Reviewer's report

Title: microRNA-99a acts as a tumor suppressor and is down-regulated in bladder cancer

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Reviewer: Takahiro Ochiya

Reviewer's report:

In this manuscript, Feng et al. showed that miR-99a was down-regulated in bladder cancer patients and could be used as diagnostic marker. The overall concept of this study was correct and the experiments in this manuscript were well considered. The experimental method was pertinent and supported the conclusions. However, the work was too descriptive at this stage and major modification appeared necessary for the publication of this manuscript in “BMC Urology”.

Major Compulsory Revisions

1. The authors performed statistical analysis by using T-test. The authors should use another statistical analysis method because T-test is improper in these experiments. (e.g. Table 2 as multivariate statistics, Figure 1C and 2 as Mann-Whitney U test or Pearson’s chi-square)

2. In Figure 3B and 3D, the authors performed cell growth assay by using CCK-8 kit. The authors should check the reason why cell growth is inhibited in bladder cancer cells over-expressing miR-99a (e.g. cell cycle arrest).

3. The authors should provide detailed clinical data regarding bladder cancer patients.

4. In Figure 1B, it was unclear why the authors decided the cutoff at 1.5. The authors should explain that point.

5. It was already reported that miR-99a is down-regulated in low-grade bladder cancer patients (Catto et al., Cancer Research. 2009). The difference of this manuscript with this previously published paper is unclear for the reader. It would be valuable to discuss the differences between both studies in the introduction section.

6. In figure 3B and 3D, the authors performed cell growth assay using two cell lines, HT1376 and J82. It was unclear why the authors did not use T24 cell line, which showed down-regulation of miR-99a expression. The authors should explain that point.

7. The authors should provide histologic examination of normal and bladder cancer patients stained with HE.

8. The authors should shorten the manuscripts in the discussion section.
**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