Pyruvate Kinase and Gastric Cancer: A Potential Marker

EDITOR'S PICK

My Editor's Pick for this edition is a captivating review by Macedo et al. on a rather new topic, which concludes that PKM2 and PDK1 measured in the blood or stools of patients, when analysed in combination with CA72-4, are good markers for gastric cancer and are predictors of poor survival. This combination of two biomarkers could help in monitoring the response to treatment and detecting progression or relapse of gastric cancer. This paper opens new avenues for research into novel drugs targeting PKM2.

Dr Sorin T. Barbu

"Iuliu Haţieganu" University of Medicine and Pharmacy, Romania

Authors: Filipa Macedo,[1] Kátia Ladeira,[2-4] Adhemar Longatto-Filho,[3-6] *Sandra F. Martins[3,4,7]

1. Portuguese Oncology Institute - Coimbra, Coimbra, Portugal
2. Portuguese Oncology Institute - Lisbon, Lisbon, Portugal
3. Life and Health Science Research Institute, School of Health Sciences, University of Minho, Braga, Portugal
4. ICVS/3B's-PT Government Associate Laboratory, Braga, Portugal
5. Molecular Oncology Research Center, Barretos Cancer Hospital, São Paulo, Brazil
6. Laboratory of Medical Investigation 14, Faculty of Medicine, University of São Paulo, São Paulo, Brazil
7. Surgery Department, Coloproctology Unit, Braga Hospital, Braga, Portugal

*Correspondence to sandramartins@ecsaude.uminho.pt

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Abstract

Gastric cancer is the second most common cause of cancer-related deaths worldwide, and the 5-year overall survival rate for advanced gastric cancer is ≤25%. Metabolism is a critical process for maintaining growth and other functions in cancer cells; in these cells, the metabolic process shifts from oxidative phosphorylation to aerobic glycolysis and the expression of pyruvate kinase (PK) splice isoform M2 (PKM2) is upregulated. A PubMed search focussing on PK in gastric cancer was conducted and 32 articles were initially collected; 12 articles were subsequently excluded from this review. PKM2 is responsible for tumour growth and invasion and correlates with short survival times and cancer differentiation. Pyruvate dehydrogenase kinase 1 is associated with cell proliferation, lymph node metastasis, and invasion. Measurement of PKM2 or pyruvate dehydrogenase kinase 1 in

the blood or stools could be a good marker for gastric cancer in combination with the glycoprotein CA72-4. The review arose from the need for new biomarkers in the management of gastric cancer and had the primary objective of determining whether PK could be used as a marker to diagnose and monitor gastric cancer.

INTRODUCTION

Gastric cancer is the fourth most common cancer in men worldwide and the second most common cause of cancer-related deaths worldwide.[1,2] In 2008, approximately 700,000 deaths were related to gastric cancer, equating to 10% of all cancer-related deaths, and 989,600 new cases of gastric cancer were diagnosed, representing 8% of all new cancer cases.[1] The number of gastric cancer patients diagnosed in the early stage of the condition is increasing due to improvements in early diagnosis; however, many cases are still found in the advanced stage.[1,2] For advanced gastric cancer, the 5-year overall survival rate is ≤25%.[3] Therefore, identification of molecular therapeutic targets and novel biomarkers for early diagnosis and individualised therapy is of great importance.

Metabolism is a critical event in maintaining growth and other functions in cancer cells. Tumour cells require high metabolism rates to sustain active proliferation and other biological events that require a large amount of energy. In contrast with non-cancerous mammalian cells, cancer cells are always in a hypoxic environment due to their fast growth and the limited oxygen supply. The metabolism in tumour cells shifts from oxidative phosphorylation to aerobic glycolysis, known as the Warburg effect.[4] For aerobic glycolysis, pyruvate kinase (PK) is a rate-limiting enzyme[5,6] that catalyses dephosphorisation of phosphoenolpyruvate to pyruvate to yield one molecule of ATP. This molecule has four isoforms: PK type M1 (PKM1), PK type M2 (PKM2), PK type L, and PK type R; PKM1 is found in muscle and brain tissue, PKM2 in embryonic and tumour cells, PK type L in the liver and kidneys, and PK type R in red blood cells.

In tumour tissues, the expression of PKM2 is upregulated since it is essential for the process of aerobic glycolysis.[5] Polypyrimidine tract-binding protein 1 (PTBP1) regulates alternative splicing of PK, resulting in PKM2 (and can lead to switching of the PKM isoform from PKM1 to PKM2), which is expressed in a broad range of human cancers.[7] PKM2 expression was shown to be involved in early tumourigenesis,[8] and an increase in PKM2 level correlates with tumour size and stage.[9] This high expression indicates that active aerobic glycolysis occurs and regulates numerous cell functions in these cells.[10] Moreover, PKM2 expression is strongly correlated with gastric cancer differentiation; differentiated types of cancers express more PKM2 protein than undifferentiated cancers.[11] Clinical studies have demonstrated that PKM2 is released into the bloodstream and levels of PKM2 in ethylenediaminetetraacetic acid (EDTA) plasma samples are increased in gastrointestinal cancers.[12]

However, the clinical and prognostic implications of PKM2 as a marker for gastric cancer are still unclear. Abnormal glucose metabolism in cancers can be used as a target for cancer treatment. Several agents targeting glycolysis have been reported to have significant cytotoxicity for cancer cells in preclinical studies, with some of these agents having advanced into clinical studies.[13] However, the use of these agents for the treatment of gastric cancer has not been reported. The objective of this literature-based review was to determine whether PK could be used as a marker to diagnose and monitor gastric cancer.

METHODS

A PubMed search was conducted focussing on PK in gastric cancer. The PubMed database was searched with the terms ("stomach neoplasms"[MeSH Terms] OR ("stomach"[All Fields] AND "neoplasms"[All Fields]) OR "stomach neoplasms"[All Fields] OR ("gastric" [All Fields] AND "cancers"[All Fields]) OR "gastric cancers" [All Fields]) AND (("pyruvic acid"[MeSH Terms] OR ("pyruvic"[All Fields] AND "acid"[All Fields]) OR "pyruvic acid"[All Fields] OR "pyruvate"[All Fields] OR "pyruvates" [MeSH Terms] OR "pyruvates" [All Fields]) AND "phosphotransferases"[MeSH Terms])".

Table 1: The main results of selected studies.

Study	Sample	Conclusion
Sugiyama et al.,[15] 2016	20 gastric cancer tissues, adjacent non-tumour tissues, and gastric mucosal epithelial cells; 3 human gastric cancer cell lines.	• Expression levels of miR-133b were downregulated in gastric cancer cells ($p<0.005$). • Gastric cancer cells transfected with miR-133b presented downregulation of PTBP1 expression and switching of PK isoform from PKM2 to PKM1, which resulted in suppression of tumour growth.
Zhang et al.,[16] 2016	18 gastric cancer tissues and 10 superficial gastritis specimens (controls); 4 cell lines (2 adenocarcinoma cell lines, 1 normal gastric cell line, and 1 gastric carcinoma cell line).	• miR-128b was downregulated in gastric cancer tissues ($p<0.01$). • PDK1 is a direct target of miR-128 resulting in a reduction of PDK1 expression and induction of apoptosis.
Tang et al.,[17] 2016	20 gastric cancer specimens and 20 adjacent non-tumour tissues; 4 gastric cancer cell lines and 1 normal gastric cell line.	• miR-let-7a expression was downregulated in gastric cancer tissues ($p=0.0002$). • miR-let-7a overexpression inhibited the expression of PKM2 ($p<0.05$) leading to decreased proliferation of gastric cancer ($p=0.038$), suppressed migration and invasion of gastric cancer cells ($p<0.001$), and inhibited tumour growth ($p<0.05$).
Gao et al.,[18] 2015	124 gastric adenocarcinoma samples and 124 non-neoplastic gastric mucosa samples.	• PKM2 was upregulated in cancer tissues. • Upregulation of PKM2 significantly correlated with both nodal metastasis and advanced TNM stage. • Overexpression of PKM2 was associated with decreased median survival durations (42.6 months versus 80.7 months) ($p<0.001$).
Lin et al.,[19] 2015	58 gastric cancer tissues specimens, 2 cell lines, and 2 mice.	• MACC1 enhanced the Warburg effect by upregulating the expression of PDK1-4, which facilitates the replenishment of energy in gastric cancer cells to overcome metabolic stress and keep growing.
Wang et al.,[20] 2013	3 human gastric cancer cell lines (2 with expression of E-cadherin and 1 without expression of E-cadherin).	• Gastric cancer cell lines showed a high level of PKM2 expression. • In cell lines with PKM2 knockdown, decreases in proliferation and E-cadherin expression levels were observed. • PKM2 attenuates cell motility and invasion when E-cadherin is present, like in the early stages of gastric cancer. When the tumour progresses, the lack of E-cadherin induces an aggressive function of PKM2 in the tumour.
Yin et al.,[21] 2013	142 tissue blocks from patients with advanced gastric cancer who underwent curative surgery.	• The level of PKM2 correlated with tumour size ($p=0.0001$), depth of invasion ($p=0.002$), and lymph node metastasis ($p=0.036$). • The expression levels of PKM2 and VEGF in gastric cancer tissues correlated ($p<0.01$). • In patients with advanced gastric cancer, PKM2 and VEGF expression were significant prognostic factors. • The 5-year overall survival rate in patients expressing lower levels of PKM2 and VEGF was significantly better than in those expressing higher levels of both proteins ($p<0.01$).
Hur et al.,[22] 2013	152 tissue blocks from patients with gastric adenocarcinoma who underwent curative surgery; 6 gastric carcinoma cell lines and 1 non-cancerous kidney cell line.	• Positive staining for HIF1α was significantly correlated with positive PDK1 expression ($p=0.029$). • PDK1 staining significantly correlated with tumour invasion ($p=0.020$), the presence of positive metastatic lymph node ($p=0.040$), and larger tumour size ($p=0.006$). • PDK1 expression was significantly correlated with the disease-free and overall survival rates. • The cell lines with the highest level of PDK1 expression demonstrated decreased responsiveness to 5-FU treatment ($p<0.001$).
Lim et al.,[23] 2012	60 gastric cancer tissues, 19 non-cancer gastric tissues, and a tissue microarray from 368 gastric cancer patients.	• PKM2 levels were increased in primary gastric cancers ($p<0.001$) and in differentiated type cancers ($p<0.001$). • PKM2 expression strongly correlated with gastric cancer differentiation ($p<0.001$) but was not related to stage ($p=0.811$). • PKM2 expression correlated with shorter overall survival ($p<0.042$) in signet-ring cell cancers.
Kwon et al.,[24] 2012	Cell lines and 188 tumour samples.	• There was a positive correlation between PKM2 expression and the tumour size. • The cell lines transduced with PKM2 shRNA presented growth inhibition due to the apoptotic pathway. • There was a positive correlation between PKM2 and *Bcl-xL*: inhibition of PKM2 downregulated the *Bcl-xL* gene, resulting in increased apoptosis and reduced cell growth.
Tsukamoto et al.,[25] 2010	22 gastric carcinoma tissues and 5 non-neoplastic gastric epithelia; 3 gastric cell lines and a miR microarray platform covering a total of 470 human miRNA.	• miR-375 was the most downregulated miR in gastric carcinoma, especially in the early phase of tumourigenesis. • Ectopic expression of miR-375 caused reduction of PDK1 expression and induced apoptosis in gastric carcinoma cells.

Table 1 continued.

Study	Sample	Conclusion
Kumar et al.,[26] 2007	A total of 56 references relevant to tumour PKM2.	• Tumour PKM2 can be quantified in blood with a specificity of 90–95% at a diagnostic cut-off value of 15.0–17.5 U/mL and in stool with a specificity of 83–95% at a cut-off value of 3.33–4.00 U/mL. • The stability of tumour PKM2 is best in EDTA plasma for 24 hours at room temperature and is not influenced by any mechanical stress. • Tumour PKM2 can be elevated in benign conditions. • Its diagnostic accuracy was comparable to CA72-4 in gastric cancer.
Schneider et al.,[27] 2005	122 gastric cancer patients and 53 controls (persons without any malignant disease).	• At 95% specificity, tumour detection was possible by the best single marker (CA72-4) in gastric cancer in 61% of cases. • A tumour marker panel increased sensitivity significantly in gastric cancers to 81% with CA72-4 and tumour PKM2 ($p<0.001$). • Adding a third marker further improved the sensitivity only marginally. • The highest sensitivity of 91% was seen in gastric cancer patients with distant metastasis by using the fuzzy classification and the markers CA72-4 and PKM2.
Zhang et al.,[12] 2004	54 patients with confirmed gastric cancer and 20 healthy volunteers.	• The mean tumour PKM2 concentration allowed a significant discrimination of gastric cancer (26,937 U/mL) from controls (10,965 U/mL) ($p<0.05$). • In gastric cancer, tumour PKM2 showed a sensitivity of 50.47%, while CA72-4 showed a sensitivity of 35.37%.
Schneider and Schulze,[28] 2003	122 gastric cancer patients and 76 control persons without any malignant disease.	• In gastric cancers, the sensitivity of tumour PKM2 (57.0%) and CA72-4 (60.7%) were comparable and higher than CA19.9 (45.5%) and CEA (23.8%).
Yoo et al.,[29] 2004	11 human gastric carcinoma cell lines and 2 cell lines resistant to cisplatin or 5-FU.	• PKM2 showed a decrease in both activity and expression in cisplatin-resistant cell lines compared to parental cell. • When PKM2 expression and activity were suppressed by administration of antisense oligonucleotide, the cells displayed increased drug resistance. • PKM2 activity showed a positive correlation with cisplatin sensitivity ($p=0.044$).
Hardt et al.,[30] 2003	15 patients with colorectal cancer, 9 patients with gastric cancer, 3 patients with inflammatory bowel disease, and 15 controls.	• Compared to healthy subjects, samples of patients with inflammatory bowel disease or colorectal tumours did not show a statistically significant difference. • In contrast, 80% of the patients with gastric cancer had elevated PK levels in their stools ($p=0.005$).
Hardt et al.,[31] 2003	8 patients with colorectal adenomas, 49 healthy controls, 9 patients with colon cancer, 7 with rectal cancer, and 5 with gastric cancer.	• Concentrations of faecal tumour PKM2 were pronounced in colorectal cancer patients compared to the other groups.
Spellman and Fottrell,[32] 1973	Human placentae and biopsy specimens from carcinomas of the lung, stomach carcinoma, and carcinoma of jejunum, and PK purified from 5 tumours: muscle, liver, stomach, intestine, and lung.	• Human placental PK is different from other human tissues, but it is similar to tumour PK.

CA: cancer antigen; CEA: carcinoembryonic antigen; EDTA: ethylenediaminetetraacetic acid; FU: fluorouracil; HIF1α: hypoxia-inducible factor 1-alpha; miR: microRNA; PDK: pyruvate dehydrogenase kinase; PK: pyruvate kinase; PKM1: pyruvate kinase isoform M1; PKM2: pyruvate kinase isoform M2; PTBP1: polypyrimidine tract-binding protein 1; shRNA: short hairpin RNA; TNM: tumour, node, metastasis; VEGF: vascular endothelial growth factor.

A total of 32 articles were initially collected. Twelve articles were subsequently excluded from this review: 6 because the studied molecules were not PK, 2 because the studied tissues were not gastric tissue, 1 because the article was not related to gastric cancer, and 3 because they were not written in English. Ultimately, 20 studies were included in the analysis.

RESULTS

The tumour PKM2 isoform has been shown to be present not only in plasma but also in faeces, indicating that PKM2 may serve as a potential marker for screening colorectal and gastric cancers in high-risk individuals.[14] The main results of the selected studies are listed in Table 1.[12,15-32]

DISCUSSION

MicroRNA (miRNA) are small non-coding RNA molecules, 18–25 nucleotides in length, that bind to the 3' region of target messenger RNA and induce silencing of protein expression.[33-35] miRNA play important roles in a variety of processes, such as cell development, apoptosis, and cell proliferation.[33,34,36] Dysregulation of miRNA is involved in many diseases and most miRNA modulate tumour suppressor genes in various types of cancers.[33,34]

miR-133b was initially considered to be a muscle-specific miRNA involved in the development of skeletal muscle, myoblast differentiation, and myogenic-related diseases; however, a wider expression of miR-133b was found in diverse tissues.[37] miR-133b plays an important role in non-muscle-related disease, such as Parkinson's disease, cardiac failure, and cancer;[38] expression of miR-133b is downregulated in many types of cancers.[39]

Sugiyama et al.[15] concluded that miR-133b was significantly downregulated in cell lines and in gastric cancer tumour tissues compared with normal cells and tissues, respectively. Furthermore, the ectopic expression of miR-133b markedly inhibited cell proliferation through the induction of autophagy. These findings indicate that miR-133b acts as a tumour suppressor miRNA through the perturbation of the Warburg effect in gastric cancer cells. The authors also proved that, in gastric cancer cell lines transfected with miR-133b, the expression of PTBP1 was markedly downregulated, the PKM isoform expression was switched from PKM2 to PKM1 for a short duration, and the tumour growth was suppressed. The authors suggested that PTBP1 could be a target molecule for the development of anti-cancer drugs.[15]

miR-128 includes miR-128a and miR-128b,[40] and their aberrant expression was observed in many malignant tumours, but the function of miR-128b in gastric cancer is not yet known. Zhang et al.[16] found that miR-128b was downregulated in gastric cancer tissues and cell lines, suggesting that it might negatively modulate the carcinoma progression. The results showed that overexpression of miR-128b decreased cell proliferation, inhibited cell viability by arresting them in G0 or G1 phase (the proportion increased by approximately 10%; $p<0.05$), suppressed invasion, and accelerated apoptosis (the rate increased 6.5–8.8-fold) through inactivation of the Akt/nuclear factor-κB (NF-κB) axis by targeting pyruvate dehydrogenase kinase 1 (PDK1).

miR-let-7a plays a role in cell differentiation, apoptosis, proliferation, and metabolism,[41] and its levels are low in different human cancers; downregulation is associated with cancer aggressiveness.[42] Tang et al.[17] concluded that miR-let-7a was highly downregulated in gastric cancer tissues and cell lines, and its overexpression resulted in the significant decrease in cell proliferation rate, colony formation, migration, invasion, and tumourigenicity. Suppression of cell growth, migration, and invasion of gastric cancer cells was achieved by downregulating the expression of PKM2. Coexpression of PKM2 and miR-let-7a could rescue a tumour inhibited by miR-let-7a, indicating that PKM2 is the target of miR-let-7a in gastric cancer. Nevertheless, the specific mechanism by which miR-let-7a affects the expression of PKM2 was not clear. In addition, Gao et al.[18] found that PKM2 was overexpressed in gastric cancer and expression correlated with nodal metastasis, advanced tumour, node, metastasis (TNM) stages, and poor prognosis.

E-cadherin plays a critical role in maintaining epithelial integrity, and the loss of E-cadherin affects the adhesive repertoire of a cell. This molecule is also a tumour suppressor with a frequently reduced or silenced expression, and its re-expression can induce morphologic reversion.[43] Epidermal growth factor receptor (EGFR) proteins enhance cell motility and at least two distinct intracellular signalling pathways are required for EGFR-mediated cell motility: the pathways using phospholipase C-γ and the mitogen-activated protein kinase pathway.[44] Wang et al.[20] demonstrated that the knockdown of PKM2 decreased the activity of E-cadherin and enhanced the EGFR signalling pathway in the cell lines that were positive for E-cadherin expression. However, in the undifferentiated gastric carcinoma cell line, which lacked E-cadherin expression, PKM2 promoted cell migration and invasion.

The major factors that determine the prognosis of gastric cancer include lymph node metastasis,

depth of tumour invasion, and tumour size. Tumour angiogenesis plays a critical role in metastisation and tumour growth. Any increase in a tumour mass must be preceded by an increase in the microvasculature to deliver nutrients and oxygen to the tumour and remove products of tumour metabolism. Without new blood vessels, most tumours would never grow beyond 1–2 mm in diameter and would remain localised to the primary site.[45] EGFR is one of the most important regulators of angiogenesis. Yin et al.[21] concluded that PKM2 and vascular endothelial growth factor expression were positively correlated with the prognosis of advanced gastric cancer.

Hur et al.[22] concluded that glucose transporter-1 and PDK1 expression were significantly associated with tumour progression, although only PDK1 expression was an independent prognostic factor for patients who received 5-fluorouracil (FU) adjuvant treatment. Treatment with dichloroacetate, a PDK1 inhibitor, reduced lactate production and increased responsiveness to 5-FU in cell lines that expressed high levels of PDK1.

Lim et al.[23] showed that PKM2 was overexpressed in gastric cancers both at the messenger RNA and protein levels compared to non-cancerous gastric tissues. Moderately and well-differentiated adenocarcinoma showed significantly higher expression of PKM2 (60.0% PKM2-positive cells) in contrast with signet-ring cell cancers, which showed 17.7% PKM2-positive cells. NF-κB is a transcription factor that controls the expression of proteins involved in the regulation of immune response and cell survival.[46] Deregulation of NF-κB signalling is associated with oncogenesis and cancer malignancies because its activation increases the expression of many genes involved in cell proliferation, metastasis, angiogenesis, and anti-apoptosis pathways.[47]

Kwon et al.[24] identified *PKM2* as an overexpressed gene in gastric cancer patients at both the transcriptional and protein levels and showed that *PKM2* expression level affected the survival of gastric cancer cells. High *PKM2* expression was associated with poor prognosis in gastric cancer patients. PKM2-mediated NF-κB stabilisation may underlie the molecular basis for increased survival in gastric cancer cells, in part by regulating the expression of *Bcl-xL*, an apoptosis-related gene.

miR-375 has been reported to be downregulated in head and neck,[48] pancreatic,[49] and hepatocellular carcinomas,[50] but its function in cancer remains to be determined. In gastric carcinoma cells, Akt phosphorylation has been reported to promote cell survival and act against apoptotic stimuli.[51] Tsukamoto et al.[25] found that miR-375 was the most downregulated miRNA from a microarray with 470 human miRNA. Re-expression of miR-375 in gastric carcinoma cell lines resulted in induction of apoptosis and reduced cell viability. Exogenous miR-375 suppresses the expression of PDK1, resulting in decreased phosphorylation of Akt in gastric carcinoma cells. Decreased expression of miR-375 may provide a survival advantage to gastric carcinomas via activation of the PDK1/Akt survival pathway. Downregulation of miR-375 results in enhanced expression of 14-3-3ζ and provides a survival advantage to gastric carcinoma.

Currently used tumour markers have a low sensitivity for detecting cancer and their role is limited to detecting recurrence after surgery or monitoring the response to treatment. Tumour PKM2 can be measured in the stool and in the bloodstream by a highly sensitive enzyme-linked immunosorbent assay (ELISA). Kumar et al.[26] compared different tumour markers and concluded that the diagnostic accuracy of tumour PKM2 was comparable to CA72-4 in gastric cancer; using a combination of these tumour markers increased the diagnostic strength. Schneider et al.[27] found that the sensitivity of tumour PKM2 (57%) was comparable to CA72-4 (61%) in gastric cancer, and the two-marker combination increased the sensitivity to 81% ($p<0.001$). For the discrimination of malignant versus non-malignant diseases, the fuzzy classificatory (a mathematical procedure for a non-invasive analytical method) increased sensitivity by 20% compared to the best single marker in gastric cancer.[27] In another study, Schneider and Schulze[28] demonstrated that the discrimination power of tumour PKM2 was superior in colorectal, gastric, and oesophageal cancers without distant metastasis, whereas Zhang et al.[12] concluded that the sensitivity of tumour PKM2 in the diagnosis of gastric cancer was

lower than that in the diagnosis of colorectal cancer, although it was higher than that of CA72-4. In addition, Hardt et al.[30] found that stool samples of gastric cancer patients had elevated PK concentrations compared to healthy controls and inflammatory bowel disease patients. In another study by Hardt et al.,[31] the authors found a significant difference in faecal tumour PKM2 concentrations between cancer patients and controls, and the highest concentrations were observed in colorectal cancer cases. Lastly, studies also showed that the sensitivity of faecal tumour PKM2 was 73.00% and the specificity was 78.00%;[52] whereas the sensitivity and specificity of serum PKM2 in gastric cancer was 50.47% and 90.00%, respectively,[12] and 66.70% and 88.90% in colorectal cancer, respectively.[53]

Chemotherapies for advanced gastric cancer, usually containing cisplatin and/or 5-FU, have response rates of 20–40%, with between 6 and 12 months of median survival.[54] However, cancer cells can be unresponsive to drug treatment at the outset of therapy (intrinsic resistance) or they may become unresponsive after exposure to the chemotherapy agent (acquired resistance).[55] Yoo et al.[29] linked PKM2 activity and cisplatin-resistance mechanisms. They observed that cisplatin resistance correlated with decreased levels of PKM2 protein and activity in human gastric carcinoma cell lines and that lowering PKM2 expression through antisense transfection increased cisplatin resistance.

CONCLUSION

Measurement of PKM2 or PDK1 in the bloodstream or stools of patients could be a good marker for gastric cancer when analysed in combination with CA72-4; these markers are related to tumour burden, proliferation, invasion, differentiation, and lower survival. Once the diagnosis of gastric cancer is set, the combination of these two biomarkers could help monitor the response to treatment, as well as detect progression or relapse. PKM2 is also associated with poor prognosis, so patients with higher levels of PKM2 at diagnosis could have lower survival rates. A consideration for future development is that targeting PKM2 could become a new therapeutic approach for the treatment of gastric cancer patients.

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