INVITED REVIEW
doi: 10.2306/scienceasia1513-1874.2025.s004

ScienceAsia 51S (1): 2025: ID 2025s004: **1–5**

Updates on pathogenesis of *Helicobacter pylori–Opisthorchis viverrini* co-infections induced cholangiocarcinoma

Banchob Sripa[a,*,†], **Raksawan Deenonpoe**[b]

[a] Tropical Disease Research Center, Department of Tropical Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002 Thailand

[b] Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002 Thailand

*Corresponding author, e-mail: banchob@kku.ac.th

Received 16 Dec 2024, Accepted 31 Jan 2025
Available online 28 Apr 2025

ABSTRACT: Opisthorchiasis, caused by the liver fluke *Opisthorchis viverrini* (OV) infection, is a major public health issue in the Lower Mekong Basin. Chronic OV infection is associated with several hepatobiliary pathology including advanced periductal fibrosis (APF) and cholangiocarcinoma (CCA), a fatal bile duct cancer. Recent evidence implicates *Helicobacter pylori*, especially cagA-positive strains, as significant contributors to APF and CCA in co-infected individuals. This review explores the multifaceted interactions between *O. viverrini* and *H. pylori*, emphasizing the role of the liver fluke as a reservoir and vector for *H. pylori*. Evidence reveals that *H. pylori* colonizes the gastrointestinal tract of *O. viverrini* via sugar moieties, such as L-fucose, of the receptors, enabling bacterial transmission to the human biliary tree. Once in the bile ducts, *H. pylori* adheres to cholangiocytes, triggering inflammation mediated by sialic acid-binding adhesins and the release of pro-inflammatory cytokines such as IL-6 and TNF-α. The bacterium's ability to induce epithelial-to-mesenchymal transition (EMT) further enhances the invasive and malignant potential of biliary epithelial cells. This review highlights the emerging understanding of the synergistic pathogenesis of *O. viverrini* and *H. pylori* co-infections, providing insights into molecular mechanisms, host-pathogen interactions, and the inflammatory milieu that drive CCA development. These findings underscore the need for integrated therapeutic strategies targeting both pathogens to mitigate the burden of APF and CCA in endemic regions.

KEYWORDS: *Opisthorchis viverrini*, *Helicobacter pylori*, liver fluke-bacterial co-infections, parasitic disease, pathogenesis, cholangiocarcinoma

INTRODUCTION

Opisthorchiasis, caused by the fish-borne trematode *Opisthorchis viverrini*, is endemic to the Lower Mekong Basin, affecting over 12 million individuals in Thailand, Lao PDR, Cambodia, Vietnam, and Myanmar [1–3]. The infection is acquired through consumption of raw or undercooked fish containing infective metacercariae. Chronic opisthorchiasis is strongly associated with hepatobiliary abnormalities, including advanced periductal fibrosis (APF) and the fatal bile duct cancer, cholangiocarcinoma (CCA) [4, 5]. The involvement of *Helicobacter pylori* in these pathologies has gained attention during recent years, with evidence suggesting that the bacterium exacerbates the pathogenic effects of *O. viverrini* [6–10]. This review integrates findings from key studies to elucidate the interplay between *O. viverrini* and *H. pylori* in APF and CCA progression.

EPIDEMIOLOGY OF OPISTHORCHIASIS AND *HELICOBACTER* CO-INFECTION

Opisthorchiasis remains highly prevalent in the Lower Mekong Basin, with northern and northeastern Thailand reporting over 6 million cases [1, 11]. Co-infection with *H. pylori* is increasingly recognized as a contributing factor to biliary disease in endemic regions [6, 7]. Notably, *O. viverrini* serves as a reservoir for *H. pylori*, allowing the bacterium to colonize the biliary tree and persist under the alkaline conditions of bile [12]. Studies have highlighted that cagA-positive *H. pylori* strains are disproportionately found in patients with relapsed or persistent APF following praziquantel (PZQ) treatment, indicating a potential role in disease recurrence and progression to CCA [10].

RELATIONSHIP BETWEEN *O. VIVERRINI* AND *H. PYLORI*

Recent investigations have highlighted the role of the Southeast Asian liver fluke, *O. viverrini*, as a carrier of *H. pylori* [12], with implications for the pathogenesis of opisthorchiasis-associated cholangiocarcinoma (CCA) [8]. Previously, significant associations between CCA and cagA+ *H. pylori* were identified [6, 13, 14], but the mechanisms underlying this relationship were unclear. Subsequent studies uncovered evidence that *H. pylori* colonizes the gastrointestinal tract of *O. viverrini*, potentially using the fluke as a vector to reach the human biliary tract [12]. We explored the molecular interactions that enable *H. pylori* to colonize the gut of *O. viverrini* and the implications for CCA development in recent years [15].

[†]2013 Outstanding Scientist of Thailand.

Mechanism of *H. pylori* colonization in *O. viverrini*

Host receptors for H. pylori

Adhesion and colonization of *H. pylori* in host tissues involve interactions between bacterial adhesins and host receptors. Known bacterial adhesins include blood-antigen binding protein A (BabA), sialic acid-binding adhesin (SabA), and others. In the mammalian stomach, BabA binds Lewis B antigens, while SabA interacts with sialyl-Lewis X. Orthologues of these human receptors were investigated in *O. viverrini*. Immunohistochemical analyses of the fluke's gut epithelium and tegument revealed the presence of receptors for *H. pylori*: Lewis B: 3.2% of worms, Sialyl-Lewis X: 3.1% of worms, TLR4: 18.2% of worms, and L-fucose: 70.2% of worms. Among these, L-fucose exhibited the highest frequency of expression in the gut epithelium, suggesting its critical role in facilitating *H. pylori* colonization [15].

Detection of H. pylori in O. viverrini

Using a ureA gene-based nested PCR assay, *H. pylori* DNA was detected in 79% (41/49) of *O. viverrini* worms examined [15]. The frequent presence of *H. pylori* within the fluke's gut corroborates its role as a bacterial reservoir [12]. Furthermore, co-localization studies revealed adherence of *H. pylori* to L-fucose-positive regions of the gut epithelium and tegument, indicating L-fucose as a receptor for bacterial attachment [15].

Specificity of L-Fucose for H. pylori adhesion

The role of L-fucose as a receptor was confirmed through experiments using fucosidase, an enzyme that cleaves fucose residues. Treatment with fucosidase resulted in a dose- and time-dependent reduction in *H. pylori* adhesion to the fluke's gut and tegument. Quantitative PCR analysis demonstrated significantly decreased *H. pylori* ureA copy numbers following enzyme treatment, further validating the specificity of L-fucose-mediated bacterial adhesion [15].

Implications for CCA development

During migration, *O. viverrini* carrying *H. pylori* reaches the biliary tract, releases the bacteria through regurgitation and adhere to cholangiocytes [16]. This colonization could induce chronic inflammation and subsequent carcinogenesis, analogous to the gastric pathogenesis of *H. pylori* [17]. The synergistic effect of liver fluke infection and bacterial colonization likely exacerbates the inflammatory microenvironment, promoting carcinogenesis in bile ducts [9, 18].

PATHOGENESIS OF CO-INFECTIONS

Role of *O. viverrini* in biliary pathology

Chronic *O. viverrini* infection induces mechanical and immunological damage to the bile ducts [19]. The parasite's excretory-secretory (ES) products trigger inflammation, hyperplasia, and periductal fibrosis, which are critical precursors to CCA [4]. The mechanical damage caused by fluke suckers and egg deposition further exacerbates biliary epithelial injury.

H. pylori in the biliary tract

H. pylori, particularly cagA-positive strains, produces virulence factors that activate pro-inflammatory pathways in biliary epithelial cells. These include the NF-κB and IL-8 signaling cascades, which promote cellular proliferation and inhibit apoptosis [20, 21]. Additionally, *H. pylori* can form biofilms and adapt to the biliary environment, enhancing its survival and pathogenicity [22, 23]. Studies suggest that *O. viverrini* infection facilitates *H. pylori* colonization by damaging the biliary mucosa and upregulating receptors like TLR4 [8].

Recent studies have demonstrated that *H. pylori* can colonize human bile duct epithelial cells, inducing significant inflammatory responses [9, 18]. Through interactions mediated by sialic acid-binding adhesins such as SabA, *H. pylori* adheres to cholangiocytes, initiating the release of pro-inflammatory cytokines, including IL-6 and TNF-α [8]. This chronic inflammatory state disrupts the epithelial barrier, enhances cell proliferation, and exacerbates tissue and DNA damage but without apoptosis, laying the groundwork for carcinogenesis [9]. The ability of *H. pylori* to invade biliary epithelial cells further underscores its role in sustaining a pro-inflammatory microenvironment conducive to malignant transformation.

Recent studies have also revealed that *H. pylori* can induce epithelial-to-mesenchymal transition (EMT) in biliary epithelial cells, similar to its effects on gastric cells. *In vitro* experiments demonstrated that exposing human cholangiocytes to *H. pylori* induced phenotypic changes, including the formation of thread-like filopodia and loss of cell-cell contact [15]. These morphological alterations were accompanied by increased expression of EMT-related markers, such as snail, slug, vimentin, and zinc finger E-box-binding homeobox, as well as the cancer stem cell marker CD44 [24]. Functional assays showed enhanced migration, invasion, and colony formation in soft agar, indicating a malignant transformation of cholangiocytes [15]. This suggests that *H. pylori* infection, particularly in the setting of opisthorchiasis, contributes to the initiation and progression of biliary malignancies by promoting EMT and invasive cellular behaviors. *H. pylori*, particularly cagA-positive strains, produces virulence factors that activate pro-inflammatory pathways in biliary epithelial cells. These include the NF-κB and IL-8 signaling cascades, which promote cellular proliferation and

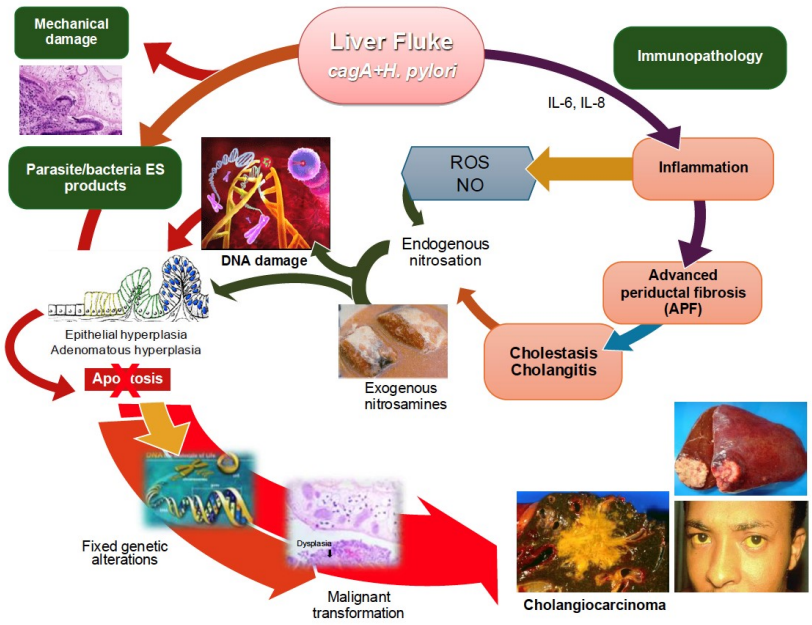

Fig. 1 Hypothesized pathways of pathogenesis of cholangiocarcinoma induced by *Opisthorchis viverrini* and *Helicobacter pylori* co-infection (adapted from Sripa et al [8]).

inhibit apoptosis [15].

Synergistic pathogenesis

The co-infection of *O. viverrini* and *H. pylori* results in a compounded pathogenic effect on the biliary tract (Fig. 1) [9, 18]. While *O. viverrini* creates a pro-inflammatory microenvironment conducive to bacterial colonization, *H. pylori* amplifies the epithelial damage and fibrosis through its virulence factors [8]. The synergy between the two pathogens is evident in the increased prevalence of high-grade APF and CCA in co-infected patients [6, 7]. Specifically, cagA-positive *H. pylori* strains exhibit unique pathogenic capabilities in the biliary system, promoting persistent inflammation and fibrosis [7].

Animal model studies further support this synergistic relationship. Hamsters co-infected with *O. viverrini* and cagA-positive *H. pylori* showed significantly higher inflammatory scores in the infected livers, including eosinophil and mononuclear cell infiltration, lymphoid aggregation, and granuloma formation, compared to those infected with either pathogen alone [9, 18]. Dysplastic changes, a precursor to CCA, were more severe and occurred earlier in the co-infected group, particularly in areas of pronounced inflammation. These findings highlight the critical role of cagA-positive *H. pylori* in exacerbating biliary pathology and predisposing to cholangiocarcinogenesis. The bromodeoxyuridine (BrdU) proliferation index, a marker of cellular proliferation, was also significantly elevated in co-infected hamster liver and biliary tracts, correlating with increased periductal fibrosis. Logistic regression analysis identified co-infection with cagA-positive *H. pylori* and infection duration as key factors driving fibrosis severity, further implicating this co-pathogenesis in disease progression [9, 18].

Neutrophils, the predominant inflammatory cells in response to *H. pylori* infection, also play a crucial role in the pathogenesis of CCA in opisthorchiasis patients. Neutrophils release neutrophil extracellular traps (NETs), composed of chromatin and proteases, which can capture pathogens but also induce tissue damage. Recent studies demonstrated that *O. viverrini* crude antigens could stimulate NET release from neutrophils in a dose-dependent manner ex vivo [25]. However, in patients with chronic *O. viverrini* infection and hepatobiliary abnormalities, NET production was dysregulated, with excessive NET formation causing collateral tissue damage and promoting fibrosis. This pro-inflammatory environment, driven by neutrophil activity, likely exacerbates bile duct injury and contributes to the progression of CCA [26].

CLINICAL IMPLICATIONS AND MANAGEMENT

The primary treatment for opisthorchiasis, PZQ, effectively eradicates the fluke but does not fully resolve APF in many patients [27]. Persistent or recurrent APF poses a significant risk for CCA [5, 10]. Adjunctive therapies targeting *H. pylori* have been proposed to reduce APF severity and prevent disease progression

[8]. Antibiotic regimens, combined with PZQ, may significantly lower the prevalence and bacterial loads of cagA-positive *H. pylori*, particularly in patients with high-grade fibrosis [8].

Recent findings underscore the necessity for stratified treatment strategies [10]. Patients with relapsed or persistent APF may benefit from tailored antibiotic regimens aimed at eradicating cagA-positive *H. pylori*. Additionally, the genetic diversity of cagA genotypes, such as the EPIYA motifs (AB, ABC, AB'C types), plays a pivotal role in driving pathogenesis. Studies reveal that EPIYA-AB'C, a Western-like genotype, is more prevalent in *O. viverrini*-infected patients and associates strongly with advanced biliary fibrosis [7]. These genotypes, particularly those containing the CagA multimerization sequence (CM), exhibit enhanced interaction with host cellular pathways, promoting fibrosis and malignancy [21].

Screening for cagA genotypes could enable personalized interventions, particularly for patients at high risk of APF progression. Clinical trials should evaluate the efficacy of targeting specific *H. pylori* virulence factors, including CM sequence-positive cagA strains, to mitigate biliary disease severity and reduce CCA incidence. Follow-up protocols incorporating diagnostic tests for *H. pylori* and APF monitoring by ultrasonography with effective targeted therapies [10, 27] could improve patient outcomes by enabling early intervention. The specific and early diagnosis in addition to effective targeted therapy focusing on tumor microenvironment are important for the treatment of cancer as reviewed in these articles [28, 29].

FUTURE PERSPECTIVES

Further research is needed to unravel the molecular mechanisms underpinning *O. viverrini* and *H. pylori* co-infections such as growth and angiogenesis [30, 31]. Developing targeted therapies that address both pathogens is essential. Advances in diagnostic tools for early detection of co-infections and stratified treatment approaches based on microbial profiles could significantly reduce the burden of APF and CCA in endemic regions. Long-term cohort studies are also warranted to evaluate the effectiveness of integrated treatment strategies.

CONCLUSION

The interplay between *O. viverrini* and *H. pylori* co-infections underscores the complexity of APF and CCA pathogenesis. CagA-positive *H. pylori* strains emerge as critical drivers of persistent fibrosis and carcinogenesis in the biliary system. A holistic approach, combining fluke eradication with bacterial management, is imperative for effective disease control and prevention in endemic populations.

Acknowledgements: This work is supported by findings from prior research conducted by the authors and collaborations with regional and international partners.

REFERENCES

1. Sripa B, Suwannatrai AT, Sayasone S, Do DT, Khieu V, Yang Y (2021) Current status of human liver fluke infections in the Greater Mekong Subregion. *Acta Trop* **224**, 106133.
2. Zhao TT, Feng YJ, Doanh PN, Sayasone S, Khieu V, Nithikathkul C, Qian M, Hao y, et al (2021) Model-based spatial-temporal mapping of opisthorchiasis in endemic countries of Southeast Asia. *Elife* **10**, e59755.
3. Sota P, Upontain S, Tangkawattana S, Punyapornwithaya V, Nakhapakorn K, Sripa B (2024) Association between *Opisthorchis viverrini* infection in cats and humans: Non-spatial and spatial analyses. *Vet Parasitol* **327**, 110150.
4. Sripa B, Kaewkes S, Sithithaworn P, Mairiang E, Laha T, Smout M, Pairojkul C, Bhudhisawasdi V, et al (2007) Liver fluke induces cholangiocarcinoma. *PLoS Med* **4**, e201.
5. Sripa B, Jumnainsong A, Tangkawattana S, Haswell MR (2018) Immune response to *Opisthorchis viverrini* infection and its role in pathology. *Adv Parasitol* **102**, 73–95.
6. Boonyanugomol W, Chomvarin C, Sripa B, Bhudhisawasdi V, Khuntikeo N, Hahnvajanawong C, Chamsuwan A (2012) *Helicobacter pylori* in Thai patients with cholangiocarcinoma and its association with biliary inflammation and proliferation. *HPB (Oxford)* **14**, 177–184.
7. Deenonpoe R, Mairiang E, Mairiang P, Pairojkul C, Chamgramol Y, Rinaldi G, Loukas A, Brindley PJ, et al (2017) Elevated prevalence of *Helicobacter* species and virulence factors in opisthorchiasis and associated hepatobiliary disease. *Sci Rep* **7**, 42744.
8. Sripa B, Deenonpoe R, Brindley PJ (2017) Co-infections with liver fluke and *Helicobacter* species: A paradigm change in pathogenesis of opisthorchiasis and cholangiocarcinoma? *Parasitol Int* **66**, 383–389.
9. Suyapoh W, Tirnitz-Parker JEE, Tangkawattana S, Suttiprapa S, Sripa B (2021b) Biliary migration, colonization, and pathogenesis of *O. viverrini* co-Infected with CagA+ *Helicobacter pylori*. *Pathogens* **10**.
10. Phung HTT, Deenonpoe R, Suttiprapa S, Mairiang E, Edwards SW, Sripa B (2022) Persistent advanced periductal fibrosis is associated with cagA-positive *Helicobacter pylori* infection in post-praziquantel treatment of opisthorchiasis. *Helicobacter* **27**, e12897.
11. Jongsuksuntigul P, Imsomboon T (2003) Opisthorchiasis control in Thailand. *Acta Trop* **88**, 229–232.
12. Deenonpoe R, Chomvarin C, Pairojkul C, Chamgramol Y, Loukas A, Brindley PJ, Sripa B (2015) The carcinogenic liver fluke *Opisthorchis viverrini* is a reservoir for species of *Helicobacter*. *Asian Pac J Cancer Prev* **16**, 1751–1758.
13. Zhou D, Wang JD, Weng MZ, Zhang Y, Wang XF, Gong W, Quan ZW (2013) Infections of *Helicobacter* spp. in the biliary system are associated with biliary tract cancer: a meta-analysis. *Eur J Gastroenterol Hepatol* **25**, 447–454.
14. Murphy G, Michel A, Taylor PR, Albanes D, Weinstein SJ, Virtamo J, Parisi D, Snyder K, et al (2014) Association of seropositivity to *Helicobacter* species and biliary tract cancer in the ATBC study. *Hepatology* **60**, 1963–1971.
15. Thanaphongdecha P, Karinshak SE, Ittiprasert W, Mann VH, Chamgramol Y, Pairojkul C, Fox J, Suttiprapa S, et al

(2020) Infection with *Helicobacter pylori* induces epithelial to mesenchymal transition in human cholangiocytes. *Pathogens* **9**, 971.
16. Thanaphongdecha P, Chamgramol Y, Pairojkul C, Deenonpoe R, Suttiprapa S, Brindle PJ, Sripa B (2022) Adherence of *Helicobacter pylori* to *Opisthorchis viverrini* gut epithelium and the tegument mediated via L-fucose binding adhesin. *Parasitology* **149**, 1374–1379.
17. Malfertheiner P, Camargo MC, El-Omar E, Liou JM, Peek R, Schulz C, Smith S, Suerbaum S (2023) *Helicobacter pylori* infection. *Nat Rev Dis Primers* **9**, 19.
18. Suyapoh W, Tangkawattana S, Suttiprapa S, Punyapornwithaya V, Tangkawattana P, Sripa B (2021a) Synergistic effects of cagA+ *Helicobacter pylori* co-infected with *Opisthorchis viverrini* on hepatobiliary pathology in hamsters. *Acta Trop* **213**, 105740.
19. Sripa B (2003) Pathobiology of opisthorchiasis: an update. *Acta Trop* **88**, 209–220.
20. Cid TP, Fernandez MC, Benito Martinez S, Jones NL (2013) Pathogenesis of *Helicobacter pylori* infection. *Helicobacter* **18**, 12–17.
21. Hatakeyama M (2014) *Helicobacter pylori* CagA and gastric cancer: a paradigm for hit-and-run carcinogenesis. *Cell Host Microbe* **15**, 306–316.
22. Tajeddin E, Sherafat SJ, Majidi MR, Alebouyeh M, Alizadeh AH, Zali MR (2016) Association of diverse bacterial communities in human bile samples with biliary tract disorders: a survey using culture and polymerase chain reaction-denaturing gradient gel electrophoresis methods. *Eur J Clin Microbiol Infect Dis* **35**, 1331–1339.
23. Gupta A, Kumar A, Singh N, Sudarshan N, Studitsky VM, Zhang KYJ, Akhtar MS (2023) The *Saccharomyces cerevisiae* SR protein Npl3 interacts with hyperphosphorylated CTD of RNA Polymerase II. *Int J Biol Macromol* **253**, 127541.
24. Baj J, Korona-Glowniak I, Forma A, Maani A, Sitarz E, Rahnama-Hezavah M, Radzikowska E, Portincasa P (2020) Mechanisms of the epithelial-mesenchymal transition and tumor microenvironment in *Helicobacter pylori*-induced gastric cancer. *Cells* **9**, 1055.
25. Watakulsin K, Chuenchom C, Thapphan C, Thai TD, Chareonsudjai S, Faksri K, Suttiprapa S, Tangkawatana S, et al (2023) Neutrophils form extracellular traps in response to *Opisthorchis viverrini* crude antigens, which are elevated in neutrophils from opisthorchiasis patients with hepatobiliary abnormalities. *Biol Open* **12**, bio059909.
26. Thai TD, Chuenchom C, Donsa W, Faksri K, Sripa B, Edwards SW, Salao K (2023) *Helicobacter pylori* extract induces purified neutrophils to produce reactive oxygen species only in the presence of plasma. *Biomed Rep* **19**, 89.
27. Mairiang E, Laha T, Kaewkes S, Loukas A, Bethony J, Brindley PJ, Sripa B (2021) Hepatobiliary morbidities detected by ultrasonography in *Opisthorchis viverrini*-infected patients before and after praziquantel treatment: a five-year follow up study. *Acta Trop* **217**, 105853.
28. Sawanyawisuth K, Wongkham C, Pairojkul C, Wongkham S (2018) Translational cancer research towards Thailand 4.0. *ScienceAsia* **44S**, 11–18.
29. Heenatigala Palliyage G, Ghosh R, Rojanasakul Y (2020) Cancer chemoresistance and therapeutic strategies targeting tumor microenvironment. *ScienceAsia* **46**, 639–649.
30. Waenphimai O, Thamrongwaranggoon U, Kidoikhammouan S, Sorin S, Klinhom-on N, Seubwai W, Saengboonmee C, Detarya M, et al (2024) CDKN3 acts as a tumor promoter enhancing proliferation of cholangiocarcinoma cells. *ScienceAsia* **50**, 2024108.
31. Vaeteewoottacharn K, Kariya R, Saisomboon S, Paungpan N, Luang S, Piyawattanamatha N, Kitkhuandee A, Okada S (2024) Lenalidomide suppresses VEGF-dependent angiogenesis and cholangiocarcinoma growth in an *in vivo* mouse model. *ScienceAsia* **50**, 2024111.