Reviewer’s report

Title: Mutational profiling reveals PIK3CA mutations in gallbladder carcinoma

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Reviewer: Kevin Halling

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In their manuscript titled “Mutational profiling reveals PIK3CA mutations in gallbladder carcinoma” Deshpande et al characterize the mutations of known oncogenes and tumor suppressor genes in 77 formalin fixed paraffin embedded archived biliary tract cancer cases using a mass spectrometric based platform. They found mutations among three genes, KRAS, NRAS and PIK3CA. They state that activating mutations in PIK3CA were identified exclusively in GBC and that KRAS mutations were only identified in intra-hepatic cholangiocarcinomas. The findings of this study are novel and add valuable new information to the literature in helping define the spectrum of oncogenetic mutations present in biliary tract cancers. This information may eventually be used to help guide therapy of biliary tract cancer patients.

The paper is well written.

Minor Essential Revisions

My greatest concern regarding this study relates to the low number of mutations that were ultimately identified after hME analysis. It would be good if the authors could provide a little more explanation on the hME methodology. In the second paragraph of the Results the authors state the hME method is “more sensitive and specific” than the OncoMap technology. I do believe that it is more specific than the OncoMap technology and that that of course is the reason that they are using to confirm the OncoMap mutations. But I suspect the hME is significantly less sensitive than the OncoMap technology (i.e. higher false negative rate). I suspect that some of the OncoMap mutations that were not confirmed by hME method were real mutations but that the hME method was not analytically sensitive enough to confirm them. Do the authors have a good feel for the analytical sensitivity of the hME assay? I do think it is good that the authors are favoring specificity over sensitivity but they may be excluding real mutations. It seems that this is especially possible given the surprisingly low number of mutations that were ultimately identified by the study.

Note: the words “sensitive and” should be removed from the sentence on page 8 that says “Candidate mutations across 12 genes (ABL1, APC, BRAF, EGFR, FGFR3, FLT3, KIT, KRAS, NRAS, PDGFRA, PIK3CA, MYC,) were then evaluated using a more sensitive and specific homologous mass extend (hME) approach on non WGA DNA using independent primers and probes…”

In the fourth paragraph of the Discussion, the authors state “Our careful
histological evaluation suggests that adenocarcinoma involving both the mid portion of the bile duct and intra-pancreatic bile duct lack KRAS mutations.” This is inconsistent with previously published findings which show that extrahepatic cholangiocarcinomas do harbor KRAS mutations. Further discussion is needed regarding this discrepant finding.

The authors should use the correct nomenclature for genes. For example, KRAS should be KRAS. Also I believe that the officially accepted nomenclature for HER2 and NEU is ERBB2.

The first sentence of page 11 (Discussion section) states that “Taken together this data points towards deregulation of PI3K signaling as a key event in the molecular pathogenesis of BTC.” I would say it is a key event in the molecular pathogenesis of some BTC.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

We are doing similar studies in our lab.