GC cells in vivo. Anti-NPTXR Abs inhibited GC peritoneal metastasis in mice. Nptxr deficient mice showed no abnormalities in reproduction, development, metabolism, or motor function. This work was published online in Molecular Cancer on August 26, 2020.

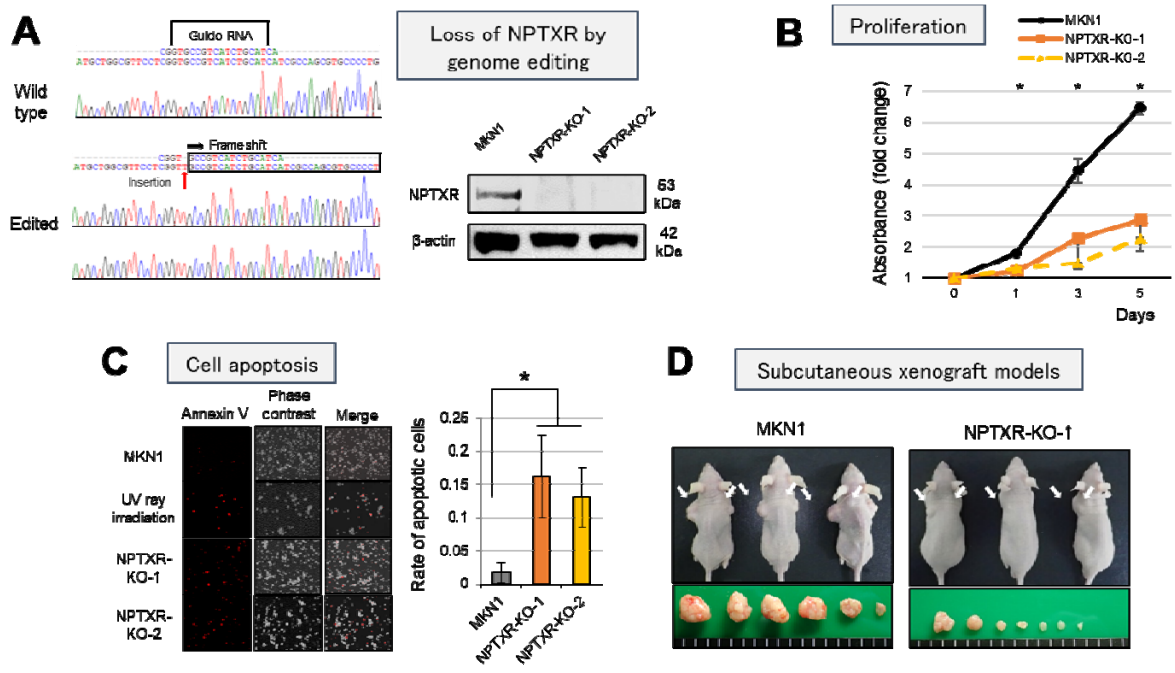
Research Background

Gastric cancer can be treated when diagnosed sufficiently early, and patients undergoing resection can expect an excellent prognosis. However, patients diagnosed with advanced cancer face a dire prognosis, mainly because of the propensity for gastric cancer to metastasize.The only therapeutic options currently available for patients with unresectable or metastatic gastric cancer are combinations of cytotoxic anti-cancer agents and targeted therapies such as monoclonal antibodies gastric cancer. However, the efficacy and prognosis of these treatments are unpredictable owing to the clinical heterogeneity and molecular complexity of gastric cancer. Moreover, some monoclonal antibodies are not well tolerated, leaving the

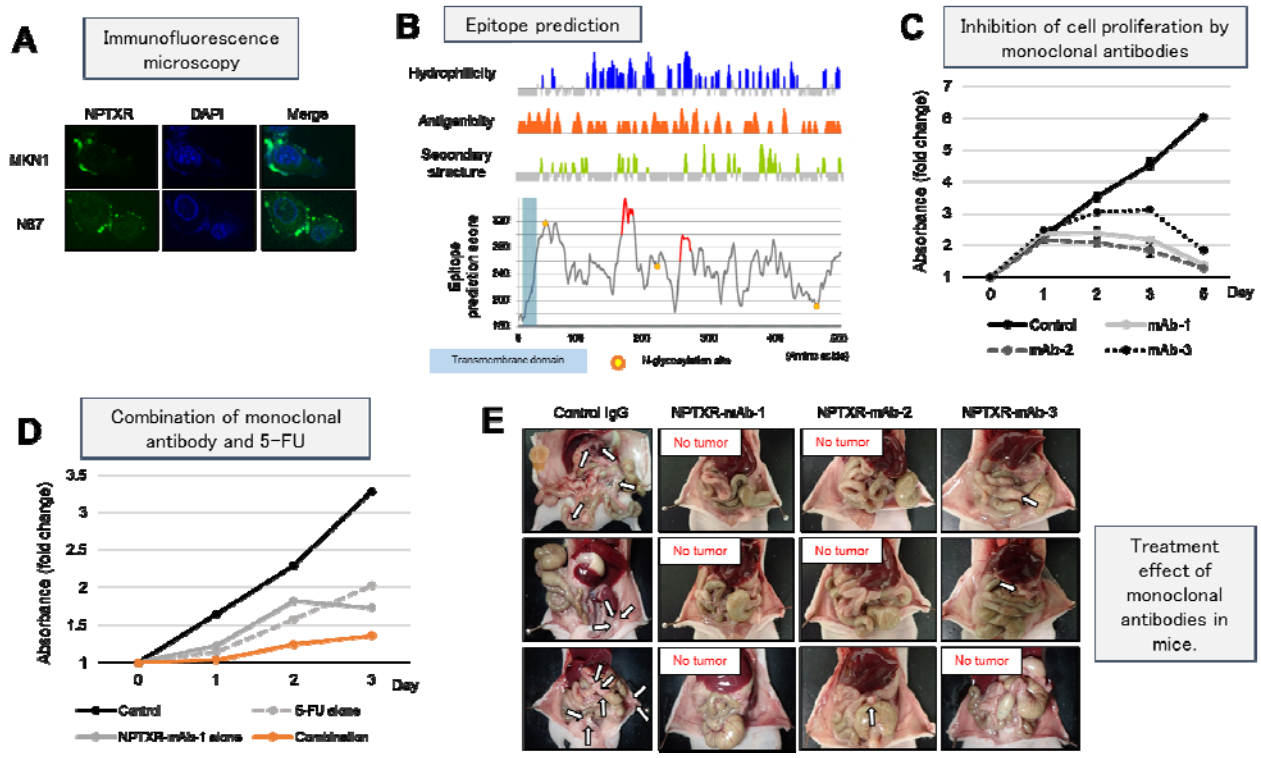
patient with few treatment options. There is thus an urgent need to identify candidate therapeutic targets for the development of agents that can control cancer metastasis through novel mechanisms. To this end, we performed transcriptome and bioinformatics analysis of gastric cancer tissues from patients with or without metastasis to identify novel candidate targets involved in metastasis. We identified neuronal pentraxin receptor (NPTXR) as being specifically overexpressed in gastric cancer tissues with metastatic potential. NPTXR is a type II transmembrane protein that functions as a trans-synaptic organizer and anchors neuronal pentraxin complexes to plasma membranes. We investigated the expression and function of NPTXR by in vitro and in vivo analysis of human gastric cancer cell lines, tumor xenograft mouse models, and Nptxr-deficient mice. We also developed monoclonal antibodies against NPTXR and evaluated their potential utility as diagnostic tools and/or therapeutic agents for gastric cancer.

Research Results

We conducted a transcriptome analysis and identified NPTXR as a candidate driver for metastasis of gastric cancer. After CRISPR/Cas9 editing, two clones with stable NPTXR knockout (KO) were generated (KO-1 and KO-2), and confirmed by direct sequence analysis to harbor the expected deletion (Figure 1A). NPTXR protein was also undetectable in both clones by western blot analysis (Figure 1A). NPTXR KO inhibited the proliferation of gastric cancer cells (Figure 1B). The Annexin V assay data revealed that cultures of NPTXR KO cells harbored significantly more annexin V-positive cells compared with MKN1 cultures, indicating that NPTXR KO cells were more susceptible to apoptosis (Figure 1C). We determined whether knockout of NPTXR influences the growth of tumors formed by gastric cancer cells in vivo. The subcutaneous tumors grew progressively in the MKN1 group while only slightly in NPTXR KO cells (Figure 1D).

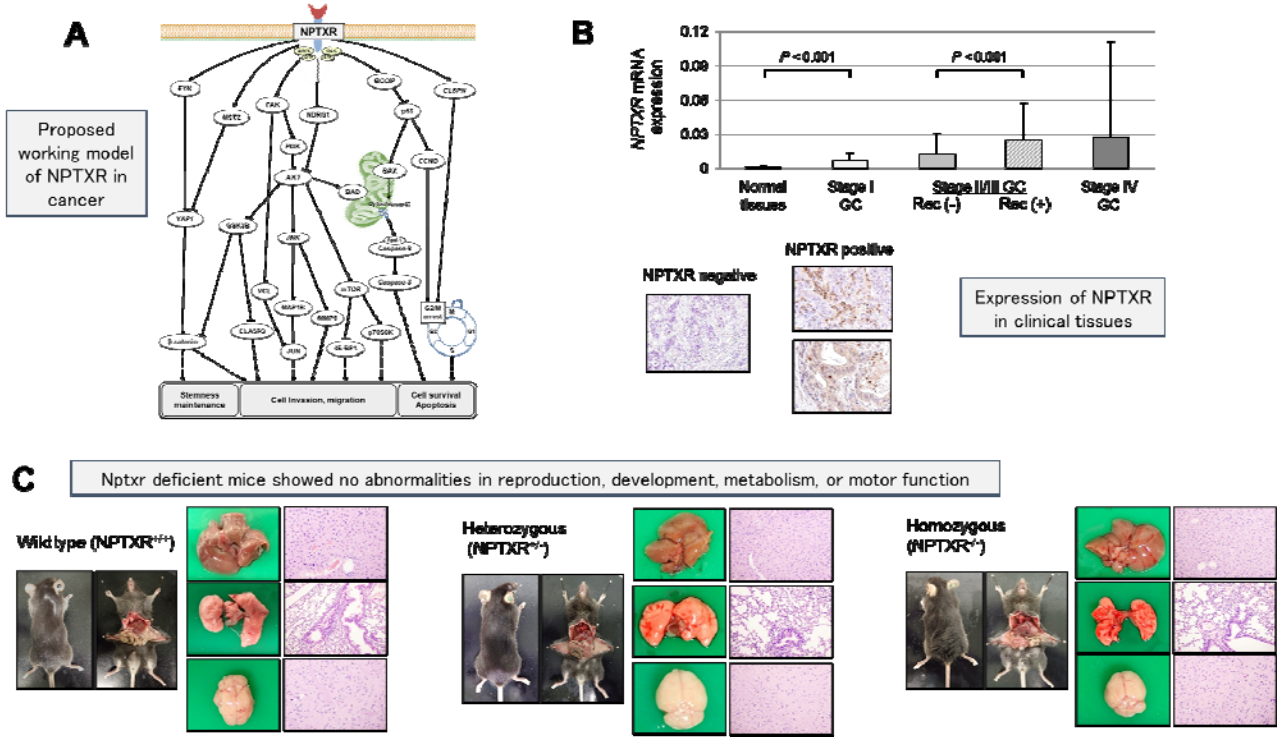


In both MKN1 and N87 cells, NPTXR was detected at the plasma membrane by immunofluorescence microscopy (Figure 2A). We generated and tested monoclonal antibodies targeting of NPTXR by immunization of mice with NPTXR peptide predicted to be immunogenic (Figure 2B). We determined whether antibody-mediated blockade of NPTXR could attenuate gastric cancer cell proliferation, and found that anti-NPTXR monoclonal antibodies significantly inhibited the proliferation of MKN1 cells compared with IgG control treatment (Figure 2C). Anti-NPTXR monoclonal antibody synergistically enhanced the antiproliferative effect of 5-FU (Figure 2D). The therapeutic potential of anti-NPTXR monoclonal antibodies was assessed using the mouse xenograft model. BALB/c nu/nu mice were injected i.p. with parental MKN1 cells and then injected i.p. with 6 μg of control IgG or anti-NPTXR monoclonal antibodies twice weekly for 4 weeks. The macroscopic appearance of peritoneal metastases 4 weeks after cell injection is shown in Figure 2E. Tumor nodules were observed in the omentum and mesenteric tissue of all mice treated with control IgG, whereas none were found in the monoclonal antibody-treated mice. No overt signs of mAb toxicity were observed in any of the mice over the 4-week treatment period.



Our functional and pathway analyses suggest that NPTXR plays numerous roles in intracellular dynamics and signaling pathways (Figure 3A). Patients with stage II/III GC who developed disease recurrence had significantly higher NPTXR mRNA levels compared with patients without recurrence (Figure 3B). In situ expression of NPTXR protein was successfully detected by immunostaining method (Figure 3B). To clarify the pathophysiological functions of NPTXR, we generated Nptxr+/+, Nptxr +/−, and Nptxr −/− mice (Figure 6C). KO of one or both Nptxr alleles (Nptxr+/− and Nptxr−/− mice) was not embryonic lethal and had no effect on the appearance or body weight of the mice, and did not result in any abnormalities in

the development of the liver, lung, and brain, metabolic parameters, or hematological parameters. Moreover, neither the Nptxr+/− nor the Nptxr−/− mice exhibited dysfunctional motor coordination or motor learning, as measured by the rotarod test.



Research Summary and Future Perspective

NPTXR plays an essential role in controlling the malignant behavior of gastric cancer cells in vitro and in vivo. NPTXR-targeting monoclonal antibodies may thus have utility as novel diagnostic tools and/or treatment modalities for GC. Since anti-NPTXR treatment is based on quite different mechanisms of action from existing molecularly targeted therapies, it can open new frontiers in treatment of gastric cancer and possibly other malignancies overexpressing NPTXR including breast, colon, lung, esophageal and pancreatic cancer.

Publication

Kanda M, Shimizu D, Sawaki K, Nakamura S, Umeda S, Miwa T, Tanaka H, Tanaka C, Hayashi M, Iguchi Y, Yamada S, Katsuno M, Kodera Y. Therapeutic monoclonal antibody targeting of neuronal pentraxin receptor to control metastasis in gastric cancer. Molecular Cancer 2020 in press
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Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Mo_Ca_200827.pdf