Human Pathology (2010) **41**, 1609–1616

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Reduced expression of Raf-1 kinase inhibitory protein is a significant prognostic marker in patients with gallbladder carcinoma

Hyun Soo Kim MD, Gou Young Kim MD, Sung-Jig Lim MD, Yong-Koo Park MD, Youn Wha Kim MD*

Department of Pathology, Graduate School of Medicine, Kyung Hee University, Seoul 130-702, Republic of Korea

Received 28 February 2010; revised 23 April 2010; accepted 30 April 2010

Keywords:
Raf kinase inhibitor protein;
Metastasis;
Immunohistochemistry;
Gallbladder cancer

Summary Gallbladder carcinoma is one of the most aggressive malignancies. It is usually diagnosed at an advanced stage, and the prognosis remains poor despite advances in imaging techniques and aggressive surgical treatment. Because of the lack of reliable prognostic markers, the aim of this study was to investigate the prognostic significance of Raf-1 kinase inhibitory protein expression in gallbladder carcinomas. Immunostaining for Raf-1 kinase inhibitory protein was performed on chronic cholecystitis, adenoma, carcinoma in situ, and primary and nodal metastatic gallbladder carcinoma. Raf-1 kinase inhibitory protein expression was reduced in 68.8% (11/16) and 42.3% (44/104) of nodal metastatic and primary gallbladder carcinoma cases, respectively, but in no case of carcinoma in situ, adenoma, or chronic cholecystitis. The differences in Raf-1 kinase inhibitory protein expression in gallbladder carcinoma versus nongallbladder carcinoma tissues ($P < .001$), and in nodal metastatic gallbladder carcinoma versus primary gallbladder carcinoma ($P = .009$), were statistically significant. Kaplan-Meier curves showed that patients with Raf-1 kinase inhibitory protein-negative or weakly positive gallbladder carcinoma had a significantly shorter overall survival than did patients with Raf-1 kinase inhibitory protein-positive gallbladder carcinoma (median, 14 versus 120 months; $P = .011$). Multivariate survival analysis showed that reduced Raf-1 kinase inhibitory protein expression was an independent prognostic predictor for overall survival ($P = .020$). Our results suggest that reduction in Raf-1 kinase inhibitory protein expression in gallbladder carcinoma contributes to invasion and metastasis and is a significant prognostic marker in patients with gallbladder carcinoma.

1. Introduction

Gallbladder carcinoma (GBCA) accounts for 2.9% of all cancers in Korea and is the sixth most common cause of cancer-related death [1]. Because of its nonspecific symptoms, the diagnosis of GBCA is usually made postoperatively on tumors at an advanced stage; almost half of patients already have metastatic disease at the time of surgery [2]. GBCA has a great propensity to directly invade the liver, and it also frequently metastasizes to the liver and pericholedochal lymph nodes [3]. Despite advances in imaging techniques and aggressive surgical treatment, the prognosis of GBCA remains poor with respect to postsurgical 5-year survival rates [1]. Thus, the search for new prognostic markers for resected patients is important to allow the assessment of metastasis and to provide the opportunity for adequate postoperative treatment in high-risk patients.

* Corresponding author.
E-mail address: kimyw@khu.ac.kr (Y. W. Kim).

0046-8177/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.humpath.2010.04.012

The mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway consists of evolutionarily conserved kinase modules that control fundamental cellular processes, such as growth, proliferation, differentiation, migration, and apoptosis. Activation of the MAPK pathway has been observed in a variety of human cancers, suggesting that this pathway plays a major role in inducing proteolytic enzymes that degrade the basement membrane, enhancing cell migration and maintaining growth, thereby promoting cancer cell invasion and metastasis [4-6]. After its activation by point mutations, Ras activates Raf-1, which in turn activates MAPK/ERK kinase (MEK). MEK then activates ERK, the prototypic MAPK [7], which translocates into the nucleus and eventually leads to cancer cell invasion via induction of matrix metalloproteinases [8,9] and migration via enhanced myosin light-chain kinase activity [10].

Raf-1 kinase inhibitor protein (RKIP) is a widely expressed and highly conserved cytoplasmic protein that belongs to the phosphatidylethanolamine-binding protein family [11,12]. RKIP was originally identified as an endogenous inhibitor of the MAPK/ERK pathway [13]; by binding to Raf-1 or MEK, RKIP interferes with MEK activation by Raf-1, inhibiting ERK activation. Recently, RKIP expression has been shown to be significantly reduced in human cancers, and a significant association between reduction of RKIP expression and invasion/metastasis has been reported in cancers of the colorectum [14], stomach [15], breast [16,17], nasopharynx [18], and ovary [19]. In addition, in a study using an orthotopic murine model, restoration of RKIP expression in metastatic prostate carcinoma cells inhibited in vitro cell invasion and lung metastasis in vivo, but did not inhibit primary tumor growth [20]. Another study using a metastatic breast carcinoma cell line also showed that RKIP expression was inversely correlated with invasiveness, but not with proliferation rate or colony-forming ability [16]. These data suggest that RKIP is a metastasis suppressor in human cancers.

Despite its documented importance in other cancers, there is no report on RKIP expression or on its prognostic significance in GBCA. In this study, we sought to evaluate RKIP expression in GBCA and to investigate the relationship between RKIP expression and clinicopathologic parameters and outcomes in patients with GBCA.

2. Materials and methods

2.1. Patient and tissue samples

This study included 104 patients with primary GBCA, 8 with carcinoma in situ (CIS), 26 with adenoma, and 11 with chronic cholecystitis. Of the 104 cases with primary GBCA, 22 had nodal metastasis, and sufficient amounts of metastatic tumor tissue (nodal metastatic GBCA) were available in 16 of these. No preoperative chemotherapy or radiotherapy had been performed. Surgical treatment for the 104 patients with GBCA was as follows: cholecystectomy with lymph node dissection and concomitant hepatic segmentectomy in 61, laparoscopic cholecystectomy with lymph node dissection in 7, cholecystectomy with concomitant hepatic segmentectomy in 25, and laparoscopic cholecystectomy alone in 11. All 45 patients with CIS, adenoma, or chronic cholecystitis underwent laparoscopic cholecystectomy alone. Two independent pathologists (H. -S. Kim and Y. W. Kim) reviewed all hematoxylin and eosin–stained slides and performed immunohistochemical staining on the most representative slide from each case. Clinicopathologic parameters, including sex, age, tumor size, histologic grade, pathologic tumor stage (pT), nodal and distant metastases, local recurrence, TNM stage group, lymphovascular invasion, perineural invasion, and resection margin status, were assessed. The tumors were postoperatively staged according to the American Joint Committee on Cancer staging system [21]. No distant metastases were identified at the time of surgery. Research protocols for the use of human tissue were approved by and conducted in accordance with the policies of the Institutional Review Board at Kyung Hee University Medical Center. Informed consent was obtained from all subjects.

2.2. Immunohistochemistry

RKIP expression was assessed by immunohistochemistry using the Bond Polymer Intense Detection System (Vision BioSystems, Mount Waverley, Victoria, Australia) according to the manufacturer's instructions with minor modifications. Briefly, 4-μm-thick sections of formalin-

Table 1 RKIP expression in chronic cholecystitis, adenoma, carcinoma in situ, and primary and nodal metastatic GBCA

	n	RKIP Expression		
		Negative (%)	Weakly positive (%)	Positive (%)
Chronic cholecystitis	11	0 (0.0)	0 (0.0)	11 (100.0)
Adenoma	26	0 (0.0)	0 (0.0)	26 (100.0)
Carcinoma in situ	8	0 (0.0)	0 (0.0)	8 (100.0)
Primary GBCA	104	16 (15.4)	28 (26.9)	60 (57.7) [a]
Nodal metastatic GBCA	16	7 (43.8)	4 (25.0)	5 (31.2) [b]

[a] Primary GBCA *vs* CIS, adenoma, and chronic cholecystitis, $P < .001$.
[b] Nodal metastatic GBCA *vs* primary GBCA, $P = .009$.