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Reviewer comments and how they are implemented:

ANSWERS TO REVIEWER 3:

Comment	What we changed	Status
The abstract and conclusion should be re-written, the reviewer thinks that a lot of focus has been given on the challenges of collecting histological samples, which might be true but is not the focus of the review.	The abstract and conclusion section have been restructured, and the challenge of obtaining the biopsies has been shortened and shifted to the introduction. We rewrote the following passage: From a clinical point of view, extrahepatic cholangiocarcinoma often presents with suspicious bile duct strictures and cholestasis, but histopathological approval with consecutive surgical treatment is often delayed due to a difficult tissue sampling and the poor outcome of histo-pathological evaluation of the fibrotic tumor samples. Thus, new easily obtainable biomarkers might help to facilitate tumor diagnosis at early stages. In this context, microRNA expression profiling of bile fluid or tumor specimens in patients with unclear biliary tumors or biliary tract stenosis might improve detection of cancer. Based on numerous studies, a panel of microRNAs listed in table 1 has been evaluated and seems to be of special value herein. Interestingly all of the molecules are associated with increased neo-vascularization. Specific MicroRNAs that should be regarded of special importance in this context, e.g., are miR141, miR-181, miR-191, and miR-200b which have also been found to be highly dysregulated in cholangiocarcinoma [33, 34]. These works have further emphasized that miRNAs have key roles for increased cellular proliferation [33, 34]. In accordance with the effects, microRNA dysregulation and even knockdown of the miR-17-92 cluster with antisense 2'-O-methyl oligoribonucleotides partly restored expression of anti-angiogenic factors <i>Tsp1</i> and <i>CTGF</i>, while miR-17-92-transduced cells formed larger, better-perfused tumors [33]. Thus, microRNA deregulation might not only induce neovascularization, but might also suppress anti-angiogenic factors. Thus, microRNA-deregulation may be a key factor in the molecular process of CCA. Evaluation of the dysregulation of defined microRNAs might	Fully implemented

	facilitate to diagnose and treat extrahepatic CCAs earlier and more successful.	
The reviewer fails to understand the relevance of the Section 2, "Role of cholangioscopy and laser scanning microscopy for detection of cholangio-carcinoma". Given the title of the review this sub-section is misplaced. In the introduction the authors can briefly say about the collection of histological samples and their inherent challenges.	As desired, this passage has been shortened in the introduction and the different section have been merged together. Section 1-clinical background, Section 2 microRNA, and section 3: neovascularization.	Restructured manuscript.
The same is true for Section 3 "MicroRNA in CCC: emerging role as predictors of cancer presence" first paragraph "Recently, a new technique has been...from 52% to 83% for biliary biopsies", how is it relevant to the review. The section 2 and 3 can be clubbed together and briefly included in the introduction/background part.	As desired, this passage has been shortened in the introduction and the different section have been merged together. Section 1-clinical background, Section 2 microRNA, and section 3: neovascularization. As desired, section 2 and 3 have now been "clubbed together", and a focus on microRNAs has been extended: MicroRNA dysregulation has been described in fluid of the biliary tract obtained by ERCP and could be of clinical interest and can easily be determined in patients with CCA. With high prognostic values, miRNAs have been screened in bile fluid and among these, miR-9 (AUC=0.975) and miR-145 (AUC=0.975) were shown to display a certain diagnostic capacity for CCA when compared with healthy individuals [15]. When comparing biliary specimens from patients with PSC- derived CCA and isolated PSC, miR-412, miR-640, miR-1537, and miR-3189 were of highly significant differentiating value [16]. miR-1537 in combination with CA19-9 resulted in higher diagnostic values than CA19-9 alone (AUC 0.91 versus 0.88; P > 0.05) [14].	Fully implemented
The authors have written statements without any details or references; for example, "Interestingly and potentially of greater specificity in the context of CCC, dysregulation of other microRNA was also reported to affect the cell cycle, epigenetics (miR-148, miR-152), inflammation (determined for miR-200, -125, and miR-605), as well as chemo-resistance (miR-200b, 204). Also, epithelial to mesenchymal transition (EMT), migration and invasion was reported for these microRNAs, allowing to explain the strong fibro-blastic transition of	We added 8 additional references for this section. Additionally we clarified how epigenetics can be affected. For that we included the following sentence including 3 further references: E.g. for epigenetic regulation miR-148a, miR-148b and miR-152 are involved in a feedback loop with DNA methyltransferase (DNMT) 1 in some tumor types[37-39]. While the miR-148/-152 family post-transcriptionally regulates DNMT1 expression, the miRNA promoter methylation is reduced.	Fully implemented

these tumors, which makes it difficult when evaluation the biopsies during histopathological evaluation". The above phrase has no detailed information about the study, their limitation etc. MiRNAs affecting epigenetics is a vague statement.		
Section 4 – "MicroRNA dysregulation and chemosensitivity of CCC" has no information about any miRNAs involved in chemosensitivity and therapy resistance. The entire manuscript fails to convey the idea that why miRNAs are important in the diagnosis and prognosis of Cholangiocarcinoma. The authors can also include how miRNAs can be useful in diagnosing different types of cholangiocarcinoma.	A more comprehensive conclusion section has been added to resolve this issue.	Please see the new conclusion section.
Several key references are missing, PMID: 31106658, 29860474, 31443224, 30887508, 30121648 and many more.	We thank reviewer 3 for leading us to these valuable further examples of miRNA involved in CCA. These key references have been integrated into the manuscript as part of table 1.	Fully implemented
The reviewer appreciates section 5, all the other sections relating to importance of miRNAs in different aspects of Cholangiocarcinoma should be as comprehensive if not more.	Section 3 - neovascularisation has been extended as desired.	Fully implemented
The authors should perform a comprehensive review of studies reporting miRNA dysregulation in cholangiocarcinoma, miRNAs as biomarkers, in therapy resistance, in-vivo studies if any and potential of miRNAs as a therapeutic approach in cholangiocarcinoma and the inherent challenges associated with it.	Almost 20 new references integrated.	Fully implemented
The study should be different and an advancement over recent articles and reviews; for example, "Dysregulation of microRNA in cholangiocarcinoma identified through a meta-analysis of microRNA profiling" by Likhitrattanapisal S. et al., World J Gastroenterol. 2020 Aug 7; 26(29): 4356–4371 and "The Role of microRNAs in Cholangiocarcinoma" by Shi T. et al., Int J Mol Sci. 2021 Jul; 22(14): 7627, respectively.	New reference have been integrated in section 2 (previous 3): A recent meta-analysis of microRNA profiling showed 70 up-regulated and 48 down-regulated miRNAs in CCA [15]. miRNAs proofed to be significantly involved in several biological processes. The most prominent dysregulated pathways included phosphatidylinositol-3 kinases/Akt, mitogen-activated protein kinase and Ras signaling pathways.	Fully implemented
The conclusion should be improved with author's point of view and future perspectives.	Changed as desired. From a clinical point of view, extrahepatic cholangiocarcinoma often present with	Fully implemented

	suspicious bile duct strictures and cholestasis, but histological approval with consecutive surgical treatment is often delayed due to a difficult approach, tissue sampling and the poor outcome of histopathological evaluation of the fibrotic tumor samples. Thus, new biomarkers that can be easily obtained might help to facilitate tumor diagnose at early stages. In this context, microRNA expression profiling of bile fluid or tumor specimens in patients with unclear biliary tumors or biliary tract stenosis might improve detection of cancer. Based on numerous studies, a panel of microRNAs listed in table 1 has been evaluated and seems to be of special value herein. Of great interest, all of the molecules have been associated with increased neo-vascularisation. Specific MicroRNAs that should be regarded of special importance in this context, e.g., are miR141, miR-181, miR-191, and miR-200b which have also been found to be highly dysregulated in cholangiocarcinoma [33, 34]. These works have further emphasized that miRNAs have key roles for increased cellular proliferation [33, 34]. In accordance with the effects, microRNA dysregulation and even knockdown of the miR-17-92 cluster with antisense 2'-O-methyl oligoribonucleotides partly restored expression of anti-angiogenic factors <i>Tsp1</i> and <i>CTGF</i>, while miR-17-92-transduced cells formed larger, better-perfused tumors [33]. Thus, microRNA deregulation might not only induce neovascularisation, but might also suppress anti-angiogenic factors, and thus, microRNA-deregulation may be a key factor in the molecular process of CCA. Evaluation of the dysregulation of defined microRNAs might thus facilitate to diagnose and treat extrahepatic CCAs earlier and more successful.	
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