Reviewer’s report

Title: Tumor and matched plasma microRNA profiling for Opisthorchis viverrini-induced cholangiocarcinoma

Version: 3 Date: 13 January 2015

Reviewer: Federica Ganci

Reviewer’s report:

The proposed manuscript “A microRNA profile for the diagnosis of Opisthorchis viverrini-induced cholangiocarcinoma” by Jordan Plieskatt et al., aims mainly at identifying microRNAs dysregulated in 14 ICC tumor tissues versus their matched controls using miRNA-seq approach. Next, the authors, by the comparison of the results obtained from this and their previous work, aims at demonstrating a good reproducibility of data obtained from the two different miRNA discovery platforms (microarray and miRNA-seq), even if they conclude that RNA-seq is more informative. Finally, the authors identify circulating microRNAs associated with ICC. In my opinion, the proposed concept by the manuscript is interesting since that to date no studies have been published on microRNAs expression profile in cholangiocarcinoma by next generation sequencing. In addition, only one study has been conducted on the association of miRNAs expression changes in plasma of ICC patients. The concepts are adequately supported by experimental evidences and the manuscript is well written. However, an additional revision of the manuscript is suggested in order to improve the understanding of the results section. Here are some suggestions:

Minor points:

1. In the introduction and discussion sections, no references were included on the previous works regarding the association of circulating miRNAs in cholangiocarcinoma. For instance, the work entitled “Upregulated circulating miR-150 is associated with the risk of intrahepatic cholangiocarcinoma”, by Wang S et al, 2014 is missing. The authors may be included it and/or other ones.

2. In the second paragraph of the results section, the authors wrote that about half (49%) of the 143 million reads obtained after the application of filters mapped to the human genome (line 120, pag.5); why only 49%? And where does the rest of reads map? Next, in the same paragraph there is an incongruence (line 121-123, pag.5); the authors wrote that 47 million of 73 reads mapping on human genome, were assigned to known miRNAs, but this number does not reflect the percentage (86%) indicated in the next sentence, why? The authors could explain better this part of results.

3. In the third paragraph of the results the authors indicate the number of the dysregulated miRNAs found in ICC versus two types of controls (67 miRNAs were found ICC vs D-NT and 316 in ICC vs N-NT), how many common miRNAs are there between these two comparisons? In other words, were all 67 miRNAs included into the 316 ones? Do they share the same direction of deregulation?
4. In the five paragraph of the results section, the authors analyzed only the deregulation of miRNAs in ICC versus D-NT according to the histological differentiation, but they did not include information on the same analysis using the other normal control (N-NT) which it is shown in figure 3. For instance, how many miRs are deregulated in ICC vs N-NT? And how many ones are common between the two subgroups? In addition, maybe a mistake is present at line 163, pag 7 where the authors wrote “(N-NT)” instead to “D-NT”.

5. In the next paragraph of the same section, an addition of a table summarizing the results obtained from the use of the two different platforms is suggested in order to help the reader in the understanding of the paragraph. For instance, how many deregulated miRs are common between the two analyses considering the comparison respect the two controls? Instead, the authors did not specified the control used in the supplementary fig.1. In addition, the authors did not find any miRs deregulated in well differentiated ICC versus D-NT, but in the cited previous work, they found 12 ones performing the same comparison; why this difference?

6. Finally in the discussion section, that authors could add additional references regarding the deregulated miRNAs identified in this work already known in literature (for instance miR-141 or miR-200b etc..) in order to support and to demonstrate the reproducibility and the importance of data obtained.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interest's below