Review
Quan Liang*

Research progress of cholangiocarcinoma induced by liver fluke infection

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Abstract: A liver fluke is a digenetic trematode parasitizing in the hepatic ducts of human beings or animals. Patients with liver fluke infection suffer from a series of hepatobiliary diseases. The prevalence of cholangiocarcinoma is significantly high in areas with a high incidence of clonorchiasis. A liver fluke is an important biocarcinogenic factor in the occurrence of cholangiocarcinoma. The secretory products of the body of this parasite and long-term mechanical stimulation induce continuous inflammation of the bile duct. Gene expression of the bile duct cells is imbalanced, leading to carcinogenesis of the bile duct. This article provides a summary of recent studies on the epidemiology, clinicopathology, and molecular biology of cholangiocarcinoma induced by liver fluke infection.

Keywords: liver fluke, cholangiocarcinoma, infection, summary

Hepatic distomiasis caused by an adult liver fluke parasitizing in the hepatic ducts of human beings or mammals is a severe parasitic zoonosis. At present, 13 liver flukes are known to infect human beings, of which the three most common pathogens are *Opisthorchis viverrini*, *Opisthorchis felineus*, and *Clonorchis sinensis* [1]. Indiscriminate ingestion of *C. sinensis* bladder worm by consuming raw fish and shrimps, among others, leads to hepatomegaly, cholangitis, cholecystitis, jaundice obstructive, hepatobiliary system tumor, and other serious diseases and complications, as well as a significant increase in the risk of cholangiocarcinoma. In February, 2009, the World Health Organization confirmed that liver fluke infection is one of the important carcinogenic factors responsible for cholangiocarcinoma [2]. Further understanding of the pathogenesis of cholangiocarcinoma induced by hepatic distomiasis can help prevent and reduce the occurrence of malignant tumor in the hepatobiliary system. This article provides a summary of recent studies on the epidemiology, clinicopathology, and molecular biology of cholangiocarcinoma induced by liver fluke infection.

1 Epidemiological study of cholangiocarcinoma induced by liver fluke infection

Tumors caused by liver fluke infection account for ~0.4% of hepatobiliary tumor cases worldwide. Meanwhile, the prevalence of hepatic distomiasis is significantly endemic such that the incidence of cholangiocarcinoma in the areas with liver fluke infection is significantly higher [3]. In epidemic areas of *C. sinensis*, the incidence of hepatobiliary system tumor is >10% [4]. *C. sinensis* is mainly common in South Korea, China, North Korea, Vietnam, and Philippines, as well as in other East Asian and Southeast Asian countries. China and Southeast Asia have recorded the highest prevalence of cholangiocarcinoma [5]. Approximately 35 million people around the world are infected with clonorchiasis, with the infected people in China accounting for the highest proportion. Liver fluke infection has become an important public health problem in China [6]. At present, ~15 million people are infected by *C. sinensis* in China, and the standardized infection rate is 2.39% [7].
The second national survey on the infection status in the epidemic area of clonorchiasis shows a significantly higher number of people infected by *C. sinensis* than the first survey. The results show increased incidences of 182%, 164%, and 630% in Guangdong, Guangxi, and Jilin, respectively, and the incidence rate is increasing annually [8]. Cases of cholangiocarcinoma associated with *C. sinensis* infection have been recorded in mainland China and Hong Kong. Similar cases have also been reported in North American immigrants in Southeast Asia [9].

*O. felineus* is prevalent in Ukraine, Kazakhstan, several areas of Western Siberia, and Eastern Europe. Meanwhile, *O. viverrini* is mainly prevalent in Cambodia, Laos, and Thailand. The highest incidence of cholangiocarcinoma has been recorded in northeast Thailand, where *O. viverrini* is prevalent, and South Korea, where *C. sinensis* is prevalent. Approximately two-thirds of all cholangiocarcinoma cases in these areas are caused by liver fluke infection. Thus, this infection shows a positive correlation with the occurrence of cholangiocarcinoma [10]. South Korea has a high incidence of cholangiocarcinoma among the countries in the world. A significant correlation was found between cholangiocarcinoma patients and liver fluke infection in South Korea [11]. Kim et al. [12] showed that regional differences in liver fluke infection are highly correlated with the prevalence and mortality of cholangiocarcinoma. The prevalence and mortality rate of cholangiocarcinoma are also high in areas with high *C. sinensis* infection rate.

### 2 Clinicopathological study of cholangiocarcinoma induced by liver fluke infection

Various liver flukes have similar life histories. The liver fluke bladder worm breaks the wall of the human duodenum to form schistosomulum. The schistosomulum goes into all levels of the bile ducts in the liver through the hepatobiliary duct to grow to be an adult worm. The adsorption and mechanical obstruction of the schistosomulum to the host and the stimulation on its metabolic products will cause a series of pathological changes in the host, resulting in a series of clinical manifestations [13]. This phenomenon will lead to obvious changes such as bile duct inflammation, thickening of the wall of the tube, and fibrosis around the bile duct. These events eventually develop into epithelial cell carcinoma in the bile duct [14,15].

Keiser et al. [16] indicated that bile duct carcinogenesis can be induced by injecting a subdose of mutagens into Syria Jintian mouse. It may be the primary antigen of the liver fluke to induce the immune response, leading to the long-term inflammation of the bile duct epithelium to accelerate tumor growth. Zhang et al. [17] showed that hepatic edema and degeneration, cholangiectasis, proliferation of bile duct epithelial cells, and peripheral fibrosis of the bile duct occurred in the host after different dosages of liver fluke bladder worm were used to infect rat. This phenomenon was accompanied by adenomatoid, papillary hyperplasia, and epithelial squamous metaplasia in several regions of nausea transformation.

Jang et al. [18] analyzed tissue subtypes of intraductal papilloma associated with clonorchiasis. The results showed tubular carcinoma, colloid carcinoma, and different degrees of differentiated carcinoma. Adult worms of the liver fluke were often found in the bile ducts surrounded by the tumor tissue.

Tangkawattana et al. [19] examined pathologically the liver tumor tissues of 27 Syria golden hamsters infected by *O. viverrini* for several months. The result showed that adenocarcinoma was the most common type of histopathology, with papillary adenocarcinoma and mucinous carcinoma accounting for 3.7% (1/27). Tubular adenocarcinoma accounted for 81.5% (22/27), whereas mixed-type carcinoma was observed in 11.1% (3/27) of the samples. Lee et al. [20] found that 73% (11/15) of hamsters fed with dimethylnitrosamine and infected by *C. sinensis* suffered from papillary or adenomatoid hyperplasia in the bile duct. Only 20% (3/15) of hamsters fed by dimethylnitrosamine exhibited this malignant change in the bile duct. These studies have shown that liver fluke infection can lead to recurrent inflammation, necrosis, and hyperplasia of the bile duct cells. Moreover, during the late stage of infection, peripheral fibrosis of the bile duct, mucoid degeneration, and adenomatoid hyperplasia of bile duct epithelium could be induced, and malignant transformation of bile duct epithelial cells was developed from atypical hyperplasia.
3 Study on the molecular biological mechanism of cholangiocarcinoma induced by liver fluke infection

A series of pathophysiological and immune responses of the host are induced by long-term liver fluke infection, which eventually leads to cholangiocarcinoma. This process contains various complex mechanisms. In February 2009, the World Health Organization and the International Agency for Research on Cancer listed *C. sinensis*, *O. felineus*, and *O. viverrini* as Class I carcinogenic factors of cholangiocarcinoma during a conference held in Lyon, France. This declaration clarified the importance of liver fluke infection in cholangiocarcinoma [2]. The pathogenesis of cholangiocarcinoma induced by clonorchiasis is a complex process. The possible mechanisms are as follows.

3.1 Chronic inflammation of bile duct caused by long-term liver fluke infection

Long-term liver fluke infection induces recurrent inflammatory response to the epithelial cells of the bile duct. Meanwhile, deformation, necrosis and exfoliation of bile duct epithelium, bile duct wall ulcer, and abundant eosinophil infiltrates are formed, leading to repeated bacterial infection that causes the thickening of the bile duct wall. Metabolites and degradation products generated during the growth and development of the worms repeatedly stimulate the monocytic giant macrophage system of the host. This process not only directly kills and damages the liver fluke but also aggravates the inflammatory damage in the epithelial cells of the hepatobiliary duct [21,22]. Cao et al. [23] observed the expression of interferon-γ, interleukin (IL)-12, and IL-4 in the liver tissue cytokine of mice infected by *C. sinensis*. The results show that Th1/Th2 cell immunization is involved in the inflammatory damage of hepatobiliary cells. An et al. [24] and Wang et al. [25] suggested that the levels of IL-12, IL-4, and Th1/Th2 in the cytokine of rats infected by liver flukes are in an imbalanced state, and cytokines participate in the pathogenesis of clonorchiasis, induce long-term inflammatory damage of hepatocytes, and eventually lead to carcinogenesis. The expression of trefoil factor 1 (TFF1) can activate the invasion of abnormal epithelial cells. Thus, TFF1 can be regarded as an agonist for tumor cell metastasis. In the study of Thuwajit et al. [26], 91.80% of hepatic distomiasis patients were accompanied by a high expression of TFF1 in cholangiocarcinoma tissues. This result further proves that long-term liver fluke infection is important for the persistent inflammation of the bile duct wall. This phenomenon may be due to the fact that liver fluke infection aggravates the long-term inflammatory reaction of the bile duct epithelium and leads to epithelial cell deformation, necrosis, and recurrent ulcer of the bile duct wall. Pinlaor et al. [27] presented that the expression levels of the mRNA of inducible nitrogen oxide synthase, antioxidase mRNA, and nuclear factor-κB detected from the host infected by *O. viverrini* were significantly increased. Similarly, the levels of nitric oxide end product, malondialdehyde, and plasma nitrate were increased significantly. This result indicates that liver fluke infection stimulates host cells to produce oxidative and nitridation reactions, resulting in the injury and repair of epithelial cells of the bile duct. This process aggravates the inflammatory reaction of epithelial cells of the bile duct, ultimately leading to the canceration of epithelial cells of the bile duct.

3.2 Carcinogenesis of the excretory–secretory products (ESPs) of liver flukes

ESPs play an important role in the ability of the worms to invade, access nutrition, and escape from immune attacks of the host, as well as in the regulation of the host immune response [28]. The ESP of the worm itself can repeatedly stimulate the mononuclear phagocyte system of the host. Long-term exposure of the epithelium of the bile duct to ESPs of genotoxic liver flukes can aggravate the damage and accelerate malignant change in the epithelial cells of proliferative bile duct. A homolog of lysophosphatidic acid phosphatase (LPAP) in ESP, with tissue located in the intestinal cavity, seminal vesicle, and egg of the adult, has been found. The sensitivity and specificity of LPAP in recombinant expression are higher than those in the
The acarine crude antigen of the worm. LPAP is also involved in the synthesis of phosphatidic acid. This enzyme is an extracellular signal molecule for regulating the growth, proliferation, and migration of cells. LPAP is closely correlated with the occurrence of cholangiocarcinoma. This result further shows that ESP of the liver flukes is one of the important factors for the carcinogenesis of epithelial cells of the bile duct [29]. Kim et al. [30] established that ESP of liver flukes induced the proliferation of human epithelial cell line HEK293 in vitro by upregulating transcription factor E2F1, leading to the changes in the cell mRNA and apoptotic protease-3 (caspase-3), among others, and aggravating the damage to epithelial cells of the bile duct. ESP can also induce the proliferation of cholangiocarcinoma cell line HuCCT1 and the expression of cyclooxygenase-2 to resist apoptosis induced by parthenolide for cholangiocarcinoma [31]. Pak et al. [32] analyzed the ESP-induced change in the gene expression in cholangiocarcinoma cell line. The results showed that the ESP can upregulate the expression of minichromosome maintenance protein 7, apoptosis-related genes (Sav), and tumor-generating gene (E2F transcription factor 5, E2F5). Moreover, the ESP can downregulate the expression of other tumor apoptosis-related genes, such as Lama3, Lamb3, Tumor Necrosis Factor (TNF), and Human chorionic gonadotropin-beta (hCGB). Proteomics methods have been adopted to analyze the impact of adult ESP on the protein expression in the cholangiocarcinoma cell line. The expression of 83 proteins was found to be affected. Among these proteins, thioredoxin 1 (Trxl) and peroxiredoxin 6 (Prdx6) help maintain the stability of cell redox and scavenge peroxides in the body. In various malignant tumors, expression is related to the occurrence and progression of tumor [33]. Kim et al. [34] found that ESP of the liver flukes can affect the expression of many genes in the cholangiocarcinoma cell HuCCT1. In particular, the increased expression of minichromosome maintenance protein 7 is activated by the transcription of histone acetyltransferase. This process plays an important role in the occurrence of cholangiocarcinoma. The malignant transformation of epithelial cells of the bile duct is facilitated by the long-term deformation and necrosis of them and exposure to a genotoxic inflammatory product [35].

3.3 Cholangiocarcinoma induced by imbalance of cell gene expression

Long-term direct mechanical stimulation of the body and egg of a liver fluke and ESPs of the worm body stimulate the epithelium of the hepatic bile duct and induce abnormal proliferation and carcinogenesis of epithelial cells of the bile duct. Inflammatory factors play an important role in cancer [36]. Smout et al. [37] found that the body and egg of a liver fluke could secrete granular protein and lead to the wanton growth of epithelial cells of the bile duct. This process showed a certain regulating effect on the development of the hepatic bile duct and directly induced abnormal hyperplasia and carcinogenesis in the epithelium of the bile duct. Tangkawattana et al. [19] showed that TP53 gene and Kras cancer gene were highly expressed in a golden hamster model with cholangiocarcinoma induced by liver fluke infection, and mutation was found in the exons 5–8 of the TP53 gene and exon 1 of the Kras gene. This mutation is possibly caused by the inactivation of the tumor suppressor gene as a result of the long-term stimulation generated by the body and egg of a liver fluke and activation of the cancer gene of the host. Liver fluke infection can cause changes in the apoptosis pathway mediated by Fas/Fas ligand, leading to abnormal changes in mRNA and caspase-3 involved in apoptosis. Thus, this process accelerates programmed cell death and the degree of malignant transformation of epithelial cells of the bile duct [38]. Moreover, the stimulation from the body and egg induces the host immune response disorder, which affects the repair of the DNA system of the host and easily leads to the carcinogenesis of epithelial cells of the bile duct. These phenomena improve the ability of tumor invasion and metastasis. Several studies have found that, after liver fluke infection, the expression of genes associated with fatty acid metabolism (PECI, CYP4A10, ACATL, EHHADH GCDH, and CYP2 family) is downregulated, while the expression of genes associated with Wnt signal transduction (WNT7b, FZD6, and PDGFRb) and cell cycle regulation genes (Cyclin-D1, CDCA3, and BCL3) is upregulated. Trxl and Prdx6 are regarded as the potential starting factors of cholangiocarcinoma induced by C. sinensis. Although cells are subject to external stimuli, Trxl and Prdx6 activate stress expression patterns to respond to the stimulation to make cells pass through a very long period of time. To date, harmful substances produced by the cells themselves and the external substances are more likely to damage the DNA, causing the cells to be tumorigenic [39].
4 Summary

Annual increase in liver fluke infection has been observed because of population flow and change in people’s eating habits. This infection has emerged as one of the main public health problems endangering human health. In addition, the incidence of cholangiocarcinoma in areas with liver fluke infection is significantly higher. Long-term stimulation by the body and egg of a liver fluke to the bile duct and ESPs of the worm body induce the immune response of the host, aggravating the inflammatory response of the hepatobiliary duct. Persistent inflammation induces the imbalance of gene expression in the bile duct cells and accelerates the carcinogenesis of bile duct cells. Inflammation is a key factor leading to cancer. With the rapid development of immunology experimental technology and molecular biology, the molecular biology of cholangiocarcinoma induced by liver flukes should also be investigated to understand their carcinogenic mechanism. This process helps reduce the incidence and mortality of cholangiocarcinoma and other hepatobiliary system diseases induced by liver flukes.

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References


