Chapter 12
Parasite-Associated Cancers (Blood Flukes/Liver Flukes)

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Abstract Parasitic infection remains as a persistent public health problem and can be carcinogenic. Three helminth parasites, namely, *Clonorchis sinensis* (liver fluke) and *Opisthorchis viverrini* as well as *Schistosoma haematobium* (blood fluke), are classified as Group 1 carcinogens by the World Health Organization's International Agency for Research on Cancer (IARC Infection with liver flukes (*Opisthorchis viverrini, Opisthorchis felineus* and *Clonorchis sinensis*), World Health Organization, International Agency for Research on Cancer, 2011). Infection by these parasites is frequently asymptomatic and is thus rarely diagnosed at early exposure. Persistent infection can cause severe cancer complications. Until now, the cellular and molecular mechanisms linking fluke infections to cancer formation have yet to be defined, although many studies have focused on these mechanisms in recent years, and numerous findings were made in various aspects of parasite-associated cancers. Herein, we only introduce the fluke-induced cholangiocarcinoma (CCA) and bladder carcinoma and mainly focus on key findings in the last 5 years.

Keywords Parasitic infection • Helminth parasites • Fluke-induced cholangiocarcinoma • Bladder carcinoma • Cancer

12.1 Cholangiocarcinoma and Liver Flukes

The small liver flukes *O. viverrini* and *C. sinensis* are helminths that can affect humans. These flukes are particularly prevalent in the Southeast and East Asia, particularly in countries such as Thailand, Lao PDR, Vietnam, and Cambodia [1–4]. These countries have a strikingly high incidence of CCA (hepatic cancer of the bile duct epithelium) and approximately 700 million people at risk of infection.

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Moreover, 10 and 35 million people are infected by *O. viverrini* and *C. sinensis*, respectively [2]. Thus, liver fluke infections are considered a serious global public health concern.

O. viverrini is represented in literature as a cause of hepatobiliary disease often referred to clinically as "opisthorchiasis," particularly CCA [5, 6]. The disease is acquired by ingesting contaminated raw or undercooked freshwater fish or fish products, such as fish sauces and fermented fishes. The flukes reside in the small intrahepatic bile ducts and cause chronic inflammation and, eventually, CCA. Similarly, *C. sinensis* is considered as an agent of CCA. However, the detailed mechanisms in the pathogenesis of CCA caused by *C. sinensis* are less clear than those caused by *O. viverrini*. Meanwhile, infections caused by *Opisthorchis felineus*, another kind of liver fluke, have no observed association with CCA so far [7]. *O. felineus* holds a highly close phylogenetic relationship with *O. viverrini*. Moreover, the biology of the former and the medical and epidemiological implications of the related infection are suggested to be essentially identical to those of *O. viverrini* and *C. sinensis*. Thus, further studies are warranted to assess the contribution of opisthorchiasis to CCA.

O. viverrini is endemic in the northeastern region of Thailand, which has the highest worldwide incidence of CCA. This geographical coincidence typically supports the liver fluke–CCA causal relationship. Pathophysiological research demonstrated the capability of *O. viverrini* to induce cancer in artificially infected laboratory animals (hamsters) [8]. The main liver fluke–CCA causal mechanisms were observed to be involved in the mechanical damage caused by parasite activities and immunopathology due to infection-related inflammation. Each of these effects has several minor counterparts. In brief, liver flukes generate tumor microenvironments that result in CCA.

12.1.1 Risk Factors for Liver Fluke-Associated Cholangiocarcinoma

To date, studies that were able to attain the valuable risk factors of liver fluke-associated CCA are few, indicating that the disease factors are exceedingly complex. A meta-analysis found that liver fluke infection is significantly associated with cholangitis, cholecystitis, cholelithiasis, hepatocellular carcinoma, and CCA [9]. Moreover, heavy infection was observed to be significantly associated with high incidence of hepatobiliary pathological changes.

Apart from heavy infection, risk factors were reported to affect the liver fluke–CCA relation. Liver fluke infections may exhibit hepatobiliary abnormalities and chronic infection that may lead to CCA development. A study on a population in the northeast of Thailand found that the prevalence of *O. viverrini* infection has decreased because of a liver fluke control program that has been implemented over the decades [10]. However, the prevalence of PDF remained high. In 55,246 subjects,

the overall prevalence of PDF was 33.0 %, and males were at higher risk in developing PDF than females. The study also showed that old age (≥70 years) and hepatitis B are associated with increased PDF risk. By contrast, the number of praziquantel treatments and diabetes mellitus cases were significantly associated with decreased PDF risk. Another report suggested that fermented food consumption can exacerbate cholangitis and cholangiofibrosis, which are risk factors for CCA-associated opisthorchiasis [11].

12.1.2 Cholangiocarcinoma-Associated Gene Variation

Genetic changes have been widely reported to be associated with liver fluke-related CCA. Ong et al. reported that whole-exome and targeted sequencing not only can verify frequent mutations in known CCA-related genes, including TP53 (44 %), KRAS (16.7 %), and SMAD4 (16.7 %), but also can identify somatic mutations in 10 newly implicated genes, including MLL3 (14.8 %), ROBO2 (9.3 %), RNF43 (9.3 %), PEG3 (5.6 %), and GNAS oncogene (9.3 %) [12]. Gene functions can be grouped according to the deactivation of histone modifiers, activation of G protein signaling, and genome stability loss. Another report compared CAA associated with *O. viverrini* with those not associated with *O. viverrini*. The study revealed mutations in novel CCA-related genes associated with chromatin remodeling (BAP1, ARID1A, MLL3, and IDH1/2), WNT signaling (RNF43 and PEG3), and KRAS/G protein signaling (GNAS and ROBO2) [13].

Genetic variations are frequently reported in liver fluke-related CCA. In addition, when the epigenetic changes and miRNAs were characterized in liver fluke-related CCA, they were found to hold potential as diagnostic or prognostic biomarkers. Sriraksa et al. reported that aberrant hypermethylation of a certain loci is a common event in liver fluke-related CCA and thus may potentially contribute to cholangiocarcinogenesis. In their results, a number of CpG islands (OPCML, SFRP1, HIC1, PTEN, and DcR1) showed frequent hypermethylation, and 91 % of CCA were methylated in at least one CpG island [14]. Furthermore, patients with methylated DcR1 exhibited significantly longer overall survival than those without. Runglawan et al. revealed that the levels of urinary miR-192 and miR-21 are higher in the risk group of subjects than those in healthy individuals. This result suggests that increased miR-192 and miR-21 levels in host urine may provide better predictive values in areas endemic for *O. viverrini* than they do in nonendemic regions [11].

Liver fluke-induced chronic inflammation plays a crucial role in cholangiocarcinogenesis through distinct signatures of genetic, epigenetic, and transcriptional alterations. These alterations indicate a unique pathogenic process in liver fluke-related CCA and thus may hold potential clinical implications in CAA diagnostics, therapeutics, and prevention.

12.1.3 Cholangiocarcinoma-Associated Parasite Proteins

Liver fluke-secreted proteins were previously shown to accelerate human cholangiocytes. The endocytosis of liver fluke proteins by host epithelial cells affects the pathways and induces the parasites to cause a highly devastating form of cancer, that is, CCA. Although the detailed mechanisms by which cells internalize liver fluke-secreted proteins remains unclear, recent studies implied that liver fluke proteins have a role in pathogenesis and highlighted an approach for vaccine development against this infectious cancer.

O. viverrini excretory/secretory products (OvESs) have been a focus of study. These products are especially internalized by biliary cells and postulated to be responsible for the chronic inflammation and proliferation of cholangiocytes. The physical attachment of *O. viverrini* to the biliary epithelium causes damage by releasing highly immunogenic OvES. When internalized preferentially by liver cell lines, OvESs induce liver cell proliferation and promote IL6 secretion [15].

Proteins secreted by *O. viverrini* accelerate wound resolution in human cholangiocytes. Smout et al. demonstrated that a gene encoding granulin-like growth factor (Ov-GRN-1), which was derived from recombinant *O. viverrini*, induces angiogenesis, an essential mechanism for malignant tumor development. In addition, Monica et al. showed that Ov-GRN-1 induces angiogenesis and accelerates wound healing in mice. In fact, wound healing and cancer progression have remarkable similarities, such as new blood vessel growth in a process called angiogenesis. Thus, determining the effect of Ov-GRN-1 in CCA progression is valuable [16].

Recent reports have highlighted the presence of secreted extracellular vesicles (EVs) in helminths. In particular, *O. viverrini* secretes EVs that induce a proinflammatory or tumorigenic phenotype in human cholangiocytes. Chaiyadet et al. demonstrated that *O. viverrini* EVs are released in the secreted products of carcinogenic liver flukes. These EVs are then internalized by cholangiocytes, subsequently driving cell proliferation and IL-6 secretion and promoting an inflammatory but simultaneously modulatory environment. These processes ultimately facilitate the CCA development [17].

Thioredoxin from *O. viverrini* was reported to inhibit the oxidative stress-induced apoptosis of bile duct epithelial cells and cholangiocytes. Immunolocalization revealed the presence of liver fluke thioredoxin within the cholangiocytes. The cells exposed to thioredoxin were observed to downregulate the apoptotic genes in mitogen-activated protein kinase pathway and upregulate antiapoptosis-related genes, including apoptosis signaling kinase 1, caspase 9, caspase 8, caspase 3, and survivin. These results suggest that *O. viverrini* thioredoxin can inhibit apoptosis and facilitate carcinogenesis. As such, thioredoxin may play an important role for liver fluke oxidoreductase in *O. viverrini*-induced CCA [18].

12.1.4 Immunopathology, Tissue Damage, and Cholangiocarcinoma

Cholangiocarcinoma (CCA) incidence related to chronic *O. viverrini* infection is a multifactorial process encompassing immunopathological mechanism, tissue damage, and signal pathway activation. Immune-mediated pathogenesis in response to liver fluke infection is a major driving force of CCA onset. Liver fluke-associated CCA involves different pro- and anti-inflammatory cytokines that may instigate cancer development. In *O. viverrini* infection, elevated total serum IL-6, and IL-6 production stimulated by *O. viverrini* in PBMC have been reported in infected individuals with advanced periductal fibrosis. Surapaitoon et al. reported 11 cytokine profiles, including those of IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF-α, and LT-α, which were increased in CCA incidence associated with *O. viverrini* infection relative to the same profiles in uninfected normal controls. The results suggest the dysregulation of immune response in liver fluke-associated CCA [19].

Oxidative tissue damage caused by the free radicals released from effector cells has been observed to surround infected bile ducts. Thanee et al. suggested that the accumulation of CD44v suppresses p38MAPK expression in transforming bile duct cells and is linked to poor prognosis in CCA patients [20]. CD44 is a single transmembrane protein involved in cancer development and expressed in a wide variety of isoforms. Moreover, the expression of CD44v on a cell surface stabilizes xCT and promotes glutathione synthesis as defense against reactive oxygen species (ROS). This process enhances cancer development and increases chemotherapy resistance. Apinya et al. identified the oxysterols Triol and 3K4 as potential mediators of cholangiocarcinogenesis. Triol and 3K4 induce DNA damage and cell apoptosis via mitochondrial-dependent mechanisms. Chronic liver fluke infection increases the production of ROS/RNS when chronic inflammation occurs in the biliary system. Oxysterols and free radicals can induce biliary epithelial cell apoptosis. Ineffective DNA repair and persistent exposure to DNA damaging agents select for resistant cells that clonally expand to become malignant.

Signaling pathways involved in chronic inflammation regulate the progression of liver fluke-associated CCA. Yothaisong et al. reported a significant activation of the PI3K/AKT signaling pathway with PTEN suppression in human CCA tissues. Watcharin et al. suggested that the PKA signaling pathway and the switching of PKA regulatory subunits from Prkar2b/PKAII to Prkar1a/PKAI are involved in the development of CCA via altered cell transformation and proliferation [13]. Furthermore, the overexpression of Prkar1a can lead to the secretion of extracellular PKA (ECPKA) outside the CCA cells. Thus, the signal pathway activation may be responsible for the CCA development induced by *O. viverrini*. Targeting the components of these particular pathways may prove beneficial for the development of effective treatment, early diagnostics, and prevention strategies for CCA.

Inflammation is a well-known key component of tumor microenvironments. CCA is induced by chronic inflammation caused by the combination of several

mechanical damage types. Immunosuppressive prednisolone was found to enhance early CCA in Syrian hamsters with liver fluke infection. This result suggests that host immune responses occur to ameliorate pathology and are also crucially associated with the pathogenesis of *O. viverrini* infection. Thus, imbalanced host immunity may enhance cancer-related inflammation.

12.1.5 Liver Fluke-Associated Microbiome and Cholangiocarcinoma

Microbial interaction with host cells can influence the health of the host considerably and has been implicated in liver fluke-associated CCA. The bacterial families *Dietziaceae*, *Pseudomonadaceae*, and *Oxalobacteraceae* are distinct microbiomes that dominate bile duct tissues. Chng et al. compared groups associated with those not associated with *O. viverrini* and then identified the enrichment for specific enteric bacteria (*Bifidobacteriaceae*, *Enterobacteriaceae*, and *Enterococcaceae*). They found that *Bifidobacteriaceae* was enriched and dominant in the *O. viverrini* microbiome and thus provided a mechanistic link to the parasite. Functional analysis revealed that altered microbiota increases the production of bile acids and ammonia in *O. viverrini* tissues and thus is linked to carcinogenesis [21]. These findings denote that parasitic infections and tissue microenvironments can influence each other and both can contribute to cancer.

12.2 Bladder Carcinoma and Blood Flukes

Schistosomiasis is a neglected tropical disease transmitted to humans from freshwater snails [22]. This disease is caused by a blood fluke of the genus *Schistosoma*. Schistosomiasis is considered as the most important helminthiasis that causes high rates of morbidity and mortality. Schistosomes affect at least 76 countries and 200–400 million people worldwide. *S. haematobium* is the causative agent of urogenital schistosomiasis and is responsible for two-thirds of the 200–400 million cases of human schistosomiasis worldwide [22, 23]. This infection is also associated with a high incidence of squamous cell carcinoma of the bladder, which is prevalent in the developing world [24, 25].

S. haematobium cercariae penetrate the skin and then transform into schistosomula. After infecting the subcutaneous tissue, the schistosomula enter the circulation and travel to the lungs and liver, where they achieve sexual maturity before entering into the vesical venous plexus. The eggs released from paired adults travel to the bladder, become trapped in the bladder wall, and release antigens and other metabolites. The eggs then lodge into the tissues and produce granulomatous inflammation that can lead to fibrosis [26, 27].

Long-term urinary schistosomiasis has been associated with the development of bladder cancer [28]. This disease is the leading cause of bladder cancer and occurs following years of chronic inflammation, fibrosis, and hyperproliferation. As a result, bladder cancer has become a significant health problem in the rural areas of Africa and the Middle East, where *S. haematobium* is prevalent [29, 30]. This information supports the association between malignant transformation and infection caused by this blood fluke. A study on an adult rural population in a Ghana region with endemic urinary schistosomiasis revealed the potential schistosome-associated bladder cancer problem and increasing association among age, severe bladder abnormalities, and occurrence of cancer biomarkers. Data on the epidemiological extent in different geographical areas estimate a schistosome-associated bladder cancer incidence of 3–4 cases per 100,000 [31]. This value suggests that schistosome-associated bladder cancer is an important public health concern in areas where *S. haematobium* is prevalent.

12.2.1 Bladder Carcinoma-Associated Parasite Genes

Bladder cancer is often difficult to diagnose without invasive measures, such as cystoscopy. However, benefitting from the development of molecular diagnostic techniques, some biomarkers were recognized as candidates for diagnosis and prognosis of this neglected tropical disease-linked cancer. In a recent study, liquid chromatography–mass spectrometry analysis was performed on urine from Angolans diagnosed with urogenital schistosomiasis and schistosome-associated bladder cancer. The metabolites were analyzed and expected to provide deep insight into the schistosome-associated bladder cancer. The analysis revealed numerous estrogen-like metabolites, including catechol estrogen quinones, CEQ-DNA-adducts, and novel metabolites derived directly from 8-oxo-7,8-dihydro-2′-deoxyguanosine, which were identified in urine of all patients [32].

A correlation was observed among the frequency of the biomarkers of bladder cancer associated with *S. haematobium*, those with p53, and sialylated glycans. The correlation highlights a missing link between infection and cancer development. The eggs of *S. haematobium* express sLea and sLex antigens in mimicry of human leukocyte glycosylation and thus may play a role in colonization and disease dissemination [33]. These results may facilitate early identification of infected patients at a high risk of developing bladder cancer and guide the future development of noninvasive diagnostic tests.

12.2.2 Bladder Carcinoma-Associated Parasite Proteins

Chronic infection with *S. haematobium* is associated with squamous cell carcinoma of the bladder. However, the molecular mechanisms underlying this association are poorly understood. Previous data revealed that the soluble extracts of mixed-sex

adult *S. haematobium* worms (SWAP) are tumorigenic [22]. Moreover, the estrogen-related molecules in SWAP downregulate the estrogen receptors (ERs) in estrogen-responsive cells [22, 34]. Schistosome estrogens present in the sera of schistosomiasis patients repress the transcription of ERs in urothelial cells [34].

The soluble egg antigens of *S. haematobium* (Sh-SEA) exhibit potent tumorigenic capacity, because the *S. haematobium* eggs are in the developmental stage wherein they can directly cause urogenital disease during schistosomiasis haematobia. The findings confirmed that Sh-SEA can stimulate cell proliferation, reduce apoptosis, and increase the oxidative stress of urothelial cells. Furthermore, the presence of catechol estrogens in Sh-SEA might induce bladder cancer. These catechol estrogens are formed by a prospective estrogen–DNA adduct-mediated pathway in the *S. haematobium* eggs [22].

12.2.3 Immunopathology, Tissue Damage, and Bladder Carcinoma

Schistosomes elicit chronic inflammatory responses in both humans and mice. In *S. haematobium* infection, the prolonged inflammatory response is thought to contribute to the development of squamous cell carcinoma. Furthermore, the dependence of schistosomes on host factors for successful infection is evolutionarily conserved in *S. haematobium*. When infecting its host, schistosomes use common host mechanisms. In addition, schistosomes use immune signals for its development. The contributions of the host genes, which are discrete from immune system genes, must be understood, because these contributions are necessary for parasite establishment and development. Previous studies addressing the host–parasite interactions during schistosomiasis focused on a subset of immune response genes used to mount a Th1/Th2 response during infection. These genes include critical immune response genes, such as IL-4, IL-6, and IL-10, which control the Th1/Th2 response. In brief, regulatory pathways accommodate host permissiveness to schistosome establishment and productive schistosome infection and parasitism [35].

Schistosomiasis haematobia is a chronic infection. The adult and egg-producing schistosomes can live for many years. Thus, reinfections frequently occur and thus can lead to bladder cancer. To discuss the basic mechanisms that are potentially common in cancers, many studies focused on the role of both estrogens and ERs on the carcinogenesis associated with urogenital schistosomiasis. Botelho et al. observed a noteworthy elevation in estradiol serum levels [34]. They also observed that the serum levels of the luteinizing and follicle-stimulating hormones remained normal. Thus, they hypothesized that excess estradiol is external to the host. The molecule responsible for the effect may be an estradiol-like molecule derived from *S. haematobium*. This molecule is an antagonist of estradiol and thus repressed the transcriptional activity of the ERs. ER transcriptional activity was suppressed in urothelial cells, and ER expression was also inhibited in the mouse bladders in

response to *S. haematobium* infection. These findings revealed the estrogen metabolism and ER signaling pathways associated with cancer induction in the context of *S. haematobium* infection [36, 37].

Another kind of hormonal and estrogenic molecule was identified in the Sh-SEA. The majority of these compounds are catechol estrogens, which are formed through the hydroxylation of steroid aromatic ring A. The hydroxylation of both C-2 and C-3 on a steroid ring is apparent and is subjected to further oxidation into an estradiol-2,3-quinone [38, 39]. The genotoxic effects of estrogen metabolites may be attributed to the oxidation of catechol estrogens to quinones, followed by redox cycling and formation of reactive oxygen species, which react with DNA. The metabolism of estrogens and production of depurinating estrogen–DNA adducts can be implicated in a pathway underlying host cell DNA damage promoted by *S. haematobium* and eventually lead to cell transformation. The carcinogenic effect of this estrogen–DNA adduct-mediated pathway may explain the link between chronic schistosomiasis haematobia and squamous cell carcinoma of the bladder [22].

At present, many mechanisms remain unclear, and further studies are necessary to understand how schistosomiasis haematobia leads to the squamous cell carcinoma of the bladder.

12.3 Other Parasite-Associated Cancers

Although certain cancer-related parasites belong to helminths, recent works have reported the association between unicellular protozoa and tumor. Despite its small size, protozoa can cause a series of diseases and public health problems worldwide. Their ability to induce tumor must be given sufficient attention. Herein, we list two kinds of tumors potentially caused by unicellular protozoa.

12.3.1 Toxoplasma and Brain Tumors

Toxoplasmosis is caused by *Toxoplasma gondii* and is a highly prevalent parasitic disease. This condition is estimated to affect a third of the world's human population [40, 41]. Two recent studies highlighted a positive correlation between the prevalence of brain tumors and *T. gondii* at the national and international scales [42, 43]. Unfortunately, these studies are correlative, and the links between *T. gondii* and cancer are complex. Thus, a causality between *T. gondii* and brain tumors was not attained. Even so, further research could shed light on the possible mechanisms underlying this association.

12.3.2 *Trichomonas and Prostate Cancer (PCA)*

Trichomonas vaginalis is an extracellular flagellated parasitic protozoan that causes a relatively common parasitic sexually transmitted infection. The disease holds an annual incidence of over 3 million. The majority of infections is asymptomatic in men and thus is often undiagnosed and untreated. These untreated cases are hypothesized to promote chronic persistent prostatic infection and resultant urethritis and prostatitis [44, 45].

T. vaginalis was previously suspected to be associated with the development of PCA [46]. For decades, a couple of clinical studies seemed to support this correlation. However, two recent studies gave negative results. A study that included 146 men with advanced prostate cancer demonstrated that *T. vaginalis* serostatus is not associated with increased risk of metastatic or fatal prostate cancer (odds ratio <1) [47]. This result does not support the increased risk of advanced or fatal prostate cancer in men infected with *T. vaginalis*. In another study, Zhu et al. suggested that the culture supernatant of *T. vaginalis* inhibits prostate cancer growth by disrupting the proliferation and promotion of apoptosis [48].

Epidemiologic evidence for the association of *T. vaginalis* with prostate cancer is inconsistent, and the role of *T. vaginalis* in PCA development remains controversial. Thus, further study may help elucidate the association between PCA and *T. vaginalis* infection.

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