Reviewer’s report

Title: Accuracy of microRNAs as markers for the detection of neck lymph node metastases in patients with head and neck squamous cell carcinoma

Version: 1 Date: 26 January 2015

Reviewer: Chamindie Punyadeera

Reviewer’s report:

Comments:
• The article is well written and it is important to the field of HNSCC.
• There is work done by number of groups highlighting the importance of miRNA to diagnose HNSSC. Please include the following recent publications that cover the importance of miRNA regulation in HNSCC. Please reference the following publications.
  1. C Salazar, R Nagadia, P Pandit, J Cooper-White, N Banerjee, N Dimitrova, ... Cellular Oncology 37 (5), 331-338
  2. C Salazar, D Calvopiña, Expert review of molecular diagnostics 14 (8), 1033-1040
  3. R Nagadia, P Pandit, WB Coman et al, Cellular Oncology 36 (1), 1-7 J
• HNSCC is 5 different subtypes of cancers and as such it is very heterogeneous. Both biologically and clinically these cancers differ based on the anatomical site of origin. Increasing there is evident that grouping these cancers as one type would not allow clinically useful biomarkers to be identified. Most of the patients used in this study are oral cancer patients. If so, recurrence is lower as opposed to HPV-positive. Please justify the selection of the sample cohort.
• Studies show that 10-40% of HNSCC patients with histopathologically negative neck lymph nodes eventually develop regional metastases, suggesting that metastatic cells present in the lymph nodes could not be detected at diagnosis Please specify which type of HNSCC? HPV +ve or negative. Recurrence both local and distant metastasis is higher in HPV+ve cancers.
• Under statistical analysis please explain whether the data set is normally distributed. If that is the case you can use T test. If not you need to use non-parametric analysis. This section needs to be justified
• Mean Ct values of U6 and U47 small nuclear RNAs were used for normalization, and the mean Ct of 10 non-metastatic lymph nodes was used as reference.
Please clarify ct of 10 ? is this the threshold? What was the average Ct for the house keeping genes between FNA and FFPE samples
• Reviewer found it very difficult to follow the study design. Initially the authors undertook a discovery study where by FFPE samples from LN positive and negative patients were compared. Followed by a retrospective study whereby the authors collected FNA and Ln from both ln+ve and Ln-ve patients. Is this the case? Supplementary table 2 indicates that: Clinical features of the 79 HNSCC patients enrolled in the FNA validation data set – 113 FNA biopsies were collected from patients? How come initially 79 and then 113?

• Explain the youden index and how this was calculated?

• Under method section also includes the RNA purity and integrity. It is difficult to isolate intact good RNA from FFPE slides. It is worth while letting the readers know what strategies were employed to yield high RNA. Also, there is no mention of DNASse treatment? Why> Is this because the procedure that you follow selectively remove large oligonucleotides?

• Minor comments: Discussion is too long. Needs to be focused.

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