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ScienceDirect

EJSO 41 (2015) 1293–1299

EJSO the Journal of Cancer Surgery

www.ejso.com

Glucose-6-phosphate dehydrogenase expression is correlated with poor clinical prognosis in esophageal squamous cell carcinoma

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Accepted 4 August 2015
Available online 24 August 2015

Abstract

Increasing evidence has demonstrated that glucose-6-phosphate dehydrogenase (G6PD), a key metabolic enzyme, participating in pentose phosphate pathway (PPP), is tightly associated with development and progression of a variety of tumors. Here, we reported expression of G6PD and its association with the prognosis of the patients with esophageal squamous cell carcinoma (ESCC). The results revealed significantly elevated G6PD mRNA and protein expressions in ESCC tissues compared with normal tissues ($P < 0.05$). Furthermore, high G6PD expression was tightly associated with histological grade, TNM staging and lymph node metastasis ($P < 0.05$), but not related to the patients' age and gender ($P > 0.05$). Importantly, the survival time of G6PD-positive patients was markedly lower than that of G6PD-negative patients ($P < 0.05$). Most notably, Cox multivariate assay demonstrated that G6PD was an independent prognostic factor for the patients with ESCC. In conclusion, G6PD may be a novel predictor for the prognosis of the patients with ESCC.

Keywords: Glucose-6-phosphate dehydrogenase; Pentose phosphate pathway; Esophageal squamous cell carcinoma; Prognostic factor

Introduction

Esophageal cancer is the eighth most common cancer and the sixth leading cause of cancer related death worldwide.[1,2] During the last three decades, there has been a markedly increasing incidence of esophageal cancer with approximately 400,000 new cases diagnosed annually in the world.[3] Esophageal cancer is mainly diagnosed as esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). Although the incidence of EAC is increasing in developed countries,[4,5] ESCC is still the major histological type with wide geographical distribution in East Asian countries.[5–7] Esophageal cancer is correlated with poor prognosis, with a 5-year survival rate from 15% to 25%.[8,9] Most notably, 50%–60% of patients with esophageal cancer exhibits advanced or metastatic phase at the time of diagnosis.[10] Recently, molecular targeting therapy has been thoroughly developed in many tumors; however, efficient therapy strategies for ESCC remain to be exploited in future. Given the dismal prognosis and metastasis for the patients with ESCC as well as the lack of efficient therapy strategies for improvement in overall survival, it is dramatically imperative to develop and identify the novel molecular target for therapy of the patients with ESCC.

The pentose phosphate pathway (PPP) is a major pathway for glucose catabolism, and its central role in tumor metabolism has attracted wide attention in recent

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http://dx.doi.org/10.1016/j.ejso.2015.08.155
0748-7983/

years.[11,12] More importantly, the inhibition of the PPP might be an attractive way to target rapidly growing tumor cells.[13] Glucose-6-phosphate dehydrogenase (G6PD), the first rate-limited enzyme of PPP, recently, has been verified to be close association with tumor development and progression.[14–16] Importantly, G6PD, a novel biomarker and potential therapeutic target for tumors,[17] plays an essential role in the synthesis of nucleic acid in rapidly proliferating cells.[18,19] However, currently, the underlying biological roles of G6PD in the development and progression of ESCC remain elusive, therefore, the purpose of the present study was to investigate expression patterns of G6PD in ESCC tissues and clarify its pivotal roles in development, progression and prognosis of ESCC. These findings suggest that G6PD may acts as an oncogenic role in the development and progression of ESCC, and thus could be an attractive target of anti-cancer drug development.

Materials and methods

Tissue samples

One hundred and twenty-eight cases of ESCC tissues and corresponding normal esophageal tissues were collected from the Department of Pathology, the First Affiliated Hospital of Zhengzhou University. All tissue samples were approved by the Institutional Review Board of our hospital, and written consent for each patient was obtained. The samples for in situ hybridization and immunohistochemistry assays were conventionally treated according to the standard procedures, whereas the samples for real-time quantitative reverse transcription PCR (qPCR) and Western blotting were immediately frozen in liquid nitrogen until analysis. All clinicopathological parameters were illustrated in Table 2.

In situ hybridization assay for G6PD mRNA expression

In situ hybridization investigation for G6PD mRNA expression was carried out according to previous publications[20,21] with G6PD probe. G6PD probe was amplified using G6PD specific primers (forward primer: 5′-CAACAGCCACATGAATGCCC-3′, reverse primer: 5′-CTTCTCCACGATGATGCGGT-3′, product size: 151 bp), and was labeled by Digoxigenin (Promega Corporation, Madison, WI) according to manufacturer's protocol. The procedures for in situ hybridization were as follows: the tissues slides were deparaffinized and placed in a 10 mM citrate buffer (pH 6.0) in a microwave oven for 15 min, followed by application of 10 μg/ml proteinase K for 30 min at room temperature. Subsequently, the slides were incubated with G6PD probe at 50 °C for 16 h in a moisturized chamber. Finally, signal development was performed with chromogen substrate NBT/BCIP stock solutions. PBS was used as negative control in stead of G6PD probe.

Table 1
In situ hybridization and immunohistochemistry scores for G6PD mRNA and protein expression in 128 ESCC patients.

Score	G6PD mRNA	%	G6PD protein	%
0–1	37	28.9	33	25.8
≥1–3	60	46.9	58	45.3
≥3	31	24.2	37	28.9
Total	128	100.0	128	100.0

Table 2
G6PD mRNA and protein expression in ESCC tissues and normal epithelial tissues.

Tissues	G6PD mRNA		X^2	*P* value	G6PD protein		X^2	*P* value
	+	−			+	−		
Normal	21	107	77.778	0.000	23	105	81.497	0.000
Tumor	91	37			95	33		

Immunohistochemistry detection for G6PD protein expression

Immunohistochemistry assay for G6PD protein expression was performed as described earlier.[20,22] Briefly, the slides were deparaffinized and hydrated according to the standard procedures. Subsequently, the slides were pretreated for antigen retrieval in 0.01 M citrate buffer in a microwave oven for 20 min, followed by incubation with G6PD primary antibody (1: 100 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C overnight. Finally, signal development was carried out with DAB. Normal horse serum was utilized as negative control in place of G6PD primary antibody.

Scoring of G6PD staining

Scoring of G6PD staining was evaluated according to our previous publication.[23] Briefly, staining intensity was recorded on a scale of 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The percentage of positive staining cells was graded as follows: 0, less than or equal to 5% positive tumor cells; 1, from 5% to less than or equal to 25%; 2, from 25% to less than or equal to 50%; 3, from 50% to less than or equal to 75%; 4, be more than or equal to 75%. The staining index (SI) was calculated according to the formula SI = percentage of positive staining cells × staining intensity. Scores less than 1 were considered negative, and the remaining cases were regarded as positive.

qPCR investigation for G6PD mRNA level

qPCR was conducted using the quant qRT-PCR (SYBR Green) Kit (Tiangen Biotech Co., Ltd, Beijing, China) as described earlier.[24] Total RNA and reverse transcription reaction were performed according to the standard procedures as described in manufacturer's instructions. Primers