Elevated Expression of Glycerol-3-phosphate Phosphatase as a Biomarker of Poor Prognosis and Aggressive Prostate Cancer

Mohamed Amine Lounis

CRCHUM: Centre Hospitalier de l'Universite de Montreal Centre de Recherche <https://orcid.org/0000-0001-7023-592X>

Veronique Ouellet

CRCHUM: Centre Hospitalier de l'Universite de Montreal Centre de Recherche

Benjamin Péant

CRCHUM: Centre Hospitalier de l'Universite de Montreal Centre de Recherche

Christine Caron

CRCHUM: Centre Hospitalier de l'Universite de Montreal Centre de Recherche

Zhenhong Li

CRCHUM: Centre Hospitalier de l'Universite de Montreal Centre de Recherche

S.R Murthy Madiraju

CRCHUM: Centre Hospitalier de l'Universite de Montreal Centre de Recherche

Marc Prentki

CRCHUM: Centre Hospitalier de l'Universite de Montreal Centre de Recherche

Anne Marie Mes Masson

CRCHUM: Centre Hospitalier de l'Universite de Montreal Centre de Recherche

Fred Saad (✉ fredsaad@videotron.ca)

Université de Montréal

Research

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Abstract

Background

The limitations of the biomarker prostate-specific antigen (PSA) necessitate the pursuit of biomarkers capable of better identifying high-risk prostate cancer (PC) patients in order to improve their therapeutic management and outcome. Aggressive prostate tumors characteristically exhibit high rates of glycolysis and lipogenesis. Glycerol 3-phosphate phosphatase (G3PP), also known as phosphoglycolate phosphatase (PGP), is a recently identified mammalian enzyme, shown to play an important role in the regulation of glucose metabolism, lipogenesis, lipolysis and cellular nutrient excess detoxification.

We hypothesized that G3PP is involved in cancer cell growth and assessed the association of its expression with PC patient prognosis.

Methods

Using immunohistochemical staining we assessed the epithelial expression of G3PP in two different radical prostatectomy cohorts with a total of 1797 patients, for whom information on biochemical recurrence (BCR), metastasis and mortality is available. Association between biomarker expression, biochemical recurrence (BCR), bone metastasis and prostate cancer specific survival was established using log-rank and multivariable Cox regression analyses.

Results

High expression of G3PP prostate cancer epithelial cells is associated with an increased risk of BCR, bone metastasis and prostate cancer specific mortality. Multivariate analysis revealed high G3PP expression in tumors as an independent predictor of BCR and bone metastasis development.

Conclusions

High G3PP expression in tumor from patients eligible for prostatectomies is a new and independent prognostic biomarker of poor prognosis and aggressive prostate cancer specifically for recurrence, bone metastasis and mortality.

Background

Prostate cancer (PC) is one of the most commonly diagnosed and lethal cancers in men (1, 2). Although detection and treatments for PC have advanced in recent years, prostate-specific antigen (PSA) remains the only widely used biomarker for PC. Combining PSA with pathologic findings help in stratifying patients in terms of risk. However, in order to optimize therapeutic decisions, there remains a need to identify biomarkers to better predict PC prognosis and aggressiveness (3). Considering that the 5-year survival rate for men with metastasized prostate cancer decreases to 31% from near 100% for localized

prostate cancer patients (4), it becomes imperative to identify a biomarker that can reliably predict which patients with localized prostate cancer are at high risk of developing metastasis.

Proliferating PC cells exhibit a higher demand for glucose and glutamine for their metabolic needs for membrane synthesis and cellular multiplication. In particular, elevated glycolysis has been considered as a hallmark of cancer cells (5). Recent evidence indicates that glycolysis and anaplerosis are not sufficient for providing the appropriate amount of energy and the necessary building blocks for cellular growth. Cancer cells also exhibit an increase in lipogenesis from glucose derived glycerol-3-phosphate for their survival and proliferation (6, 7). Chronic exposure of cancer cells to elevated concentrations of fatty acids and glucose induces glucolipotoxicity/metabolic stress, leading to cell death (8-10). To circumvent this and in order to survive under conditions of toxicity due to nutrient excess, cancer cells deploy metabolic detoxification mechanisms to prevent the accumulation of toxic metabolites. Our recent discovery of glycerol-3-phosphate phosphatase (G3PP) in mammalian cells (11) (gene name *pgp*; 2 phosphoglycolate phosphatase) adds another level of regulation of glucose and lipid metabolism, as G3PP hydrolyzes glycerol-3-phosphate (Gro3P), a critical metabolite at the crossroads of glucose and lipid metabolism. We have shown that increasing the G3PP activity in rat hepatocytes decreases the synthesis of glycerolipids, glycolysis and gluconeogenesis and protected the tumorigenic INS-1(832/13) pancreatic β -cell line from glucolipotoxicity (11). On the other hand, RNAi-knockdown of G3PP induces glucolipotoxicity in INS-1(832/13) cells and enhances glycolysis and glycerolipid synthesis in hepatocytes (11, 12).

Based on the major role of G3PP in the regulation of glycolysis and lipid metabolism it is conceivable that this enzyme plays a critical role in tumor cells and could be a potentially useful biomarker of PC aggressiveness.

In the present study, we investigated the expression level of the G3PP protein as an independent predictor of biochemical recurrence (BCR), bone metastases and PC-specific death in two independent cohorts.

Methods

Patients and tissue micro array series

Two retrospective cohorts of patients were used to create two different tissue micro array (TMA) series. The TF123-TMA cohort is composed of 285 treatment naïve PC patients who signed an informed consent to participate in the Centre de recherche du Centre hospitalier de l'Université de Montréal (CRCHUM) PC biobank, affiliated to the Cancer Research Network (RRCancer) (13-16). These patients underwent radical prostatectomy (RP) between 1992 and 2006 at the Centre hospitalier de l'Université de Montréal (CHUM). Ethics approval for biobanking and evaluation of biomarkers was obtained from the Comité d'éthique de la recherche du CRCHUM. The Canadian Prostate Cancer Biomarker Network (CPCBN)-TMA series is composed of treatment-naïve specimens from 1512 patients who underwent RP between 1990 to 2011. These patient samples came from five biobanks: CRCHUM, Research Institute of the McGill University Health Center (RI-MUHC), Centre de recherche du Centre hospitalier Universitaire de Québec – Université Laval (CRCHUQc-UL), University Health Network (UHN) of Toronto, and Vancouver Prostate Centre (VPC).

All patients signed an informed consent for the use of their prostate tissue samples in research. Local ethics review board approved the inclusion of specimens into the CPCBN multicentre resource (www.tfri.ca/cpcbn).

A pathologist reviewed hematoxylin/eosin (H&E)-stained slides from archived formalin-fixed paraffin-embedded (FFPE) RP tissue blocks and circled the area of interest. For each patient, 0.6 mm cores (2-4 cores of tumor tissue and 1-2 cores of benign tissues) were taken from the FFPE blocks and arrayed on receiver blocks.

Immunohistochemistry

We confirmed the specificity of the G3PP antibody using western blot analysis and immunohistochemistry in prostate cancer cell lines (Fig S1A). Immunohistochemistry assays for TMAs were performed on 4 µm sections of each TMA block using the Benchmark XT autostainer (Ventana Medical Systems, Tucson, AZ). Sections were subjected to antigen retrieval in Cell Conditioning 1 (#950-124, Ventana Medical System) for 90 min and then stained using the pre-diluted anti-G3PP mouse monoclonal antibody (1:100, sc-390883 (E-10), Santa Cruz Biotechnology, Santa Cruz, CA) manually added to the slides and incubated at 37°C for 60 min. Antibody binding was revealed using the UltraView universal DAB detection kit (#760-500, Ventana Medical System). Counterstaining was achieved using hematoxylin and bluing reagents (#760-2021 and #760-2037, Ventana Medical System). Tissues were dehydrated and mounted using SubX mounting media (Leica microsystems, Concord, ON, Canada). All sections were scanned using a VS-110 microscope with a 20x 0.75 NA objective and a resolution of 0.3225 µm (Olympus Canada Inc., Richmond Hill, ON, Canada).

Scanned images were imported to VisiomorphDP software (Visiopharm, Denmark). Semi-automated Analysis Protocol Packages (APPs) were established to determine the mean intensity (MI) of G3PP specifically in the epithelium (Supplementary Fig 1B). Cores with less than 5% of epithelial cells or damaged were discarded (less than 1%).

Statistical analyses

Statistical analyses were performed with SPSS software 25.0 (SPSS Inc. Chicago, IL). The correlation with clinicopathological variables was estimated with a nonparametric Spearman's correlation test. The plan of analysis was to evaluate the association of G3PP expression with PC patient clinical endpoints, which included BCR, the development of bone metastasis and PC-specific mortality. The cut-off applied for the dichotomization of the data was defined by the 75th percentile of G3PP expression. BCR (defined as PSA at 0.2 ng/mL and rising with decision for additional treatment, or PSA that did not achieve a level of <0.2 ng/mL following surgery defined as failed RP) (17), bone metastasis-free survival and PC-specific survival curves were plotted using the Kaplan–Meier estimator, and the log-rank test was used to evaluate significant differences. The univariate and multivariate proportional hazard models (Cox regression) were used to estimate the hazard ratios (HRs) for G3PP expression. In the rare occasion when clinical data

were missing, the case was withdrawn from the analyses. Results were considered statistically significant at p -values < 0.05 .

Results

Elevated epithelial expression of G3PP is associated with PC aggressiveness

We first studied the expression of G3PP in the TF123 cohort and then examined the CPCBN-TMA series (13-16). Clinicopathological characteristics of PC patients in each cohort are presented in Table 1.

High expression of G3PP is associated with an increased risk of biochemical recurrence of PC

Correlations of epithelial G3PP expression in tumor cores with clinical characteristics were assessed on continuous and dichotomized data. We used the 75th percentile of G3PP (64.77 MI for TF123-TMA and 53.84 MI for CPCBN-TMA) as the threshold to dichotomize data based on the quartile method (18). Expression of G3PP is considered low when is $\leq 75\%$ and high when is $> 75\%$. Using dichotomization, we identified 66 (21.05%) PC patients with high expression of G3PP and 201 patients with low expression in the TF123 series. In the CPCBN-TMA series, 366 (24.20%) PC patients presented a high expression of G3PP and 1098 patients with low expression of G3PP.

Kaplan–Meier survival curves showed that high expression of G3PP was associated with an increased risk of BCR within five years in both the TF123 (log-rank = 8.859, $p = 0.003$) (Fig 2A, left panel), and the CPCBN-TMA series (log-rank = 28.768, $p < 0.001$) (Fig 2B, left panel). Interestingly, these results were also confirmed in an overall follow-up for both the TF123 (log-rank = 7.758, $p = 0.005$) (Fig 2A, right panel) and CPCBN-TMA series (log-rank = 23.888, $p < 0.001$) (Fig 2B, right panel).

Univariate Cox regression analyses for 5 years and overall follow-up demonstrated that all known predictors of BCR were statistically significant in both cohorts (Table 2 and Supplementary Table 1). Univariate regression analyses of both continuous (HR 1.024 [95% CI 1.008–1.039], $p = 0.003$) and dichotomized (HR 1.932 [95% CI 0.854–2.162], $p=0.004$) values of G3PP expression were associated with an increased risk of BCR in the TF123 series (Table 2). These results were validated in the CPCBN series in continuous (HR 1.031 [95% CI 1.022–1.039], $p < 0.001$) and dichotomized G3PP (HR 1.761 [95% CI 1.427–2.173], $p < 0.001$) (Table 2). When associated with the clinical parameters of BCR, epithelial G3PP expression remained an independent parameter as indicated by the multivariate Cox regression analyses (Table 2). We also observed similar results in both cohorts after an overall follow-up (Supplementary Table 1).

High expression of G3PP is a predictor of bone metastasis within 10 years

Bone is the main site of distant metastasis that represents a major clinical endpoint of PC (19). Lack of metastasis is a strong predictor of PC survival (20, 21). Kaplan–Meier estimates demonstrated that bone metastasis-free survival was shorter for patients with a high G3PP expression in both TMA series (log-

rank = 14.437, $p < 0.001$ for TF123 and log-rank = 20.337, $p < 0.001$ for CPCBN) at 10 years (Fig 3A and B, left panels). Similar results were obtained for the overall follow-up in both the TF123 (log-rank = 7.152, $p = 0.007$) and CPCBN series (log-rank = 11.780, $p = 0.001$) (Fig 3A and B, right panels).

Multivariate analyses of the CPCBN cohort TMA showed that G3PP expression was significantly associated with development of bone metastases in continuous (HR 1.054 [95% CI 1.024–1.085], $p < 0.001$), and dichotomized data (HR 5.691 [95% CI 2.066–15.580], $p = 0.001$) within 10 years (Table 3) or overall survival (Supplementary Table2). The CPCBN-TMA series confirmed that high G3PP expression was a strong predictor of bone metastasis development within 10-year follow-up using both continuous (HR 1.052 [95% CI 1.032–1.073], $p < 0.001$) and dichotomized (HR 3.910 [95% CI 2.062–7.412], $p < 0.001$) values. Moreover, G3PP association with bone metastasis development remained significant when clinical parameters (PSA, pTNM and Gleason score) were included in the model (Table 3).

High expression of G3PP is a predictor of PC-specific mortality

Using Kaplan-Meier estimators, we found that patients with high expression of G3PP showed reduced PC-specific survival. These results were observed in the TF123 cohort (log-rank = 8.671, $p = 0.003$) and were validated in the CPCBN (log-rank = 4.644, $p = 0.031$) TMA series (Fig 4). The separation among low and high G3PP expression was particularly prominent within the first five years. Univariate Cox regression analyses showed a significant association of PC-specific mortality with continuous (HR 1.048 [95% CI 1.015–1.083], $p = 0.004$) and dichotomized (HR 3.990 [95% CI 1.477–10.774], $p = 0.006$) G3PP expression in the TF123 cohort (Table 4). These results were also recapitulated in the CPCBN series (continuous: HR 1.035 [95% CI 1.013–1.058], $p = 0.002$ and dichotomized: HR 1.995 [95% CI 1.051–3.786], $p = 0.035$) (Table 4).

Discussion

Identification of novel biomarkers that can help improve the stratification of patients with prostate cancer remains an important objective for optimal clinical management. Currently, serum PSA is the only biomarker used in screening, prognosis and therapeutic response/progression for patients with PC (22, 23). Because of the limitations of PSA, identifying additional biomarkers able to predict disease aggressiveness and aid in therapeutic decisions may lead to better outcome and reduced PC mortality. A number of additional PC biomarkers are now being explored (24).

Here, we have identified using two different cohorts, a promising intracellular biomarker, G3PP, whose elevated expression is associated with poor PC prognosis, particularly within the first five years of diagnosis. Using immunohistochemistry to assess the expression of G3PP in PC TMAs, we analyzed the expression using digital image analysis software. Immunohistochemistry is routinely used in pathology and its combination with digital pathology provides a powerful tool for the standardization of biomarker expression analysis and evaluation that can be readily translated into clinical practice (25, 26). We observed that G3PP is localized in the cytoplasm of epithelial cells from RP specimens of treatment naïve PC patients and found that high G3PP expression was associated with an increased risk of early and

overall BCR in the TF123 cohort of 285 patients and in the larger multi-center CPCBN cohort of 1512 patients. We also show that high expression of G3PP is an independent predictor of BCR (PSA >0.2 ng/mL). Importantly, bone metastasis-free survival and PC-specific survival were shorter for patients with higher G3PP expression in cancer cells. Moreover, this marker remained independent when analyses were adjusted for pathological staging of the primary tumor and Gleason score at RP for bone metastasis after 10 years of follow-up. This finding is important given that metastases has been established as a reliable surrogate for survival in localized prostate cancer (21).

Interestingly, Kaplan-Meier analysis for BCR, bone metastasis and prostate cancer specific survival showed that elevated expression of G3PP is a predictive biomarker of early relapse and poor prognosis. The implantation of G3PP as a biomarker in clinics could considerably help in the management and treatment of patients. As a predictive biomarker, patients with high expression of G3PP are more susceptible to harbor aggressive PC and may be candidates for earlier and more aggressive therapy. Patients considered at very high risk, considering clinical, pathologic and biomarker characteristics, may eventually be considered for early multimodal approach.

G3PP is an enzyme that regulates the cellular level of Gro3P, a critical intermediate at the crossroads of glucose and lipid metabolism (12). Cancer cells exhibit a high rate of glycolysis. Importantly, studies show that high expression of glycolytic markers are associated with poor prognosis of PC (27). Glycolysis generated reducing equivalents (NADH) in the cytosol that are transferred to the mitochondrial electron transport chain via the redox Gro3P-dehydrogenases shuttle. This shuttle is a major contributor of reactive oxygen species (ROS) production (28). Under conditions of accelerated glycolysis together with excess availability of glucose, there can be significant Gro3P shuttle mediated production of ROS, which have toxic effects on cells. We previously proposed that elevated activity of G3PP, by hydrolyzing excess glucose-derived Gro3P, will generate less toxic glycerol and can dampen the ROS mediated detrimental effects (12). In line with this notion, elevated Gro3P-dehydrogenases has been observed in several tumors (29). Thus, in prostate cancer G3PP could play an important role in preventing the elevated ROS production during glucose utilization in the interior of tumors and may also provide glycerol substrate for cancer cells at the tumor-front as well as to the periprostatic adipose tissue. Besides modulating ROS, G3PP regulates the glycerolipid/free fatty acid (FFA) cycle (lipogenesis followed by lipolysis) that plays an important role in cancer cell growth (30, 31). We have previously shown that oleate promotes breast cancer cell growth via the FFA receptor FFAR1/GPR40 (32) and PI-3-kinase activation (33) and that upregulation of cellular triacylglycerol - FFA cycling by oleate is associated with long-term serum-free survival of human breast cancer cells (34).

Based on these observations, we believed that the high expression of G3PP observed in PC tumors will help in the detoxification of cells and in the regulation of a higher rate of metabolism leading to a more aggressive cancer.

Conclusion

In conclusion, we have demonstrated the prognostic value of epithelial G3PP levels in two cohorts of PC patients treated with radical prostatectomy. This study is the first to highlight the value of G3PP as an early predictor of patients at high risk of recurrence, bone metastases and mortality. With further validation, the use of G3PP as an additional prognostic biomarker may eventually help clinicians in the management of early-stage prostate cancer.

Abbreviations

PSA : Prostate-specific antigen

PC : Prostate cancer

G3PP : Glycerol 3-phosphate phosphatase

PGP : Phosphoglycolate phosphatase

BCR : Biochemical recurrence

Gro3P : Glycerol-3-phosphate

TMA : Tissue micro array

CRCHUM : Centre de recherche du Centre hospitalier de l'Université de Montréal

RR CANCER : Cancer Research Network

RP : Radical prostatectomy

CHUM : Centre hospitalier de l'Université de Montréal

CPCBN : Canadian Prostate Cancer Biomarker Network

RI-MUHC : McGill University Health Center

CRCHUQc-UL : Centre de recherche du Centre hospitalier Universitaire de Québec – Université Laval

UHNT : University Health Network of Toronto

VPC : Vancouver Prostate Centre

H&E : Hematoxylin/eosin

FFPE : Formalin-fixed paraffin-embedded

APP : Analysis Protocol Packages

MI : Mean intensity

HR : Hazard ratio

ROS : Reactive oxygen species

FFA : Free fatty acid

Declarations

Ethics approval and consent to participate

All patients included in the TF123 and the CPCBN cohorts signed an informed consent form to participate in one of the biobank (CE12.216). Each biobank received the approval from their local ethics review board for their inclusion into the CPCBN resource (CE 09.173, and CE 15.366). The comité d'éthique de la recherche of the Centre de recherche du CHUM approved the project at the lead institution (CE 09.173, and CE 15.366).

Consent for publication

“Not applicable”

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

Dr Saad is a Consultant and honoraria received from Astellas, Astrazeneca, Bayer, Janssen, Myovant, Pfizer, Sanofi, and institutional research funding received from Astellas, AstraZeneca, Bayer, Janssen, Merck, Myovant, Pfizer, Sanofi, BMS. All other authors declare no potential conflicts of interest.

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Authors' contributions

MAL designed and performed the experiments and wrote the manuscript. VO analyzed the data and reviewed the manuscript. CC and ZL participated in the visiomorph analysis. BPSRMM, MP, AMMM and FS supervised the experiments and reviewed the manuscript.

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Tables

Due to technical limitations, table 1, 2, 3 and 4 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Epithelial expression of G3PP in prostate cancer tissues. (A) Representative images of G3PP immunostaining on TMA cores of prostate cancer. Images represent expression levels of G3PP at less than the 25th percentile, between the 25th and 50th, between 50th and 75th and more than 75th percentile. Scale bars, 200 μ m (top) and 50 μ m (bottom). (B) Epithelial expression of G3PP in prostate cancer tissues versus benign adjacent tissues of the TF123 and CPCBN TMAs and (C) expression of

epithelial G3PP in TF123 and CPCBN TMA's based on Gleason score categories (1, 2, 3 or 4). (Mann–Whitney U test, Mean $\pm$ SEM).

Figure 2

High expression of G3PP is associated with increased risk of biochemical recurrence within 5 years and overall follow-up. Kaplan-Meier biochemical recurrence (BCR)-free survival curves at 5 years and overall for the TF123 (A) and CPCBN (B) cohorts TMAs.

Figure 3

High expression of G3PP is associated with increased risk of bone metastases after 10 years and overall follow-up. Kaplan-Meier bone metastasis-free survival curves after 10 years and overall follow-up for the TF123 (A) and CPCBN (B) cohorts TMAs.

G3PP epithelial expression

Low ($\leq 75\%$)

High ($> 75\%$)

Figure 4

High expression of G3PP is associated with increased risk prostate cancer-specific mortality. Kaplan-Meier bone metastasis-free survival curves after overall follow-up for the TF123 and CPCBN cohorts.

Supplementary Files

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