Enhanced expression of the M2 isoform of pyruvate kinase is involved in gastric cancer development by regulating cancer-specific metabolism

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Recent studies have indicated that increased expression of the M2 isoform of pyruvate kinase (PKM2) is involved in glycolysis and tumor development. However, little is known about the role of PKM2 in gastric cancer (GC). Therefore, we examined the expression and function of PKM2 in human GC. We evaluated PKM1 and PKM2 expression by quantitative RT-PCR in gastric tissues from 10 patients who underwent gastric endoscopic submucosal dissection, 80 patients who underwent gastrectomy, and seven healthy volunteers, and analyzed the correlation with clinicopathological variables. To assess the function of PKM2, we generated PKM2-knockdown GC cells, and investigated the phenotypic changes. Furthermore, we examined the induction of PKM2 expression by cytotoxin-associated gene A (CagA), a pathogenic factor of Helicobacter pylori, using CagA-inducible GC cells. We found that PKM2 was predominantly expressed not only in GC lesions but also in the normal gastric regions of GC patients and in the gastric mucosa of healthy volunteers. The PKM2 expression was significantly higher in carcinoma compared to non-cancerous tissue and was associated with venous invasion. Knockdown of PKM2 in GC cells caused significant decreases in cellular proliferation, migration, anchorage-independent growth, and sphere formation in vitro, and in tumor growth and liver metastasis in vivo. The serine concentration-dependent cell proliferation was also inhibited by PKM2 silencing. Furthermore, we found that PKM2 expression was upregulated by CagA by way of the Erk pathway. These results suggested that enhanced PKM2 expression plays a pivotal role in the carcinogenesis and development of GC in part by regulating cancer-specific metabolism.

Cancer cells produce an increased amount of lactate by glycolysis even in the presence of oxygen, a phenomenon termed “aerobic glycolysis” by Otto Warburg.⁶ This “Warburg effect” was thought not only to give cancer cells a great advantage under hypoxic conditions, but also to reduce reactive oxygen species.ⁱ,² Recent studies showed that the M2 type of pyruvate kinase (PK) is a key glycolytic enzyme for the Warburg effect.⁴,⁵ Pyruvate kinase regulates the final rate-limiting step of glycolysis by transferring the high-energy phosphate from phosphoenolpyruvate to ADP to generate ATP and pyruvate. Mammals have four isoforms of PK (L, R, M1, and M2), which are present in different cell types. Both PKL and PKR are encoded by the PKLR gene, and are expressed in the liver and red blood cells, respectively.⁶,⁷ The PK in other tissues is produced from the PKM gene, through a pre-mRNA that is alternatively spliced to yield the PKM1 or PKM2 isoform, which contains exon 9 or 10, respectively.⁶,⁷ Pyruvate kinase M1 is expressed in most differentiated tissues, whereas PKM2 is found primarily in embryonic tissues and tumor cells.⁶ The PKL, R, and M1 PK types are present as highly enzymatically active tetrameric forms. In contrast, PKM2 can exist as either an active tetramer or inactive dimer, and in tumor cells it is reported to be present as a dimer with low activity.⁴,⁷,⁹ As a result of this low activity of PKM2, glycolysis slows, and the subsequent pools of glycolytic intermediates are used to generate the nucleotides and amino acids that are essential for cellular growth.¹¹,¹² In some cancer cells, PKM2’s knockdown and replacement with PKM1 reverses the Warburg effect and reduces the cells’ ability to form tumors in nude mice.¹⁰ In addition, elevated PKM2 expression is found in various human cancers, suggesting that a switch of the PK (M) isoform from PKM1 to PKM2 is a pivotal event in carcinogenesis and/or cancer development.

Although the prevalence rate of gastric cancer (GC) has decreased,²⁻⁴² globally, it is the fourth most common cancer in men and the fifth in women, and its death rate is second only...
Expression of PKM2 is associated with a poor prognosis in GC patients, and PKM2 was shown to promote GC cell growth through the regulation of Bcl-xL expression or epidermal growth factor/epidermal growth factor receptor signaling. However, little is known about the shift from PKM1 to PKM2 or the role of PKM2 in GC carcinogenesis and development through Warburg-effect regulation.

In this study, we show that PKM2 is upregulated in human GC tissues without a switch in isoform expression and that PKM2 knockdown in GC cells reduced their sphere-formation ability, tumorigenicity, and metastasis by altering the Warburg effect. Furthermore, we found that PKM2 was induced by cytotoxin-associated gene A (CagA) through the MAPK signaling pathway, suggesting that increased PKM2 expression is also involved in the early stage of gastric tumorigenesis.

![Fig. 1.](image)

**Fig. 1.** Pyruvate kinase isoform M1 (PKM1) and PKM2 mRNA in normal gastric mucosa and gastric cancer (GC) tissues. (a) Expression levels of PKM1 and PKM2 were analyzed by quantitative RT-PCR in the normal gastric mucosa of healthy volunteers, and in early GC and advanced GC tissues. A significantly higher expression of PKM2 than PKM1 was found in all examined samples. (b) PKM2 mRNA levels were confirmed by quantitative RT-PCR in gastric mucosa from normal volunteers (NV), and in cancerous lesions (C) and non-cancerous tissue (NC) from early and surgically resected advanced GCs. PKM2 expression was significantly higher in GC lesions than in the corresponding non-cancerous tissues. *P < 0.05; **P < 0.01.

![Fig. 2.](image)

**Fig. 2.** Dominant pyruvate kinase M2 (PKM2) expression in gastric tissues. (a) cDNAs from normal gastric mucosa and from cancerous and non-cancerous tissues of gastric cancer patients who underwent endoscopic submucosal dissection or surgery were amplified by PCR using primers that anneal to exons 8 and 11, respectively. The alternative exons that encode the distinctive segments of PKM1 and PKM2 are exons 9 and 10, respectively. To distinguish between the PKM1 (exon 9 included) and PKM2 (exon 10 included) isoforms, the PCR products were cleaved with NcoI, PstI, or both. (b) After amplification, the products were separated into four aliquots and digested with NcoI (N), PstI (P), both enzymes (NP), or neither (uncut control, U). The products were separated by electrophoresis and visualized under UV light. The cDNA amplicons were completely cleaved with PstI but little with NcoI, indicating that PKM2 was the dominant isoform in both normal and cancerous gastric tissue.
Materials and Methods

Tissues. A total of 187 gastric tissues, including specimens from seven healthy volunteers (biopsy) and 90 matched normal and carcinoma pairs, were evaluated in this study. The GC tissues and their corresponding normal gastric tissues were obtained from patients who underwent surgery (n = 80) or endoscopic submucosal dissection (n = 10) at Miyagi Cancer Center (Natori, Japan), between 2007 and 2013. The samples obtained surgically or endoscopically were immediately frozen or immersed in RNA Later Stabilization Solution (Life Technologies, Carlsbad, CA, USA), respectively, and stored at −80°C. From the seven healthy volunteers, normal gastric mucosa biopsy specimens were obtained and immediately placed into RNA Later Stabilization Solution and stored at −80°C. The pathologic TNM status of these GCs was histopathologically classified according to the TNM Classification of Malignant Tumours, 6th edition of the Union for International Cancer Control (2002). No patient received chemotherapy or radiotherapy before the endoscopic resection or surgery. The healthy volunteers were confirmed to have no Helicobacter pylori infection by 13C-urea breath test (Otsuka Pharmaceuticals, Tokyo, Japan) or/and the measurement of serum H. pylori IgG antibody. This study was approved by the local ethics committee of Miyagi Cancer Center, and informed consent was obtained from each patient.

RNA preparation, reverse transcription, and quantitative real-time PCR. Total RNA was prepared using the RNeasy Mini Kit (Qiagen, Tokyo, Japan) for surgically resected GC tissues, or the mirVana miRNA Isolation Kit (Life Technologies) for endoscopically resected GC tissues, normal gastric biopsy specimens from healthy volunteers, and cultured cell lines, according to the manufacturers’ protocols. First-strand cDNA was generated from 0.5–1 μg total RNA using the PrimeScript 1st strand cDNA Synthesis Kit (TaKaRa Bio, Shiga, Japan) following the manufacturer’s protocol. PKM1 and PKM2 expressions were quantified by the LightCycler 480 Probes Master qRT-PCR kit (Roche Applied Science, Indianapolis, IN, USA) following the manufacturer’s protocol with probes (Universal Probe Library; Roche Applied Science). The primer sets used were: PKM1, forward 5′-cagccaaaggggactatcct-3′ and reverse 5′-gagctcgcacaagttcttc-3′; PKM2, forward 5′-ctatcctggaggctgtgc-3′ and reverse 5′-gtggggtcgctggtaatg-3′; and β-actin, forward 5′-ccacecgagaagatga-3′ and reverse 5′-

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Table 1. Association of pyruvate kinase M2 (PKM2) expression with clinicopathological features of 80 resected gastric cancer specimens

| Low-PKM2 (n = 58) | High-PKM2 (n = 22) | P-value† |
|-------------------|-------------------|----------|
| Age, years<br>&lt;70 | 32 | 9 | 0.254 |
| ≥70 | 26 | 13 | 0.539 |
| Gender<br>Male | 38 | 16 | 0.494 |
| Female | 20 | 6 | 0.152 |
| Differentiation<br>Intestinal | 32 | 14 | 0.001 |
| Diffuse | 26 | 8 | 0.227 |
| Primary tumor (T)<br>T1 | 7 | 5 | 0.761 |
| T2–4 | 51 | 17 | 0.845 |
| Regional lymph nodes (N)<br>N0 | 26 | 6 | 0.141 |
| N1–3 | 36 | 26 | 0.152 |
| Distant metastasis (M)<br>M0 | 48 | 21 | 0.001 |
| M1 | 10 | 1 | 0.761 |
| Stage<br>I | 19 | 8 | 0.152 |
| II–IV | 39 | 14 | 0.227 |
| Lymphatic invasion<br>ly0 | 24 | 6 | 0.245 |
| ly1–3 | 34 | 16 | 0.001 |
| Venous invasion<br>v0 | 35 | 4 | 0.001 |
| v1–2 | 23 | 18 | 0.539 |

†Analyzed by χ²-test. The pathologic TNM status of gastric cancers was histopathologically classified according to TNM Classification of Malignant Tumours, 6th edition (Union for International Cancer Control, 2002).
tccatcagatgc-3'. The PKM1 and PKM2 expression levels were normalized to that of β-actin.

**Polymerase chain reaction–RFLP.** Analysis with PCR-RFLP was carried out to accurately evaluate the relative levels of PK isoforms as described previously. Complementary DNAs synthesized as described above were amplified by PCR using primer sets as described previously. The primer sets annealed to exons 8 and 11, and had the following sequences: hPKMF, 5'-AGAAACAGCCAAAGGGGACT-3'; and hPKMR, 5'-CATTCATGGCAAAGTTCACC-3'. After amplification, the reactions were separated into four aliquots for digestion with: (i) NcoI (Roche Applied Science); (ii) PstI (Roche Applied Science); (iii) NcoI and PstI; or (iv) neither. The products were separated by electrophoresis on a 3% agarose gel with 0.5 μg/mL ethidium bromide, and visualized by UV light.

**Cell lines.** The GC cell lines MKN7, MKN74, MKN45, and KATO III were obtained from the Riken BioResource Center (Tsukuba, Japan) in 2011. These cell lines were not tested or authenticated after they were obtained. All cell lines were maintained in RPMI-1640 (Wako Pure Chemical Industries, Osaka, Japan) containing 10% inactivated FBS (EuroClone, Milano, Italy) with 100 U/mL penicillin and 100 μg/mL streptomycin (Nacalai Tesque, Kyoto, Japan) and cultured in a humidified 5% CO2 incubator at 37°C.

**RNA interference.** To knock down PKM2 in GC cell lines, we used the Knockout RNAi Systems (Clontech

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**Fig. 4.** Effect of pyruvate kinase M2 (PKM2) expression on tumor cell growth in vitro and in vivo. (a) PKM2 expression level was measured by quantitative RT-PCR and Western blot (WB) analyses in stably shPKM2-expressing gastric cancer (GC) cells (MKN7, MKN74, MKN45, and KATOIII). PKM2 expression was effectively reduced in the shPKM2-expressing GC cells. The quantitative RT-PCR results are shown in the bar graph, and Western blot results are shown below. (b) MTT assays showed that cell growth was significantly inhibited in all of the PKM2 knockdown (sh1 and sh2) GC cell lines. MTT assays were carried out in triplicate, and the data were analyzed by Dunnett’s test. *P < 0.05; **P < 0.01. (c) MKN45-sh1 (n = 6) and MKN-control (n = 6) cells were injected s.c. into NCG mice (1 × 106 cells/injection), and then the volume of s.c. tumors was measured once a week for 6 weeks. Tumor formation was significantly delayed (left panel), and tumor growth significantly suppressed (right panel) in the mice treated with PKM2-knockdown MKN474 cells (sh1) compared with control cells. In addition, smaller tumors were formed by the PKM2-knockdown MKN 74 cells compared to control cells. Tumors were resected from NCG mice (lower panel). Tumor formation ability was analyzed by Kaplan–Meier methods and evaluated by the log-rank test (left panel). Tumor volume was calculated by the formula: ([long diameter] × [short diameter]) × 0.5 (middle panel). *P < 0.05; **P < 0.01. NS, not significant.
Laboratories, Mountain View, CA, USA) according to the manufacturer’s protocol. We designed five shRNA sequences that targeted PKM2 according to a previous study. After annealing the complementary shRNA oligonucleotides, the oligonucleotides were ligated into a pSIREN vector (sh1 and 2). The Platinum-A packaging cell lines (provided by Professor Kitamura, Division of Cellular Therapy/Division of Stem Cell Signaling, The Institute of Medical Sciences, The University of Tokyo) were then transfected with shPKM2 or pSIREN Vector (control) to produce recombinant retroviruses. Gastric cancer cells were then infected with the recombinant retroviruses, and stably infected cell lines were selected with puromycin. Cells transduced with the sh1 and sh2 vector showed an effective reduction of PKM2, and were selected for further study. The sequences of the shRNAs that targeted PKM2 were: sh1, sense 5'-gaggca-gaggtgtccacatc-3' and anti-sense 5'-ctcgtctcgcctgtaga-3'; and sh2, sense 5'-agcgaggtgctcacatc-3' and anti-sense 5'-tcgtctcgcctgtagat-3'. All of the experiments in vitro were carried out in triplicate and repeated three times, and representative data are shown. Supplementary methods are included in Documents S1–S11.

Results

Pyruvate kinase M2 is dominantly expressed in normal gastric mucosa, and its expression is enhanced in tumor tissues. The PKM1 and PKM2 mRNA expression in normal mucosa (n = 7), early gastric cancer (n = 10), and resected gastric tumor (n = 80) was investigated by quantitative (q)RT-PCR, Fig. 5. Invasion capacity and anchorage-independent cell growth of pyruvate kinase M2 (PKM2)-downregulated gastric cancer (GC) cells. (a) Invasion capacity was evaluated by a two-chamber assay in which 1 × 10^5 or 5 × 10^5 cells were plated in each insert in triplicate. After 72 h, the cells on the bottom of the insert were stained and counted. Significantly fewer PKM2 downregulated migrated cells were observed compared to control cells (MKN7: 9 × 100, bar = 100 μm; MKN74 and MKN45: ×40, bar = 100 μm). *P < 0.05. (b) Anchorage-independent growth potential of GC cells was evaluated by soft agar assay in which 1 × 10^4 cells in 1× media and 0.3% low-melting-point agarose were plated in triplicate. Three weeks later, the cells were stained with MTT and colonies were counted. Representative wells are shown. Significantly fewer colonies were observed with the PKM2-knockdown GC cells (sh1 and sh2) compared with control GC cells. *P < 0.05; **P < 0.01. (c) To examine metastasis, 1.0 × 10^5 cells of MKN74-sh1 (n = 10) and MKN-control (n = 9) cells were injected into the tail vein of mice. After 8 weeks, the mice were killed and the number of liver metastases was counted macroscopically. The number of metastases to the liver was significantly decreased for sh1-MKN74 cells compared to control cells. *P < 0.05 (left panel). Metastatic tumors could be recognized macroscopically (middle panel), and formed tumor glands in the liver (right panel) (HE; original magnification, ×40, bar = 100 μm).
PKM2 expression in human gastric cancer

with normalization to the β-actin expression. As shown in Figure 1(a), PKM2 was expressed at a much higher level than PKM1 in the gastric mucosa of healthy volunteers as well as in GC tissues. To confirm the dominant expression of PKM2 against PKM1, we undertook PCR-RFLP analysis, which measures the relative level of PKM alternatively spliced mRNAs more accurately than qRT-PCR. (9) Using cDNAs as templates, PCR was carried out using a single pair of primers corresponding to constitutive exons 8 and 10 (Fig. 2a). As exons 9 and 10 are identical in length (167 bp), the resulting PCR products were digested with restriction enzymes that cleave either exon 9 (NcoI) or 10 (PstI) to distinguish the two isoforms (Fig. 2b). These analyses showed that PKM2 (containing exon 10) is the predominant isoform in both normal mucosa and carcinoma of the stomach. In contrast, PKM1 was under the detection limit in all the tissues examined.

Furthermore, the expression level of PKM2 was significantly higher in cancerous lesions than in non-cancerous tissue (Fig. 1b). We next assessed the association between PKM2 expression and clinicopathological factors for the 80 surgically resected GC specimens. For this analysis, we classified GC tissues into a High-PKM2 group (PKM2/β-actin ≥ 1.0) and a Low-PKM2 group (PKM2/β-actin < 1) according to the PKM2 expression level (Fig. 3). The association of PKM2 expression with the clinicopathological features of 80 resected GC tissues is summarized in Table 1. A significant relationship was found between PKM2 expression and venous invasion (P < 0.01).

Knockdown of PKM2 in GC cells significantly decreased cellular proliferation in vitro and in vivo. To assess the functional role of PKM2 in GC development, we generated stably shPKM2-expressing GC cells (MKN7, MKN74, MKN45, and KATOIII). Quantitative RT-PCR and Western blot analyses revealed that the PKM2 expression levels were effectively reduced in the knockdown compared to control cells (Fig. 4a). The MTT assays showed that all of the PKM2-knockdown GC cell lines had significantly impaired growth (Fig. 4b). Similarly, the s.c. tumor formation was significantly delayed (Fig. 4c), and the tumor growth was significantly reduced (Fig. 4c) in NOG mice treated with PKM2-knockdown versus control GC cells.

Decreased PKM2 expression inhibits GC cell migration and anchorage-independent cell growth in vitro and the metastatic capacity of GC cells in vivo. We next evaluated the role of PKM2 in GC cell migration using a two-chamber assay. The number of migrated cells was significantly decreased in PKM2-downregulated compared to control GC cells (Fig. 5a). To examine the function of PKM2 in the anchorage-independent growth of GC cells, we undertook a soft agar assay. The PKM2-downregulated MKN74 and KATOIII cells formed fewer colonies than their respective control cells (Fig. 5b).

As cell migration and anchorage-independent cell growth are generally correlated with the metastatic ability of tumor cells, we examined the metastatic capacity of PKM2-knockdown GC cells by a tail vein assay. As expected, the MKN74-sh1 cells formed fewer macroscopic liver metastases in NOG mice compared with MKN74-control cells (Fig. 5c, P < 0.01).

Involvement of PKM2 in sphere formation. Morfouace et al. (20) reported that PKM2 plays a role in glioma spheroid differentiation by interacting with Oct4, a major regulator of the self-renewal and differentiation of stem cells. To examine PKM2’s role in the self-renewal of GC cells, we carried out a sphere formation assay. The PMK2-knockdown cells formed fewer spheres compared to control cells (Fig. 6). In addition, as the number of passages increased, control cells formed more spheres while the PKM2-knockdown cells formed fewer spheres.

Involvement of PKM2 in serine metabolism and the Warburg effect in GC cells. Many studies have reported that PKM2 plays a role in cancer metabolism, but little is known about its function in GC. To address the global regulatory functions of PKM2 in GC cell metabolism, we undertook metabolomic analyses of PKM2-knockdown (MKN74-sh2) and control GC cells. As shown in Figure 7(a), glycolytic intermediates were reduced in PKM2-knockdown compared to control GC cells (Fig. 7a). Decreased PKM2 expression inhibits GC cell migration and anchorage-independent cell growth in vitro and the metastatic capacity of GC cells in vivo. To assess the functional role of PKM2 in GC development, we generated stably shPKM2-expressing GC cells (MKN7, MKN74, MKN45, and KATOIII). Quantitative RT-PCR and Western blot analyses revealed that the PKM2 expression levels were effectively reduced in the knockdown compared to control cells (Fig. 4a). The MTT assays showed that all of the PKM2-knockdown GC cell lines had significantly impaired growth (Fig. 4b). Similarly, the s.c. tumor formation was significantly delayed (Fig. 4c), and the tumor growth was significantly reduced (Fig. 4c) in NOG mice treated with PKM2-knockdown versus control GC cells.

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observed in the PKM2-sh2 cells, although NEAA treatment induced the proliferation of these cells (Fig. 7c). These findings suggest that PKM2 is involved in GC cell growth, at least partly, by regulating serine metabolism.

Pyruvate kinase M2 expression is induced by CagA through the Erk signaling pathway. As PKM2 was upregulated in early GC tissues, as described above, we speculated that it is involved in carcinogenesis of the stomach. To address this possibility, the relationship between PKM2 and CagA, an important pathogenic protein of *H. pylori*, was examined using a CagA-inducible GC cell line (MKN28-WT-A10). As shown in Figure 8(a), both the protein and mRNA levels of PKM2 were enhanced by the induction of CagA. To explore the mechanism of the CagA-induced PKM2 expression, we investigated the effect of U0126, specific inhibitor of the Erk pathway, because CagA was previously reported to activate this pathway. Under U0126 treatment, CagA could not upregulate PKM2 expression, suggesting that CagA induces PKM2 expression through the Erk signaling pathway (Fig. 8b).

Discussion

In this study we examined the involvement of PKM2 in gastric carcinogenesis, development, and metabolism. We clearly revealed that: (i) PKM2 was predominantly expressed not only in GC tissues but also in normal gastric mucosa; (ii) PKM2...
expression in GC tissues was higher than in normal gastric tissues, tended to increase with GC progression, and was significantly associated with venous invasion; (iii) PKM2 knockdown in GC cells attenuated their proliferation, migration, metastatic ability, and cancer stem cell-like properties; (iv) PKM2 suppression impaired the serine metabolism and aerobic glycolysis; and (v) CagA induced PKM2 expression through the Erk signaling pathway.

Pyruvate kinase M2 (PKM2) expression is induced by cytotoxin-associated gene A (CagA) through the Erk pathway. (a) CagA expression was induced in MKN28-TetOFF-CagA cells by doxycycline (Dox) removal. After CagA induction, the cells were harvested, and RNA or protein was extracted at the times indicated. Western blot analysis showed that PKM2 was upregulated 6 h after Dox removal. The intensity of the PKM2 bands was normalized to that of α-tubulin, and values are expressed relative to the expression at 0 h (left panel). PKM2 mRNA expression was examined 3 h after CagA induction, by quantitative RT-PCR analysis in triplicate (right panel). A significant induction of PKM2 mRNA was observed (right panel). (b) To examine the role of Erk signaling in CagA-induced PKM2, the CagA-inducible cells were treated with 1 μM U0126, a MAPK inhibitor, 1 h prior to the removal of Dox. The treated cells were collected 6 h after CagA induction, and RNA or protein was extracted. The band densities for HA (CagA), PKM2, pErk, and Erk were normalized to that for α-tubulin. The PKM2 level was expressed relative to that of U0126- and Dox+ cells. The CagA-induced PKM2 expression disappeared when the MAPK inhibitor U0126 was added prior to CagA induction (left panel). PKM2 mRNA was also examined after CagA induction under Erk signaling blockade, by quantitative RT-PCR analysis in triplicate. Upregulation of PKM2 mRNA by CagA was abrogated when the cells were treated with U0126 (right). *P < 0.05.
so-called Warburg effect. Here we showed that PKM2 knockdown increased the glycolytic intermediates and reduced the lactic acid production in GC cells, indicating that PKM2 suppression reversed the Warburg effect. We also found that, among amino acids, serine was specifically increased in the PKM2-downregulated GC cells. Moreover, adding serine increased the cell growth of GC cells but not of PKM2 knockdown GC cells, suggesting that GC cells with impaired PKM2 function cannot utilize serine for cell proliferation in a normal environment. This possibility is compatible with recent reports that serine activates PKM2 through direct interaction.\(^{22,34}\)

Collectively, these findings suggest that PKM2 promotes GC cell proliferation by regulating serine metabolism in addition to the Warburg effect.

In this study, PKM2 was found to be overexpressed at the early stage of GC compared with adjacent non-tumorous tissue. We therefore hypothesized that PKM2 is involved in gastric carcinogenesis, and investigated the correlation between \(H.\) pylori, one of the most important risk factors of GC, and PKM2. We revealed that CagA, a major pathogenic protein of \(H.\) pylori, increased PKM2 expression through the Erk signaling pathway. However, we did not observe a higher expression of PKM2 in non-cancerous lesions of GC tissues that were infected with \(H.\) pylori compared to the normal gastric mucosa of healthy volunteers. The reason for this discrepancy is not clear. In human gastric mucosa, CagA is degraded by autophagy shortly after \(H.\) pylori infection, but it is specifically accumulated in gastric cells expressing the variant form of CD44 (CD44v9), a cell-surface marker associated with cancer stem cells.\(^{35}\) The amount of PKM2 induced by CagA in such \(H.\) pylori-infected mucosa might be below the detection level, as the proportion of cancer stem cells among cancer cells is very low. This situation may partially explain our discrepant findings. Although further examination is required to address this issue, the CD44v9-positive stem-like cells where CagA accumulates may promote gastric carcinogenesis at least partly through CagA’s induction of PKM2.

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**Disclosure Statement**

The authors have no conflict of interest.

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**Supporting Information**

Additional Supporting Information may be found online in the supporting information tab for this article:

*Doc. S1.* Antibodies.

*Doc. S2.* Western blot analysis.

*Doc. S3.* Cell growth assay.

*Doc. S4.* Cell migration assay.

*Doc. S5.* Soft agar colony formation assay.

*Doc. S6.* Sphere formation assay.

*Doc. S7.* Metabolome analysis.

*Doc. S8.* Confirmation of the serine requirement for gastric cancer cell growth.

*Doc. S9.* Animal experiments.

*Doc. S10.* Association between cytotoxin-associated gene A (CagA) and pyruvate kinase M2 (PKM2) expression.

*Doc. S11.* Statistical analysis.