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Investigation of the Association between FGL1 Expression and Prognosis in Gastric Cancer Patients

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Abstract

Gastric cancer is the fourth most common cancer worldwide, and it ranks second leading cause of cancer deaths. Several studies have shown that FGL2 contributes to the pathogenesis of a number of infectious diseases. However, little is known about its biological functions in cancer development and metastasis. In this study, the association between FGL1 expression and prognosis was investigated in GC patients. Gastric cancer and adjacent normal tissues (n=20) were obtained from patients diagnosed with gastric cancer aged between 30 and 50. Total RNA was extracted, reverse transcription and qPCR were performed, and Relative expression level was calculated using the 2-ΔΔCq method. It was found that FGL1 expression in gastric cancer tissues was obviously higher than adjacent tissues at mRNA levels (P<0.003).

INTRODUCTION

Gastric cancer is the fourth most common cancer worldwide, and it ranks as the second leading cause of cancer deaths [1]. Metastatic spread is fatal, causing mass-effect and failing of physiological homeostasis. Achieving a detailed molecular understanding of GC pathogenesis is pivotal to improving patient outcomes for this complex disease [2]. Environmental factors play critical roles in GC pathogenesis, with major risk factors being H *pylori* infection, diet, and smoking. Besides environmental agents, GC pathogenesis also involves host genetic factors [3]. Studies from several groups over the past decade have now produced a near-comprehensive catalog of GC-associated "driver" alterations, including gene mutations, somatic copy number alterations (sCNAs), structural variants, epigenetic changes, and transcriptional changes involving mRNAs and noncoding RNAs (ncRNAs) [4]. Fibrinogen-like protein 2 (fgl2)/fibroleukin, also called fg12 prothrombinase, has recently been identified as a new member of the fibrinogen-related protein superfamily, with the serine protease activity [5]. Mouse fgl2 (mfgl2) and human fgl2 (hfgl2) are localized in chromosomes 5 and 7, respectively. The biological activity of fgl2 prothrombinase, similar to coagulating factor Xa, can directly catalyze prothrombinase into activated thrombinase, thereby initiating a cascade coagulating reaction [6]. It has been identified as a novel effector molecule of Treg cells and plays a critical role in regulating innate immunity and adaptive immunity [7]. Several studies have shown that FGL2 contributes to the pathogenesis of a number of infectious diseases. However, little is known about its biological functions in cancer development and metastasis [8]. Previous studies have demonstrated that FGL1 expression was increased in the regenerated liver and could stimulate 3Hthymidine uptake in primary hepatocytes, implying that FGL1 facilitates hepatocyte proliferation.

Moreover, recombinant FGL1 protected against liver injury in rats with fulminant hepatic failure. These observations suggest that FGL1 plays a role in liver regeneration and liver protection [9]. In addition to its expression in the liver, FGL1 expression has also been reported in adipose tissue. In this study, the association between FGL1 expression and prognosis was investigated in GC patients.

METHOD AND MATERIALS

Gastric cancer and adjacent normal tissues (n=20) were obtained from patients diagnosed with gastric cancer aged between 30 and 50 without any therapy. These tissue samples were collected with the consent of the patients. Total RNA was extracted from gastric tissues of the patients using QIAzol reagent (Qiagen- Hilden, Germany). Reverse transcription was performed by BioFact™ RT Series (Biofact, South Korea). RT-qPCR was performed with RealQ

Plus Master Mix Green (Ampliqon, Denmark). PCR cycles were run on the Rotorgene 6000. The cycling conditions were 95˚C for 30 sec, 1 cycle, 95˚C for 5 sec, and 62˚C for 20 sec, 40 cycles. The primers used for amplification were forward 5'-GCA AGG AGTCTGCTTCTG CT-3', reverse 5'-TGCCATGTTCCCCCTTGAAA-3', GAPDH forward 5'-GGAGCGAGATCCCTCCAAAA T-3', and reverse 5'-GGCTGTTGTCATACTTCTC ATGG-3'. The relative expression level was calculated using the 2-ΔΔCq method [15]. GAPDH was employed as an endogenous control. The data analysis was performed based on the sample threshold cycle (Ct) value from three independent experiments.

RESULTS

In this study, the FGL1 expression of 20 patients was analyzed in the gastric cancer tissues and the corresponding adjacent tissues by qPCR. It was found that FGL1 expression in gastric cancer tissues was obviously higher than the adjacent tissues at mRNA levels (P<0.003, Fig. 1). The results showed no significant association between FGL1 expression and age and sex.

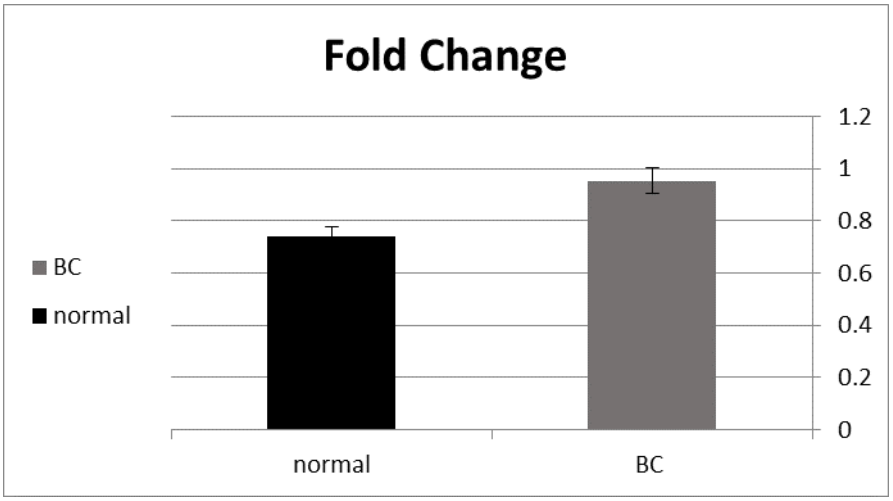

Fig. 1. FGL1 expression in gastric cancer tissues was obvi¬ously higher than that in the adjacent tissues

DISCUSSION

Although the global incidence of gastric cancer has been decreased dramatically in recent decades, it is the most common cancer in north and northwest Iran [10]. The wide variation in incidence across different geographical areas and the higher proportion of cardia cancer are two main characteristics of gastric cancer in Iran [11]. Current investigations indicate that a high prevalence of H.pylori infection, high dietary intake of salt, and smoking are the main environmental factors of gastric cancer in Iran [12]. The role of genetic factors in gastric cancer risk has been of increasing interest in the last several years, probably because of advances in DNA analysis technologies and human genome knowledge [13]. The fgl2 prothrombinase, a member of the fibrinogen superfamily, was primarily reported to be produced by activated macrophages, T cells, and endothelial cells. Human fgl2 (hfgl2) were localized in chromosomes 7. Fgl2 is a 64-70 kDa, type 2 transmembrane protein containing a C-terminal FRED (fibrinogen-related extracellular domain) [14]. In previous studies, it has been reported that FGL1 was also expressed in brown adipose tissue, and the expression was enhanced following liver injury, suggesting an association between the injured liver and adipose tissues [15].

Further experiments indicated that FGL1 plays a role in metabolism and liver regeneration [16]. In the present study, the upregulation of FGL1 was reported in GC. It was demonstrated that FGL1 expression in gastric cancer tissues was obviously higher than the adjacent tissues at mRNA levels (P<0.003, Fig. 1). The results showed no significant association between FGL1 expression and age and sex. It is recommended to perform this test on a larger population to ensure the results. It is also possible to knock out this gene in some traces and evaluate the results.

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