Reviewer’s report

Title: The expression of microRNA-375 in plasma and tissue is matched in human colorectal cancer

Version: 2 Date: 18 June 2014

Reviewer: Joana Carvalho

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This manuscript by Xu et al, aimed at investigating the expression and clinical significance of plasma and tissue miRNAs in CRC patients. Although, the expression levels of miRNAs have been extensively reported in CRC either by analysing tissues or plasma samples, there are few reports addressing the expression of miRNAs in tissue and matched plasma samples of CRC patients. From this point of view alone, this manuscript is interesting. However, after having carefully reviewed the manuscript as well as figures and tables, I feel that some points should be clarified and the data should be more carefully presented.

The authors should consider the following:

Major Compulsory Revisions

1- Abstract

The authors should better clarify the aim of the study. For instance, they should mention what they mean by significance in CRC. Are they looking for non-invasive diagnostic, prognostic or predictive biomarkers?

“In the screening phase, a set of 42 miRNAs were gained which showed…” With this sentence, it seems that all 42 miRNAs were upregulated or gained at the genomic level… Please re-phrase.

2- Background

In general, the introduction is well organized although some references should be added particularly in sentences 95-96; 98. The last paragraph of the background section should be more carefully written without detailed information concerning materials and methods. For instance the aims of the study should be clearly written and a brief description of the main findings should be pinpointed.

3-Materials and Methods

It would be nice to see the clinicopathological characteristics of all patients and tumours summarized in a table. It would be nice to have more clinical data on table 1 and consider presenting it in this section.

4- Results

Circulating miRNA microarray profiling

The authors have mentioned that 42 miRNAs were differentially expressed when
comparing plasma samples of 6 CRC patients with plasma samples of 6 healthy controls. Besides this description, it would be nice to see hierarchical clustering images of the differentially expressed miRNAs. In the legend of table 2, it would be nice to explain what is the meaning of fold change.

Validating of selected miRNAs by qRT-PCR

Upon the screening phase, the authors decided to validated 5 miRNAs by using a larger cohort of plasma samples from CRC patients (n=88) and healthy individuals (n=40). Overall, the results of this section should be described in a more organized way, to better lead to conclusions and to their importance.

Concerning this, the authors should consider the following:

1- Mention if they could in fact validate the expression of the selected miRNAs in this larger cohort. It would be nice to see the plots representing the expression levels of the 5 miRNAs in the screening and validation series. By comparing the data described in table 2 and table 3 (plasma), only miR-206 was in fact validated and found to be highly expressed in the plasma of CRC patients. Do the authors have any explanation for this?

2- The authors should clarify what was the purpose of using a screening and validation series, since they have not pursue the only miR that was in fact validated.

3- Although, the authors have mentioned that the expression levels of miR-150, miR-125b and miR-126b were not statistically different when comparing plasma of CRC patients and plasma of controls, it would be nice to add these plots to figure 1. In the same line, please add the plot of miR-206 expression in tissues to figure 2.

The legend of x-axis of figure 1 is confusing. Please consider instead of “T” writing CRC patients (n=88) and instead of “N” writing healthy controls (n=40).

4- It would be nice to analyse the expression levels of miR in CRC tissues and matched normal mucosas by using the $2^{\Delta\DeltaCT}$ in order to better understand how the expression of each selected miR varies when comparing tissue carcinoma and matched normal mucosa of each patient.

5- Discussion

In this section, the authors mentioned that miR-150, miR-125b, miR-206, miR-126* as well as miR-375 were previously quantified and associated with clinicopathological characteristics of CRC patients. Concerning the current study, did the expression levels of these miRNAs associate with some of the clinicopathological variables of CRC patients?

It was recently identified a panel of miRNAs that could be of potential use in the development of a multi-marker blood test for early detection of CRC (PLoS One. 2013 May 14;8(5):e62880. doi: 10.1371/journal.pone.0062880) The authors should comment on this in comparison with the results obtained in their study.

Did the authors have any possible explanation for the higher expression levels of
miR-125b and miR-206 in the plasma of CRC patients in comparison with the corresponding tumour tissue?

Did the authors know which mechanism or mechanisms are underlying miR-150, miR-125b, miR-206, miR-126* as well as miR-375 de-regulation in CRC?

Minor issues not for publication
Typographical errors:
- phase instead of “phrase” (abstract and results sections)
- target instead of “targeted” (materials and methods section)
- verify instead of “verity” (results section)

Stylistic suggestion: the overall manuscript should be uniformed in terms of American or British English.

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

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests