Reviewer's report

Title: Small RNAs in metastatic and non-metastatic oral squamous cell carcinoma

Version: 2 Date: 12 March 2015

Reviewer: Luciano Cascione

Reviewer's report:

The manuscript of Dr. Patricia Severino and co-workers analyzed sequencing data from metastasized and non-metastasized OSCC. They identified several microRNAs differentially expressed between the two classes of study, they evaluated them in plasma samples, identified other class of small RNAs involved to metastatic phenotype and a new putative microRNA molecule.

The findings are based on a limited number of samples (18 samples, 8 non-metastatic and 10 metastatic tumor samples) and are not further validated on a larger cohort, even they used a bigger cohort of plasma samples. In general, the manuscript is nicely written and the analysis strategy is straightforward. The findings are relevant in this field of research and do not only support previous findings of the group, but also gain novel insights into the relevance of miRNAs in oral squamous cell carcinoma metastatic cascade. Nevertheless,

I have some doubts that should be clarified before considering the manuscript for publication.

Major:

- The last sentence of the abstract is confusing "...suggest the evaluation of miRNA detection for the evaluation of oral squamous cell carcinoma metastatic potential.". Please, clarify this sentence.

- Please use the new microRNA nomenclature. The two arms of a microRNA were previously annotated as mir/mir*, with the general idea that mir*(mir-star) were degraded. But as we started sequencing at higher coverage, previously thought mir* were also found to be expressed at reasonable depth (specifically in developmental stages/tissues). miR-Base changed the nomenclature denoting the two arms as mir-5p/mir-3p.

- They sequenced 18 OSCC samples, the author wrote "Eighteen small RNA libraries were constructed, one for each OSCC sample: 8 samples presented lymph node metastasis at the time of diagnosis and 8 samples did not present metastasis.". How do they come from N0=8+N1=8 to a total of 18 samples (Table 1)?

- Please, specify in Table 1, (add a column) which sample presented lymph-node
metastasis and which were metastasis free.

- Please upload your data to SRA (http://www.ncbi.nlm.nih.gov/sra) or specify where your sequencing data is available.

- The authors wrote "Six miRNAs were found to be upregulated in metastatic OSCC with statistic significance.". They did not mention the p-value cutoff used to consider a miRNA statistically differentially expressed.

- The p-values reported in table 2, are they adjusted for multiple testing?

- The authors evaluated the expression of the differentially expressed miRNAs in N0 vs N1 patients in plasma of 30 patients by real-time. Did they performed any statistical test on the real-time data? Do they have a p-value of each miRNA? If so, did they adjust the p-values for multiple testing?

Minor:

- Why do you only focus on miRNAa predictions in your miRNA target analysis

- Page 8, "...comprehension of it mechanistic role " should be "...comprehension of its mechanistic role"

- Page 16 "Characterizing this putative precurso miRNA..." should be "Characterizing this putative precursor miRNA..."

- The author should cite and use miRandola, the Extracellular/Circulating microRNAs database. miRandola: Extracellular Circulating microRNAs Database PLoS ONE 7(10): e47786 doi:10.1371/journal.pone.0047786

**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:**

I declare that I have no competing interests