Reviewer 1

The present manuscript shows that phosphatidylcholine (PC) can pass by a paracellular transport from systemic sources to the apical side of cholangiocytes, thanks to an apical negative electrical potential generated by CFTR and AE2, with consequent binding to membrane-localized mucin 3 and an equilibrated shift to secretory mucin 2.

Many thanks for reading our paper and your helpful comments. In the following we have outlined how we addressed the points you raised.

It is very interesting but there are some points to elucidate and to enrich:
- The Authors, in the Abstract, in the Introduction and in the Discussion sections, have mentioned the primary sclerosing cholangitis (PSC) as a disease in which PC translocation into mucus via a paracellular transport across the apical/lateral tight junction (TJ) barrier may have implications for its pathogenesis. Therefore my question is: why did they use a cholangiocarcinoma (CCA) cell line, in particular a typical extrahepatic CCA cell line? The Authors need to clarify this point.

As suggested, we have added some comments to clarify why we think that our study has implications in the pathogenesis of primary sclerosing cholangitis and why we used the Mz-ChA-1 cell line in our study. The cell line Mz-ChA-1 was originally described as an adenocarcinoma cell line. However, many subsequent studies showed that the cell line has many features of human cholangiocyte cells (e.g. Braconi et al., PLoS ONE 2010;5(12):e15195; Scharmpf et al., Hepatology 2015;62:1249-1259; Onori et al., Int J Cancer 2010;127:43-54; Zach et al., J Stem Cell Res Transplant 2015;2(1):103). We have added a comment on the phenotypic features and cited the mentioned references supporting our statements.

- Related to the previous point, I am not completely agree with the sentence “These cells share indeed the characteristics of physiologic biliary epithelial cells” (line 59). In fact, we have the reference but the sentence remains too general, the authors should explain better which are the physiologic characteristics of Mz-ChA-1. For that reason, I suggest to add a non-malignant cholangiocyte cell line, also to compare the important data obtained.

We fully agree with your concern. The cell line was established in 1985 and derived from a mechanically dissociated gallbladder adenocarcinoma metastasis (Knuth et al., J Hepatol. 1985;1:579-596). Already shown in the mentioned paper, the cell line shows TJ complexes and has a highly differentiated phenotype producing large quantities of mucus. In comparison to many other human biliary tract carcinoma cell lines, such as TGBC-1, TBC-51, Mz-ChA-2 and SK-ChA-1, the cell line Mz-ChA-1 is 10-1000 times less invasive through both the collagen and the basement membrane (Koike et al., Int J. Oncol 1998;13:1269-1274). These properties make this cell line an adequate tool for our study because its phenotype is not as malignant as that of other cholangiocellular cell lines.

To address your concern, we have rephrased our description of that cell line more critical and added comments on its phenotype. The shortcoming of the tissue culture model is discussed in the last paragraph of the discussion.

- It should be really interesting also to add some images of PC movement across the polarized and unpolarized cholangiocytes.

This is a comment, we highly appreciate. Unfortunately this time we did not have the technical requirement to perform these studies as it was shown in CaCo2 cells in 2016 (Stremmel et al., Biochim Biophys Acta 2016;1861:1161-1169). This shortcoming is discussed in the last paragraph of the discussion.

- In the Material and Methods section, the titles 4.1 and 4.3 are the same, they could change and specify one or put together just in one paragraph.

Thanks for this comment. We have shifted the information of 4.3 into paragraph 4.1 and lined up all following paragraphs.

We once would like to thank you for the time you spent in reading our manuscript and your valuable comments and hope that you will agree that the revised version is now suitable for publication in the International Journal of Molecular Sciences.