Author’s response to reviews

Title: Distinct molecular etiologies of male and female hepatocellular carcinoma

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Version: 1 Date: 16 Aug 2019

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Response to reviewers’ comments

We want to thank Dr Bejugam and Dr Ruiz for thoughtful comments and feedback regarding our manuscript. We have revised the manuscript accordingly.

Reviewer reports:

Pruthvi Raj Bejugam, Ph.D (Reviewer 1):
"After reviewing your manuscript "Distinct molecular etiologies of male and female hepatocellular carcinoma" my first thoughts are this study is novel and interesting. I am quite satisfied with this quite unconventional study design. The overall methodology of the study is novel and obtained results and relevant conclusions are satisfactory. There are no major revisions are required but we have a few minor queries in our mind.

Minor queries,
1) Your entire study focussed on the analysis of QTLs, Why QTLs? you could have explained your reasons for choosing QTLs in an elaborative manner for common readers or non-genomics readers."

We have laid out our rationale on focusing on gene expression and regulation in the introduction as follows:

• It is known that the sexes differ in terms of risk, disease progression, and somatic alteration in HCC.
• It is known that sex-specific regulation of gene expression (sex-specific eQTLs) may contribute to sex differences in disease prevalence and severity.
• Previous studies have identified sex-biased gene expression signatures (sex-biased expression in tumors) in HCC and other cancer. However, these studies focused on only comparing tumor samples, and did not account for sex-differences in non-diseased tissues, did not examine the dysregulation of genes in tumors in comparison to adjacent tissues in each sex (sex-specific DEGs), and did not explore sex-specific regulation of gene expression (sex-specific eQTLs).
Our study fills in these gaps and provides much-needed information in the sex-specific etiology of HCC.

"2) in HCC tumors you have found 34 genes which were expressed in a sex-biased way and you have briefly mentioned about few genes viz. DTX1, CD24, and PI3K/AKT. these are all well-characterized and widely studied genes, in your point of view which among those 34 can be a novel target. you could have elaborated on that."

We have expanded the discussion to cover genes that are known to be cancer-associated but have not been studied in the context of HCC (GGT6). We have also added information on the roles of CXCL14, ATF5, GPR37, HAMP, NTS, FGFR2 (all under-expressed in male tumors in this study) in HCC.

"3) In the page, no. 10 (line no.228) you have mentioned 'due to limited power', what is this power phrase means, is it regarding statistical power or computing power? Please explain."

We have revised the sentence to explicitly state “statistical power”.

Fiona Ruiz (Reviewer 2):

"When performing the eQTL analysis to identify germline genetic effects on the tumor gene expression did you take into consideration effects of race? In your Female and Male cohorts you showed a significant difference in race between the two cohorts. I think it is informative to show whether these germline SNP’s are enriched in different racial groups or if they are solely gender specific."

In our eQTL analyses, we accounted for ancestry and population structure by including the first 3 genotype PCs as covariates. This is a standard approach for accounting for population structure in association studies on multi-ethnic cohorts.

"In your eGene results you mentioned POGLUT1 being a male specific eGene and that it is an essential regulator of NOTCH signaling. However, NOTCH1 signaling was found to be a shared pathway between both sexes. Did any of the male specific eGenes contribute to male specific enriched pathways? Making the connection between the differentially expressed genes and pathways in the sexes and the sex specific eGenes would add additional insight into the different underlying biology of male and female HCC tumors."

We have expanded the discussion regarding Notch and PI3K/AKT signaling pathways. Notch and PI3K/AKT are known to cooperate: concurrent activation of Notch and PI3K/AKT pathways can trigger tumorigenesis and is prevalent in aggressive cancers. We find simultaneous activation of PI3K/AKT and Notch pathways in male HCC, and sex-specific genetic effects on regulation of genes involved in these pathways. These results point to a major role of the Notch/PI3K/AKT axis in the development of HCC in males.