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Original contribution

Expression of microsomal prostaglandin E2 synthase-1 and its role in human hepatocellular carcinoma☆,☆☆

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Summary Microsomal prostaglandin E2 synthase-1 (mPGES1), an inducible enzyme similar to cyclooxygenase-2, functions downstream of cyclooxygenase-2 in the synthesis of prostaglandin E2. It contributes to carcinogenesis in a variety of tumors. Here, mPGES1 expression was assessed using immunohistochemistry of tissue microarrays containing a total of 100 hepatocellular carcinoma (HCC) tissue samples, 100 peritumoral liver tissue samples, and 13 normal liver tissue samples. The expression of mPGES1 was significantly increased in the HCC tissue samples ($P < .001$), relative to normal liver tissue. Second, there was a significant positive correlation between mPGES1 expression and the Barcelona Clinic Liver Cancer stage ($P < .001$) in HCC tissue samples. This correlation was also observed with encapsulation ($P = .004$) and portal vein thrombosis ($P < .001$). In addition, the lentiviral vector (Lv-mPGES1-shRNA), which down-regulates mPGES1, inhibited tumor growth in an HCC animal model. Taken together, mPGES1 expression was associated with multiple malignant characteristics and enhanced tumorigenesis in HCC and may serve as an important clinical and pharmacologic biomarker.

1. Introduction

Hepatocellular carcinoma (HCC) results in hundreds of thousands of deaths worldwide every year, half of which occur in China [1]. In addition, although overall cancer-related mortality has been declining in the United States, the HCC mortality rate is increasing [2]. Thus, novel molecular targets for prevention and treatment will be crucial to improve this poor outcome.

Numerous studies indicate that the enzymes involved in arachidonic acid metabolism and their products are implicated in various cancers [3–6]. A recognized strategy for inhibiting carcinogenesis is to suppress prostaglandin (PG) production in premalignant and malignant tissues [3–6]. Increased levels of prostaglandin E2 (PGE2) have been detected in a variety of malignancies and have been shown to play a role in the development and progression of cancer including HCC [7]. Therefore, it is important to identify the upstream enzymatic pathways that are affected in neoplastic tissues, resulting in increased PGE2 production. Microsomal PGE2 synthase-1 (mPGES1), an inducible enzyme, acts downstream of cyclooxygenase-2 in the synthesis of PGE2.

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It contributes to tumorigenesis in the stomach [8], penis [9], breast [10], liver [11], lung [12], pancreas [13], bile duct [14], prostate [15], and colon [16].

Takii and colleagues [11] first demonstrated overexpression of mPGES1 in HCC. However, their study was conducted on a relatively small sample size (40 cases), in which detailed clinical data such as patient stage, tumor size, and metastasis were not provided. In this study, the expression of mPGES1 was evaluated in 100 HCC samples on tissue microarrays (TMAs) using immunohistochemistry (IHC), and its expression was correlated with HCC clinicopathologic parameters. Overexpression of mPGES1 correlated with the progression of HCC. Loss-of-function analysis to determine whether mPGES1 down-regulation affects HCC cancer biology revealed that tumor growth was inhibited by decreasing mPGES1 expression in an HCC animal model. These findings suggest that mPGES1 may be an important HCC therapeutic target.

2. Materials and methods

2.1. Patient samples

Samples were taken from 100 patients with HCC and 13 patients with hepatic hemangioma at the Liver Center, First Affiliated Hospital of Fujian Medical University, between 2009 and 2010. All HCC tissue samples and corresponding peritumoral liver tissue samples were obtained from patients who had undergone surgical hepatectomy (86 men and 14 women; age range, 27-77 years; average age, 51 years). Thirteen normal liver tissue samples were obtained from patients who underwent liver resection because of hepatic hemangiomas. None of the patients received preoperative chemotherapy or radiation therapy. Peritumoral liver tissues were obtained from regions more than 3 cm in distance from the tumors. The tissue samples were immediately fixed in neutral-buffered formalin and embedded in paraffin for immunohistochemical studies. The diagnoses of HCC were confirmed by pathologic studies, as was the normalcy of the peritumoral liver tissue samples. In addition, the peritumoral tissue samples were evaluated to ensure the absence of tumor and inflammation. Clinical information was collected from patient records and incorporated in Table 1. Tumor stage was determined by the Barcelona Clinic Liver Cancer (BCLC) staging system [17], and tumor differentiation was graded by the grading system of Edmondson and Steiner [18]. This study was approved by the Institute Research Ethics Committee of Fujian Medical University, and informed consent was obtained from each patient according to the committee's regulations.

2.2. Tissue microarrays

According to the TMA methods described by Kononen et al [19], a modified protocol for preparing paraffin TMAs was developed as previously described [20]. A total of 100 formalin-fixed, paraffin-embedded, HCC tissue samples and their corresponding peritumoral liver tissue samples were placed on the TMAs, along with 13 normal liver tissue samples.

Table 1 Relationship between mPGES1 expression and clinicopathologic parameters

Variable	n	mPGES1 expression		χ^2	*P*
		Positive, n (%)	Negative, n (%)		
Sex					
Male	86	55 (64.0)	31 (36.0)	0.296	.587
Female	14	10 (71.4)	4 (28.6)		
Age (y)					
≤51	62	38 (61.3)	24 (38.7)	0.987	.320
>51	38	27 (72.2)	11 (27.8)		
Size (cm)					
>3	83	54 (65.1)	29 (34.9)	0.001	.978
≤3	17	11 (64.7)	6 (35.3)		
Encapsulation					
None or incomplete	81	58 (71.6)	23 (28.4)	8.175	.004
Complete	19	7 (36.8)	12 (63.2)		
Histomorphologic type					
Trabecular	51	30 (58.8)	21 (41.2)	2.428	.488
Pseudoglandular	11	9 (81.8)	2 (18.2)		
Solid	32	22 (68.8)	10 (31.2)		
Sclerosis	6	4 (66.7)	2 (33.3)		
Differentiation					
Well/Moderate	36	19 (52.8)	17 (47.2)	3.694	.055
Poor	64	46 (71.9)	18 (28.1)		
BCLC stage					
0/A	21	6 (28.6)	15 (71.4)	15.506	.000
B/C	79	59 (74.7)	20 (25.3)		
Portal vein thrombus					
No	73	39 (53.4)	34 (46.6)	15.924	.000
Yes	27	26 (96.3)	1 (3.7)		

2.3. Immunohistochemistry

IHC was performed using the EliVision Plus Two-step System (Maixin Incorporation, Fuzhou, China) according to the manufacturer's instructions, as previously described [20]. The TMA slides were blocked with 10% normal goat serum at 37°C for 30 minutes and incubated with a 1:500 dilution of rabbit polyclonal antihuman mPGES1 antibody (Cayman, Ann Arbor, MI) for 1 hour at 37°C, followed by 3 washes with phosphate-buffered saline. The slides were incubated with polymerized HRP-Anti Ms/Rb lgG (Maixin Incorporation, Fuzhou, China), followed by 3,3′-diaminobenzidine, and counterstained with hematoxylin. Negative control sections were incubated with preimmune serum, or with antiserum preabsorbed with a 100-fold excess of mPGES1-blocking peptide (Cayman).

The percentage of tumor cells showing cytoplasmic staining was scored for each case. Moderate to strong cytoplasmic staining observed in more than 10% of tumor cells