Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2008;21:429-436

Accepted: January 22, 2008

PGK1 a Potential Marker for Peritoneal Dissemination in Gastric Cancer

Derek Zieker[1,2*], Ingmar Königsrainer[1*], Frank Traub[1], Kay Nieselt[3], Bettina Knapp[3], Christian Schillinger[3], Christian Stirnkorb[1], Falko Fend[4], Hinnak Northoff[2], Susan Kupka[1], Björn L. D. M. Brücher[1] and Alfred Königsrainer[1]

[1]Department of General, Visceral and Transplant Surgery, Comprehensive Cancer Center, Tuebingen, [2]Department of Transfusion Medicine, Tuebingen, [3]Department of Information and Cognitive Sciences, Center for Bioinformatics Tuebingen and [4]Department of Pathology, Tuebingen, [*]Equally contributing authors

Key Words
PGK1 • CXCR4 • CXCL12 • Peritoneal carcinomatosis • Gastric cancer

Abstract
Background/Aims: Peritoneal carcinomatosis, which is caused by the dissemination of cancer cells into the abdominal cavity is a frequent finding in patients with primary gastric cancer, and it is associated with a poor prognosis. The mechanisms that mediate peritoneal carcinomatosis in diffuse primary gastric tumours require definition. Methods: We therefore compared the gene expression profile in diffuse primary gastric cancer patients with and without peritoneal carcinomatosis (n=13). Human specimens from consecutive gastric cancer patients with and without peritoneal carcinomatosis were investigated using oligonucleotide microarrays. Differentially expressed genes of interest were further evaluated using quantitative real-time polymerase chain reaction (qRT-PCR). Results: The results reveal a significant overexpression of phosphoglycerate kinase 1 (PGK1), the chemokine CXCR4 and its ligand CXCL12 in specimens from diffuse gastric cancer patients with peritoneal carcinomatosis. Overexpression of PGK1 is known to increase the expression of CXCR4. CXCR4 on its part can increase CXCL12 expression. Elevated levels of CXCR4 and CXCL12 are associated with an increase in the metastatic rate and play an important role in the metastatic homing of malignant cells. Conclusion: The overexpression of PGK1 and its signalling targets may be a expression-pathway in diffuse primary gastric carcinomas promoting peritoneal dissemination and may function as prognostic markers and/or be potential therapeutic targets to prevent the migration of gastric carcinoma cells into the peritoneum.

Introduction

Gastric cancer is the fourth most common cancer and is the second leading cause of cancer-related death worldwide [1]. Diffuse gastric adenocarcinoma shows a different pattern compared to the intestinal type, such as lymphatic permeation, Krukenberg tumours and peritoneal metastases in particular [2]. Peritoneal carcinomatosis is a common finding in diffuse and advanced gastric cancer patients and is associated with a poor prognosis. The molecular mechanism causing peritoneal dissemina-

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015-8987/08/0216-0429$24.50/0

Accessible online at:
www.karger.com/cpb

Dr. Derek Zieker
Department of GeneralVisceral and Transplant Surgery, University of Tuebingen
Hoppe-Seyler-Strasse 3, 72076 Tuebingen (Germany)
Tel. +49 7071 2981658, Fax +49 7071 295459
E-Mail derek.zieker@med.uni-tuebingen.de

tion of cancer cells is still poorly understood and up to now there have been no valid prognostic markers indicating which primary gastric tumour is likely to develop peritoneal dissemination.

Recently elevated concentrations of the chemokine CXCR4 in gastric cancer tissue and its ligand CXCL12 in ascitic fluids have been associated with peritoneal carcinomatosis in gastric cancer [3]. The human chemokine system contains about 50 known ligands and 20 G protein–coupled receptors that control migration and activation of leukocytes and influence angiogenesis and tumour growth [4]. It is assumed that tumour cells themselves are able to express chemokines [4-6]. Disseminated tumour cells that express members of the CXCR family and invade the circulation are thus attracted and arrested by their corresponding ligand. These cells therefore acquire an ability to infiltrate into distinct organs. CXCR4 and its ligand CXCL12 are reported to be involved in the metastasis of prostate cancer, non–small-cell lung cancer, pancreatic and breast cancer [3, 7-9]. To date, only one recently published article has demonstrated involvement of CXCR4 and its ligand CXCL12 in gastric cancer [3].

Wang and his group showed a close relationship between the regulation of the CXCR4/CXCL12 axis and phosphoglycerate kinase 1 (PGK1) [10]. PGK1 is an ATP-generating enzyme of the glycolytic pathway and is regulated by hypoxia-inducible factor-1α (HIF-1α) [10, 11]. In addition to its role as a glycolytic enzyme and as a suppressor of proangiogenic factors such as VEGF, PGK1 is also thought to be involved in the onset of malignancy [10]. Thus, overexpression of PGK1 has been observed in breast and pancreatic carcinoma and multi-drug resistant ovarian cancer, but has not previously been studied in gastric cancer [12-14].

Another molecule known to be involved in early invasiveness into surrounding tissues and the peritoneal cavity is β-catenin [15, 16]. β-Catenin, a 92-kDa protein, is the central downstream effector of Wnt signalling, but it is also a potential downstream target of PGK1 [10]. Targets of β-catenin are known to be associated with cell proliferation, tumour invasion and metastasis, angiogenesis and drug resistance [16, 17]. At present is unknown whether PGK1 and β-catenin are linked in gastric cancer development and/or peritoneal dissemination.

PGK1 signalling through CXCR4, CXCL12 and β-catenin has as yet only been investigated *in vitro,* but not *in vivo* with human samples. In vitro, it was shown that PGK1, CXCR4 and CXCL12 reciprocally regulate each other expression. This was demonstrated in PGK1 overexpressing cells and SiRNA investigations by indirect immunofluorescence and Wetern blot analysis in prostate cancer cell lines [10]. β-catenin as a downstream target of PGK1 was evaluated using gene expression arrays in overexpressed PGK1 prostate cancer cell lines [10]. As diffuse gastric cancer is a major cause of peritoneal dissemination, we compared the gene expression profile in human samples of diffuse primary gastric cancer patients with and without peritoneal carcinomatosis. In this study, we investigated the expression of PGK1 and its signalling targets, such as CXCR4, CXCL12, β-catenin and HIF-1α. On the basis of the findings, we present a potential promising expression-pathway in the development of peritoneal carcinomatosis from diffuse gastric cancer through PGK1 signalling.

Materials and Methods

Patients, tissue specimens and RNA extraction

Primary tumour samples from 13 patients with histologically confirmed diffuse gastric cancer (untreated and with no previous neoadjuvant therapy) who underwent laparotomy for gastrectomy were investigated. Peritoneal carcinomatosis in diffuse gastric cancer patients was histologically confirmed in eight of the 13 patients (5 women, 3 men; mean age 58 years, range 27–78; TNM-categories according to the UICC [18]: pT2-category (n=2), pT3-category (n=6), pN0-category (n=2), pN1-categories (n=2), pN2-category (n=4), M1-category (n=8)). No peritoneal carcinomatosis in diffuse gastric cancer patients was observed in five of the 13 patients (4 women, 1 man; mean age 70 years, range 60–76; TNM-categories according to the UICC: pT2-category (n=2), pT3-category (n=3), pN1-categories (n=3), pN2-category (n=2), M0-category (n=5)). All of the tumour specimens were obtained at the Department of General, Visceral and Transplant Surgery at the University of Tübingen, Germany. The specimens were snap-frozen in liquid nitrogen and stored at –80 °C until use. Each tumour sample was subjected to frozen sectioning, stained with haematoxylin and eosin, and re-evaluated by an experienced surgical pathologist. Manual microdissection was performed to ensure that the tumour cell content was greater than 80%.

All of patients provided informed consent to participate in the study, which was approved by the local ethics committee (168/2005). RNA was extracted using the NucleoSpin RNA II Kit (Macherey-Nagel, Dueren, Germany). The RNA quality and quantity were confirmed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, California, USA) and the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA).

Microarray data generation

Microarray data were collected using oligonucleotide microarrays (65mer) produced at the Max Planck Institute, Tübingen, Germany. The array contains oligonucleotides for

Table 1. Primer sequences (human) in qRT-PCR.

Gene product	Sense primer	Antisense primer
Phosphoglyceratekinase 1 (PGK1)	CATACCTGCTGGCTGGATGG	CCCACAGGACCATTCCACAC
Chemokine receptor 4 (CXCR4)	CAGTTTCAGCACATCATGGTTGG	GTGACAGCTTGGAGATGATAATGC
Chemokine ligand 12 (CXCL12)	GTCAAGCATCTCAAAATTCTC AACAC	CACTTTAGCTTCGGGTCAATGC
Hypoxia-inducible factor 1, alpha (HIF1A)	CAACCTCAGTGTGGGTATAAGAAAC	AAATTTCATATCCAGGCTGTGTCG
Vascular endothelial growth factor (VEGF)	GCCTTCGCTTACTCTCAC	GCTGCTTCTTCCAACAATG
E-Cadherin 1 (CDH1)	TGATGTGAACACCTACAATGC	CTCCTGTGTTCCTGTTAATGG
Catenin, beta 1 (CTNNB1)	GTCTTACCTGGACTCTGGAATCC	GGTATCCACATCCTCTTCCTCAG

about 900 transcripts as well as buffer, control and empty spots, and each feature was printed twice. Further details of the array can be obtained from the National Center for Biotechnology Information's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession number GPL5676 and GSE8657. Using nine oligonucleotide microarrays we compared six human primary tumours in specimens from consecutive diffuse gastric cancer patients with (n=3) and without (n=3) development of peritoneal carcinomatosis among each other. A double-loop design was used in order to minimize the variability introduced by the two dyes. Amplification of the sample RNA was performed using Ambion's Amino Allyl MessageAmp™ II aRNA Amplification Kit (Ambion Inc., Austin, Texas, USA). Dye-coupling reaction was performed using Amersham CyDye Post-labelling Reactive Dye Pack (GE Healthcare, Buckinghamshire, UK). After an aRNA fragmentation using Ambion's Fragmentation Reagents (Ambion Inc., Austin, Texas, USA), hybridization was carried out at 48 °C for 14 hours. The slides were scanned in a microarray scanner (Genetix Limited, Hampshire, UK). The photomultiplier tube voltage was set to 100% for both red and green channels. The two resulting green and red images were overlaid using ImaGene 5 (BioDiscovery, Inc., El Segundo, California, USA).

Quantitative real-time PCR

Sample RNA was transcribed using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany). Selected cDNAs were quantified using real-time PCR on a LightCycler® instrument (Roche, Mannheim, Germany). For PCR-assisted amplification, the SYBR Green Jump Start TAQ ReadyMix (Sigma, Taufkirchen, Germany) was used in accordance with the manufacturer's instructions. The specificity of the PCR conditions was assured by determining the melting temperature and product sequencing. Quantification of gene expression levels was done by analysing an external standard curve together with the samples of interest, a method that has been recently described in detail elsewhere [19]. Primer sequences are listed in table 1.

Statistical analysis

Raw data collection was performed using ImaGene v.5.0. Further statistical and bioinformatics analyses were performed using the R language (www.r-project.org) and the 'limma' (Linear Models for Microarray) package from the Bioconductor project (http://www.bioconductor.org/). As a first step in signal extraction, for each channel we used the mean of the pixel distribution for the foreground signal and the median for the background of each spot as estimators of the raw signal values. All spots were used, regardless of their flag status. The data were normalized using loess normalization on the normexp-background corrected expression values, followed by a dye-swap normalization and in-between-array quantile normalization. Both the loess and quantile normalization methods were used as provided in the limma package. As a result, the log-ratio M of samples with peritoneal carcinomatosis versus samples without peritoneal carcinomatosis for each spot was computed. All arrays, including both normalized and raw data, can be obtained from the National Center for Biotechnology Information's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE8657. On the basis of the M values computed, differentially expressed genes were detected by applying the Welch one-sample t-test as implemented in R. Based on the complete normalized expression matrix with all 18 samples, quartet mining as implemented in GeneMining (Schillinger, C. Diploma thesis University of Tübingen, 2007) was applied to identify the top-ranked 100 genes that separate the two patient groups, diffuse gastric cancer with peritoneal and without peritoneal carcinomatosis. From these 100 features, a phylogenetic sample tree, using NeighborJoining was computed [20]. As a distance function we used the Pearson Correlation distance. In addition, a k-means clustering was performed on the set of 100 features and the clusters were visualized subsequently using the Enhanced Heatmap of Mayday [21, 22].

Results

Microarray data analysis and validation

Gene expression analysis was carried out using a custom-designed oligo microarray. The comparison of the normalized complete expression data of each diffuse gastric cancer patient with or without peritoneal carcinoma-

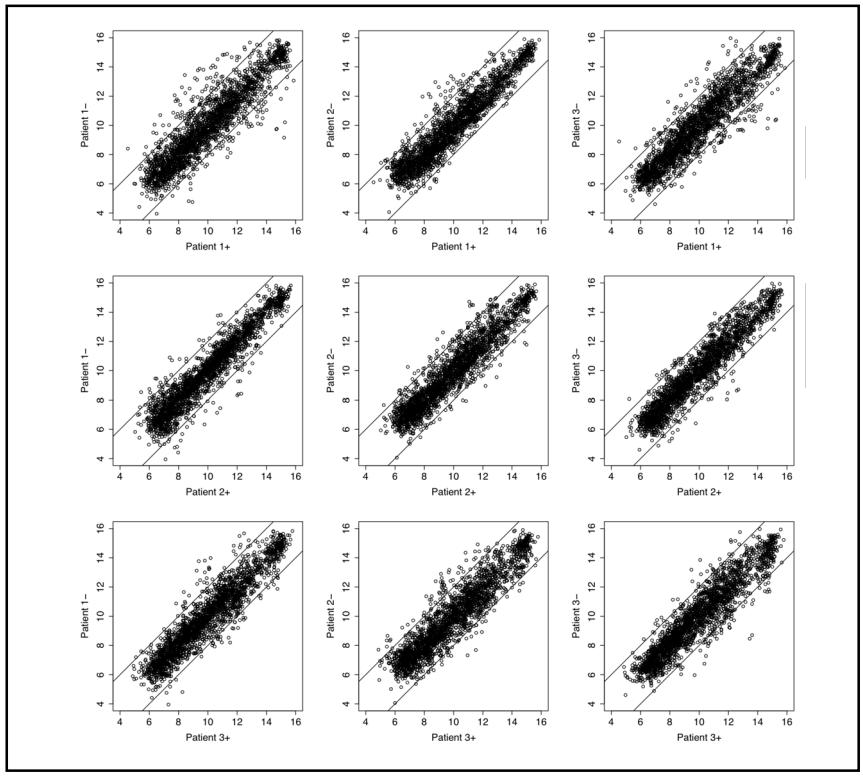


Fig. 1. Patient expression data. Scatterplot of log-expression values of each patient with diffuse gastric cancer with peritoneal carcinomatosis (three samples, +) against each patient with diffuse gastric cancer without peritoneal carcinomatosis (three samples, -). The lines in each scatter plot indicate the border of 2-fold differential expression.

tosis shows a large coherence between the two tumour groups, with up- or down-regulated genes within each patient (see figure 1). To specify the commonly differentially regulated genes between diffuse gastric cancer samples with or without peritoneal carcinomatosis, we applied the t-test. At a significance level of $P < 0.05$, we found 57 genes to be differentially expressed. 25 genes were up- and 32 genes were down-regulated in diffuse primary gastric cancer with peritoneal carcinomatosis (see supplementary file 1 for the complete list, including annotation). To validate the array results, 8 genes were selected for evaluation using quantitative real-time PCR (Phosphoglycerate kinase 1 (PGK1), Cell division cycle associated 7-like (CDCA7L), Cyclin-dependent kinase 8 (CDK8), Interleukin-1, beta (IL-1B), Tumour protein p53 (TP53), S100 calcium binding protein A8 (S100A8), Heat shock 70 protein 1A (HSPA1A) and Sphingosine-1-phosphate phosphatase 2 (SGPP2). These data confirm the expression changes detected by the microarrays, except for S100A8 (data not shown).

Quantitative real-time PCR with additional genes associated to PGK1

Significant P values on the microarray and mRNA overexpression via quantitative real-time PCR was observed for PGK1 ($P = 0.00163$ on the microarray, 4.6-fold in real-time PCR) in diffuse primary gastric cancer specimens with known peritoneal carcinomatosis in comparison to diffuse primary gastric cancer specimens without peritoneal carcinomatosis. Based on this finding, we decided to evaluate additional genes associated to PGK1 using quantitative real-time PCR. These genes were HIF-1α, CXCR4, CXCL12, β-catenin, VEGF, and E-cadherin. Specific mRNA overexpression in diffuse primary gastric cancer specimens with peritoneal carcinomatosis was detected for PGK1 (4.6-fold), HIF-1α (3.8-fold), CXCR4 (2.9-fold), CXCL12 (11.3-fold) and β-catenin (3.8-fold), whereas 5-fold lower expression of VEGF mRNA was detected in diffuse primary gastric cancer tumours with peritoneal dissemination (see figure 2). When primary tumours with peritoneal carcinomatosis were compared with primary tumours without peritoneal carcinomatosis, no differential E-cadherin mRNA expression was observed. The results for the quantitative real-time PCR are listed in table 2. The phylogenetic tree built from the 100 top-ranked features as determined by quartet mining, shows the separation between the two patient groups (here indicated by the labels + for diffuse gastric cancer samples with peritoneal carcinomatosis, - for samples without peritoneal carcinomatosis) (see figure 3 left). In addition, except one sample replicate of patient 2+, all patient replicates cluster, confirming the reliability of the chip and the methods applied. The heatmap (see figure 3 right) shows the expression profiles of the 100 chosen

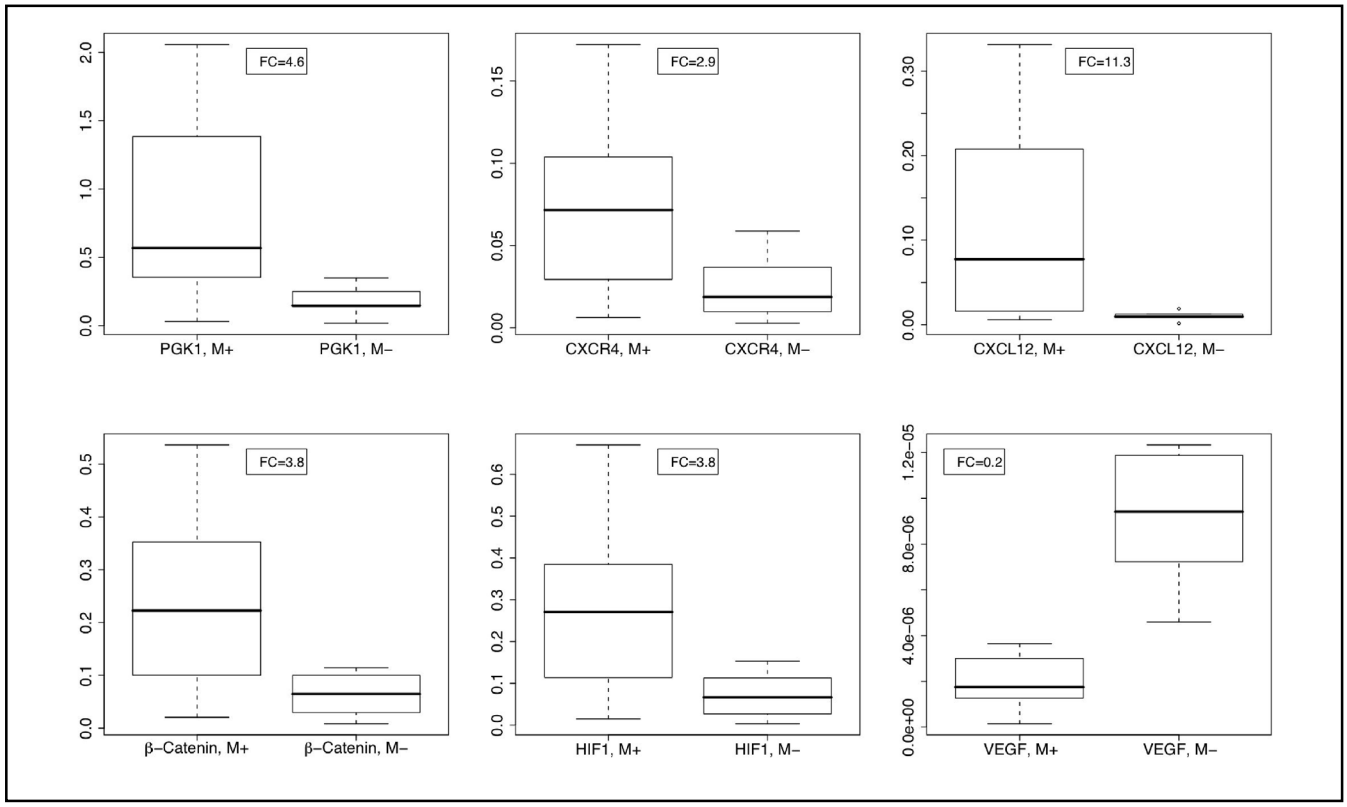


Fig. 2. Box plots of qRT-PCR measurements of individual patients' sample RNA. The box plot shows the minimum, the first quartile, the median, the third quartile and the maximum of the underlying distribution of qRT-PCR ratio values. The y-scale of each boxplot shows the qRT-PCR ratio of measured expression values versus values of an internal standard for each respective gene. For each gene, we computed two box plots; the left one refers to gastric tumour samples with peritoneal carcinomatosis (M+) and the right one to samples without (M–). In addition, the mean fold change of peritoneal carcinomatosis–positive samples versus the negative samples is indicated for each gene.

Table 2. Selected genes were evaluated with qRT-PCR of individual specimen samples (n=13). The fold changes listed relate to differentially regulated genes that are specific for diffuse primary gastric cancer tumours with peritoneal carcinomatosis.

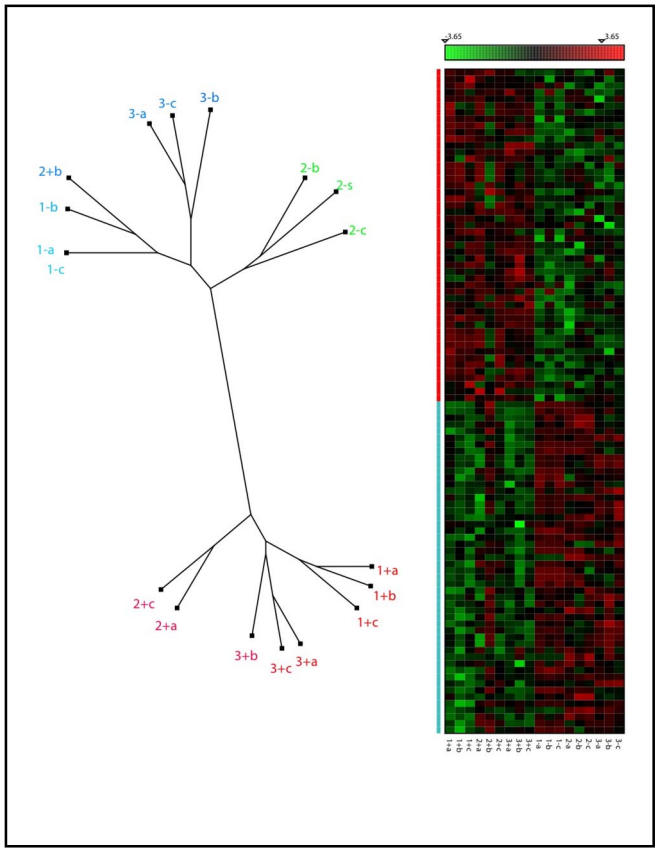
Gene product	Accession number	qRT-PCR fold change
PGK1	NM_000291	4.6
HIF1A	NM_001530	3.8
CXCR4	NM_003467	2.9
CXCL12	NM_001033886	11.3
VEGF	NM_003376	0.2
β-catenin	NM_001904	3.8
E-Cadherin	NM_004360	Unchanged

features. Again it is clearly visible that patient sample 2+b is most probably misclassified. There are two prominent clusters, indicated by the colors orange and cyan, respectively, for which features in the orange labelled cluster are upregulated in diffuse gastric cancer patients with peritoneal carcinomatosis, while features in the cyan labelled cluster are downregulated in diffuse gastric cancer patients without peritoneal carcinomatosis. Features in the violet labelled cluster are also generally down-regulated in diffuse gastric cancer patients with peritoneal carcinomatosis.

Discussion

We evaluated the differential expression of genes in specimens from consecutive patients with diffuse gastric cancer with and without peritoneal carcinomatosis, using oligonucleotide microarrays and quantitative real-time PCR. To our knowledge, this is the first study that has shown highly specific overexpression of PGK1 mRNA in diffuse primary gastric cancer with histologically proven peritoneal carcinomatosis. In addition to its function as a glycolytic pathway enzyme, solid tumour cells exert gly-

Fig. 3. Left: Patient sample tree of the top 100 genes separating the two patient classes (+ = diffuse gastric cancer with peritoneal carcinomatosis, - = diffuse gastric cancer without peritoneal carcinomatosis). The top 100 genes were computed using GeneMining. Right: Heatmap of the 100 genes clustered using k-means (with k=2).



colytic enzymes including PGK1 to produce ATP when their supply of oxygen is limiting [23]. Further overexpression of PGK1 is related to elevated levels of CXCR4 and CXCL12 that are in turn associated with metastatic homing of malignant cells. PGK1 is already known to play an important role in some malignancies, such as breast, ovarian and pancreatic carcinoma, but not so far in gastric cancer [12-14]. These results suggest that PGK1 overexpression might function in promoting dissemination into the peritoneum (model, figure 4).

A molecule known to regulate the expression of PGK1 is HIF-1α [11]. Silencing of HIF-1α abolishes the induction of PGK1, which confirms PGK1 as a downstream target of HIF-1α [11]. HIF-1α is the major factor involved in the cellular response to hypoxia. During hypoxia HIF-1α is central in modulating the tumor microenvironment, sensing nutrient availability, controlling anaerobic glycolysis, intracellular pH and cell survival [11]. Stimulating PGK1 expression via HIF-1α during hypoxia supplies tumour cells to make ATP when their supply of oxygen is limiting. An overexpression of PGK1 relates to increased necessity of energy in fast growing tumours due to protein synthesis and degradation pathways [14]. The results of the present study show overexpression of HIF-1α mRNA in primary gastric cancer tumours with peritoneal carcinomatosis, in correlation with PGK1 upregulation.

Wang and his group demonstrated a significant association between PGK1 regulation and the CXCR4/CXCL12 axis in prostate cancer cell lines [10]. They reported that overexpression of PGK1 increased the metastatic rate due to its effect on increased CXCR4 and CXCL12 expression. On the other hand, enhanced expression of PGK1 also led to decreased secretion of pro-angiogenic factors, such as VEGF. These findings indicate that there is a reciprocal relationship between CXCR4/CXCL12 signalling and PGK1 expression [10]. In addition PGK1 also influences DNA replication and repair in mammalian cell nuclei and can induce multidrug resistance (MDR) through an MDR-1-independent mechanism [12, 24]. Although the detailed mechanism

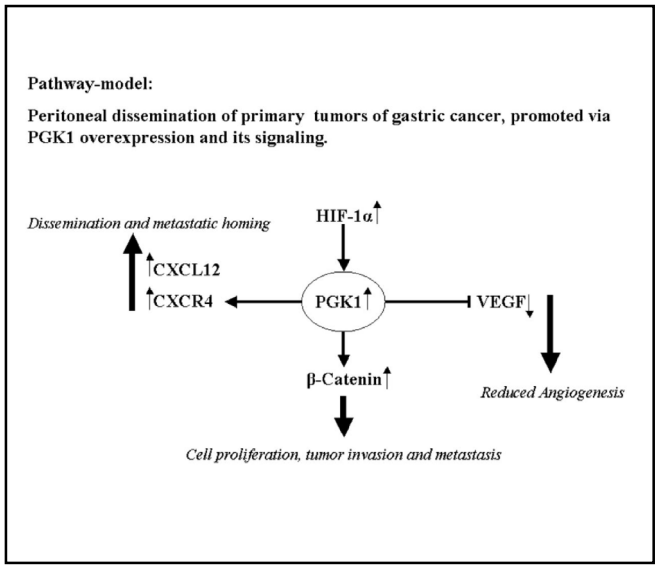


Fig. 4. Model of peritoneal dissemination via PGK1 signaling in primary tumours of gastric cancer. High sites of HIF-1α lead to overexpression of PGK1. Overexpression of PGK1 signals through increased expression of the CXCR4/CXCL12 axis and β-catenin, which are considered to enhance tumour growth, invasion, dissemination of malignant cells and metastatic homing. On the other hand, overexpression of PGK1 reduced angiogenesis through decreased expression of VEGF.

how PGK1 advances migration, adhesion and growth control is yet unclear, it is recognized that enhanced glycolytic metabolism will provide more energy (ATP) for cell proliferation and growth and will increase the concentration of metabolites such as lactate and pyruvate to regulate hypoxia-inducible gene expression and actuate the expression of CXCR4, its ligand CXCL12 and the downstream effector of Wnt signalling β-catenin that are known to be involved in migration, adhesion and growth [10, 14, 25-27].

Concerning CXCR4 and its ligand CXCL12 they are known to be involved in the metastasis of prostate cancer, non–small-cell lung cancer and breast cancer [7, 28]. CXCR4 thus appears to play an important role in the metastatic homing of malignant cells [10, 28, 29]. Elevated CXCR4 levels in gastric tumours and high concentrations of CXCL12 in malignant ascitic fluids were recently reported to be associated with peritoneal carcinomatosis [3]. The results of the present study revealed a high increase in CXCR4 and CXCL12 mRNA and strong suppression of VEGF mRNA expression in primary gastric cancer tissue from patients with peritoneal carcinomatosis. In addition to confirm the results described above, our results are the first showing a strong association between PGK1 regulation and the CXCR4/CXCL12 axis in diffuse primary gastric cancer with known peritoneal carcinomatosis.

Recently reported downstream targets of PGK1 are E-cadherin and β-catenin [10]. In contrast, our E-cadherin mRNA expression results did not suggest that there is differential regulation, whereas overexpression of β-catenin mRNA in primary tumours with peritoneal carcinomatosis was observed. These findings are in contrast with reports investigating prostate cancer cell lines, in which high levels of PGK1 led to decreased expression of β-catenin. However, *in vivo,* increased expression of β-catenin is associated with cell proliferation, tumour invasion and metastasis, angiogenesis and drug resistance in gastric cancer [16, 17]. Hence, an association between PGK1 and β-catenin expression in gastric cancer with peritoneal dissemination is a novel and promising finding and may explain the invasion, dissemination and metastasis of tumour cells in gastric cancer.

With regard to predictors for peritoneal carcinomatosis, no valid prognostic markers/predictors are yet available that indicate that a primary gastric tumour is likely to develop peritoneal dissemination. There are several quantitative prognostic indicators that serve as guidelines in the selection of treatment to maximize the benefits of therapy, but not for prediction of dissemination [30]. Although the present study was conducted with relatively few samples, the very clear differential expression of PGK1, HIF-1α, CXCR4, CXCL12, β-catenin and VEGF is nonetheless highly specific. Additionally, using the phylogenetic sample tree described, microarray analysis might predict through its specific clustering of the investigated specimen, if a tumour belongs to the group with or without peritoneal dissemination. Therefore, the mentioned molecules and the phylogenetic sample tree might serve as an excellent marker panel for detecting primary tumours that are likely to develop peritoneal dissemination.

In conclusion, the results presented here broaden the existing data and confirm previous investigations concerning PGK1 and its signalling. In addition, the data indicate for the first time the extended mechanism for PGK1 in primary diffuse gastric cancer tumours. The differential expression detected in diffuse primary gastric cancer tissue in patients with peritoneal carcinomatosis strongly suggests that this expression-pathway may promote peritoneal dissemination. We are aware of the fact that our obtained results concerning gene expression can not be directly transferred to protein expression. At this stage the post-translational modifications and protein expressions levels of PGK1 and its signalling targets were not investigated. Nevertheless regarding the recently detected results in prostate cancer cell lines we assume that the protein levels of PGK1 and its signalling targets may be rather similar to our presented expression data in diffuse gastric cancer. Thus, correlations of gene transcript abundance with corresponding protein levels will have to be done in the future.

PGK1 and its signalling genes may be able to serve as future prognostic markers with therapeutic implications, such as neoadjuvant chemotherapy and hyperthermic peritoneal perfusion after gastrectomy. They might also serve as potential therapeutic targets to prevent the migration of gastric carcinoma cells into the peritoneum.

Acknowledgements

The authors gratefully acknowledge the input and contributions to the present study made by the participating technologists and staff, including Melanie Hauth, Jürgen Winter, Silvia Wagner and Caroline Zug for their help in sampling the tissues, as well as Georg Otto and Robert Geisler (members of the Max Planck Institute in Tübingen, Germany) for their encouragement with regard to microarray manufacture.

References

1 Parkin DM, Pisani P, Ferlay J: Global cancer statistics. CA Cancer J Clin 1999;49:33-64 1.

2 Duarte I, Llanos O: Patterns of metastases in intestinal and diffuse types of carcinoma of the stomach. Hum Pathol 1981;12:237-242.

3 Yasumoto K, Koizumi K, Kawashima A, Saitoh Y, Arita Y, Shinohara K, Minami T, Nakayama T, Sakurai H, Takahashi Y, Yoshie O, Saiki I: Role of the CXCL12/CXCR4 axis in peritoneal carcinomatosis of gastric cancer. Cancer Res 2006;66:2181-2187.

4 Gerard C, Rollins BJ: Chemokines and disease. Nat Immunol 2001;2:108-115.

5 Bottazzi B, Polentarutti N, Acero R, Balsari A, Boraschi D, Ghezzi P, Salmona M, Mantovani A: Regulation of the macrophage content of neoplasms by chemoattractants. Science 1983;220:210-212.

6 Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A: Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50-56.

7 Oonakahara K, Matsuyama W, Higashimoto I, Kawabata M, Arimura K, Osame M: Stromal-derived factor-1alpha/CXCL12-CXCR 4 axis is involved in the dissemination of NSCLC cells into pleural space. Am J Respir Cell Mol Biol 2004;30:671-677.

8 Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK: Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res 2002;62:1832-1837.

9 Saur D, Seidler B, Schneider G, Algul H, Beck R, Senekowitsch-Schmidtke R, Schwaiger M, Schmid RM: CXCR4 expression increases liver and lung metastasis in a mouse model of pancreatic cancer. Gastroenterology 2005;129:1237-1250.

10 Wang J, Wang J, Dai J, Jung Y, Wei CL, Wang Y, Havens AM, Hogg PJ, Keller ET, Pienta KJ, Nor JE, Wang CY, Taichman RS: A glycolytic mechanism regulating an angiogenic switch in prostate cancer. Cancer Res 2007;67:149-159.

11 Dayan F, Roux D, Brahimi-Horn MC, Pouyssegur J, Mazure NM: The oxygen sensor factor-inhibiting hypoxia-inducible factor-1 controls expression of distinct genes through the bifunctional transcriptional character of hypoxia-inducible factor-1alpha. Cancer Res 2006;66:3688-3698.

12 Duan Z, Lamendola DE, Yusuf RZ, Penson RT, Preffer FI, Seiden MV: Overexpression of human phosphoglycerate kinase 1 (PGK1) induces a multidrug resistance phenotype. Anticancer Res 2002;22:1933-1941.

13 Hwang TL, Liang Y, Chien KY, Yu JS: Overexpression and elevated serum levels of phosphoglycerate kinase 1 in pancreatic ductal adenocarcinoma. Proteomics 2006;6:2259-2272.

14 Zhang D, Tai LK, Wong LL, Chiu LL, Sethi SK, Koay ES: Proteomic study reveals that proteins involved in metabolic and detoxification pathways are highly expressed in HER-2/neu-positive breast cancer. Mol Cell Proteomics 2005;4:1686-1696.

15 Kurayoshi M, Oue N, Yamamoto H, Kishida M, Inoue A, Asahara T, Yasui W, Kikuchi A: Expression of Wnt-5a is correlated with aggressiveness of gastric cancer by stimulating cell migration and invasion. Cancer Res 2006;66:10439-10448.

16 Lowy AM, Clements WM, Bishop J, Kong L, Bonney T, Sisco K, Aronow B, Fenoglio-Preiser C, Groden J: beta-Catenin/Wnt signaling regulates expression of the membrane type 3 matrix metalloproteinase in gastric cancer. Cancer Res 2006;66:4734-4741.

17 Yamada T, Takaoka AS, Naishiro Y, Hayashi R, Maruyama K, Maesawa C, Ochiai A, Hirohashi S: Transactivation of the multidrug resistance 1 gene by T-cell factor 4/beta-catenin complex in early colorectal carcinogenesis. Cancer Res 2000;60:4761-4766.

18 Sobin LH: TNM, sixth edition: new developments in general concepts and rules. Semin Surg Oncol 2003;21:19-22.

19 Zieker D, Fehrenbach E, Dietzsch J, Fliegner J, Waidmann M, Nieselt K, Gebicke-Haerter P, Spanagel R, Simon P, Niess AM, Northoff H: cDNA microarray analysis reveals novel candidate genes expressed in human peripheral blood following exhaustive exercise. Physiol Genomics 2005;23:287-294.

20 Saitou N, Nei M: The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406-425.

21 Dietzsch J, Gehlenborg N, Nieselt K: Mayday-a microarray data analysis workbench. Bioinformatics 2006;22:1010-1012.

22 Gehlenborg N, Dietzsch J, Nieselt K: A Framework for Visualization of Microarray Data and Integrated Meta Information. Information Visualization 2005;4(3):164-175.

23 Daly EB, Wind T, Jiang XM, Sun L, Hogg PJ: Secretion of phosphoglycerate kinase from tumour cells is controlled by oxygen-sensing hydroxylases. Biochim Biophys Acta 2004;1691:17-22.

24 Popanda O, Fox G, Thielmann HW: Modulation of DNA polymerases alpha, delta and epsilon by lactate dehydrogenase and 3-phosphoglycerate kinase. Biochim Biophys Acta 1998;1397:102-117.

25 Matigian N, Windus L, Smith H, Filippich C, Pantelis C, McGrath J, Mowry B, Hayward N: Expression profiling in monozygotic twins discordant for bipolar disorder reveals dysregulation of the WNT signalling pathway. Mol Psychiatry 2007;12:815-825.

26 Yeh CS, Wang JY, Chung FY, Lee SC, Huang MY, Kuo CW, Yang MJ, Lin SR: Significance of the glycolytic pathway and glycolysis related-genes in tumorigenesis of human colorectal cancers. Oncol Rep 2008;19:81-91.

27 Unwin RD, Craven RA, Harnden P, Hanrahan S, Totty N, Knowles M, Eardley I, Selby PJ, Banks RE: Proteomic changes in renal cancer and co-ordinate demonstration of both the glycolytic and mitochondrial aspects of the Warburg effect. Proteomics 2003;3:1620-1632.

28 Sun YX, Wang J, Shelburne CE, Lopatin DE, Chinnaiyan AM, Rubin MA, Pienta KJ, Taichman RS: Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) in vivo. J Cell Biochem 2003;89:462-473.

29 Wang J, Wang J, Sun Y, Song W, Nor JE, Wang CY, Taichman RS: Diverse signaling pathways through the SDF-1/CXCR4 chemokine axis in prostate cancer cell lines leads to altered patterns of cytokine secretion and angiogenesis. Cell Signal 2005;17:1578-1592.

30 Harmon RL, Sugarbaker PH: Prognostic indicators in peritoneal carcinomatosis from gastrointestinal cancer. Int Semin Surg Oncol 2005;2:3.