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Original contribution

Aberrant activation of Sonic hedgehog signaling in chronic cholecystitis and gallbladder carcinoma☆,☆☆

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Summary Sonic hedgehog (Shh) signaling has been extensively studied and is implicated in various inflammatory diseases and malignant tumors. We summarized the clinicopathological features and performed immunohistochemistry assays to examine expression of Shh signaling proteins in 10 normal mucosa, 32 gallbladder carcinoma (GBC), and 95 chronic cholecystitis (CC) specimens. The CC specimens were classified into three groups according to degree of inflammation. Compared with normal mucosa, CC, and GBC specimens exhibited increased expression of Shh. The immunoreactive score of Shh in the GBC group was higher than that in the mild to moderate CC groups but lower than that in the severe CC group ($P < .05$). Expression of Patched (Ptch) and Gli1 gradually increased from non-malignant cholecystitis to malignant tumors. Compared with CC specimens, GBC specimens showed higher cytoplasmic and membranous expression for Ptch ($P < .05$). Gli1 staining showed cytoplasmic expression of Gli1 in both CC (60% for mild, 77% for moderate, and 84% for severe) and GBC specimens (97%). Nuclear expression of Gli1 was detected in 16% of severe CC specimens with moderate to poor atypical hyperplasia, and in 62.5% of GBC specimens. Shh expression strongly correlated with expression of Ptch and Gli1. Furthermore, patients with strongly positive Gli1 staining had significantly lower survival rates than those with weakly positive staining. Our data indicate that the Shh signaling pathway is aberrantly activated in CC and GBC, and altered Shh signaling may be involved in the course of development from CC to gallbladder carcinogenesis.

Abbreviations: Shh, Sonic hedgehog; Ptch, Patched; GBC, Gallbladder carcinoma; CC, Chronic cholecystitis.

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1. Introduction

Gallbladder cancer (GBC) accounts for nearly two thirds of biliary tract tumors, and is the fifth most lethal cancer of the gastrointestinal tract [1,2]. GBC generally occurs in the sixth to seventh decade of life, and shows a strong female predominance [2]. The prognosis of GBC is poor, and the 5-year survival rate of patients with GBC is extremely low. This poor prognosis is attributable to the tendency for late presentation, development of early lymph-node metastases, adjacent organ invasion, and poor response to chemotherapy. To improve the prognosis of GBC, it is important to screen out the risk factors or precursor diseases relevant to gallbladder carcinogenesis, and establish appropriate prognostic markers, favoring early diagnosis and prevention.

Recent studies have revealed that the development sequence from chronic cholecystitis (CC) to carcinoma is relevant for GBC. A mass of clinical data suggests that CC causes limited or diffused hypertrophy of the gallbladder wall, atypical hyperplasia of the mucous layer, and metaplasia of the intestinal epithelium. In addition, the pathological changes occurring in CC are commonly seen in GBC tissue. Although it is not proven that CC has a direct carcinogenic effect, an extremely close relationship between CC and GBC has been confirmed [3], similar to that seen between inflammatory bowel disease and colon cancer [4]; in the chronic viral hepatitis to liver cirrhosis to hepatocellular carcinoma sequence [5]; and between chronic pancreatitis and pancreatic cancer [6]. Therefore, in the chronic carcinoma sequence, severe CC is considered to be a possible precursor lesion for neoplasia under certain circumstances [7].

The Sonic hedgehog (Shh) signaling pathway is known to be an essential pathway in the growth and patterning of various tissue types during embryonic development [8,9]. Recent studies have confirmed that Shh signaling transduction is mainly mediated by a complex interaction between the Shh, Smoothened (Smo), and Patched (Ptch) proteins, with the two latter proteins functioning as transmembrane receptors. In the absence of Shh, Ptch inhibits the activity of the Smo protein, thus repressing activation of the Hedgehog signaling pathway. When Shh binds to and inactivates Ptch, the inhibitory effect of Ptch on Smo is removed, and Smo is released to activate the expression of Gli1 and promote it to translocate to the nucleus [10,11].

Gli1 is a member of the Gli family, and is a downstream transcriptional target molecule of the Shh pathway. In addition, accumulation of full-length Gli1 results in activation of Shh pathway target genes, thus expression of Gli1, and especially its translocation to the nucleus, is a marker of Shh signaling activation [10,12]. Moreover, with induction of downstream Ptch transcription by Shh–Gli pathway activation, which forms a negative feedback loop to the Shh–Gli pathway, various members of the Shh signaling pathway link into a coherent circuit. It is widely accepted that aberrant activation of Shh signaling is implicated in a variety of cancers and disease states, such as pancreatic intraepithelial neoplasias, which occur in both chronic pancreatitis and pancreatic cancer [13–19]. However, there has been little research into the expression of Shh signaling molecules in the mucosa of CC and GBC.

In this study, we classified CC into three groups mainly according to the degree of hypertrophic wall, then explored the relationship between the expression of Shh signaling proteins and the different stages of CC to GBC. The aim of the study was to investigate the role of Shh signaling molecules in the development and progression of GBC.

2. Materials and methods

2.1. Ethics approval

This study was approved by the medical ethics committee of Nanfang Hospital, and specimens were treated anonymously according to ethical and legal standards.

2.2. Patients and specimens

We obtained 10 normal mucosa, 95 CC, and 32 GBC specimens. The 95 formalin-fixed and paraffin wax–embedded cholecystectomy specimens taken from patients with CC were obtained from Nanfang Hospital of Southern Medical University (Guangzhou, China) during the period 2009–2010. All specimens were stained with hematoxylin and eosin and were reviewed by a senior pathologist, who assessed and defined the degree of CC. Based on the degree of inflammatory cell infiltration, the degree of hyperplasia, and the thickness of the muscular layer, chronic inflammation was classified as follows: mild cholecystitis (a few lymphocytes and mononuclear cells present in the mucosa and submucosa, with mild thickening, defined as muscular layer thickness less than one-third that of the whole wall); moderate cholecystitis (moderate infiltration of lymphocytes and mononuclear cells, and moderate thickening, defined as muscular layer thickness of between one-third and two-thirds that of the wall); and severe cholecystitis (lymphocytes and mononuclear cells present throughout the wall, along with severe thickening, defined as muscular layer thickness more than two-thirds that of the wall).

The 32 cases of surgically resected primary GBCs were collected from April 2006 to June 2010. The American Joint Committee on Cancer primary tumor classification and the World Health Organization histological typing [20] were applied to all 32 cases, which were staged as follows: 3 at stage 0, 2 at stage I, 5 at stage II, 16 at stage III, and 6 at stage IV.

The 10 normal mucosa specimens were collected from surgical specimens for comparison. The clinical data on these patients were available from hospital records.