News Release

Title

Blockade of CHRNB2 signaling with a therapeutic monoclonal antibody attenuates the aggressiveness of gastric cancer cells

Key Points

- We identified cholinergic receptor nicotinic beta 2 subunit (CHRNB2) as being specifically overexpressed in gastric cancer tissues with metastatic potential from global expression profiling of 57749 molecules
- Stable knockout of CHRNB2 significantly attenuated survival and functions associated with metastasis of cancer cells.
- Anti-CHRNB2 monoclonal antibodies inhibited gastric cancer metastasis in mouse models.

Summary

Prof. Yasuhiro Kodera and Dr. Mitsuro Kanda (Department of Gastroenterological Surgery) in Nagoya University Graduate School of Medicine (Dean: Dr. Kenji Kadomatsu) identified cholinergic receptor nicotinic beta 2 subunit (CHRNB2) as being specifically overexpressed in gastric cancer tissues with metastatic potential from a global expression profiling of 57749 molecules. Knockdown of CHRNB2 attenuated gastric cancer cell proliferation, whereas forced overexpression of CHRNB2 increased cell proliferation. Knockout of CHRNB2 significantly influenced cell survival and functions associated with metastasis. The effects of polyclonal antibodies targeting the C- and N-termini of CHRNB2 guided the development of anti-CHRNB2 monoclonal Abs that inhibited the growth of gastric cancer cells in vitro and in vivo. Pathway analysis revealed that CHRNB2 interfered with signaling through the PI3K-AKT and JAK-STAT pathways. Chrnb2-deficient mice exhibited normal reproduction, organ functions, and motor functions. CHRNB2 regulates multiple oncological phenotypes associated with metastasis, and blockade of CHRNB2 expression using specific antibodies shows promise for controlling metastasis in gastric cancer. This work was published online in Oncogene on July 30, 2021.

Research Background

Gastric cancer is one of the leading causes of cancer mortality worldwide. The phenotypic heterogeneity of gastric cancer cells significantly influences patient outcomes, which primarily depend on whether the tumors metastasize. Chemotherapy is the mainstay treatment for advanced disease, which frequently fails due to the acquisition of chemoresistance. Therefore, identification of novel molecular targets is vitally important for efforts to develop targeted therapies.

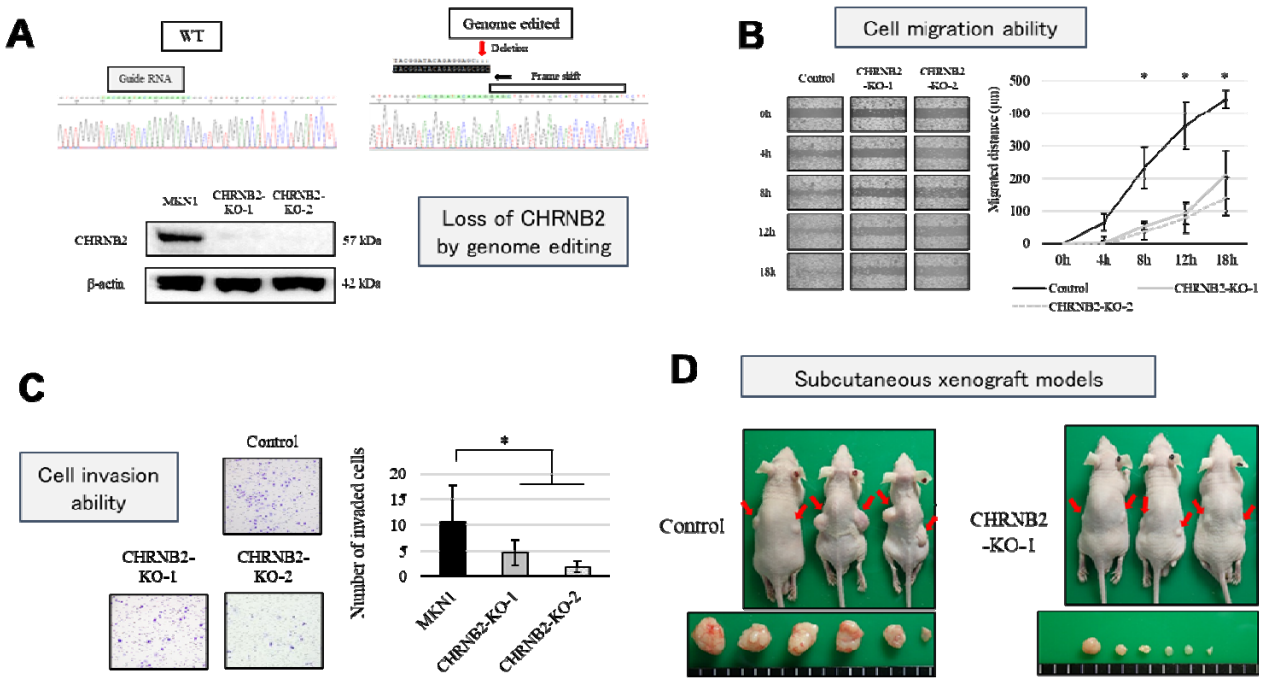
Monoclonal antibody-based immunotherapy has become a primary component of cancer therapy. Such targeted therapies may significantly improve treatment efficacy with minimal adverse

effects on normal cells and tissues. Several therapeutic monoclonal antibodies improve the prognosis of patients with advanced gastric cancer. However, their efficacy is unpredictable and ultimately unsustainable due to the clinical heterogeneity and molecular complexity of gastric cancer. Therefore, new therapeutic targets that control the metastasis of gastric cancer must be identified.

The selection of cancer-specific cell surface targets is required to generate clinically efficacious antibodies. For this purpose, we performed transcriptomic and bioinformatics analyses of cell surface antigens overexpressed in gastric cancers that have metastatic potential. We found that the gene encoding CHRNB2 was overexpressed specifically in gastric cancer tissues and had metastatic potential. CHRNB2 is a member of the nicotinic acetylcholine receptor family, and little evidence is available concerning its oncological roles. We therefore evaluated the functions and expression of CHRNB2 in human gastric cancer cell lines, a mouse tumor xenograft model, and in Chrnb2-deficient mice. We generated antibodies targeting CHRNB2 and assessed their abilities to treat gastric cancer.

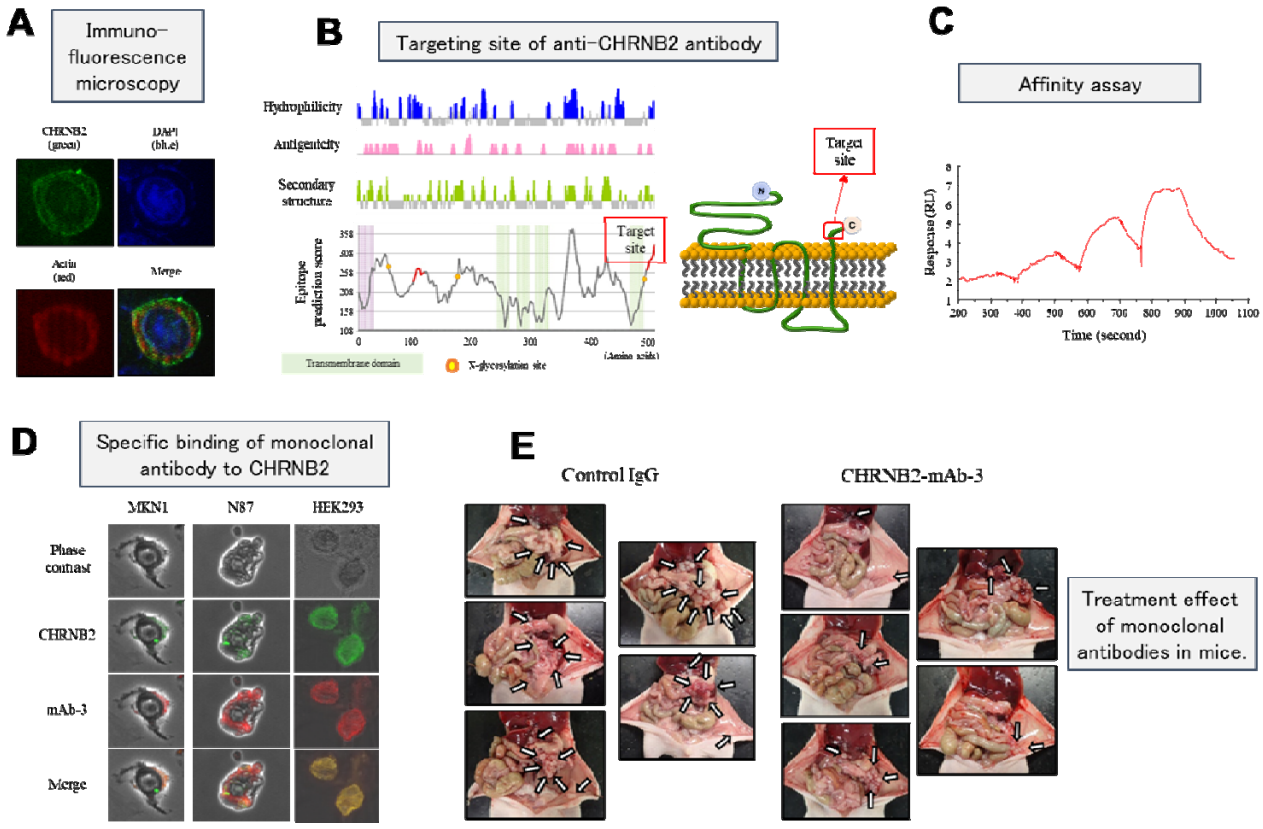
Research Results

We conducted a transcriptome analysis and identified CHRNB2 as a candidate driver for metastasis of gastric cancer. After CRISPR/Cas9 editing, two clones with stable CHRNB2 knockout (KO) were generated (KO-1 and KO-2), and confirmed by direct sequence analysis to harbor the expected deletion and flame shift (Figure 1A). Western blotting did not detect CHRNB2 protein in either clone (Figure 1A). The KO-1 and KO-2 cells exhibited attenuated proliferation compared to the controls. Migration (Figure 1B) and invasion of the CHRNB2-KO cells (Figure 1C) were decreased compared to controls. Furthermore, adhesive properties of five extracellular matrix proteins were reduced in CHRNB2-KO cells compared to controls. We next determined the influence of CHRNB2 KO on tumor growth in nude mice. While subcutaneous tumors formed by the control cells progressively grew over 56 days, the growth of CHRNB2-KO-1 cells was significantly reduced (Figure 1D).



We next measured ALDH levels to determine whether CHRNB2 KO affected the presence of subpopulations of gastric cancer cells with stem cell-like properties and found that CHRNB2 KO-1 cells exhibited decreased ALDH levels compared to controls. Furthermore, the proliferation of CHRNB2-KO MKN1 cells was more sensitive to the cytotoxic effects of 5-fluorouracil and cisplatin.

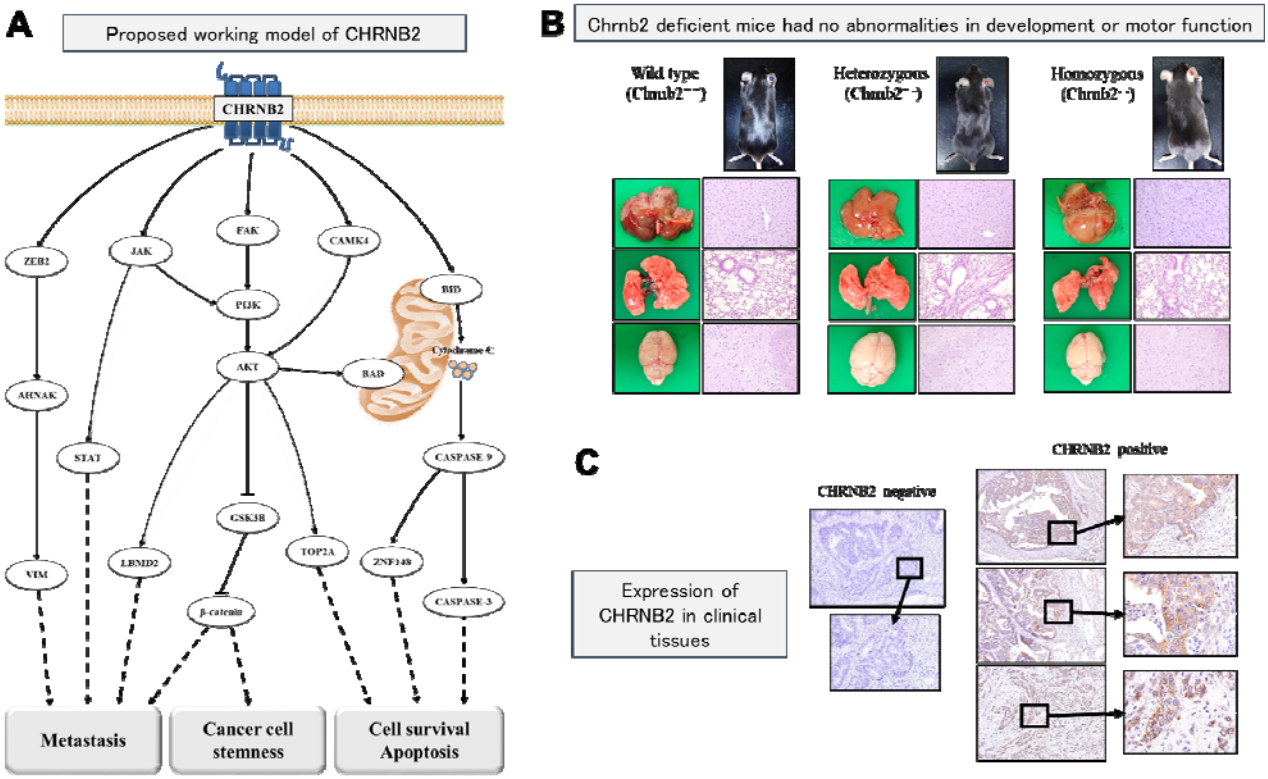
CHRNB2 was expressed on the surface of MKN1 cells (Figure 2A). We generated and tested monoclonal antibodies targeting of CHRNB2 by immunization of mice with CHRNB2 peptide predicted to be immunogenic (Figure 2B). A sensorgram of the analyte (peptide CTFLHSDHSAPSSK) to anti-CHRNB2 monoclonal antibody is shown in Figure 2C. The affinity constant of monoclonal antibody was determined to be 5.0 nM. To define the specificity of the anti-CHRNB2 monoclonal antibodies, fluorescent cell staining was performed. We also observed that the localization of CHRNB2 proteins on the cell surface and monoclonal antibody overlapped in both MKN1 and N87 cells (Figure 2D). After forced expression of labeled endogenous CHRNB2 in HEK293 cells and addition of anti-CHRNB2 monoclonal antibody, we found that localization of CHRNB2 and monoclonal antibody overlapped (Figure 2D). Next, the in vivo therapeutic effect of the CHRNB2 monoclonal antibody was evaluated in a mouse xenograft model of metastatic gastric cancer. Four weeks after implantation of gastric cancer cells, a number of nodules on the omentum and mesenteric tissue were observed in all mice treated with control IgG (Figure 2E). In contrast, no nodules were observed in mice treated with CHRNB2 monoclonal antibody (Figure 2E).



Suppression of CHRNB2 expression led to decreased phosphorylation of PI3K and AKT and enhanced phosphorylation of GSK3B. Phosphorylation of the components of the JAK-STAT signaling pathway was suppressed in response to blockade or silencing of CHRNB2 expression. In contrast, inhibiting CHRNB2 expression had little effect on the phosphorylation of components of the MAPK, Hippo, or NFkB pathways. Decreased phosphorylation of vimentin, microtubule-associated protein 1B, and catenin beta 1 was observed when CHRNB2 expression was inhibited. The proposed working model of CHRNB2 is shown in Figure 3A.

Chrnb2$^{+/+}$, Chrnb2$^{+/-}$, and Chrnb2$^{-/-}$ mice were generated to identify the pathophysiological functions of CHRNB2. No embryonic lethality was induced by KO of one or both Chrnb2 alleles. Moreover, KO of Chrnb2 had no effect on the appearance or body weight of mice and was not associated with abnormal development of the brain, lungs or liver (Figure 3B). Moreover, neither Chrnb2$^{+/-}$ nor Chrnb2$^{-/-}$ mice exhibited dysfunctional motor coordination or motor learning.

In situ expression of CHRNB2 protein was successfully detected by immunostaining method (Figure 3C).



Research Summary and Future Perspective

CHRNB2 plays an essential role in controlling the malignant behavior of gastric cancer cells in vitro and in vivo. CHRNB2-targeting monoclonal antibodies may thus have utility as treatment modalities for gastric cancer. Since anti-CHRNB2 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 CHRNB2 including colon, lung, breast and pancreatic cancer.

Publication

Kanda M, Shimizu D, Nakamura S, Sawaki K, Umeda S, Miwa T, Tanaka H, Inokawa Y, Hattori N, Hayashi M, Tanaka C, Nakayama G, Iguchi Y, Yamada S, Katsuno M, Kodera Y. Blockade of CHRNB2 signaling with a therapeutic monoclonal antibody attenuates the aggressiveness of gastric cancer cells. Oncogene 2021 in press
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Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Onco_210730.pdf