Molecular Genetics and Targeted Therapeutics in Biliary Tract Carcinoma

We wish to thank the reviewers for the constructive comments. The authors' responses are as follows:

Reviewer #1

This review article by Eric I Marks et al. reported "Molecular Genetics and Targeted Therapeutics in Biliary Tract Carcinoma". They reviewed the pathogenesis, genetic targeted pathways (RAS-RAF-MEK-MAPK pathway, PI3K-AKT-mTOR pathway and Inflammation-associated pathway) and targeted therapeutics (EGFR inhibitors, HER2 inhibitors, VEGF inhibitors, MEK inhibitors and Multi-kinase inhibitors) in biliary tract carcinoma including cholangiocarcinoma and gallbladder carcinoma. This article is interesting and helpful for readers to understand the pathogenesis and genetic targets of biliary tract carcinoma.

Minor points: Biliary tract carcinoma is rare malignancy, but it is difficult to diagnose before invasion and metastasis. Although the authors mentioned about clinical information including incidence, the table with such information is very helpful for readers.

Authors' response:
A new Table 1 regarding the epidemiology and clinical features of the primary malignancies of the biliary tract is being added.

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Reviewer #2
In this article, Eric I Marks et al. provided an updated review of the molecular genetics of BTC. The pathogenesis and cellular patho-physiology of these malignancies is described, with emphasis on those molecular abnormalities that could be targeted for intervention. The mechanism of action of each targeted agent under investigation for use in treating BTC is discussed, as well as data from pre-clinical and clinical studies. Ongoing clinical trials of these molecularly targeted agents in BTC are also presented. This paper contains interesting information which merit publication.

For the benefit of the reader, however, a number of points need clarifying and certain statements require further justification. They are given below. 1. It is better to cite newly published paper with concern to the 5-year survive rate of BTC (Page 4). 2. Since cholangiocarcinoma is seldom discovered early in its development, there is limited data on its pathogenesis. Therefore, data obtained from studies of pancreatic adenocarcinoma have been used to generate working models of BTC. It is better to provide evidence of this point (Page 7). 3. Table 3 showed the ongoing clinical trials of targeted therapeutics in cholangiocarcinoma or gallbladder adenocarcinoma, however, the outcome of these trials has limited information (Page 19-20).

Authors' response:

1. The reference #6 for the SEER cancer statistics as accessed on September 12, 2015 is being cited.

2. The references #7 and 8 are being cited.

3. The Table 3 (current Table 4), the ongoing clinical trials are listed, and the outcome of these studies is pending their completion and report.

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Reviewer #3

This is a well-written and well-documented review on biliary tract cancer.

My main comment relates to the fact that the distinction between intrahepatic cholangiocarcinoma and extrahepatic bile duct cancer is lacking throughout the review. This distinction is important in view of significant differences in aetiology, presentation, clinical (differential) diagnosis, morphology and treatment. Similarly, the references include articles that pertain to either or both tumour groups. Careful separation of information related to any of the three groups – intrahepatic cholangiocarcinoma, extrahepatic bile duct cancer or gallbladder cancer, should be maintained throughout the manuscript, including tables and references.

Authors' response: In the new Table 1, Table 2 (previous Table 1), and Table 3 (previous Table 2), as well as in the text, the three subtypes of biliary tract carcinoma are being listed and described.

"Furthermore, nomenclature as recommended by WHO 2010 should be used. Further comments: - the nomenclature used for the precursor lesions in the gallbladder are not in line with those of the WHO. "Dysplasia" is no longer included in the WHO, but has been replaced by BilIN (although in clinical practice the term "dysplasia" may still be used). The authors should clarify the basic difference between IPN (macroscopically visible tumour, often with polypous or carpet-like appearance), BilIN (microscopic lesion) and adenoma (a macroscopically visible, localized polypous neoplastic lesion). "

Authors' response: The precursor lesions including IPN. BilIN, and adenoma are being amended in the section "Pathogenesis of Biliary Tract Carcinoma".

- The authors may wish to include adenomas in their discussion of precursor lesions, and comment on the prevalence and risk of malignant transformation of adenomas in the extrahepatic biliary tree, including the gallbladder.

Authors' response: This has been addressed in the first paragraph of the section titled "pathogenesis of biliary tract carcinoma".

- the progression model for gallbladder cancer includes as a first step "hyperplasia/metaplasia". The authors may wish to reflect upon the fact that epithelial hyperplasia is rare in the gallbladder, while dysplasia – and invasive adenocarcinoma – is not uncommon in gallbladders with mucosal atrophy."

Authors' response: This was addressed in the text by clarifying that the transformation most likely occurs through metaplasia to dysplasia.

- introduction: the statement "When patients present with a localized biliary tumor without vascular invasion, surgical resection may be attempted…." is misleading. Meant here is invasion in neighbouring large blood vessels, not (microscopic) vascular invasion.

Authors' response: This is being amended as suggested.

- page 4 (introduction): replace "Genetic studies of BTC have shed new insights into …" by " have shed light upon…".

Authors' response: This is being amended as suggested.

- page 7 (Cholangiocarcinoma): replace "BilIN, appears as a flat or microscopically papillary mass of …" by "microscopic papillary proliferation of ….".

Authors' response: This is being amended as suggested.

- page 8 (Cholangiocarcinoma): replace "biliary epithelia that is accompanied by rods of fibrovascular tissue surrounding the bile duct" with "epithelial that is supported by cores of fibrovascular tissue.".

Authors' response: This is being amended as suggested.

Reviewer #4

1. The title of the Table 1 is "Mutational frequency of genetic alterations in biliary tract carcinoma". However, in the text, when discussed the HER2 abnormality, it was described "Increased expression of this receptor is quite common in biliary tumors (Table 1)"(line 2, page 11) . Thus, it is not clear the percentage listed in Table 1 (16%, 5%, 0) is indeed the

mutation rate or the abnormal expression rate of HER2 . 2. It's better add the genetic alteration type of a certain gene (e.g. point mutation site, copy number variation, etc) in Table 1.

Authors' response: The title of the previous Table 1 and new Table 2 is being amended as follows: Table 2 Frequency of mutation or abnormal expression of molecular targets in biliary tract carcinoma.

2. It's better add the genetic alteration type of a certain gene (e.g. point mutation site, copy number variation, etc) in Table 1.

Authors' response: In the new Table 2, the type of genetic alteration is being amended as "Point mutation", "Increased expression", or "Increased activation".