Reviewer’s report

Title: Overexpression of miR-21-5p as a predictive marker for complete tumor regression to neoadjuvant chemoradiotherapy in rectal cancer patients

Version: 1 Date: 29 July 2014

Reviewer: Pedro Borralho

Reviewer’s report:

Major Compulsory Revisions:

1. General approach by the authors is sound, and the clinical question is relevant. The identification of biomarkers to predict complete pathological response in rectal tumors, allowing the identification of patients not requiring surgery, would be extremely relevant. However, the present study presents some limitations, particularly relating to the size of sample populations used both for training and validation sets, which is quite limited. The inclusion of additional samples in both sets is highly recommended. The inclusion of non-responders in the analysis (therapy resistant) would also be interesting.

2. In addition, it would be important to validate the data by an additional independent method, for instance qRT-PCR, for hsa-miR-21 expression, to validate and develop a faster, more easily implemented, and cheaper method compared to deep-sequencing. (alternatively, in situ hybridization for mature miR-21 in FFPE sections may also be an alternative).

3. Regarding data from the “potential role of miR-21-5p on treatment response”, the analysis of the miR-21-5p putative target expression should be included, at least as supplementary data. Since it is widely known that miR-21 expression is increased in the vast majority of human cancers, and it is also widely associated with decreased response to chemotherapy, it is somewhat counter-intuitive that increased expression of miR-21 may be a biomarker for complete pathological response following neoadjuvant chemoradiotherapy. Nevertheless, the suggested link between increased expression of miR-21 from incomplete responders to complete responders, associated to a concomitant decreased expression of SATB1 protein from incomplete responders to complete responders, may be relevant to provide mechanistic explanation to the data here reported by the authors. In this regard, the authors evaluated SATB1 expression by qRT-PCR in 11 samples, obtaining concordant results, although not reaching statistical significance. This data is relevant and should be complemented by evaluation of STAB1 protein expression (e.g. by western blot in total protein extracts obtained from tumor biopsies of incomplete and complete responders), and also by increasing the sample universe to use. Evaluation of protein expression is particularly relevant since miRNA target gene regulation in some instances does not lead to major changes in RNA abundance, but does in fact lead to abundant reduction of the target protein. If possible, parallel evaluation of
RNA and protein from the same sample would be ideal, and currently there are simple methods allowing this simultaneous isolation and evaluation of both molecular species from the same tumor specimens.

Discretionary Revisions:

4. The authors data would be importantly complemented if they could perform experiments to demonstrate in vitro that miR-21 directly binds to SATB1 3`UTR (e.g. luciferase reporter assays) and if possible, that increased miR-21 expression in rectal cancer cell lines does increase tumor cell response to chemoradiotherapy used in rectal cancer management. These aspects would importantly enrich the present manuscript, and increase its relevance. In addition, it would be important to include whole transcriptome data mentioned by the authors, from these samples regarding the expression of known and validated miR-21 direct targets, to better grasp all possible changes occurring in these miR-21 target genes.

5. The manuscript by Drebber et al, 2011 (INTERNATIONAL JOURNAL OF ONCOLOGY 39: 409-415, 2011) should be included in the discussion of the present manuscript.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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.