Reviewer’s report

Title: Establishment and characterization of pleomorphic adenoma cell systems: an in-vitro demonstration of adenoma-carcinoma sequence in the salivary gland

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Reviewer: William C Kisseberth

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This is a generally very well written manuscript by Maruyama et al. In this study, the authors report the establishment of five cell “systems” derived from primary cultures from a single benign (based on morphology) pleomorphic adenoma. Each of these cell systems is clonally derived from limiting dilution of a low passage culture of the original tumor. The cell systems are characterized based on morphology, S-100 and cytokeratin IHC, classical cytogenetics, p53 protein expression and exon sequencing, and FISH. The authors conclude that pleomorphic adenoma contains cells with genetic alteration even when its histology is benign, and that carcinoma cells may develop from some of the population of benign forms. Therefore, an adenoma-carcinoma sequence could be considered to exist in the salivary gland.

This is a largely descriptive paper. These findings do not really clarify whether benign adenoma cells were transformed in vitro, or whether instead “atypical” cells that were present within the histologically benign tumor in vivo merely expanded in vitro.

Major Compulsory Revisions:

With respect to methodology:

1. It would be useful to culture cells from normal salivary tissue, too, to determine if the benign adenoma’s growth in culture was really different than that of normal tissue.

2. Xenografts should be done with pieces of the original adenoma specimen and/or primary culture, too. If the cell systems are being defined as malignant based on growth as xenografts, then you should also establish that the original tumor was biologically benign, not just morphologically benign.

3. Tumorgenicity should be based on injections into more than just 2 mice per cell system. No statement was given as to whether or not tumors were established in both mice for each cell system. A table showing the number of tumor "takes"/number mice injected and tumor latency for each cell system would be helpful.

Minor Essential Revisions:

“Conclusions” in the Abstract is misspelled.
Figure 3: Panel A. histograms a and c indicates chromosome numbers less than 97, contradicting the legend.

Figure 5: Need to indicate from which sample (primary or which cell system) THESE results are from.

**Level of interest:** An article of limited interest

**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.