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ORIGINAL ARTICLE

Genetic variants in glucose-6-phosphate isomerase gene as prognosis predictors in hepatocellular carcinoma

Zhuomin Lyu[a,1], Yibing Chen[b,1], Xu Guo[b], Feng Zhou[c], Zhaoyong Yan[a], Jinliang Xing[b], Jiaze An[d,], Hongxin Zhang[a,*]**

[a] *Department of Pain Treatment, Tangdu Hospital, Fourth Military Medical University, 169, Changle West Road, Xi'an, Shaanxi, 710038, China*
[b] *State Key Laboratory of Cancer Biology, Experimental Teaching Center of Basic Medicine, Fourth Military Medical University, Xi'an, Shaanxi, 710032, China*
[c] *Department of General Surgery, Tangdu Hospital, Fourth Military Medical University, Xi'an, Shaanxi, 710032, China*
[d] *Department of Hepatobiliary Surgery, Xijing Hospital, Fourth Military Medical University, 169, Changle West Road, Xi'an, Shaanxi, 710032, China*

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Summary

Background: Metabolic reprogramming is an important hallmark of cancer cells, including the alterations of activity and expression of enzymes in glucose metabolism. Previous studies have demonstrated the critical role of glucise-6-phosphate isomerase (GPI) in cancer initiation, metastasis and progression. However, the significance of single nucleotide polymorphisms (SNPs) in *GPI* gene has not been investigated in hepatocellular carcinoma (HCC).

Methods: In this study, a total of 3 functional SNPs in *GPI* gene were genotyped in 492 HCC patients with surgical treatment. Multivariate Cox proportional hazards model and Kaplan—Meier curve were used for the analysis of overall survival (OS) and recurrence-free survival (RFS).

Results: The homozygous variant genotypes of rs7248411 in mRNA splice sites of *GPI* gene were significantly associated with an increased risk of death in the multivariate analysis (Hazard ratio [HR], 2.07; 95% confidence interval [95% CI]: 1.16—3.68 in a recessive model). In stratified analysis, the association remained significant in patients with high α-fetal protein (AFP) level (HR = 2.37, 95% CI 1.25—4.49). Moreover, we identified the interaction between rs7248411 and AFP level in predicting the prognosis of HCC patients (*P* for interaction < 0.001).

Abbreviations: HCC, hepatocellular carcinoma; SNPs, single nucleotide polymorphisms; HR, hazard ratio; CI, confidence interval.

* Corresponding author.
** Co-corresponding author.
E-mail addresses: anchen@fmmu.edu.cn (J. An), zhhxtdjr@163.com (H. Zhang).

[1] Zhuomin Lyu and Yibing Chen contributed equally to this work.

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Conclusions: Our data suggest that *GPI* gene polymorphism may serve as potential biomarkers to predict the OS of HCC. Further studies with different ethnicities are needed to validate our findings and generalize its clinical utility.

Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies worldwide, and its morbidity and mortality rates have escalated in recent years [1]. Despite improvements in surveillance and clinical treatment strategies, the prognosis of HCC patients remains poor [2]. Traditional clinicopathological parameters such as tumor morphology, histopathological features, concentration of serum alpha-fetoprotein (AFP) and tumor stage offer limited information for prognosis prediction and fail to guide the therapeutic schedule for individual patient [3]. Therefore, it is extremely urgent to explore novel biomarkers to discriminate patient groups with different clinical outcomes and direct the treatment for HCC patients.

In normal tissue, the vast majority of differentiated cells use oxidative phosphorylation (OXPHOS) for ATP production. These cells metabolize glucose into pyruvate through glycolysis, then oxidize the pyruvate through the tricarboxylic acid cycle, then generate ATP through ATP synthase [4]. In contrast, rapidly proliferating tumor cells consume glucose at a higher rate compared to normal cells and part of their glucose carbon is converted into lactate, even in oxygen-rich conditions; this is referred to as the Warburg effect or aerobic glycolysis. Glucose-6-phosphate isomerase (GPI) is a second glycolytic enzyme that catalyzes the isomerisation of G6P into fructose-6-phosphate. GPI has other biological roles when secreted as an extracellular cytokine with properties that include autocrine motility factor (AMF), also referred to as GPI/AMF [5,6].

A series of studies have reported that the overexpression of GPI/AMF is associated with an aggressive phenotype and the increased mortality in many cancer types, including gastrointestinal [7], kidney, lung and breast cancers [6,8,9]. Increased expression of the GPI/AMF is also associated with poor prognosis in patients with clear cell-renal cell carcinoma [10]. Moreover, GPI/AMF could reduce apoptosis through PI3 K/Akt signaling pathway activation. This activation is generally associated with increased tumor progression, tumor cell invasiveness and antiapoptosis [11]. Down-regulation of GPI/AMF enhanced the potent anticancer action of ginsenoside Rh2 on leukemia KG1α cells [12]. Silencing of GPI/AMF induces mesenchymal-to-epithelial transition and suppression of osteosarcoma pulmonary metastasis [13]. In HCC, Torimura et al. have demonstrated that GPI enhances cell invasion in an autocrine manner by stimulating the adhesion, motility, and matrix metalloproteinase-2 (MMP-2) secretion of these cells through activation of β1 integrins [14]. Yu et al. have demonstrated that GPI promotes the migration of hepatoma cells through the upregulation of MMP-3 [15]. Moreover, Shih et al. have shown that autocrine GPI prevents the apoptosis of hepatoma cells through PI3 K/Akt and STAT3 signaling pathways [16]. These data collectively suggest the important roles of GPI in the development and progression of HCC.

Single nucleotide polymorphism (SNP), a common typical genomic variation, can be used as a stable biomarker of genetic background to predict therapeutic response and prognosis [17]. However, whether functional SNPs in *GPI* gene have any influence on HCC patients' clinical outcomes remains unclear. In this study, we assessed the effects of three functional SNPs in *GPI* gene on recurrence and survival in a cohort of 492 Chinese HCC patients.

Subjects and methods

Study population

Between January 2009 and January 2012, HCC patients were enrolled at Eastern Hepatobiliary Surgery Hospital, Secondary Military Medical University (SMMU) in Shanghai, China. The enrollment was based on the following criteria:

- histologically confirmed with primary HCC and no history of other cancers;
- received curative surgical resection treatment, but without any preoperative anticancer treatment;
- with complete clinical and follow-up data, as well as common epidemiological data.

Finally, 492 patients were included in the present study. Blood sample (5 mL) was obtained from each participant before any treatment for genomic DNA extraction using E.Z.N.A. Blood Midi Kit (Omega Bio-Tek, Norcorss, GA, USA). This study was approved by the Ethic Committee of SMMU, and a signed informed consent was obtained from each participant.

Epidemiologic and clinical data collection

Demographic and personal data were collected through in-person interview using a standardized epidemiological questionnaire, including gender, ethnicity and residential region. Detailed clinical information was collected through medical chart review or consultation with treating physicians, including time of diagnosis, time of surgery, time of recurrence and/or death, tumor stage, differentiation, HBsAg, lymph node invasiveness, serum AFP and TACE treatment after surgery. A standard follow-up was performed at 6-month intervals by a trained clinical specialist through on-site interview, direct calling or medical chart review. The