Received: 11 April 2023 | Revised: 8 December 2023 | Accepted: 22 December 2023

DOI: 10.1111/cas.16087

Cancer Science WILEY

ORIGINAL ARTICLE

Potential of Mac-2-binding protein glycan isomer as a new therapeutic target in pancreatic cancer

Takahiro Yamanaka[1] | Kenichiro Araki[1] | Takehiko Yokobori[2] | Ryo Muranushi[1] | Kouki Hoshino[1] | Kei Hagiwara[1] | Dolgormaa Gantumur[1] | Norihiro Ishii[1] | Mariko Tsukagoshi[1] | Akira Watanabe[1] | Norifumi Harimoto[1] | Atsushi Masamune[3] | Haruki Uojima[4] | Masashi Mizokami[4] | Kiyoaki Ito[5] | Ken Shirabe[1]

[1]Division of Hepatobiliary and Pancreatic Surgery, Department of General Surgical Science, Gunma University, Graduate School of Medicine, Maebashi, Gunma, Japan

[2]Gunma University Initiative for Advanced Research, Maebashi, Gunma, Japan

[3]Division of Gastroenterology, Tohoku University, Graduate School of Medicine, Sendai, Japan

[4]Genome Medical Science Project, National Center for Global Health and Medicine, Ichikawa, Japan

[5]Department of Gastroenterology, Aichi Medical University, Nagakute, Aichi, Japan

Correspondence
Kenichiro Araki, Division of Hepatobiliary and Pancreatic Surgery, Department of General Surgical Science, Gunma University, Graduate School of Medicine, 3-39-22 Showamachi, Maebashi 371-8511, Gunma, Japan.
Email: karaki@gunma-u.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Number: 22H03143

Abstract

Pancreatic cancer (PC) is a challenging malignancy to treat. Mac-2-binding protein glycan isomer (M2BPGi) is a novel serum marker of liver fibrosis and hepatocellular carcinoma and is secreted by hepatic stellate and stroma cells. Serum M2BPGi levels are upregulated in PC patients. We measured the expression of M2BPGi in the serum of 27 PC patients and determined whether M2BPGi affects the malignant potential of PC cells in vitro. We also examined the effect of M2BP on PC tumor growth and gemcitabine sensitivity in vivo. Serum M2BPGi levels in PC patients were higher compared with those of healthy subjects. M2BPGi extraction in cancer-associated fibroblasts (CAFs) was higher compared with that of PC cells. M2BPGi treatment promoted the proliferation and invasion of PC cells. The suppression of galectin-3, which binds to M2BPGi, did not affect the proliferation-promoting effect of M2BPGi in PC cells. The suppression of M2BP reduced tumor growth and enhanced gemcitabine sensitivity in PC-bearing xenograft mice. CAF-derived M2BPGi promotes the proliferation and invasion of PC cells. Targeting M2BPGi may represent a new therapeutic strategy to circumvent refractory PC.

KEYWORDS
cancer-associated fibroblasts, galectin-3, Mac-2-binding protein glycan isomer, pancreatic cancer, stroma cells

1 | INTRODUCTION

Pancreatic cancer (PC) is one of the most malignant human tumors. The prognosis of PC is very poor because of its rapid progression, early metastasis, and limited response to chemotherapy and radiotherapy.[1] Therefore, it is necessary to identify the underlying mechanisms of PC progression to develop new therapeutic targets.

Prostate tumors contain extensive fibrosis, which is caused by cancer-associated fibroblasts (CAFs). CAFs play an important role

Abbreviations: CAF, cancer-associated fibroblast; CM, culture medium; HCC, hepatocellular carcinoma; HSC, hepatic stellate cells; M2BPGi, Mac-2-binding protein glycan isomer; PC, pancreatic cancer; WFA, Wisteria floribunda agglutinin.

in the tumor microenvironment and promote malignant behavior, such as proliferation, invasion, metastasis, and resistance to therapy through interactive signaling pathways.[2,3] Some studies indicate that CAFs promote the malignant behavior of cancer cells by secreting cytokines, growth factors, and exosomes that contain microRNAs.[4,5] These findings suggest that targeting extracellular secretion from CAFs into the tumor microenvironment may be a novel strategy for controlling PC aggressiveness.

Wisteria floribunda agglutinin (WFA) lectin binds to terminal N-acetylgalactosamine motifs on carbohydrate structures and with Mac-2-binding protein (M2BP, also known as galectin-3-binding protein) glycoforms during liver fibrosis.[6] Therefore, WFA-binding M2BP is known as a Mac-2-binding protein glycan isomer (M2BPGi).[6] M2BPGi is considered a novel serum marker of liver fibrosis and HCC development associated with various pathologies, including hepatitis B and C virus infection, nonalcoholic fatty liver disease, and primary sclerosing cholangitis.[7,8] Serum M2BPGi levels may be a prognostic indicator for HCC patients with hepatitis B and C virus infection.[7,9,10] Bekki et al. reported that M2BPGi is secreted by hepatic stellate cells (HSCs) in the microenvironment of the cirrhotic liver. Moreover, the subsequent binding of galectin-3 with M2BPGi, known as galectin-3-binding protein glycoform, activates Kupffer cells.[11] We previously reported that M2BPGi is secreted into the tumor microenvironment from stromal cells in HCC tissues and the aggressiveness of HCC cell lines is upregulated following M2BPGi treatment.[12] Similar to HCC and fibrotic liver, the serum levels of M2BPGi are upregulated in patients with PC and chronic pancreatitis[13,14]; however, few studies have addressed whether M2BPGi in the tumor microenvironment with CAFs is a promising therapeutic target in refractory PC.

In the present study, we determined whether CAFs in the tumor microenvironment of PC tissues produce M2BPGi and examined the effect of M2BPGi on PC cells. Therefore, we measured M2BPGi levels in the culture medium (CM) of PC cells and CAFs and the effects of M2BPGi on the proliferation and invasion of PC cells. In addition, an in vivo xenograft model coimplanted with CAFs and PC cells was established to assess the therapeutic efficacy of combining gemcitabine with the suppression of M2BP by siRNA.

2 | MATERIALS AND METHODS

2.1 | Patient sample

We retrospectively reviewed PC patients who underwent an operation at Gunma University Hospital (Maebashi, Japan). Serum M2BPGi levels were measured in preoperative laboratory tests of 27 PC patients in our cohort and 150 healthy subjects from the previous report.[15] FFPE samples from PC patients were used for RNA in situ hybridization. All clinical samples and patient data were analyzed in accordance with our institutional guidelines and the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Gunma University (approval number: 2018–280).

2.2 | RNA in situ hybridization assay

Paraffin-embedded tissue sections on slides were subjected to RNA in situ hybridization to determine the localization and expression of *M2BP* RNA using an *M2BP*-specific probe and an RNAscopeR2.5 HD Detection kit (Advanced Cell Diagnostic, ACD). The technique was performed manually according to the manufacturer's protocol. The resulting images were evaluated by light microscopy.

2.3 | Immunofluorescence staining

Immunofluorescence staining was performed on clinical PC samples as previously described[12] using primary antibodies, including mouse anti-αSMA (A2547; 1:1000; Sigma), goat anti-M2BP (A2226; 1:200; R&D System), and biotinylated-WFA lectin (FL1351; 1:1000; Vector Laboratories), as well as appropriate Alexa Fluor-conjugated secondary antibodies and fluorescent streptavidin conjugates.

2.4 | Cell culture and cell isolation

We established primary CAFs (CAF1 and CAF2) from surgically resected PC tissues at Gunma University using a previously described method.[16] The resected PC samples were used in accordance with the Helsinki Declaration and Institutional Review Board of Gunma University (approval number: 2016-118) after obtaining written informed consent. We used the CAFs between passage numbers 4 and 8. The human PC cell lines, Suit2 and BXPC3, were obtained from the JCRB Cell Bank and SW1990 was obtained from the ATCC. PANC1 and the human pancreatic stellate cell line hPSC5, derived from PC, was provided by RIKEN BRC. The human HSC line LX-2 was purchased from Millipore. Cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin (Thermo Fisher Scientific) and maintained at 37°C in a humidified incubator with 5% CO_2 atmosphere.

2.5 | Preparation of CM

Cells were cultured in DMEM supplemented with 10% FBS until 80% confluent. Next, the medium was changed to serum-free DMEM and the cells were cultured for an additional 72 h. At the end of the incubation, the media was collected and centrifuged for 10 min at 1500 *g* and designated as CM for this study.

2.6 | Protein extraction and Western blot analysis

Protein extraction was performed using RIPA buffer (Wako) according to the manufacturer's instructions. To evaluate the production of M2BP protein in CAFs, hPSC5 and CAF1 cell lines were treated with the protein transport inhibitor GoldiPlug (6 h) before

protein harvest. Proteins were separated using SDS–PAGE with 10% Bis–Tris gels and transferred to nitrocellulose membranes. Membranes were blocked with 5% skim milk and incubated overnight at 4°C with the following primary antibodies: anti-M2BP goat antibody (1:1000; R&D Systems), anti-galectin-3 rat antibody (1:1000; Cedarlane), and anti-β-actin mouse monoclonal antibody (1:1000; Sigma-Aldrich) as a loading control. Membranes were then treated with horseradish peroxidase (HRP)-conjugated secondary antibodies. Protein bands were detected using ECL™ Prime Western Blotting Detection Reagent and an ImageQuant™ LAS 4000 imager (GE Healthcare).

2.7 | Cell proliferation assay

The cell proliferation assay was performed using a Cell Counting Kit-8 assay (CCK-8; Dojindo Laboratories). The PC cells were seeded in 96-well plates. After an overnight incubation, the media was changed to serum-free medium containing M2BPGi and glycoform-deleted M2GPGi (GD-M2BPGi) (Sysmex Co.) at concentrations ranging from 0 to 3 μg/mL. GD-M2BPGi was produced from M2BPGi by the PNGase F according to the manufacturer's instructions (New England Biolabs). The GD-M2BPGi was purified using gel filtration columns. Cell proliferation was evaluated after 48 h. The absorbance of each well was measured using a spectrophotometer (Bio-Rad) at 450 nm with a reference wavelength of 650 nm.

2.8 | Invasion assay

The cell invasion assay was performed using 24-well Corning® BioCoat™ Matrigel Invasion Chambers (Corning). PC cells were seeded into the upper chamber in FBS-free medium and the lower chamber was filled with medium supplemented with 3% FBS containing M2BPGi at concentrations ranging from 0 to 3 μg/mL. After incubation for 48 h, the cells were fixed and stained with Diff-Quik (Sysmex). After staining, the cells that migrated through the pores to the lower surface of the membrane were counted under a microscope. In total, five randomly selected fields were evaluated.

2.9 | SiRNA transfection in vitro

Pancreatic cancer cells (1.0×10^6/100 μL) were suspended in Opti-MEM I reduced serum media (Thermo Fisher Scientific) and mixed with 200 μM M2BP siRNA (sense: AGGUACUUCUACUCCCGAA, antisense: UUCGGGAGUAGAAGUACCU) or 200 μM galectin-3 siRNAs #1 (sense: GGAGAGUCAUUGUUUGCAA, antisense: UUGCAAACAAUGACUCUCC), siRNA #2 (sense: GGGAAUGAUGUUGCCUUCCACUUUA, antisense: UAAAGUGGAAGGCAACAUCAUUCCC), and control siRNA (Dharmacon) respectively. SiRNA transfection was performed with electroporation using a CUY21 EDIT II electroporator (BEX) with poring and transfer pulses applied at 150 and 10 V. The proliferation and invasion assays were then performed as described above.

2.10 | In vivo experiments

We used in vivo tumor-bearing mouse models inoculated with PC cell lines alone and in combination with CAFs as previously reported.[17] The mice had free access to water and food and were housed in specific pathogen-free cages and bedding in a 12-h light/dark regimen with controlled room temperature. Briefly, suspensions of Suit2 PC cells (3×10^6 cells) alone or Suit2 PC cells (3×10^6) plus hPSC5 cells (1×10^6) were bilaterally subcutaneously injected into the flanks of isoflurane-anesthetized 7-week-old female NOD-SCID mice (CLEA Japan, Inc). Two weeks after implantation, the mice were randomly divided into control, siRNA of M2BP (siM2BP), gemcitabine, and siRNA of M2BP plus gemcitabine (siM2BP + gemcitabine) groups. In vivo silencing of M2BP was performed as described by Tsukagoshi et al.[18] After the mice were deeply anesthetized with isoflurane, a fork-type electrode was inserted into the subcutaneous tumors, scooping from the bottom of the tumor, and then M2BP siRNA (2000 pmol/100 μL, sense: AGGUACUUCUACUCCCGAAtt, antisense: UUCGGGAGUAGAAGUACCUga) or PBS (100 μL) was injected into each tumor. The plate-type electrode was immediately put in contact with the surface of the tumor and electric pulses were delivered to each tumor using the CUY21EDIT II Next-Generation Electroporator (BEX). Treatment with siRNA was repeated every 4 days, and 50 mg/kg gemcitabine was administered intraperitoneally every 4 days under isoflurane anesthesia. Each group contained four xenografts and the treatments were continued for 2 weeks (Figure S1A). The suppression of M2BP was confirmed in the xenograft tumors (Figure S1B). Tumor diameters and body weight were measured twice a week, and tumor volume was calculated using the following formula: $S\times S\times L/2$, where S is the short diameter of the tumor, and L is the long diameter of the tumor. To harvest the tumors, the mice were anesthetized with isoflurane and euthanized by cervical dislocation. All mouse experiments were performed in compliance with the guidelines of the Institute for Laboratory Animal Research at Gunma University, Maebashi, Japan (approval number: 18-024).

2.11 | Immunohistochemistry

Immunohistochemical analysis of in vivo tumor samples was performed as previously described[19] using the goat anti-M2BP antibody (A2226; 1:1000; R&D System).

2.12 | Statistical analysis

Data for continuous variables were expressed as the mean ± standard deviation (SD). Differences among three or four groups were

evaluated using ANOVA with Tukey's multiple comparison tests. For the in vivo study, differences between groups were evaluated using repeated-measures ANOVA with Bonferroni's post hoc tests. All $p < 0.01$ or 0.05 was considered statistically significant. All statistical analyses were performed using Easy R (EZR) statistical software.

3 | RESULTS

3.1 | Expression and production of M2BPGi in PC patients

The mean value of serum M2BPGi level in 27 patients with PC was 0.89 (cutoff index [COI], range 0.25–2.63) (Figure 1A). The mean M2BPGi COI in Japanese healthy subjects ($n = 150$) was 0.54, which was significantly lower than that of patients with PC ($p < 0.01$) (Figure 1A). Furthermore, Tangvoraphonkchai et al.[20] reported M2BPGi in healthy subjects was <0.25 COI. In situ hybridization revealed that the M2BP mRNA was present not only in tumor cells, but also in the stromal cells of PC tissues, suggesting the production of M2BPGi by these cells (Figure 1B). *M2BP* mRNA was expressed in all 17 PC tissues assessed using RNAscope. However, no relationship between *M2BP* mRNA and serum M2BPGi levels was observed (data not shown). Immunofluorescence staining with M2BP, WFA, and αSMA revealed stromal cells in PC tissue which had M2BPGi (Figure S2). Moreover, M2BPGi was observed not only in the CM of PC cells, but also in the CM of CAFs of PC (Figure 1C). Also, M2BPGi levels in the CM of some CAFs were higher compared with those of LX-2, which secretes M2BPGi[11] (Figure 1C). Finally, M2BP protein levels were increased in CAFs of PC following treatment with GoldiPlug as a representative protein transport inhibitor, which indicates the existence of a rapid M2BP-release system from M2BP-producing cells (Figure 1D).

3.2 | M2BPGi promotes the proliferation and invasion of PC cells

To determine the effect of M2BPGi on PC cell lines, we examined the effect of M2BPGi on the proliferation of PC cell lines. M2BPGi treatment of PC cell lines significantly promoted the proliferation and invasion ability in a concentration-dependent manner compared with the control (Figure 2A,B). Interestingly, the proliferation-promoting

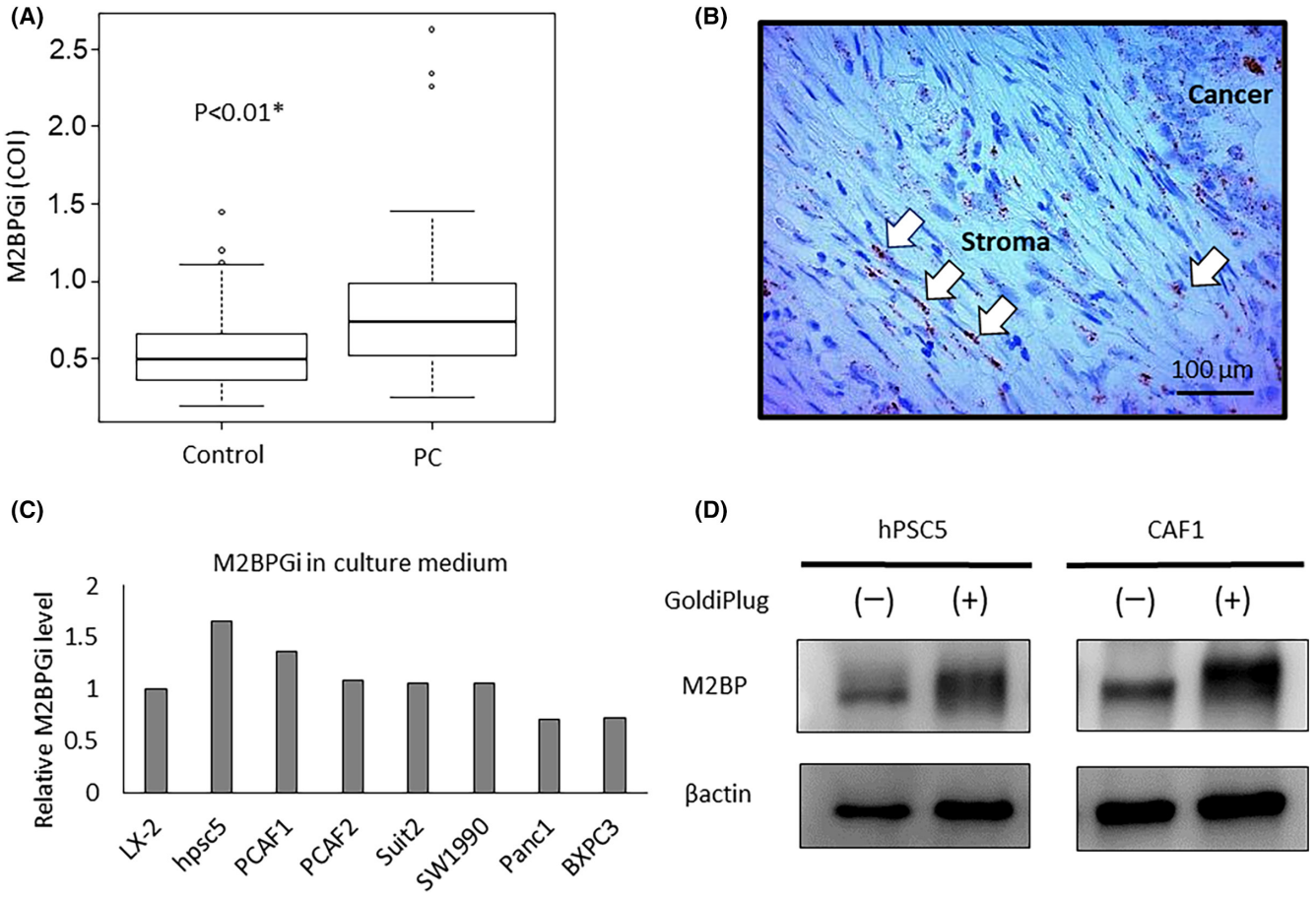

FIGURE 1 Mac-2-binding protein glycan isomer (M2BPGi) levels in pancreatic cancer (PC) patients' samples and cell lines. (A) Serum M2BPGi values (cutoff index [COI]) in PC patients ($n = 27$) were higher compared with those of healthy subjects ($n = 150$) ($p < 0.01$). (B) The expression of M2BP mRNA in a representative PC tissue is present not only in cancer cells but also in cancer-associated fibroblasts (CAFs) by in situ hybridization. The white arrows indicate M2BP mRNA production in CAFs. (C) Relative M2BPGi levels in the medium of LX-2, CAFs, and PC cells. (D) Changes in M2BP accumulation by GoldiPlug treatment of the CAF cell line hPSC5 and primary CAFs.

effect of M2BPGi in PC cells was inhibited by treatment with GD-M2BPGi, which suggests the functional importance of glycoform on M2BPGi (Figure 2C).

3.3 | The significance of M2BP in cancer cells

To determine the importance of M2BP in cancer cells on PC aggressiveness, we measured the effect of proliferation and invasion of M2BP-suppressed PC cell lines. The results indicated that M2BP suppression did not affect the proliferation of PC cells, but it suppressed the invasion of PC cells (Figure 3A–C).

3.4 | Significance of galectin-3 on the cancer-promoting mechanism of M2BPGi

We previously reported that the effect of M2BPGi against HCC cell lines was mediated by galectin-3, a representative binding partner of M2BPGi.[12] Therefore, to determine the importance of galectin-3 on M2BPGi-induced PC aggressiveness, we examined the effect of M2BPGi in galectin-3-suppressed PC cell lines. Galectin-3 suppression did not affect M2BPGi-induced proliferation in PC cells, which is contrary to the relationship between M2BPGi and galectin-3 in HCC cells (Figure 4A,B).

3.5 | The combination of M2BP suppression with gemcitabine significantly inhibits tumor growth in a tumor-bearing mouse model

Using a tumor-bearing mouse model, we determined the combined effect of M2BP suppression plus gemcitabine, a standard drug used to treat patients with PC in clinical practice. The M2BP suppression and gemcitabine alone significantly inhibited tumor growth compared with the control. Of note, the combination of M2BP suppression with gemcitabine showed the most significant tumor growth inhibition compared with the other groups (Figure 5). Moreover, we determined the effect of the combined therapy on PC cells alone in xenografts, but M2BP suppression did not significantly affect tumor growth (Figure S3). Although we evaluated adverse events associated with the treatments in vivo, there were no significant differences among the groups regarding the body weight of the mice.

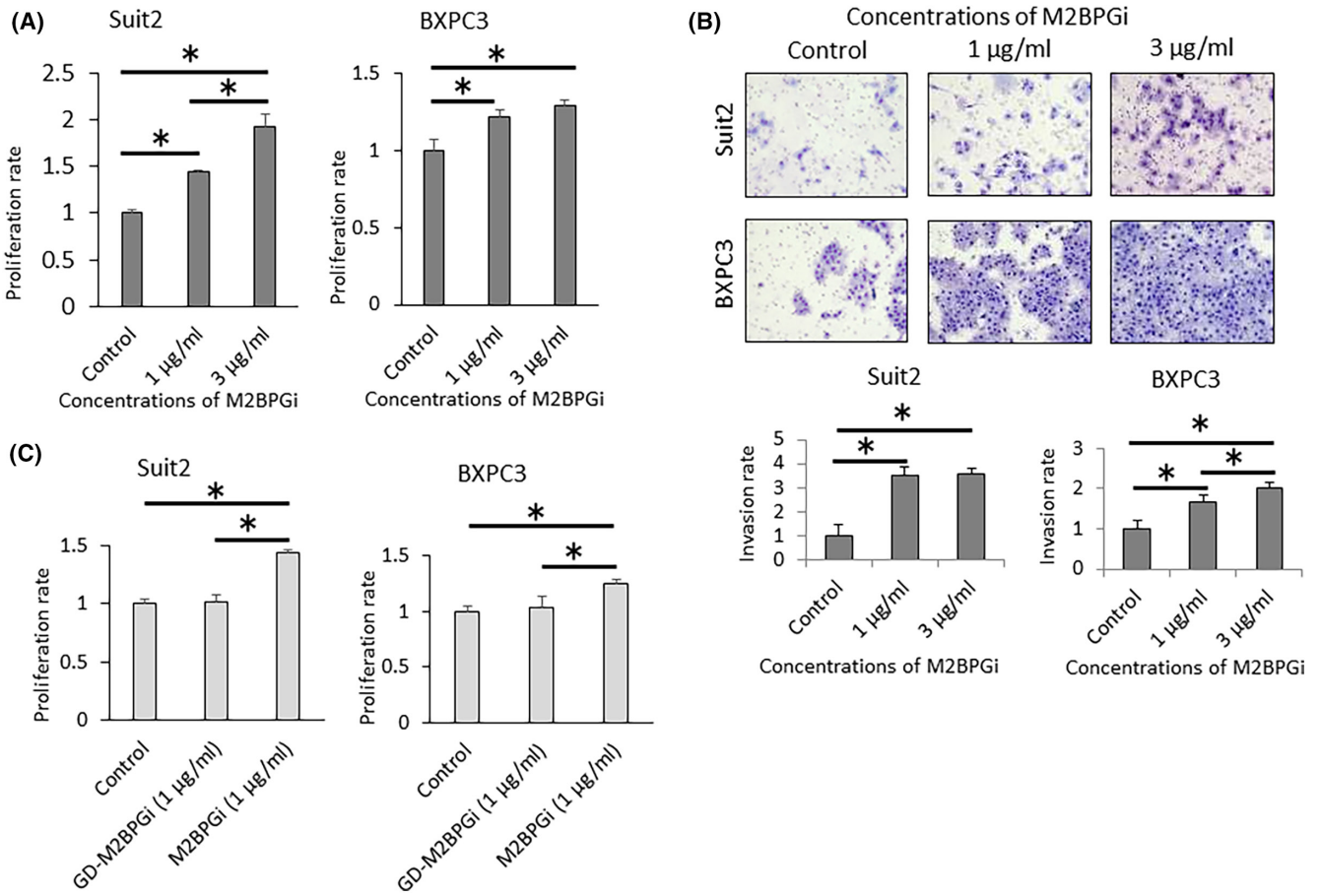

FIGURE 2 Effects of Mac-2-binding protein glycan isomer (M2BPGi) on pancreatic cancer (PC) cells. (A) M2BPGi promotes the proliferation of PC cells in a concentration-dependent manner ($n = 5$). *$p < 0.01$. (B) M2BPGi promotes the invasion of PC cells in a concentration-dependent manner ($n = 5$). *$p < 0.01$. (C) The effect of M2BPGi and glycoform-deleted M2GPGi (GD-M2BPGi) on proliferation potency in PC cells. *$p < 0.01$.

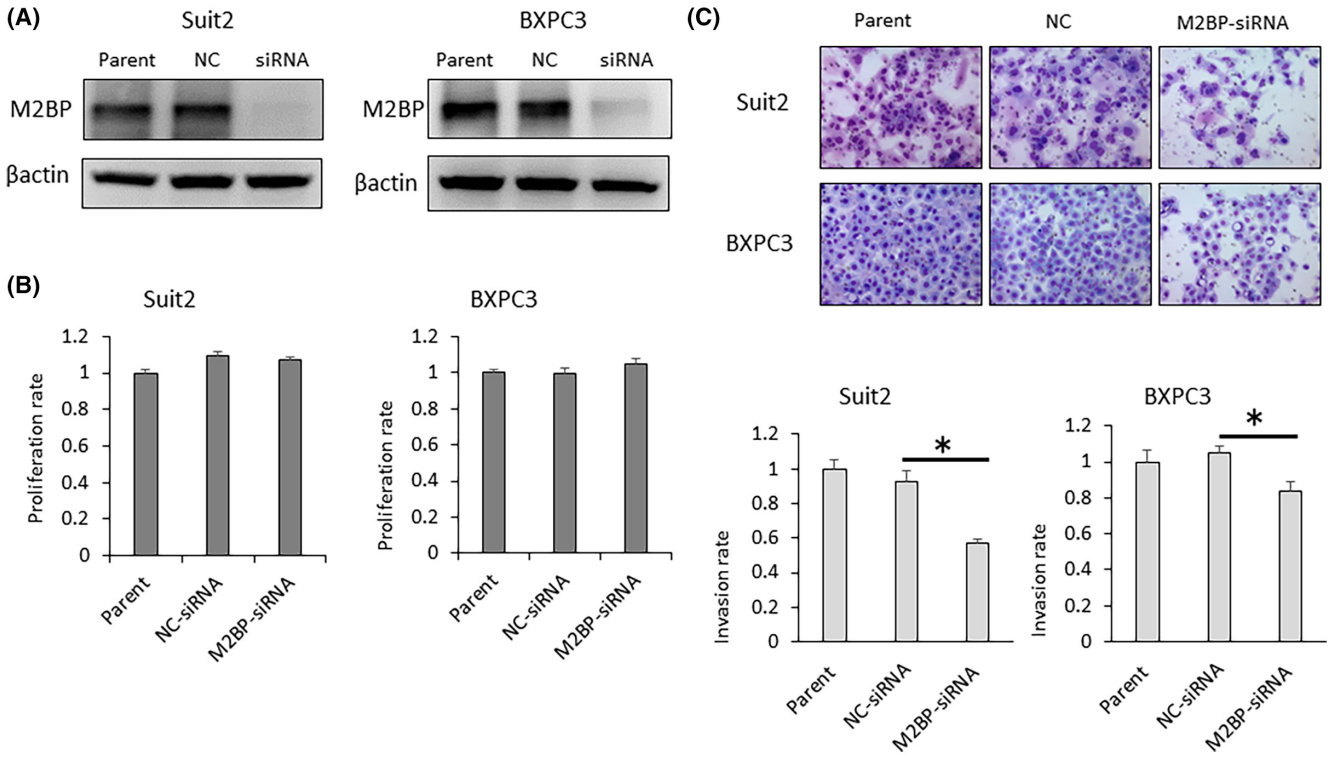

FIGURE 3 Effects of M2BP on the aggressiveness of pancreatic cancer (PC) cells. (A) The expression of M2BP in Suit2 and BXPC cells is suppressed using specific siRNA. (B) Suppression of M2BP does not affect the proliferation of PC cell lines ($n=5$). (C) Suppression of M2BP inhibits the invasion of PC cell lines ($n=5$). $^*p<0.01$.

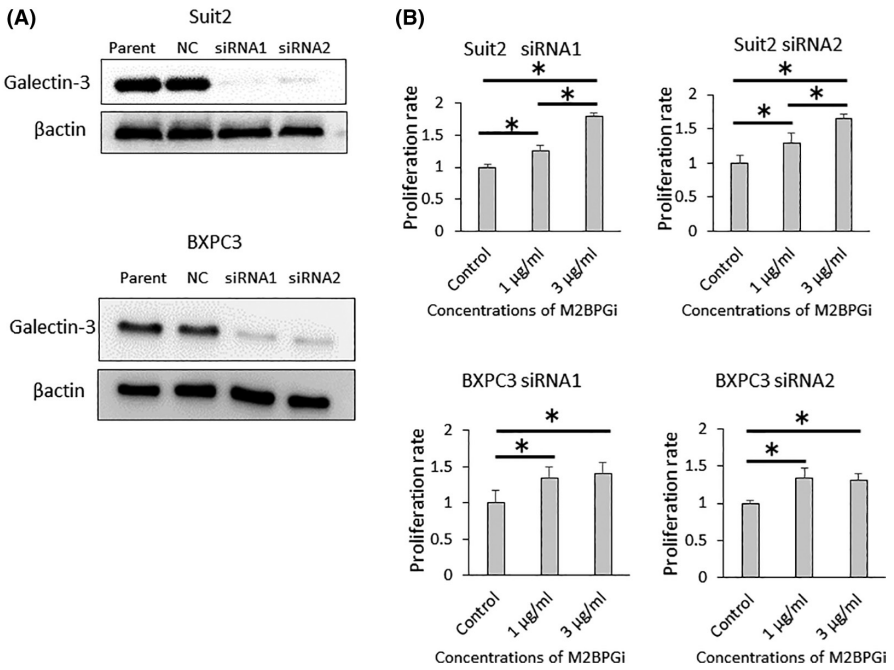

FIGURE 4 Effects of Mac-2-binding protein glycan isomer (M2BPGi) on galectin-3-suppressed pancreatic cancer (PC) cells. (A) The expression of galectin-3 in Suit2 and BXPC cells is suppressed by siRNA1 and siRNA2. (B) M2BPGi promotes the proliferation of galectin-3-suppressed PC cells in a concentration-dependent manner ($n=5$). $^*p<0.01$.

4 | DISCUSSION

This study is the first report to show that CAF-secreted M2BPGi promotes the malignant potential of PC cell lines. Moreover, in vivo experiments revealed that M2BP suppression inhibited tumor growth and enhanced gemcitabine sensitivity to an even greater extent compared with single treatment. This suggests that M2BPGi may represent a novel therapeutic target to treat refractory PC.

Recently, tumor–stromal interactions in cancer tissues have been recognized as one of the important factors in tumor progression, and CAFs have been observed to promote invasive and metastatic characteristics of several cancers, such as PC.[16,17,19] Furthermore,

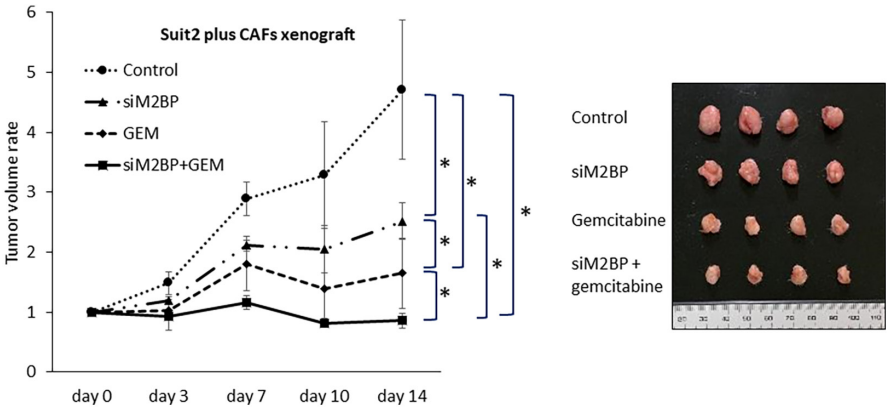

FIGURE 5 The combination of M2BP suppression with gemcitabine markedly inhibits xenograft tumor growth. Tumor growth curve shows that combination therapy with siM2BP plus gemcitabine suppresses the growth of xenograft tumors consisting of Suit2 plus cancer-associated fibroblasts (CAFs) to the largest extent. Photograph of tumors from the four treatment groups (control, siM2BP, gemcitabine, siM2BP + gemcitabine) ($n = 4$); $^*p < 0.05$.

stromal cells have been reported to secret M2BPGi and promote HCC.[12] In the present study, we clarified that CAFs of PC secrete greater amounts of M2BPGi compared with PC cells. M2BPGi was expected to be secreted immediately after production by CAFs because a large amount of M2BP accumulated in CAFs following Goldiplug treatment. M2BPGi treatment promoted the proliferation and invasion of PC cells; however, the suppression of M2BP in PC cells significantly affected only the invasion of PC cells in vitro but not proliferation in vitro or the growth of PC xenografts. Therefore, M2BP in PC cells may have little effect on cancer progression in PC. In the present study, CAFs in the stroma of PC were expected to produce and immediately secrete large amounts of M2BPGi, similar to that of HSC, which produced and secreted M2BPGi in the stroma of liver fibrosis.[11] Thus, CAF-secreted M2BPGi into the PC microenvironment may play an essential role in the regulation of cancer aggressiveness.

The physiological activity of M2BPGi is regulated by binding with galectin-3.[11] Moreover, we also reported that M2BPGi promotes HCC aggressiveness by binding with galectin-3.[12] In the present study, M2BPGi promoted the proliferation and invasion of PC cells in vitro, whereas suppression of M2BP inhibited PC tumor growth plus CAF xenografts in vivo. Unexpectedly, galectin-3 suppression did not affect M2BPGi-induced proliferation in PC cells, indicating that, in contrast to our HCC results, M2BPGi-induced PC aggressiveness is not mediated by galectin-3 regulation. Therefore, the effect of M2BPGi on PC cells may be independent of binding with galectin-3. The effects of M2BPGi on other cancer cells and normal cells have not been reported so far. Further studies are needed to clarify the functional mechanisms of M2BPGi.

M2BPGi was identified as a serum biomarker that is increased in liver virus infection, fibrosis, and cancer.[7] Moreover, we discovered a new function of hepatic stromal cells as the origin of M2BPGi production.[12,21] Few studies have evaluated whether M2BPGi levels are changed in other diseases, excluding liver disorders. In PC patients, we clarified that the serum level of M2BPGi was higher compared with that in healthy individuals. Moreover, a retrospective observational study in a small number of PC patients showed that the serum levels of M2BPGi were higher compared with those of noncancerous chronic pancreatitis, whereas high M2BPGi levels were associated with the detection of metastatic lesions and poor prognosis.[14] Further studies are needed to clarify the clinical meaning and value of serum M2BPGi in PC because the present M2BPGi data were limited to a small number of PC cases.

Chemotherapy for PC is largely ineffective; therefore, developing new therapeutic targets is necessary.[1] We found that M2BPGi promotes PC aggressiveness and may represent a new therapeutic target. Interestingly, the direct suppression of M2BP protein translation by RNA interference inhibited the growth of PC xenografts in vivo, whereas combination therapy with gemcitabine enhanced the suppressive effect. Moreover, in this study, GD-M2BPGi did not promote the proliferation of PC cells in vitro, suggesting the importance of glycoform in M2BPGi and PC aggressiveness. Therefore, our data indicate that therapeutic strategies to inhibit M2BP expression and WFA glycoform conjugation is promising because both components of M2BPGi are essential for M2BPGi function in PC cells.

Our study has some limitations. First, it is unclear whether isolated hPSC5 cells can fully maintain the phenotype of CAFs in cancer tissue because CAFs are thought to maintain homeostasis by interacting with cancer cells. Second, mouse models of subcutaneous tumors using hPSC5 cells and PC cell lines may not fully reproduce the stroma of PC, as human PC is composed of not only cancer cells and CAFs but also immune cells and vascular endothelial cells.

In conclusion, we demonstrated that M2BPGi is primarily secreted by CAFs and promotes the aggressiveness of PC cells. Moreover, we showed that the combination of M2BP suppression with gemcitabine significantly inhibited the tumor growth in PC xenograft models. Therefore, CAF-derived M2BPGi may be important for PC aggressiveness and the combination of M2BPGi targeting with conventional chemotherapy may represent a new therapeutic strategy to circumvent refractory PC.

AUTHOR CONTRIBUTIONS

Takahiro Yamanaka: Investigation; writing – original draft. **Kenichiro Araki:** Investigation; methodology. **Takehiko Yokobori:** Investigation; methodology. **Ryo Muranushi:** Investigation; methodology. **Kouki Hoshino:** Investigation; methodology. **Kei Hagiwara:** Investigation; methodology. **Dolgormaa Gantumur:** Investigation; methodology. **Norihiro Ishii:** Investigation; methodology. **Mariko Tsukagoshi:** Investigation; methodology. **Akira Watanabe:** Investigation; methodology. **Norifumi Harimoto:** Investigation; methodology. **Atsushi Masamune:** Resources. **Haruki Uojima:** Investigation. **Masashi Mizokami:** Investigation. **Kiyoaki Ito:** Investigation. **Ken Shirabe:** Methodology; project administration; supervision.

ACKNOWLEDGMENTS

The authors would like to thank Sysmex Co. for providing M2BPGi and GD-M2BPGi. The authors would also like to thank Enago (www.enago.com) for the English language review.

FUNDING INFORMATION

The present study was supported by a Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (22H03143).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: The measurement of serum levels of M2BPGi in pancreatic cancer patients and the research protocol were approved by the Institutional Review Board (IRB) of Gunma University (approval number: 2018–280). Informed Consent: The establishment of cancer-associated fibroblasts from surgically resected pancreatic cancer tissues was approved by the IRB of Gunma University (Approval number: 2016-118), and consent to participate was obtained from all patients. The study was performed in accordance with the Declaration of Helsinki. Registry and the Registration No. of the study/trial: N/A. Animal studies: All mouse experiments were performed in compliance with the guidelines of the Institute for Laboratory Animal Research at Gunma University, Maebashi, Japan (approval number: 18-024).

CONSENT FOR PUBLICATION

Approval.

ORCID

Takahiro Yamanaka https://orcid.org/0000-0002-2587-0083
Norihiro Ishii https://orcid.org/0000-0001-6591-2439

REFERENCES

1. Ilic M, Ilic I. Epidemiology of pancreatic cancer. *World J Gastroenterol*. 2016;22(44):9694-9705.
2. Apte MV, Wilson JS, Lugea A, Pandol SJ. A starring role for stellate cells in the pancreatic cancer microenvironment. *Gastroenterology*. 2013;144(6):1210-1219.
3. Boju P, Quan L, Zheyu N, Li Z, Yupei Z. Cancer-associated fibroblasts in pancreatic adenocarcinoma. *Future Oncol*. 2015;11(18):2603-2610.
4. Rosa FH, Todd M, Thiruvengadam A, et al. Cancer-associated stromal fibroblasts promote pancreatic tumor progression. *Cancer Res*. 2008;68(3):918-926.
5. Katherine ER, Ann EZ, Melissa LF, Junmin W, Laurie EL, Reginald H. Cancer-associated fibroblast exosomes regulate survival and proliferation of pancreatic cancer cells. *Oncogene*. 2017;36(13):1770-1778.
6. Kuno A, Sato T, Shimazaki H, et al. Reconstruction of a robust glycodiagnostic agent supported by multiple lectin-assisted glycan profiling. *Proteomics Clin Appl*. 2013;7(9–10):642-647.
7. Shirabe K, Bekki Y, Gantumur D, et al. Mac-2 binding protein glycan isomer (M2BPGi) is a new serum biomarker for assessing liver fibrosis: more than a biomarker of liver fibrosis. *J Gastroenterol*. 2018;53(7):819-826.
8. Umetsu S, Inui A, Sogo T, Komatsu H, Fujisawa T. Usefulness of serum Wisteria floribunda agglutinin-positive Mac-2 binding protein in children with primary sclerosing cholangitis. *Hepatol Res*. 2018;48(5):355-363.
9. Toyoda H, Kumada T, Tada T, et al. Serum WFA+ -M2BP levels as a prognostic factor in patients with early hepatocellular carcinoma undergoing curative resection. *Liver Int*. 2016;36(2):293-301.
10. Harimoto N, Itoh S, Yamanaka T, et al. Mac-2 binding protein glycosylation isomer as a prognostic marker for hepatocellular carcinoma with sustained virological response. *Anticancer Res*. 2022;42(1):245-251.
11. Bekki Y, Yoshizumi T, Shimoda S, et al. Hepatic stellate cells secreting WFA+ -M2BP: its role in biological interactions with Kupffer cells. *J Gastroenterol Hepatol*. 2017;32(7):1387-1393.
12. Dolgormaa G, Harimoto N, Ishii N, et al. Mac-2-binding protein glycan isomer enhances the aggressiveness of hepatocellular carcinoma by activating mTOR signaling. *Br J Cancer*. 2020;123(7):1145-1153.
13. Fujiyama T, Ito T, Ueda K, et al. Serum levels of Wisteria floribunda agglutinin-positive Mac-2 binding protein reflect the severity of chronic pancreatitis. *J Dig Dis*. 2017;18(5):302-308.
14. Waragai Y, Suzuki R, Takagi T, et al. Clinical significance of serum Wisteria floribunda agglutinin-positive Mac-2 binding protein in pancreatic ductal adenocarcinoma. *Pancreatology*. 2016;16(6):1044-1050.
15. Uojima H, Nakabayashi K, Yamasaki K, et al. New chemiluminescent enzyme immunoassay for quantitative measurement of Mac-2 binding protein glycosylation isomer in chronic liver disease. *J Gastroenterol*. 2023;58:1252-1260.
16. Yamanaka T, Harimoto N, Yokobori T, et al. Nintedanib inhibits intrahepatic cholangiocarcinoma aggressiveness via suppression of cytokines extracted from activated cancer-associated fibroblasts. *Br J Cancer*. 2020;122(7):986-994.
17. Ishii N, Araki K, Yokobori T, et al. Conophylline suppresses pancreatic cancer desmoplasia and cancer-promoting cytokines produced by cancer-associated fibroblasts. *Cancer Sci*. 2019;110(1):334-344.
18. Tsukagoshi M, Araki K, Yokobori T, et al. Overexpression of karyopherin-α2 in cholangiocarcinoma correlates with poor prognosis and gemcitabine sensitivity via nuclear translocation of DNA repair proteins. *Oncotarget*. 2017;8(26):42159-42172.
19. Yamanaka T, Harimoto N, Yokobori T, et al. Conophylline inhibits hepatocellular carcinoma by inhibiting activated cancer-associated fibroblasts through suppression of G protein-coupled receptor 68. *Mol Can Ther*. 2021;20(6):1019-1028.

20. Tangvoraphonkchai K, Suttichaimongkol T, Kularbkaew C, Sangaimwibool P, Sukeepaisarnjaroen W. Application of Mac-2 binding protein glycosylation isomer as a non-invasive biomarker for probing liver disease. *Sci Rep*. 2022;12(1):6757.
21. Gantumur D, Harimoto N, Muranushi R, et al. Hepatic stellate cell as a Mac-2-binding protein-producing cell in patients with liver fibrosis. *Heptol Res*. 2021;51(10):1058-1063.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Yamanaka T, Araki K, Yokobori T, et al. Potential of Mac-2-binding protein glycan isomer as a new therapeutic target in pancreatic cancer. *Cancer Sci*. 2024;115:1241-1249. doi:10.1111/cas.16087